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PREVALENCE OF SOME AEROBIC AND ANAEROBIC PSYCHROTROPHS IN VACUUM-PACKED MEAT WITH SPECIAL REFERENCE TO *L. MONOCYTOGENES*

(With 3 Tables)

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مدى تواجد الميكروبات المحبة للبرودة والميكروبات اللاهوائية فى منتجات اللحوم المعبآة تحت التفريغ مع أهمية خاصة لميكروب الليستيريا مونوسيتوجينز

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تم جمع عدد 40 عينة من منتجات اللحوم المعبآة تحت التفريغ بواقع عشرة عينات من كل من الفرانكفورتر، البسترامى، الروزبيف ، البيف باكون وقد آجرى الفحص الظاهرى بالنسبة للون والرائحة وظهور عصارة اللحم وكذلك متوسط التركيز الهيدروجينى للعينات وقد أوضح الفحص البكتيرى أن متوسط العد الكلى للميكروبات المحبة للبرودة فى الفرانكفورتر، البسترامى، الروز بيف، البيف باكون $9,4 \times 10^7 \pm 3,8 \times 10^7$ ، $2,9 \times 10^8 \pm 6,4 \times 10^7$، $1,3 \times 10^8 \pm 4 \times 10^7$ و $3 \times 10^8 \pm 1,6 \times 10^8$ على التوالى وكان متوسط عد بكتيريا حمض اللاكتيك $2 \times 10^7 \pm 4,4 \times 10^7$ ، $4,6 \times 10^8 \pm 9,1 \times 10^7$، $1,4 \times 10^7 \pm 3,3 \times 10^8$ على التوالى وكان متوسط العد الكلى للميكروبات اللاهوائية $9,7 \times 10^7 \pm 1,8 \times 10^7$ ، $2,2 \times 10^7 \pm 3,2 \times 10^8$، $4,7 \times 10^8 \pm 9,8 \times 10^7$، $2,3 \times 10^7 \pm$ كما تم فحص عدد ثلاثين عينة من منتجات اللحوم المعبآة من الفرانكفورتر،البسترامى، الروز بيف بدون تفريغ ومقارنتها بمثيلاتها المعبآة تحت التفريغ لتحديد مدى تواجد الميكروب المكور العنقودى الذهبى وميكروب الليستيريا مونوسيتوجينز وميكروب الباسيلس سيريس وميكروب الكلوستريديم بيرفرينجينز وكانت النتائج سلبية لعينات البسترامى والروز بيف بينما تم عزل الميكروب المكور العنقودى الذهبى من الفرانكفورتر والبيف باكون فى منتجات اللحوم المعبآة تحت التفريغ كما تم عزل الميكروب المكور العنقودى الذهبى من الفرانكفورتر والروزبيف وميكروب الباسيلس سيريس من الفرانكفورتروالبسترامى فى منتجات اللحوم المعبآة بدون تفريغ

SUMMARY

Forty samples of four vacuum-packed meat products (ten each of frankfurter, pastrami, rose beef and beef bacon) were collected from different wholesale retail outlets at Cairo and Giza Governorates. The samples were assessed in terms of colour, odour, appearance of released

meat juice and pH measurement and examined for psychrotrophic, lactic acid bacteria and anaerobic counts. The mean psychrotrophic counts were $9.4x10^7 \pm 3.8x10^7$, $2.9x10^8 \pm 6.4x1o^7$, $1.3x10^8 \pm 4x10^7$ and $10^8 \pm 3x10^7$/g respectively, while the mean counts for lactic acid bacteria were $1.6x10^8 \pm 4.4x\ 10^7$, $2x10^8 \pm 4.6x10^7$, $9.1x10^7 \pm 1.4x\ 10^7$, and $10^8 \pm 3.3x10^7$/g respectively. Also the mean anaerobic counts were $9.7x10^7 \pm 1.8x10^7$, $1.8x10^8 \pm 3.2x10^7$, $2.2x\ 10^8 \pm 4.7x10^7$ and $9.8x10^7 \pm 2.3x10^7$/g respectively. Another thirty samples of packed frankfurter, pastrami, rose beef were examined and compared with the aforementioned vacuum frankfurter, pastrami, rose beef products for detection of *S.aureus, L. monocytogenes, B. cereus and Cl. perfringens*. *S. aureus* could be detected in vacuum packed frankfurter and beef bacon samples while in resembling packed samples *S. aureus* and *B. cereus* could be isolated.

