ANAEROBIC SPORE FORMING BACTERIA CONTAMINATING SOME VACUUM PACKAGED MEAT PRODUCTS

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ABSTRACT

The present study was designed to determine the anaerobic spore forming bacteria contaminating some vacuum packaged meat products. A total of 45samples of Cocktail, Salami and Pepperoni (15 of each) were collected randomly from different supermarkets in Alexandria governorate. The obtained results revealed that the mean values of total anaerobic and C.perfringens counts were $9.97x10^3 \pm 2.58$and $5.13x10^3 \pm 1.08$ cfu /g) for Cocktail, $4.12x10^3 \pm 0.89$ and $1.68x10^3 \pm 0.41$ (cfu /g) for Salami and $1.55x10^3 \pm 0.38$ and $6.05x10^2 \pm 1.27$ (cfu /g) for Pepperoni, respectively. Anaerobic spore forming Clostridial spp. which isolated in different percentages from the examined samples,included C.perfringens, C.absonum, C.bifermentans, C.butyricum, C.histolyticum, C.pasteurinum, C.putrefaciens, C.sordelli, C.sporogenes. Moreover, Lecithinase activity of C.perfringens isolated from the examined samples of vacuum packaged meat products indicated that 5 samples out of 9 Cocktail samples contaminated with C.perfringens were lecithinase +ve while the remaining 4 samples were lecithinase -ve. while, 4 Salami samples contaminated with C.perfringens were lecithinase +ve and one sample was lecithinase -ve and 2 samples out of 4 Pepperoni samples contaminated with C.perfringens were lecithinase +ve while the remaining 2 samples were lecithinase -ve.Typing of lecithinase +ve of C.perfringens isolated from the examined samples of vacuum packaged meat products by intradermal inoculation test of Guinea pig was shown type A,C and D in Cocktail,type A,B and C were detected in Salami and type A and D were detected in Pepperoni with diffpercentages. The public health importance of the isolated organisms were discussed.

INTRODUCTION

Packaging plays a key role in protecting the product from contamination by external sources, and reducing damage during its transportation and handling in the supply chain from the producer and manufacture to the consumer **(Jay and Paul, 2007)**.

Vacuum packing is a very simple procedure, the meat product is placed inside a heavy duty plastic bag, which in turn is put into a special machine which expels all the air from the bag and then seals the pouch, making it air tight. This simple procedure is very effective one which prevents the outside air from reacting

meat. Moreover, prevent the growth of aerobic bacteria, shrinkage, oxidation and color discoloration of meat **(Genigeoris, 1985)**.

Anaerobic bacteria are important groups of microorganisms responsible for many health hazards to consumers used processed vacuum packed meat products in their meals. *Clostertdium perfringens* are the most important and strictly anaerobic organism. Isolation of this organism from food is generally considered to bc of less significance than the detection of its toxin **(Hobbs et al.,1982).**

Spores of some bacterial species can survive pressures abovc 1.1000 Map (Mega Pascal), at least at refrigerated or at room temperature. Gram-positivc bacteria are generally assumed to be more resistant than Gram-negative bacteria **(Cheftel, 1995 and Gould, 1995)**.

The ubiquitous distribution of the *C. perfringens*, makc it difficult to exclude its spores during thc processing of various meat products and its presence must be assumed **(Bean and Griffin, 1990)**. The contamination may be oceurred at any time during cooling of meat below 70-80°C this tempcrature not kill sporcs but shocked them and allow rapid germination **(Hall and Angelotti, 1965)**.

The processing, handling and storagc of most mcat products may be constitute a public health hazard either due to presence of spoilagc bacteria responsible for unfavorable changes, or pathogenic bacteria leading to harmful effects as food infection or intoxication among consumers **(Eley, 1992)**.

C.perfringens strains produce five types of toxins from A to E, based on their production of four lethal extracellular toxins (alpha, beta, epsilon and iota). Actually, most of food poisoning outbreaks usually due to the strain type A **(Labbe, 1988)**. Only type A were recorded as part of the microflora of both soil and intestinal tract of man and animals **(Smith, 1975)** .

C. perfringens food poisoning occurred from 8-20 hours after consumption of the contaminated food **(Hobbs and Roberts, 1987)**. The symptoms include abdominal pain, profuse diarrhea, nausea and rarely vomiting and fever are unusual , these symptoms may continue for 12-48 hours due to thc activities of a large dose of the swallowed bacteria in food and produce an enterotoxin in the intestine. **(Warrel et al., 2003 and Ryan et al., 2004)**. Type A is the most important one which produce gastroenteritis in man and a more serious but rare illness is caused by food contaminated with type C strain **(Brynestad and Granum, 2002)**.

