

**EVALUATION OF MYCOLOGICAL STATUS AND DETECTION OF ITS TOXINS
IN BASTERMA AND LUNCHEON IN ASSUIT CITY**

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ABSTRACT

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A total of 60 samples of luncheon and basterma (30 from each) were gathered randomly from different supermarkets in Assuit Governorate. They analyzed for determining of the total fungi and yeast count using direct plate method on dichloran rose-bengal agar medium as well as identification of the isolated fungi. Mycotoxins production were detected using Thin Layer Chromatographic technique. The results revealed that total mould count / gm of examined samples of luncheon and basterma were 1.86×10^2 and 1.29×10^2 respectively, The total yeast count / gm was 1.8×10^1 in luncheon and 1.1×10^1 in basterma. *Aspergillus* was the most prevalent genus contaminating 96.7% and 53.3 % of luncheon and basterma samples respectively and comprising 30.9 % and 23.6% of the total fungi in both types of samples respectively, the predominant species of *Aspergillus* in luncheon were *A.flavus* (56.7%), *A.Fumigatus* (23.3), *A.oryzae* (20%), and *A.niger* (20%). While those recovered from basterma were *A.Flavus* (10%) *A.niger* (36.7%) and *A.tamari* (6.6%). *Penicillium* occupied the second prevalent genus, it was encountered in (40%) and (33.3%) of samples of luncheon and basterma respectively and matching (8.3%) and (13.6%) of total fungi on two types of samples respectively, the predominant species of *Penicillium* recovered from luncheon were *P.oxalicum* (23.3%), *P.janthinellum* (10%) and *P.glabrum* (6.6%), and those recovered from basterma were *P.oxalicum* (16.7%), *P.expanus* (10%) and *P.brevicompatum* (6.6%). *Fusarium* was the third frequent genus Contaminating (23.3%) of both luncheon and basterma and constituting (4.9%) and (7.9%) of total fungi on luncheon and basterma respectively, one spp. of *Fusarium* could be detected from luncheon, it was *F. verticillioides* (23.3%), and two spp. from basterma (*F.nygamai*6.7%) and (*F.verticillioides*13.3%). The remaining fungi was *Rhizopus* spp. which contaminate 13.3 % and 6.7% of luncheon and basterma respectively and comprising 2.9% and 2.1% of the total fungi of two types of samples respectively. In this study 4 types of mycotoxins were isolated from 26.7% of luncheon and from 13.3% of basterma in which Aflatoxin B1, Ochratoxin A, *Stregmatocyein* and *Zaeralenone* were detected in 13.3%, 3.3%, 6.7% and 3.3% of luncheon respectively. The public health hazard associated with its consumption are discussed.

تقييم الحالة الفطرية وتحديد مدي افرازها للسموم في البسطرمة واللاتشون في مدينة اسيوط