Key words: *Meat products, vacuum-packed meat, psychrotrophs, L.monocytogenes*

INTRODUCTION

Food packaging serves to protect products against deteriorative effects, contain the product, communicate to the consumer as a marketing tool, and provide consumers with ease of use and convenience (Yam *et al.*, 2005). The display of meat in plastic materials allows consumer evaluation of the product in an attractive, hygienic and convenient package (Renerre and Labadie, 1993). Packaging of food now performs beyond the conventional protection properties and provides many functions for the contained product (Han, 2005a).

Modern meat packaging techniques are intended to maintain microbial and sensory quality of the product. Product shelf-life can be extended by inhibiting or retarding the growth of undesirable microflora. This can be achieved by manipulation of the meat microenvironment (Hotchkiss, 1988). It is well known that packaging makes food more convenient and gives the food greater safety assurance from microorganisms, biological and chemical changes so that the packaged foods may have a longer shelf life. As a result, packaging has become an indispensable element in the food manufacturing process in order to meet the huge demand of the food industry (McMillin, 2008).

Modified atmosphere packaging (MAP) is the removal and/or replacement of the atmosphere surrounding the product before sealing in vapor-barrier materials. MAP can be vacuum packaging (VP), which

removes most of the air before the product is enclosed in barrier materials, or forms of gas replacement, where air is removed by vacuum or flushing and replaced with another gas mixture before packaging sealing in barrier materials. The headspace environment and product may change during storage in MAP, but there is no additional manipulation of the internal environment (McMillin *et al.,* 1999).

Packaging ranged from overwrap packaging for short-term chilled storage and/or retail display to barrier packages for longer terms of chilled storage or display (Kerry *et al.,* 2006). In such packaging, an initial atmosphere is generated by either permitting air to be enclosed or by injecting a desired initial gas mixture. This blend then changes as a result of multiple variables including: (i) permeation of oxygen, carbon dioxide, and water vapor through the package material; (ii) transmission of oxygen, carbon dioxide, and water vapor through the seal and defective structural areas; (iii) temperature of the package material which may lead to small changes in permeation; (iv) surface area of the package material; and (v) thickness of the package material (Tsigarida and Nychas, 2001). Such changes may influence/affect the contribution of different members of microbial association and as a consequence, an extension of shelf life can be achieved. Despite the extended shelf life of refrigerated products stored under vacuum pack/modified atmosphere packaging conditions there is an increased concern about the growth/survival of microaerophilic psychrotrophic pathogens (Garcia de Fernando *et al.,* 1995).

Success and continuation of the many different retail MAP formats has been dependent upon product, package, and system interactions, relationships of processors and retailers, and consumer acceptance of the merchandising format (Brody, 2002). Meat spoilage is a complex process in which microorganisms present in the muscular tissue due to secondary contamination during processing are involved, and which depends on ambient temperature. The storage of meat is associated with changes in quality resulting from microbial activity, shift of pH, production of toxic substances, and aberrant odor (Huis, 1996). Lactic acid bacteria appeared to be more resistant and they became the dominant component of the microflora during chilled storage (Farkas *et al.,* 1996).

Typical microbial flora responsible for anaerobic spoilage of meat is lactic acid producing bacteria (LAPB) of the genus *Lactobacillus* (Huis, 1996). LAPB, including the genera *Lactobacillus* and *Leukonostoc*, predominate in meat stored under anaerobic condition.

Anaerobic decomposition of meat is a slow process setting in as soon as the bacterial population reaches 10^8 to 10^9 CFU/g. Shelf life can be shorter if the hydrogen sulphide-producing species *Lactobacillus sakei* prevails (Pipek, 1995). The lactobacillar population on meat usually includes the species of *L. sakei, L. curvatus, and L. plantarum* (Hugas, 1998).