MATERIAL AND METHODS

1- Collction of samples:

Fourty five random samples of vacuum packaged meat products represented by Cocktail, Salami and Pepperoni (15 of each) were collected from different supermarkets in Alexandria governorate fo detection of anacrobie spore forming bacteria contaminating such samples.

2- Preperation of samples (A.P.H.A., 1992):

Accurately, 25 gram of eaeh samples were homogenized in 225 ml of sterile peptone water under complete aseptic conditions.

Further, decimal serial dilutions were prepared.

3- Enumeration and identification of anaerobic bacteria (I.C.M.S.F.,1996):

Enumeration of total anaerobic was applied by using reinforced clostridial agar plates which incubated anaerobically at 37°C for 48 hours. However, the developed colonies were enumerated and the average number was recorded as total anacrobic count / g .On the other hand, the suspected colonies were picked up and purified in nutrient agar slopes for further identification according to **Krieg and Holt (1984)**.

4- Enumeration, identification and typing of C.perfringens:

Closteridium perfringens count was carried out according to the technique adopted by **Bcarnes et al., (1980)** using peptone water tubes incubated anaerobically at 37°C for 24 hours followed by subculturing into tryptose sulfite cycloserine agar plates which were incubated in anaerobic jar at 37°C for 24 hours. All black colonies were enumerated and the average numbers of *C.perfringens* were recorded.

Suspected isolates of *C.perfringens* were tested for lecithinase activity by using egg yolk agar plates (Naglar reaction). Moreover, the isolated strains were typed by dermonecrotic reaction through intradermal inoculation test in Guinea pigs according to **Sterne and Batty (1975)**.

5- Statistical analysis:

The obtained results were statistically evaluated by application of Analysis of Variance (ANOVA) according to **Feldman et al; (2003)**.

RESULTS & DISCUSSION

Extension of the shelf-life of the meat products is one of the technology needs to meet the demands of consumers. In this respect, increasing attention is put on packaging techniques. Vacuum packaged are recent innovation that have been gaining importance as preservation technique to improve the shelf-life of the meat, the products became more attractive and easily handled **(Naraimha and Sachindra, 2002)**.

The present results recorded in Table (1) revealed that the total anacrobic count of such examined samples varied from $4x10^2$ to $6.3x10^4$ with a mean value $9.97x10^3 \pm 2.58x10^3$/g for Cocktail, $2x10^2$ to $1.9x10^4$ with a mean value $4.12x10^3 \pm 0.89x10^3$/g for Salami and $1x10^2$ to $7.6x10^3$ with a mean value$1.55x10^3 \pm 0.38x10^3$/g for Pepperoni. Furthermore, the differences associated with such examined samples were significant ($p \leq 0.05$). Higher results were obtained by **Edris et al., (1992); AbEl-Rahman et al., (1996) and Shaltout (1999)**.

The results indicated that the presence of anaerobes in samples may attributed to the bad quality of raw meat used for manufacturing (anaerobes are mainly derived from soil, human intestine contents and animal excreta contaminating meat) as well as additives and spices **(Kukharkova, 1972)**. Spices often act as important vector for various microorganisms, espeetally spore formers implicating possible health problems for consumers, the food support the growth of these bacteria and

the production of enterotoxin (Mousumi and **Prabir, 2004**).

Furthermore, inadequate thermal treatment during processing of meat products may be a cause of the high anaerobic count **(FAO, 1992)** and cold storage of meat products usually lowers the rate of growth of anaerobic spore-forming bacteria than ambient storage temperature **(Hersum and Hulland, 1969)**.

Table (1) declared that the total *C.perfringens* counts of such examined samples was ranged from $2x10^3$ to$1.7x10^4$ with an average count $5.13x10^3 \pm 1.08x10^3$ /g for Cocktail, $1x10^2$ to $4.8x10^3$ with an average $1.68x10^3 \pm 0.41x10^3$ /g for Salami and $1x10^2$ to $9x10^2$ with an average $6.05x10^2 \pm 1.27x10^2$/g for Pepperoni and the differences associated with such examined samples were significant ($p \leq 0.05$).

Lower results were obtained by **El-Kelish et al., (1987)**, nearly similar results were recorded by **Eleiwa (2003)**, while higher results were obtained by **Hassan (1999)**. This differences may be due to the different of the hygienic level in the processing plants from which the samples were taken.

C.perfringens is widely distributed in the environment. Spores persist in soil, sediments and areas subjected to human or animal fecal pollution **(Brynestad and Granum, 2002)**. Many organism that compete with *C.perfringens* are killed when meat are cooked, but not its spores which are heat-shocked during cooking, So more of them germinate when temperature is favorable for growth and vegetative cells multiply, So, food must be reheated to temperature sufficient to kill vegetative forms **(Bryan, 1980)**. Subsequent growth must be prevented by cooling the meat below15°C as soon after cooking as possible, if the meat is to be stored **(Mead, 1994)**.