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لقد تم جمع ٦٠ عينة من اللاتشون والبسطرمة بواقع ٣٠ عينة من كل نوع جمعت عشوائيا من محلات السوبر ماركت المختلفة بمحافظة اسيوط ، وتم تحليل هذه العينات لمعرفة العدد الكلي للفطريات والخمائر وذلك باستخدام طريقة الزرع علي الوسط الغذائي داي كلوران روز بنجال وكذلك التعرف علي الفطريات المعزولة. ولقد تم معرفة السموم المفترزة باستخدام طريقة (Thin Layer Chromatographic). أظهرت النتائج ان العدد الكلي للفطريات لكل جرام في كلا من اللاتشون والبسطرمة $10^2 \times 1.86$ ، $10^2 \times 1.29$ علي التوالي وكان العدد الكلي للخمائر لكل جرام في اللاتشون $10^1 \times 1.1$ وفي البسطرمة $10^1 \times 1.1$ وكان فطر الأسبرجلس هو الأكثر إنتشاراً فكانت نسبته 96.7 % ، 53.3 % في اللاتشون والبسطرمة علي التوالي وكان يمثل 30.9 % ، 23.6 % بالنسبة للعدد الكلي للفطريات المعزولة من نوعي العينات علي التوالي وكانت الأنواع السائدة لهذا الفطر في اللاتشون هي أسبرجلس فلافس 56.7 % ، أسبرجلس فيوميجاتس 23.3 % ، أسبرجلس أوريزا 20% وأسبرجلس نيجر 20% بينما الأنواع التي أكتشفت في البسطرمة كانت أسبرجلس فلافس 10% ، أسبرجلس نيجر 36.7% وأسبرجلس تماري 6.6%. ويعتبر فطر البنسيليم وهو الثاني إنتشاراً موجود بنسبة 40% ، 33.3% في عينات اللاتشون والبسطرمة علي التوالي وكان يمثل 8.3% ، 13.6% بالنسبة للعدد الكلي للفطريات المعزولة من نوعي العينات علي التوالي وكانت الأنواع السائدة لهذا الفطر في اللاتشون هي بنسيليم أوكزاليك 23.3% ، بنسيليم جانتيليم 10% وبنسيليم جلاسيريم 6.6% بينما الأنواع التي اكتشفت في البسطرمة كانت بنسيليم أوكزاليك 16.7% ، بنسيليم إكسبانييس 10% وبنسيليم بريقي كومباكتم 6.6%. فطر الفيوزيريم هو الثالث إنتشاراً حيث كان موجود بنسبة 23.3% في كلاً من عينات اللاتشون والبسطرمة وكان يمثل 4.9% ، 7.9% بالنسبة للعدد الكلي للفطريات المعزولة من اللاتشون والبسطرمة علي التوالي. ولقد وجد نوع واحد من الفيوزيريم في عينات اللاتشون وهو فيوزيريم سيلويدس 23.3% 23.3% ونوعان في عينات البسطرمة وهما فيوزيريم نيجامى 7.6% ، فيوزيريم فيرتى سيلويدس 13.3% وكان فطر الريزوبس موجود بنسبة 13.3% ، 6.7% في عينات اللاتشون والبسطرمة علي التوالي وهذا يمثل 2.9% ، 2.1% من العدد الكلي للفطريات المعزولة. في هذه الدراسة تم عزل ٤ أنواع من السموم الفطرية بنسبة 26.7% في اللاتشون ، 13.3% في البسطرمة وكانت هذه السموم هي أفلاتوكس B ، أوكراتوكس A ، ستريجما توسينين ، زيرالينون بواقع 13.3% ، 3.3% ، 6.7% ، 3.6% في اللاتشون علي التوالي بينما وجدت هذه السموم بنسبه 3.3% لكلاً منها في عينات البسطرمة. ولقد تم مناقشة النتائج واهمية الفطريات والخمائر التي تم عزلها للحفاظ على الصحة العامة.

Key words:

INTRODUCTION

Meat products are the most palatable and fast food meat. They are considered the best alternative for the fresh meat due to their low price, easily preparation and palatability (Frazer and Westhoff, 1988).

Luncheon meat usually consists of finely chopped meat and fat, with or without some added cereal and using, cured with salt and nitrite and heat processed (Ranken, 1984), while basterma was prepared from fresh salted meat coated with spice pasta and

stored at room temperature (Refai *et al.*, 2003).

Although meat products are available sources of protein but they are also an important potential sources of serious diseases if they contaminated by different moulds which are widely distributed in nature (Mossel, 1982; Foster *et al.*, 1983).

Mycotoxins comprise a structurally diverse family of fungal toxins, many of which have been strongly implicated as chemical progenitors of toxicity in man and animal (Ramos *et al.*, 1996).

Further more, afltoxins are relatively stable compounds, not destroyed by processing and even be concentrated (Carvajal *et al.*, 2003; Honikel, 2003).

The presence of toxinogenic moulds in a meat product does not automatically mean the presence of mycotoxins, especially if growth has not occurred, but rather that a potential for mycotoxin contamination exists. On the other hand, the absence of toxinogenic moulds doesn't guarantee that the meat products are free of mycotoxins, since the toxins may persist long after the moulds have disappeared (Ismail *et al.*, 1994).