As a result of dominating of flora by lactic acid bacteria, the shelf life might be extended. That is because lactic acid bacteria are considered harmless, although adventitious growth of certain types of lactic acid bacteria may cause spoilage due to accumulation of undesirable metabolites (Gould, 1996).

Vacuum-packed meat products supported *S.aureus* growth and cannot be considered shelf stable. The product pH and moisture-protein ratio were the compositional factors best correlated with *S. aureus* survival and growth, but pH and a_w, or pH and water-phase salt also may provide useful predictive guidance for *S. aureus* growth (Ingham *et al.,* 2005).

Alisarlim *et al.* (2005) stated that because of high level of *L. monocytogenes* contamination in vacuum packaged processed meat products, it is essential to make sure that all necessary sanitary requirements be met to avoid any contamination. Inadequate cooking practices or raw consumption of these contaminated products may cause potential risks for public health.

B. cereus is a common contaminant of food processing environment and is, therefore, of concern to the meat industry. It can produce a heat sensitive enterotoxin, which produce diarrheal syndrome and a heat resistant one induces a vomiting syndrome (Hayes, 1992).

Ray *et al.* (1989) added that a *Clostridium sp.* was associated with spoilage of the vacuum-packed beef and that it produced extensive proteolysis, loss of texture, foul odor and bright red to reddish-green color in meat.

MATERIALS and METHODS

Collection of samples

Forty samples of four vacuum-packed meat products (ten of each, Frankfurter, Pastrami, Rose beef and Beef bacon) were collected from different wholesale retail outlets at Cairo and Giza cities. The collected samples were transferred immediately to the laboratory in an icebox with minimum period of delay to be subjected to the following examinations:

1- Sensory assessment:

The samples were assessed in terms of colour, odour and appearance of released meat juice using three description for each characteristic with the following verbal definitions (Napravnikova *et al.*, 2002):

Colour 1- fresh, light pink
2- moderately altered
3- markedly altered, grayish to greenish

Odour 1- typical of meat product
2- agreeable, milky
3- repulsive, typical of beginning spoilage

Meat juice 1- no released juice
2- small amount, lightly opalescent
3- markedly turbid, dense to sticky

pH: It was determined using pH meter (Digital, Jenco 609)

2- Bacteriological examination:

2.1 Preparation of samples: The technique recommended by FDA (2001) was followed where ten grams of samples to which 90 ml of 1% sterile buffered peptone water (Oxoid CM9) were aseptically added to provide a dilution of 1/10, then the content of the bag was stomached for 60 seconds using stomacher (stomacher lab. Blender 400, Seward Lab. Serial No. 30469 type BA 7021, London), then 10 fold decimal dilutions were prepared up to (10^{-7}).

2.2 Bacterial count:

2.2.1 Pyschrotrophic count was done using plate count agar at 17˚C for 16 hours then at 7˚C for 3 days according to APHA(1992)

2.2.2 Lactic acid bacteria count using MRS agar (de Man-Rogosa-Sharpe agar) at 30˚C for 72 hours in anaerobic jar was determined according to APHA (1992)

2.2.3 Anaerobic count was done using Reinforced Clostridia agar at 37˚C for 48 hours in anaerobic jar according to Gibbs and Freame (1965).

2.3 Isolation and 1dentification of some pathogens:

2.3.1 Isolation and identification of *Staphylococcus aureus* was carried out according to Food and Drug Administration FDA (2001)

2.3.2 Isolation and identification of *Listeria monocytogenes* was done according to FDA (2003)

2.3.3 Isolation and identification of *Bacillus cereus* outlined by FDA (2001)

2.3.4 Isolation and identification of *Clostridium perfringens* according to the recommended methods described by APHA (1992) was carried out.

Another thirty samples of packed frankfurter, pastrami, rose beef were examined for detection of *S. aureus, L. monocytogenes, B. cereus* and *Cl. perfringens* and compared with aforementioned examined vacuum products of the same types for presence of these microorganisms.