Table (2)showed the percentage of isolated anaerobic spore forming bacteria in Cocktail, Salami and Pepperoni which were *C.perfringens* 60%, 33.33% and 26.67, *C.bifermentans* 13.33%, 26.67% and 33.33%, *C.butyricum* 26.67%, 46.67% and13.33%, *C.putrefaciens* 53.33%, 26.67% and 33.33%, *C.sporogenes*, 33.33%, 53.33% and 40%, respectively, *C.absonum* and *C.pasteurinum* isolated from Cocktail only in the same percent 6.67% and not detected in the other samples, *C.histolyticum* 6.67% in Salami samples only and not detected in the other samples, *C.sordelli* were detected in Cocktail and Salami samples only by a percentage of 26.67% and 13.33%, respectively.

Raw meat served as a vehicle for carrying bacteria when the animals under stress condition of transport or (during/or after) slaughter into kitchen and workers hands **(Bryan, 1969)**. The contamination by these organisms may occurs also through knives, hands, cloths of workers and water used in washing carcasses, surfaces, cutting board and equipments **(Bally, 1972 and Hobbs and Gilbert, 1978)**.

Most food poisoning cases involving *C.perfringens* are reported from restaurants, hospitals and homes for elderly people. Proper cleaning and disinfection should be relatively easy to control food borne diseases

caused by *C.perfringens* **(Labb, 2000)**. The spore of this organism is heat resistant and may survive some cooking procedure **(Tranter et al., 1987)**.

Table (3) showed Lecithinase activity of *C.perfringens* isolated from the examined samples of vacuum paekaged meat products indicated that 5 samples out of 9 samples contaminated with *C.perfringens* were lecithinase +ve while the remaining 4 samples were lecithinase -ve for Cocktail. while, all the examined samples contaminated with *C.perfringens* were lecithinase +ve except one sample of Salami out of 5 were lecithinase -ve and 2 samples out of 4 samples contaminated with *C.perfringens* were lecithinase +ve while the remaining 2 samples were lecithinase -ve for Pepperoni.

C.perfringens causes human gas gangrene and two different food borne diseases, the relatively mild form is the type A (diarrhea) which is the more common among the industrialized world and the other serious, but rare is the type C which has implicated as a cause of human necrotic enteritis **(Granum, 1990)**.

Table (4) declared typing of lecithinase +ve of *C.perfringens* isolated from the examined samples of vacuum packaged meat products by intradermal inoculation test of Guinea pig was shown type A, C and D were detected in Coektail (with percent 20%, 6.67% and 6.67% respectively) , type A, B and C were detected in Salami (with percent 6.67%, 6.67% and 13.33% respectively) and type A and D were detected in Pepperoni (with percent 6.67% and 6.67%, respectively). Moreover, the majority of isolated strain was type A which the most important one it produee gastroenteritis in man **(Hobbs and Roberts, 1987)**.

We can control the growth of *C.perfringens* type A and its ability to enterotoxin production by using Nisine as food preservatives and its general dose is 100-200 mg/kg **(Eleiwa, 2009)**.

Table (1): Statistical analytical results of total anaerobic and *C.perfringens* counts(cfu /g) in the examined samples of vacuum packaged meat products (n = 15).

	Total anaerobic count			Total *C.perfringens* count		
	Min.	Max.	Geometric Mean± S.E.	Min.	Max.	Geometric Mean ± S.E.
Coektail**	4×10^2	6.3×10^4	$9.97\times10^3 \pm 2.58$	2×10^2	1.7×10^4	$5.13\times10^3 \pm 1.08$
Salami	2×10^2	1.9×10^4	$4.12\times10^3 \pm 0.89$	1×10^2	4.8×10^3	$1.68\times10^3 \pm 0.41$
Pepperoni	1×10^2	7.6×10^3	$1.55\times10^3 \pm 0.38$	1×10^2	9×10^2	$6.05\times10^2 \pm 1.27$

**ANOVA test indieated high significant differences ($p < 0.01$) between the different types of vacuum packaged meat products.

Table (2): Incidence of anaerobic spore forming bacteria in the examined samples of vacuum packaged meat products (n = 15).

