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INCIDENCE OF PROTEOLYTIC AND LIPOLYTIC MOULDS AND YEASTS IN SOME READY TO EAT MEAT PRODUCTS

(With 7 Tables)

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مدى تواجد الفطريات والخمائر المحللة للبروتينات والدهون فى منتجات اللحوم المعدة للأكل

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تم تجميع عدد مائة وخمسة عينة عشوائية من منتجات اللحوم (15 عينة من كل من اللانشون البقرى، لانشون الدواجن، لانشون رومى، سلامى، روزبيف، بسطرمة، بسطرامى) وذلك لفحصها ميكولوجيا وكان متّوسط العد الكلى للفطريات / جم هو 1.6 X 10^2 ±29.6 ، 2.8 X 10^2 ±37.4 لكل من منتجات اللانشون البقرى والبسطرمة على التوالى بينما كانت باقى منتجات اللحوم خالية من الفطريات. وكان متوسط العد الكلى للخمائر / جم 1.7 x 10^4 ±3.6 x 10^3 ، 2.8 x 10^4 ±8 x 10^3 ، 8.8 x 10^3 ±3.7 x 10^3 ، 9.4 x 10^3 ±1.4 x 10^3 ، 1.2 x 10^4 ±3.8 x 10^3 ، 4.8 x 10^2 ± 10^2 و 2.6 x 10^4 ± 3.1 x 10^3 لكل من عينات اللانشون البقرى، لانشون الدواجن، لانشون الرومى، سلامى، روزبيف، بسطرمة، بسطرامى على التوالى ووجد ان اعلى عد فى العينات الايجابية للفطريات هو 4 x 10^2 /جم ، وكان اقل عد هو 10^2 بينما كان اعلى عد للخمائر فى العينات التى تم فحصها 5.8 x 10^4 /جم وكان اقل عد 10^2 . وكانت نسبة تواجد الفطريات فى عينات منتجات اللحوم تتبع أجناس الميكور، البنسيليم فركوزم، الكلادوسبوريم بنسب مختلفة تتراوح بين 16.6 % إلى 50 %. كما أن أعلى نسب لتواجد الخمائر فى عينات منتجات اللحوم تتبع أجناس الكانديدا يليها رودوتوريلا ثم كربتوكوكس على التوالى. تم إختبار قدرة العترات المعزولة للفطريات من عينات منتجات اللحوم لإفراز الإنزيمات المذيبة للبروتين والدهون. كما تم ذكر الاهمية الصحية للعترات المعزولة من الفطريات والخمائر وكذلك الإحتياطات الصحية اللازمة.

SUMMARY

A total of one hundred and five random samples of meat products (15each of beef luncheon, chicken luncheon, turkey luncheon, salami, rose beef, basterma and pastrami) were collected and subjected to mycological

evaluation. Moulds could be detected only in beef luncheon and basterma where their mean counts were 1.6 $X10^2$ ±29.6 and 2.8 X 10^2 ±37.4/gm respectively. The mean total yeasts counts/gm were $1.7X10^4$ ± $3.6x10^3$, $2.8X10^4$ ± 8x 10^3, 8.8 $X10^3$ ± 3.7 x 10^3, $9.4x10^3$± 1.4 x 10^3 , $1.2x10^4$ ± 3.8 x 10^3 , 4.8X 10^2 ±10^2 and 2.6 X 10^4 ± 3.1 x 10^3 for beef luncheon, chicken luncheon, turkey luncheon, salami, rose beef, basterma and pastrami samples respectively. It was found that the highest count of all examined positive samples for moulds was $4x10^2$ / gm and the lowest count was 10^2 while for yeasts the highest count of the examined samples was $5.8x10^4$ /gm and the lowest count was 10^2. *Mucor* spp., *Penicillium vercosaum and Cladosporium* spp. were isolated from the examined samples at varying percentages ranged from 16.6 - 50 %. The predominant species of yeasts isolated from beef luncheon, chicken luncheon, turkey luncheon, salami, rose beef, basterma and pastrami were *Candida* spp. followed by *Rhodotorula* spp. and then *Crypotococcus* species. The isolated moulds and yeasts from the examined samples were tested and evaluated for proteolytic and lipolytic activities. The public health significance of isolated moulds and yeasts as well as the sanitary precautions were mentioned.

Key words: *Meat products, moulds, yeasts, proteolytic and lipolytic activities.*

INTRODUCTION

A wide variety of meat products of reduced water activity (a_w) are manufactured around the world. These products are more or less shelf stable depending on the ingredients and the process used (APHA, 1992).