RESULTS

Table 1: Mean values of sensory descriptors of the examined vacuum packed meat products (No=10)

Sensory Descriptor	Meat Products											
	Frankfurter			Pastrami			Rose Beef			Beef Bacon		
	Storage period(28)*			Storage period(53)*			Storage period(38)*			Storage period(60)*		
	pH			pH			pH			pH		
	5.2-5.51	5.53-5.81	5.84-6.16	5.2-5.61	5.53-5.81	5.84-6.16	5.2-5.51	5.53-5.81	5.84-6.16	5.2-5.51	5.53-5.81	5.84-6.16
Colour	2	1.5	1.5	1	1	1.5	1.5	1	2	2.5	2	2
Odour	2	2	2	1	1	1.5	1.5	1	1.5	2	1.5	2
Meat juice	2.5	1.5	2	1.5	1.5	2	2	1.5	2	2	2	2

* Mean time (in days) between production date and examination date

Table 2: Statistical analytical results of the different bacterial counts in the examined vacuum packed meat products.

Microbial counts	Vacuum packed meat products											
	frankfurter			pastrami			rose beef			beef bacon		
	Min	Max	Mean ± S.E	Min	Max	Mean ± S.E	Min	Max	Mean ± S.E	Min	Max	Mean ± S.E
Psychro-trophic	$5x10^6$	$8x10^8$	$9.4x10^7 \pm 3.8x10^7$	10^7	$8x10^8$	$2.9x10^8 \pm 6.4x1o^7$	$4x10^6$	$7x10^8$	$1.3x10^8 \pm 4x10^7$	$2.1x10^6$	$6x10^8$	$10^8 \pm 3x10^7$
Lactic acid bacteria	$2x10^6$	$8x10^8$	$1.6x10^8 \pm 4.4x\ 10^7$	$3.1x10^7$	$7x10^8$	$2x10^8 \pm 4.6x1o^7$	$3x10^6$	$3x10^8$	$9.1x1o^7 \pm 1.4x\ 1o^7$	$1o^7$	$7x10^8$	$10^8 \pm 3.3x10^7$
Anaerobic bactria	$3.6x10^6$	$3x10^8$	$9.7x10^7 \pm 1.8x10^7$	$3.4x10^7$	$4x10^8$	$1.8x10^8 \pm 3.2x10^7$	$4x10^7$	$6x10^8$	$2.2x10^8 \pm 4.7x10^7$	$3x10^6$	$4x10^8$	$9.8x10^7 \pm 2.3x10^7$

Table 3: Incidence of isolated microorganisms from the examined vacuum-packed and packed meat products (No=10)

Products / Isolates	Frankfurter				pasterami				rose beef				beef bacon	
	Vacuum packed		Packed		Vacuum packed		Packed		Vacuum packed		Packed		Vacuum packed	
	No	%	No	%	No	%	No	%	No.	%	No	%	No	%
S. aureus	1	10	1	10	ND	0	ND	0	ND	0	2	20	2	20
L.monocytogenes	ND	0	ND	0	ND	0	ND	0	ND	0	ND	0	ND	0
B. cereus	ND	0	1	10	ND	0	1	10	ND	0	ND	0	ND	0
Cl. perfrengines	ND	0	ND	0	ND	0	ND	0	ND	0	ND	0	ND	0

DISCUSSION

Vacuum packing is one of the modified atmosphere packing methods that decrease oxygen content to very low limits while increase carbon dioxide contents to a relatively high content. This modification of the atmosphere results in changes in the predominant microflora and so the rate and pattern of spoilage. The spoilage may be caused by a wide range of reactions, including physical, chemical, enzymatic and microbial interactions (Gould, 1996).

The package protects products against deteriorative effects (Yam *et al.,* 2005), which may include discoloration, off-flavor and off-odor development, nutrient loss, texture changes, pathogenicity, and other measurable factors (Skibsted *et al.,* 1994). Modified atmosphere packaging (MAP) techniques are used in the food industry to extend the product shelf-life. MAP can be classified into two main categories, namely, low oxygen modified atmosphere (including vacuum packaging, CO_2 gas flushing, N_2 gas flushing) and high oxygen modified atmosphere (Robertson, 1993).