Fungal contamination is considered as one of the important spoilage agent of meat and meat products, occurring during slaughtering of animals, transportation, processing of meat products, and using of contaminated equipment, or other additives and spices (Misra, 1983; Abd el- Rahman, 1987).

However, the spices of mycotoxins in human food may be directly from fungal contamination or indirectly through contamination of feed stuffs consumed by the animal (Hussien *et al.*, 1997).

Mould contamination not only causes deterioration of food and feed but also can

adversely affected the healthy of humans and animals since they are capable of producing toxic metabolic known as mycotoxins causing cases of food poisoning, liver cancer in human (Mossel, 1982 and Foster *et al.*, 1983).

Under adversity of moisture, PH, temperature conditions economic losses and various degrees of food decomposition were taken place, they can cause lipolytic and proteolytic spoilage (Besancon *et al.*, 1992; Jakobsen and Narvhus, 1996).

Since fungi influence, the biochemical character and flavour of the product and its appearance is commercially undesirable and often result in down grading of the product (Bouton and Grappin 1995; Beuvier *et al.*, 1997).

However, mould and yeast have no role in mould spoilage or toxin production if the garlic content in the pasta in basterma has not less 35% from the total content (El-Khateib *et al.*, 1987).

The main objective of this study is to study the incidence of the incidence of mould and yeast in meat products (luncheon and basterma).

to obtain a spore suspension in 0.2% (v/v) aqueous tween 80 (Smith and Onions, 1983).

2-Cultivation of fungal isolates and extraction of mycotoxins:

According to El-Kady and Moubasher, 1982.

3- Purification of the crude extracts:

The extract was purified by column chromatography, which described by Josefsson and Moller, 1977.

4- Thin-Layer Chromatographic determination of mycotoxins:

For qualitative analysis of mycotoxins, Thin Layer Chromatographic (TLC) technique was applied (AOAC 1975, El-Kady and Moubasher, 1982; Dorner 1998).

5- Statistical analytical results was carried by standard error of proportion (Z).

MATERIALS and METHODS

Collection of samples:

Sixty samples of luncheon and basterma (30 for each) were collected from different supermarkets in Assiut city. The samples were placed in a sterile plastic bags and transferred to laboratory and kept at 4°C until fungal analysis.

Enumeration and isolation of fungi:

The direct plating technique using Dichloran – Ros – Bengal media (Pitt and Hocking, 1985) was employed for isolation of fungi from luncheon and basterma.

Determination of mycotoxins produced by fungal isolates applied in the following steps:

1- Fungal isolates:

Seven days old culture of each isolate on PDA (Potato dextrose agar) slope was used

RESULTS

Table 1: Incidence of isolated fungi in examined luncheon and basterma samples:

Fungal spp.	Luncheon		Basterma	
	No. of +ve samples	Percentage	No. of +ve samples	Percentage
<i>Aspergillus</i>	29	96.7	16	53.3
<i>A. flavus</i>	17	56.7	3	10
<i>A. fumigatus</i>	7	23.3	-	-
<i>A. oryzae</i>	6	20	-	-
<i>A. niger</i>	6	20	11	36.7
<i>A. Tamarri</i>	-	-	2	6.6
<i>Fusarium</i>	7	23.3	7	23.3
<i>F. verticillioides</i>	7	23.3	4	13.3
<i>A. nygamai</i>	-	-	2	6.7
<i>Penicillium</i>	12	40	10	33.3
<i>P. glabrum</i>	2	6.6	-	-
<i>P. oxalicum</i>	7	23.3	2	6.6
<i>P. janthinellum</i>	3	10	-	-
<i>P. expansum</i>	-	-	3	10
<i>P. brevicompactum</i>	-	-	5	16.7
<i>Rhizopus spp.</i>	4	13.3	2	6.7
<i>Yeast spp.</i>	7	23.3	18	60