Red meat processors are actively looking for reasonable interventions that minimize the risk of introducing undesirable microorganisms and bacterial pathogens from contaminated raw carcasses into processed meats (Surve *et al.*, 1991).

Pastrami is a process that involves spicing of meat, brine curing, smoking it and, finally, steaming it until the connecting tissues within the meat break down into gelatins. Although beef navels are the traditional cut of meat for making pastrami, it is now common to see pastrami made from beef brisket, beef round and turkey. Pastrami was created as a method for preserving meat before modern refrigeration. Traditional New York pastrami is made from the navel end of the brisket. It is cured in brine, coated with a mix of spices such as garlic, coriander, black pepper, paprika, cloves, allspice, mustard seed and smoked.

Salami was thus all kind of salted meats. The Italian tradition of cured meats including several styles, the word salami soon specialized to

indicate only the most popular kind, made with ground, salted and spiced meat forced into animal gut with an elongated and thin shape, then left to undergo some kind of fermentation process. A traditional salami, with its typical marbled appearance, is made from one or more of the following meats: pork, chopped beef (particularly veal), venison, poultry (especially turkey), and horse (chester). Additional ingredients may include: minced fat, wine, wheat, corn starch, salt, various herbs, spices and vinegar. The raw meat mixture is usually allowed to ferment for a day and then the mixture is either stuffed into an edible natural or non-edible artificial casing then hung to cure. The casings are often treated with an edible mold (*Penicillium*) culture as well. The mould is desired as it imparts flavor and prevents spoilage during the curing process. Salami is cured in warm, humid conditions in order to encourage growth of the bacteria involved in the fermentation process. Sugar is added as a food source for the bacteria during the curing process. Lactic acid is produced by the bacteria as a waste product, lowering the pH and coagulating and drying the meat.

Spoilage development is a complicated biological event, which needs to be studied at the species and biotype level. Certain microbial taxa may be differently influenced by the specific storage conditions, and different microbial species may unpredictably develop during meat storage, thus influencing the time and type of spoilage development (Ercolini *et al.*, 2006).

Moulds and yeasts grow at a wide range of temperature and pH values, resulting in spoilage of the product (Pitt and Hocking, 1997). Their count is used as an index of storability and sanitary quality of the product. Such moulds and yeasts can cause gas, off flavor and rancidity or other flavor defects due to their proteolytic activity (Viljoen and Greyling, 1995).

The traditional source of moulds on raw dry sausages is the natural house mycoflora. This often consistes of heterogeneous molds composed of representatives of different genera and species (Berwal and Dinchev, 1991). Many of these moulds are undesirable and may lead to serious problems for both the consumer and the producer. Some of these molds are capable of producing mycotoxins (Lopez Diaz *et al.*, 2001).

Yeast, like moulds, contributes a small, but definite part of the natural microflora of meat, although dry-cured meat products are frequently contaminated with yeast, with counts usually low in comparison to those of bacteria (Cook, 1995).

Growth of commonly occurring filamentous fungi in foods may result in production of toxins known as mycotoxins, which can cause a variety of diseases in humans, from allergic responses to immunosuppression and cancer. The most important mycotoxins are

aflatoxins , ochratoxin A and zearalenone. (Pitt, 2000; CAST, 2003 and Garcia and Heredia, 2006).

Mycotoxins are widespread in many countries, especially in tropical and subtropical regions where temperature and humidity conditions are optimal for growth of moulds and for production of toxins, so they are found in a wide variety of agricultural products (such as corn, wheat, soya beans and spices), and animal feeds, as well as meat products (including cured meats, sausages and chicken meat) as a result of carryover from contaminated animal feed (Takahashi-Ando *et al.,* 2004 and Cavaliere *et al.,* 2006).

All AFs are chronically toxic to varying degrees. Aflatoxin B_1 is considered to be among the most potent carcinogens known and has been linked epidemiologically with cases of human liver cancer in a number of developing countries (Aikins and Norman, 1998).

Carcinogenic, mutagenic and teratogenic effects of AF B_1 have been reported for several animal species, and in humans (Sabbioni and Sepai, 1998).

This study was undertaken to secure the prevalence of moulds and yeasts in some meat products as well as to monitor the proteolytic and lipolytic activities of such moulds and yeasts.