The results of sensory assessment presented in Table (1) showed variable degrees of colour, odour and meat juice but most of the examined samples showed light pink colour, typical odour of meat, and only negligible amount of released juice. The sensory changes were markedly observed in frankfurter and beef bacon. The pH ranged between 5.2 and 6.16, most of the examined samples had pH under 6. Fermentation of saccharides by lactobacilli results in the production of lactic acid associated with a decrease of pH and an odour change. Lactic Acid Producing Bacteria are a component of the original microbial flora of meat that readily propagates on cooled and vacuum-packed meat. Their growth in red vacuum-packed meat stored in cooling rooms inhibits the propagation of G-bacteria. LAPB, via various mechanisms, inhibit the propagation of pathogenic bacteria (Hugas, 1998).

The results in Table (2) showed that the mean psychrotrophic counts for frankfurter, pastrami, rose beef and beef bacon were $9.4x10^7 \pm 3.8x10^7$, $2.9x10^8 \pm 6.4x1o^7$, $1.3x10^8 \pm 4x10^7$ and $10^8 \pm 3x10^7$/g respectively. The mean counts for lactic acid bacteria were, $1.6x10^8 \pm 4.4x\ 10^7$, $2x10^8 \pm 4.6x1o^7$, $9.1x1o^7 \pm 1.4x\ 1o^7$, and $10^8 \pm 3.3x10^7$/g respectively. Nearly similar results for psychrotrophic counts were reported by Blikstad and Molin (1983); El-Khawas (2001), also for lactic acid bacteria counts, results agreed with that reported by Samelis and Georgiadou (2000); El-Khawas (2001) in Frankfurter, Rose beef and Beef bacon**.**

Raw material was distinguished as the source of the major spoilage strains. Contamination of the product surfaces after cooking was shown to

be airborne. The removal of the product from the cooking forms was localized as a major site of airborne lactic acid bacteria (LAB) contamination. Food handlers and some surfaces in contact with the product during the manufacture were also contaminated with the spoilage strains. Some strains were also able to resist cooking. These strains may have an effect on the product shelf life by contaminating the slicing machine (Bjorkroth and Korkeala 1997)

The microorganisms that are most commonly associated with refrigerated foods and cause food spoilage are psychrotrophs and not psychrophilis (Cousin e*t al.*, 1992).

Gill (1983) identified *L. curvatus* and *L. sake* as representatives of psychrotrophic microbial flora on vacuum–packed meat. Samelis *et. al.* (1998) revealed *Lactobacillus sake* and *Leuconostoc mesenteroides* spp. as the major causative agents of spoilage due to recontamination in the cutting room. Dykes *et al.* (1991) recorded that contamination of sausage surface by lactic acid bacteria occurred as a result of manufacturing and handling process.

Samelis and Georgiadou (2000) stated that mainly *Lactobacillus sakei*, dominated the microbial flora of industrially manufactured sausage. The isolation frequency of *Lactobacillus sakei* / *curvatus* from sausages stored anaerobically was as high as 92-96%, on the other hand Ahn and Stiles (1990) isolated bacteriocins-producing lactic acid bacteria from vacuum-packaged meat stored at 4 ^{o}C, which are antagonistic substances against *Enterococcus* spp. and *Listeria monocytogenes*. Bacteriocins differed in characteristics between strains, most of them still active after heat treatment at 62 ^{o}C for 30 min, except for that of Leuconostoc strains and there is evidence that more than one bacteriocin-like substance may be produced by some strains. Growth medium, pH and growth temperature all affected the production of the inhibitory substances.

Regarding anaerobic bacteriae, Table 2 showed that their mean counts were $9.7x10^7 \pm 1.8x10^7$, $1.8x10^8 \pm 3.2x10^7$, $2.2x10^8 \pm 4.7x10^7$ and $9.8x10^7 \pm 2.3x10^7$/gm of frankfurter, pastrami, rose beef and beef bacon respectively. Nearly similar results for anaerobic counts in frankfurter, rose beef and beef bacon were reported by Hitchener *et al.* (1982); Blikstad and Molin (1983); El-Khawas (2001).

Number of bacteria on pastrami were slightly greater than frankfurter, rose beef and beef bacon, this agree with Holley and McKellar (1996) who found that numbers of bacteria on pastrami were significantly greater than on ham and bologna (pastrami > ham > bologna).