Table 2: Total count of isolated fungi in examined luncheon basterma samples: and basterma samples:

Fungal spp.	Luncheon		Basterma		Z
	No. of +ve samples	Percentage	No. of +ve samples	Percentage	
<i>Aspergillus</i>	63	30.9	33	23.6	0.28*
<i>A. flavus</i>	29	14.2	5	3.6	
<i>A. fumigatus</i>	10	4.9	-	-	
<i>A. oryzae</i>	10	4.9	-	-	
<i>A. niger</i>	14	6.9	26	18.6	
<i>A. Tamarri</i>	-	-	2	1.4	
<i>Fusarium</i>	10	4.9	11	7.9	0.12*
<i>F. verticillioides</i>	10	4.9	8	5.7	
<i>A. nygamai</i>	-	-	3	2.1	
<i>Penicillium</i>	17	8.3	19	13.6	0.21*
<i>P. glabrum</i>	3	1.5	-	-	
<i>P. oxalicum</i>	10	4.9	3	2.1	
<i>P. janthinellum</i>	4	1.96	-	-	
<i>P. expansum</i>	-	-	6	4.3	
<i>P. brevicompactum</i>	-	-	10	7.1	
<i>Rhizopus spp.</i>	6	2.9	3	2.1	0.03*
<i>Yeast spp.</i>	18	8.8	11	7.9	0.22*
Total	186		129		

* No significant difference.

Z: standard errier of propotion

Table 3: Detection of mycotoxins produced by fungi isolated from Luncheon and Basterma using (TLC) technique:

Types of samples	No. of +ve isolates	No. of tve isolates producing toxin	Fungal spp.	Detected toxin
Luncheon	17	4	<i>Aspergillus flavus</i>	Aflatoxin B ₁
Luncheon	7	2	<i>Aspergillus fumigatus</i>	Stregmatocystein
Luncheon	6	0.0	<i>Aspergillus oryzae</i>	- ve
Luncheon	6	0.0	<i>Aspergillus niger</i>	- ve
Luncheon	7	1	<i>Fusarium verticilliodes</i>	Zearalenone
Luncheon	2	0.0	<i>Penicillum glabrum</i>	- ve
Luncheon	7	1	<i>Penicillum oxalicam</i>	Ochratoxin A
Luncheon	3	0.0	<i>Penicillum janthinellum</i>	- ve
Luncheon	4	0.0	<i>Rhizopous spp.</i>	- ve
Basterma	3	1	<i>Aspergillus flavus</i>	Aflatoxin B ₁
Basterma	11	0.0	<i>Aspergillus niger</i>	- ve
Basterma	2	1	<i>Aspergillus tamari</i>	Stregmalocystin
Basterma	2	0.0	<i>Fusarium nygamai</i>	- ve
Basterma	4	1	<i>Fusarium verticillioides</i>	Zearalenone
Basterma	2	0.0	<i>Penicillum brevicompatum</i>	- ve
Basterma	3	0.0	<i>Penicillum expansum</i>	- ve
Basterma	5	1	<i>Penicillum oxalicum</i>	Ochratoxin A
Basterma	2	0.0	<i>Rhizopous spp.</i>	- ve

DISCUSSION

Earlier studies reporting environment as a source of food contaminant moulds (Kure *et al.*, 2001; Mizakova *et al.*, 2002; Battilani *et al.*, 2007; Sorensen *et al.*, 2008). In this study the results revealed that all examined samples (100%) were contaminated with moulds.

Results given in Tables (1&2) revealed that total mould count / gm of examined samples of luncheon and basterma were 1.86×10^2 and 1.29×10^2 respectively. The count in luncheon samples were lower than that reported by Abdel-Rahman *et al.* (1995) (9.2×10^3), Roushdy *et al.* (1996) (1.5×10^6), Hussien *et al.* (1997) (2.7×10^5), Sayed *et al.* (2000) (2.68×10^2); El-Tabiy (2006) (2.4×10^3). The higher total mould count / gm of basterma were reported by Abdel-Rahman *et al.* (1995) (1.4×10^4), Hussien *et al.* (1997) (9.2×10^4) and Refai *et al.* (2003), (the count varied from 10 (2) to 10 (5)).