MATERIALS and METHODS

1 - Collection of samples:

One hundred and five samples (15 each of packed and unpacked beef luncheon, chicken luncheon, turkey luncheon, salami, rose beef, basterma and pastrami)) were collected from groceries and supermarkets in Cairo and Giza governorates. The samples were transferred to the laboratory with undue delay, where they were subjected to mycological investigation.

2 - Preparation of the samples:

The technique adopted was that recommended by APHA (1992). Ten gms from each sample were carefully and aseptically weighed and homogenized with 90 ml of sterile o.1 % peptone water(Oxoid CM9) for about 2 min. using sterile homogenizer bags (in stomacher lab. Blender 400, Seward Lab. Serial No. 30469 type BA 7021, London). Ten fold serial dilutions were done in a manner to obtain a suitable number of fungal colonies which could be easily counted.

3 - Estimation of total mould and yeast count:

The technique described by Bailey and Scott (1998) was adopted**.** Duplicate plates of Sabouraud dextrose agar medium (containing 0.05 mg of chloramphenicol per ml) were inoculated each with 1 ml from the

prepared serial dilutions. Inoculated plates were incubated at 25˚C for 5 days. The total yeast and mould counts per gram of the products were then calculated and recorded.

4 - Isolation and identification of mould and yeast

Suspected mould isolates were identified according to Pitt and Hocking (1997). Isolated moulds were cultured onto Malt extract plates for 3-5 days at 25˚C then identified macroscopically and microscopically. Suspected yeasts isolates were identified according to Koneman *et al.* (1978); Finegold and Martin (1982).

5 - Proteolytic activity of moulds and yeasts (O'reilly and Day, 1983):

Each mould or yeast isolate was inoculated on the surface of skim milk agar in which skim milk was added just before pouring the medium into the Petri- plates. The plates were incubated at 28˚C for 7 days. After the incubation period, the clear zones of hydrolysis were measured and recorded.

6 - Lipolytic activity of moulds and yeasts was determined according to the technique recommended by Koburger and Jacger (1987). Each mould or yeast isolate was inoculated on the surface of Tributyrin agar plates. The plates were incubated at 30˚C for 3 days, the medium appeared opaque but lipolytic colonies were surrounded by a clear zone.

RESULTS

Table 1: Statistical analytical results of moulds and yeasts count of meat products samples (n=15)

samples	Fungi							
	Mould				Yeast			
	Positive samples		Level		Positive samples		Level	
	No	%	Range	Mean±SE	No	%	Range	Mean±SE
Beef luncheon	3	20	10^2-$2x10^2$	$1.6x10^2$ ±29.6	15	100	$8x10^2$-$3.1x10^4$	$1.7x10^4$ ± $3.6x10^3$
Chicken luncheon	-	-	-	-	10	66.6	$1.5x10^3$-$7x10^4$	$2.8x10^4$ ± $8x\ 10^3$
Turkey luncheon	-	-	-	-	15	100	$5.4x10^3$-$7x10^4$	$8.8x10^3$ ± $3.7\ x\ 10^3$
Salami	-	-	-	-	15	100	$4.4x10^3$-$2.5x10^4$	$9.4x10^3$± $1.4\ x\ 10^3$
Rose beef	-	-	-	-	15	100	$1.5x10^3$-$5x10^4$	$1.2x10^4$± $3.8\ x\ 10^3$
Basterma	5	33.3	$2x10^2$-$4x10^2$	$2.8x10^2$± 37.4	14	93.3	10^2-$1.3x10^3$	$4.8x10^2$±10^2
Pastrami	-	-	-	-	15	100	10^4-$5.8x10^4$	$2.6x10^4$± $3.1\ x\ 10^3$

Table 2: Incidence of moulds isolated from the examined meat products samples

Mould species	Meat Products															
	Beef luncheon		Chicken luncheon		Turkey Luncheon		Salami		Rose beef		Basterma		Pastrami		Total	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
Penicillium varcoasum	-	-	-	-	-	-	-	-	-	-	4	26.66	-	-	4	26.66
Mucor spp.	2	13.33	-	-	-	-	-	-	-	-	-	-	-	-	2	13.33
Cladosporium spp.	3	20	-	-	-	-	-	-	-	-	3	20	-	-	6	40
Total	5	33.33	-	-	-	-	-	-	-	-	7	46.66	-	-	12	79.99

Table 3: Incidence of yeasts isolated from the examined meat products samples (n=15)