The obtained results illustrated that psychrotrophic count, lactic acid bacteria count and anaerobic count resembling each other to a great

extent, this agree with Mcdaniel *et al.* (1984) who recorded that the total aerobic count was about the same as that of psychrotrophic count in all storage stages. In the same manner, psychrotrophic and anaerobic counts were almost identical with the total aerobic count (Blikstad and Molin, 1983). They added that the similarity of the results of different counts on different media and incubation conditions can be referred to the predominance of microflora by lactic acid bacteria. In vacuum packaging the microflora shift from predominance of *Pseudomonas* spp. to *Lactobacillus* spp. (95% of flora)**.**

Table (3) revealed that *S. aureus* could be isolated from vacuum packed frankfurter (10%) and beef bacon (20%) while *L. monocytogenes* could not be detected. Staphylococcal food poisoning due to vacuum-packed cooked meat products is relatively rare, although cases are known. *S. aureus* is frequently found in small numbers (about 10^2 cfu/g) on cooked meat (Varnam and Evans, 1991). Carmo *et al.* (2002) recorded that the food handlers are one of the most important sources of contamination by S. aureus which cause food poisoning outbreaks**.**

These results disagree with Abd Al-Hafize (1995); Mohamed (2000); El-Khawas (2001) who failed to detect *S. aureus* and attribute this result to the inhibition of *S. aureus* by lactic acid bacteria. Results agree with Sheridan, *et al.* (1995) who failed to detect *L. monocytogenes***.** Also Samelis *et al.* (2000) pointed out that *L. monocytogenes* was always absent from the vacuum-packed product provided that heating to a core temperature of 70^oC occurred and recontamination during slicing and packing was prevented, otherwise.

Krockel (1992) added that, *L. monocytogenes* was successfully inhibited in various culture media or in meat products by means of bacteriocin-forming *Lactobacillus sake* strain. Vacuum packing and storage under refrigeration is effective in controlling the growth of *L. monocytogenes* in sliced vacuum packed mortadella, indicating that good practices and implemented HACCP programs are essential to assure safety of this product (Bersot *et al.,* 2008). On the other hand Katsaras and Dresel (1994) stated that *L. monocytogenes* counts increased throughout storage of the vacuum-packed sliced bologna-type sausage, in spite of development of high counts of lactic acid bacteria. Tobia *et al.* (1997) could identify five isolates as *L. monocytogenes*. They further added that the presence of this microorganism in this kind of product suggests environmental post-process contamination or insufficient thermal process.

Neither *B. cereus* nor *Cl. perfrengines* could be detected in vacuum packed frankfurter, pastrami, rose beef and beef bacon respectively (Table 3). Similar results were reported by Ouf (2001) who failed to detect it in

any of the examined heat-treated (frankfurter and luncheon) meat products. Moreover the presence of *Lactobacillus* spp. inhibit the growth of *B. cereus* and this mainly due to the presence of bacteriocin produced by the members of this species, which predominate in vacuum-packaged meat products (Suma *et al.*, 1998).

On the other hand Juneja and Marmer (1996) stated that vegetative cells *of Cl. perfringens* were not detected from a spore inoculum in vacuum-packaged even after 28 days of storage in the presence of 3% salt. *Cl. perfringens* growth was not observed at 4^{o}C regardless of salt levels.

Also it is clear from Table (3) that *S. aureus* could be isolated from packed frankfurter and rose beef (10% and 20% respectively). *B. cereus* could be isolated from packed frankfurter and pastrami (10% for each). The low percentage of *L.monocytogenes* had been explained by Thevenot *et al.* (2006) who stated that the inefficiency of cleaning procedures to remove *L. monocytogenes* has associated with its ability to adhere to stainless steel and form biofilms.

Modified atmosphere packaging (MAP) has led the evolution of fresh and minimally processed food preservation, especially in meat and meat products for the past two decades. Packaging technology innovations and ingenuity will continue to provide MAP that is consumer oriented, product enhancing, environmentally responsive, and cost effective (McMillin, 2008).

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