Results in Tables (2) indicated that there was no significance difference in count between luncheon and basterma.

Although the total mould count of any food article is not indicative of its safety for consumption yet it is of supreme importance in judging the hygienic condition under which it has been produced, handled and stored (Martin and Lowery, 1992). The variation in quantitative estimation of mould counts might be attributed to improper sanitation during slaughter, preparation, manufacturing, additives specially using spices of low quality or during transportation, storage and marketing of the products (Abobaker, 1986; Refai *et al.*, 1990; Roushdy *et al.*, 1996).

The mould count in both products samples may be attributed to the widespread distribution of mould in nature. Fungi are normal inhabitant of wool of the animal, also have ability to be adopted at wide range of temperatures (Nasser *et al.*, 1998). The most common fungal genera in both luncheon and

basterma (Tables 1&2) were *Aspergillus* then *Penicillium*. This observation was relatively agree with Wu *et al.* (1974); Abdel-Rahman *et al.* (1984); Beuchat (1987); Lotfi *et al.* (1987), Sayed *et al.* (2000); Mohamed and Hussien (2004); Aideia (2005); El-Tabiy (2006), while Roushdy *et al.* (1996) and Hussein *et al.* (1997) reported that *penicillium* was the common fungal genera followed by *Aspergillus* in different meat products.

Tables (1& 2) revealed that *Aspergillus* was the most prevalent genus contaminating 96.7% and 53.3% of luncheon and basterma respectively and comprising 30.9% and 23.6% of the total fungi of the same samples respectively, while Azza *et al.* (1997) detected *Aspergillus* in 32.6% of luncheon samples and in 31.1% of basterma.

From Table (1, 2) The predominant species of *Aspergillus* recovered from luncheon samples were *A. flavus* (56.7 %), *A. Fumigatus* (23.3%), *A.oryzae* (20%) and *A. niger* (20%), while species of *Aspergillus* recovered from samples of basterma were *A. flavus* (10%), *A. niger* (36.7%) and *A. tamari* (6.6%). The results obtained by Hussien *et al.* (1997) that *A. niger* was 12.5 % in luncheon and 10.9% in basterma, while Sayed *et al.* (2000) reported that *A. flavus* was moderately occurred in luncheon.

A.fumigatus has a marginal xerophile nature ranges from 12°C to 55°C Ayerst, (1966), he also reported that *A.niger* is more prevalent in warmer climates in field saturation and stored foods Al-Doory (1980) revealed that *Aspergillus* spp. were incriminated in pulmonary aspergillosis, skin infection, sinusitis and otitis for food handlers.

Also Tables (1, 2) showed that *Penicillium* occupied the second prevalent genus. It was encountered in 40% and 33.3% of the samples of luncheon and basterma respectively and matching 8.3% and 13.6% of the total fungi on two types of samples respectively, Hussien *et al.* (1997) could detect *penicillium* in 26.4% of luncheon and in 42.7% of basterma, Dereje *et al.* (2010) indicated that most of the *P. nalgiovense* have the ability to produce toxins on the

products and can become potential food safety hazards.

The predominant species of *Penicillium* recovered from luncheon samples were *P.oxalicum* (23.3%), *P.janthinellum* (10%) and *P.glabrum* (6.6%), and those recovered from basterma samples were *P.oxalicum* (16.7%), *P.expanus* (10%) and *P. brevicompactum* (6.6%). Some investigators could detect another spp. of *penicillium* in luncheon Abdel-Rahman and El-Bassiony, (1984) found *P. verrucosum var cyclopium* in 94.5%, Sayed *et al.* (2000) isolated *P.viridicatum* from 20%.

Penicilloles are diseases induced by pathogenic strains of *Penicillium* in human involving the upper respiratory tract and lungs, carcinogenic effects are also caused by mycotoxins produced by many *Penicillium* species (Mossel, 1982).