	Cocktail		Salami		Pepperoni	
	No.	%	No.	%	No.	%
C.perfringens	9	60.00	5	33.33	4	26.67
C.absonum	1	6.67	---	---	---	---
C.bifermentans	2	13.33	4	26.67	5	33.33
C.butyricum	4	26.67	7	46.67	2	13.33
C.histolyticum	---	---	1	6.67	---	---
C.pasteurinum	1	6.67	---	---	---	---
C.putrefaciens	8	53.33	4	26.67	5	33.33
C.sordelli	4	26.67	2	13.33	---	---
C.sporogenes	5	33.33	8	53.33	6	40.00

Table (3) :Lecithinase activity of *C.perfringens* isolated from the examined samples of vacuum packaged meat products (n = 15).

	Lecithinase +ve *C.perfringens*		**Lecithinase -ve *C.perfringens***		**Total**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Cocktail	5	33.33	4	26.67	9	60.00
Salami	4	26.67	1	6.67	5	33.33
Pepperoni	2	13.33	2	13.33	4	26.67
Total (n=45)	11	24.44	7	15.56	18	40.00

Table (4): Typing of lecithinase +ve of *C.perfringens* isolated from the examined samples of vacuum packaged meat products (n = 15).

	A		**B**		**C**		**D**	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Cocktail	3	20.00	---	---	1	6.67	1	6.67
Salami	1	6.67	1	6.67	2	13.33	---	---
Pepperoni	1	6.67	---	---	---	---	1	6.67
Total (n=45)	5	11.11	1	2.22	3	6.67	2	4.44

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الملخص العربى

الميكروبات اللاهوائية المتجرثمة الملوثة لبعض منتجات اللحوم المعبأة تحت التفريغ

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أجريت هذه الدراسة لاستبيان مدى تلوث بعض منتجات اللحوم المعبأة تحت التفريغ بالبكتيريا اللاهوائية المتجرثمة وسمومها مثل الكلوستريديوم بيرفرينجنز التى تؤثر بدورها على الصحة العامة للمستهلك، لذلك أجريت هذه الدراسة على عدد ٤٥ عينة من كل منتجات الكوكتيل والسلامى والبيرونى (بواقع ١٥ عينة من كل نوع) وتم تجميعها عشوائياً من السوبر ماركت بمحافظة الإسكندرية وقد دلت النتائج إلى أن متوسط العدد الكلى للبكتيريا اللاهوائية والكلوستريديوم بيرفرينجنز / جرام كالتالى ٩٫٩٧ × ١٠^٣ ± ٢٫٥٨ × ١٠^٢ و ٥٫١٣ × ١٠^٣ ± ١٫٠٨ × ١٠^٣ / جرام فى عينات الكوكتيل، ٤٫١٢ × ١٠^٣ ± ٠٫٨٩ × ١٠^٣ و ١٫٦٨ × ١٠^٣ ± ١٠٫٤١ × ١٠^٣ / جرام فى عينات السلامى، ١٫٥٥ × ١٠^٣ ± ٠٫٣٨ × ١٠^٣ و ٦٠٥ × ١٠^٢ ± ١٫٢٧ × ١٠^٢ / جرام فى عينات البيرونى على التوالى، أما بالنسبة لأنواع الميكروبات اللاهوائية المعزولة من العينات فقد تم عزل عترات من بكتيريا الكلوستريديوم بنسب مختلفة وهى كلوستريديوم بيرفرينجنز وكلوستريديوم أبسوميوم وكلوستريديوم بايفرمنتاس وكلوستريديوم بيوتريكم وكلوستريديوم هيستوليتيكم وكلوستريديوم باستير يم وكلوستريديوم بيوتريفاشيانت وكلوستريديوم سورديلى وكلوستريديوم سبورجينس وبالإضافة إلى أن ٥ عينات من ٩ عينات موجبة للكلوستريديوم بيرفرينجنز كانت موجبة للسيسيناز و٤ عينات الباقية كانت سالبة فى منتجات الكلوكتيل وبينما كل العينات الموجبة للكلوستريديوم بيرفرينجنز كانت موجبة للسيسينازما عدا واحدة من ٥ عينات موجبة للكلوستريديوم بيرفرينجنز كانت سالبة فى منتجات السلامى وكما أن ٢ عينة من ٤ عينات موجبة للكلوستريديوم بيرفرينجنز كانت موجبة للسيسيناز و٢ عينة الباقية كانت سالبة فى منتجات البيرونى، كما دلت النتائج على وجود عترات موجبة السيسيناز فى بكتريا الكلوستريديوم بيرفرينجنز بعد حقنها فى خنزير غينيا وجدت بنسب مختلفة فكانت عترات A,C,D فى عينات الكوكتيل وعترات A,C,D فى عينات السلامى وعترات A,D فى عينات البيرونى، وقد تم تحدد مدى خطورة هذه الميكروبات على الصحة العامة للمستهلك وإعطاء بعض النصائح لمحاولة التخلص من خطورتها.