Yeast species	Meat Products															
	Beef luncheon		Chicken luncheon		Turkey Luncheon		Salami		Rose beef		Basterma		Pastrami		Total	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
Candida tropicalis	10	6	5	3	3	1.8	9	5.4	7	4.2	3	1.8	7	4.2	44	26.5
Candida albicans	9	5.4	4	2.4	2	1.2	3	1.8	8	4.8	3	1.8	3	1.8	32	19.3
Candida lipolitical	7	4.2	3	1.8	4	2.4	5	3	5	3	3	1.8	5	3	32	19.3
Cryptococcus spp.	-	-	2	1.2	3	1.8	6	3.6	5	3	-	-	-	-	16	9.6
Rhodotorula spp.	9	5.4	6	3.6	6	3.6	7	4.2	5	3	6	3.6	3	1.8	42	25.3
Total	35	21	20	12	18	10.8	30	18	30	18	15	9	18	10.8	166	100

Table 4: Proteolytic activity of isolated moulds from examined meat products samples (N0 of isolates=12)

Mould species	Meat Products													
	Beef luncheon		Chicken luncheon		Turkey luncheon		Salami		Rose beef		Basterma		Pastrami	
	No	+ ve	No	+ ve	No	+ ve	No	%	No	+ ve	No	+ ve	No	+ ve
Penicillium vercoasum	-	-	-	-	-	-	-	-	-	-	4	3	-	
Mucor spp.	2	1	-	-	-	-	-	-	-	-	-	-	-	-
Cladosporium spp.	3	2	-	-	-	-	-	-	-	-	3	1	-	-
Total	5	41.6	-	-	-	-	-	-	-	-	7	58.4	-	-

Table 5: Lipolytic activity of isolated moulds from the examined meat products samples (N0 of isolates=12)

Mould species	Meat Products													
	Beef luncheon		Chicken luncheon		Turkey luncheon		Salami		Rose beef		Basterma		Pastrami	
	No	+ ve	No	+ ve	No	+ ve	No	+ ve	No	+ ve	No	+ ve	No	+ ve
Penicillium vercoasum	-	-	-	-	-	-	-	-	-	-	4	4		
Mucor spp.	2	2	-	-	-	-	-	-	-	-	-	-	-	-
Cladosporium spp.	3	3	-	-	-	-	-	-	-	-	3	1	-	-
Total	5	41.6	-	-	-	-	-	-	-	-	7	58.4	-	-

Table 6: Proteolytic activity of isolated yeasts from the examined meat products samples (n=15)

Yeast species	Meat Products															
	Beef luncheon		Chicken luncheon		Turkey luncheon		Salami		Rose beef		Basterma		Pastrami		Total	
	No	+ ve	No	+ ve	No	+ ve	No	+ve	No	+ve	No	+ve	No	+ve	No	+ve
Candida tropicalis	10	7	5	4	3	2	9	5	7	5	3	2	7	4	44	29
Candida albicans	9	4	4	3	2	1	3	1	8	7	3	3	3	2	32	21
Candida lipolitical	7	6	3	2	4	2	5	2	5	3	3	2	5	4	32	21
Cryptococcus spp.	-	-	2	1	3	2	6	5	5	3	-	-	-	-	16	11
Rhodotorula spp.	9	8	6	5	6	4	7	5	5	3	6	4	3	2	42	31
Total	35	25	20	15	18	11	30	18	30	21	15	11	18	12	166	113

Table 7: Lipolytic activity of isolated yeasts from examined meat products samples (n=15)

Yeast species	Meat Products															
	Beef luncheon		Chicken luncheon		Turkey luncheon		Salami		Rose beef		Basterma		Pastrami		Total	
	No	+ve	No	+ve	No	+ve	No	+ve	No	+ve	No	+ve	No	+ve	No	+ve
Candida tropicalis	10	10	5	4	3	3	9	6	7	6	3	3	7	6	44	38
Candida albicans	9	8	4	4	2	1	3	2	8	5	3	2	3	2	32	24
Candida lipolitical	7	7	3	3	4	4	5	4	5	4	3	2	5	5	32	29
Cryptococcus spp.	-	-	2	2	3	2	6	5	5	4	-	-	-	-	16	13
Rhodotorula spp.	9	9	6	5	6	6	7	3	5	5	6	2	3	1	42	31
Total	35	34	20	18	18	16	30	20	30	24	15	9	18	14	166	135

DISCUSSION

Protein hydrolyzing microorganisms may produce a variety of odour and flavor defects particularly when contamination is high so it is necessary to give full consideration of spoilage microorganisms not only bacteria but also the mould and yeasts. The same consideration must be given to lipolytic fungi as it widely spread in nature and are heat resistant and their activity withstand in foods for long period even at low temperature (Lashin, 2003).