Fusarium was the third frequent genus contaminating 23.3% of both luncheon and basterma and constituting 4.9% and 7.9% of total fungi on luncheon and basterma respectively, one spp. of *Fusarium* was detected in luncheon samples it was *F. verticillioides* (23.3%), and two spp. were detected in basterma samples, they were *F. nygamai* (6.7%) and *F. verticillioides* (13.3%). (Tables 1&2).

The remaining fungi was *Rhizopus* spp. which contaminat 13.3% and 6.7% of the samples of luncheon and basterma comprising 2.9%and 2.1%of the total fungi, respectively, (Tables 3&4). Most of these fungi had been isolated previously, but with different frequencies from meat products, Hefnawy (1980); Hegazi *et al.* (1992); Zaki *et al.* (1995); Nagat, (1997).

Yeasts derived from fungal analysis of luncheon samples revealed 7(23.3%) which comprised 8.8% in luncheon samples (Table 1) and revealed 18 (60%) in basterma samples which comprised 7.8% of the total fungi (Table 2). While the total yeast/gm in luncheon and basterma were 1.8X10 and 1.1X10 respectively.

The growth of microbes, such as bacteria, yeasts and moulds deteriorate the safety and

quality of food products and cause significant economic loss (Fittenborg *et al.*, 1996; Pitt and Hocking, 1999; Samson *et al.*, 2004), also growth of fungal particles can be facilitated by chains of production processes that each and every process in the chain should be considered to determine where to act (FAO, 2008).

A.flavus is the most important natural source of aflatoxins in the world's food supplies of which are B₁, B₂, G₁ and G₂. The risk of B₁ and B₂ aflatoxins starts when they ingested by a lactating animal and a proportion is hydroxylated to M₁ and M₂ aflatoxin derivatives of lower toxicity but their significant due to the wide spread consumption of mycotoxicated milk by infants (Frobish *et al.*, 1986). Also Aflatoxins produced by *A.flavus* exposed man and animals to acute and chronic toxicity distinguished in four forms: liver damage, liver cirrhosis, induction of tumours and teratogenic effects (Frisvad and Samson, 1991).

As shown in Table (3) in this study 4 types of mycotoxins were detected of which aflatoxin B₁ was detected in four samples luncheon and one sample of basterma, that 23.5% and 33.3% of isolated *A.flavus* could produce this toxin in both luncheon and basterma samples respectively. AFB₁, is highly toxic compound and its occurrence poses a threat to the health of consumers specially young children (Pierides *et al.*, 2000). In addition to aflatoxin Stregmatocyste in toxin was found in two samples of luncheon which produced by 28.5% of isolated *A. fumigatus*, also this toxin was found in one sample of basterma produced by 50% of isolated *A. tamari*.

OchratoxinA was detected in two samples, one in luncheon and the other in basterma in which 14.3% and 20% of *P.oxalicum* could produce this toxin in luncheon and basterma respectively, also zearalenon toxin was detected in one sample of luncheon and one sample of basterma in which 14.3% and 25% of *F.verticillioides* could produce this toxin in luncheon and basterma respectively.

Other species of fungi could not produce mycotoxin.

These data showed that mycotoxin was isolated from 26.7% of luncheon and from 13.3% of basterma in which Aflatoxin B₁, Ochratoxin A, Stregmatocystein and Zaeralenone were detected in 13.3%, 3.3%, 6.7% and 3.3% of luncheon samples respectively and were detected in 3.3% in all samples of basterma. (Ismail and Zaky, 1999) could detect aflatoxin B₁ in 14% of luncheon samples which is nearly similar to our results.

In conclusion, It could be concluded that luncheon and basterma are liable to be contaminated with several fungal species as they are normal inhabitants of air and adapt themselves at a wide range of environmental conditions. strict hygienic measures must be applied during manufacturing. Application of HACCP system (Hazard Analysis Critical Control Point) along the line of production must be paralleled with the end product inspection.

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