Presence of mould and yeast in meat and meat products indicates bad hygienic measures in the processing and handling of fresh meat and meat products (Abdel-Rahman *et al.*, 1984).

Table 1 showed that moulds could be detected only in beef luncheon and basterma where the mean count values were $1.6 X10^2 \pm 29.6$ and $2.8 X 10^2 \pm 37.4$ respectively On the other hand the mean total yeast count/gm were $1.7X10^4 \pm 3.6x10^3$, $2.8X10^4 \pm 8x 10^3$, $8.8 X10^3 \pm 3.7 x 10^3$,$9.4x10^3 \pm 1.4 x 10^3$, $1.2x10^4 \pm 3.8 x 10^3$, $4.8X 10^2 \pm 10^2$ and $2.6 X 10^4 \pm 3.1 x 10^3$for beef luncheon, chicken luncheon, turkey luncheon, salami, rose beef, basterma and pastrami samples respectively.

Contamination with yeasts and moulds and their toxins constitute a public health hazard, obviously it is of an importance to prevent mould growth to stop toxin production through preventing the natural contamination of raw materials, storage food under conditions which prevent mould growth, strict hygienic measures and regulations should be imposed during processing, packing and transportation (El-Diasty and Salem 2007).

From Tables 2 and 3 it was clear that *Mucor* spp., *Penicillium vercosaum* and *Cladosporium* spp. were isolated from the examined meat products samples at varying percentages ranged from 13.33 – 26.66 %.The growth of *Penicillum* spp. on the surface of meat-based dry fermented sausage provides them with a protective effect against some undesirable microorganisms. *Penicillium* also acts as antioxidant, minimizes the risk of excessive drying, and it is responsible for flavor development due to the decomposition of proteins, free fatty acids and lactic acid (Ludemann, *et al.*, 2004). The predominant species of yeasts isolated from beef luncheon, chicken luncheon, turkey luncheon, salami, rose beef, basterma and pastrami were *Candida* spp. followed by *Rhodotorula* spp., and then *Crypotococcus* spp. From Tables 4 and 5 for mould isolates it was clear that out of 4 isolates of *Penicillium vercoasum* 3 showed proteolytic activity while 4 isolates showed lipolytic activity. Two isolates of *Mucor* spp., 1 showed proteolytic activity while the two isolates showed lipolytic

activity and out of 6 isolates of *Cladosporium* spp. 3 showed proteolytic activity while 4 isolates showed lipolytic activity. On the other hand from tables 6 and 7 it is evident that out of 108 isolates of *Candida* spp., 71 showed proteolytic activity while 91 isolates showed lipolytic activity. Out of 16 *Cryptococcus* spp., 11 showed proteolytic activity while 13 isolates showed lipolytic activity and out of 42 *Rhodotorula* spp., 31 showed proteolytic activity while 31 isolates showed lipolytic activity. From the previous results it is obvious that lipolytic activity is more predominant than proteolytic activity. Sayed (1999); Nasser (2002); El-Diasty(2004) stated that both *Aspergillus* and *Penicillium* spp. as well as *Candida* spp. were of proteolytic and lipolytic activities. Most isolates of *A.flavus*, *A.niger*, *Cladosporium* spp., *Mucor* spp. and *Penicillium* were having a proteolytic activity with different strength. *Geotrichium* spp. where having a lipolytic activity. Also most isolates of *Candida lipolytica, C.parapasillosis* were having lipolytic activity (El-Diasty and Salem, 2007).

Exposure to mycotoxins can produce both acute and chronic toxicities ranging from death to deleterious effects upon the central nervous, cardiovascular and pulmonary systems and upon the alimentary tract. The ability of some mycotoxins to compromise the immune response and consequently, to reduce resistance to infectious disease is now widely considered to be the most important effect of mycotoxins particularly in developing countries (FAO, 2001).

CONCLUSION

In spite of presence of different mould and yeast species from meat products having been isolated these kinds of food appear to be favourable for all people of all ages. The spoilage potential of yeast and mould appears to be related to their proteolytic and lipolytic activity so, mould and yeast contaminations as well as their proteolytic and lipolytic activity should be monitored routinely for food safety and to reduce human risk in public health**.**

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