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ANTIMICROBIAL EFFECT OF SOME PRESERVATIVES ON BACILLUS CEREUS ISOLATED FROM SOME MEAT PRODUCTS

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ABSTRACT

Four types of ready to eat meat products represented by minced meat, Beef burger, sausage and luncheon (20 of each) were collected from different shops and supermarkets in Gharbia governorate. Each sample was subjected to bacteriological examination for demonstration of B. cereus and examined the antimicrobial effect of some preservatives on isolated B. cereus from the different samples. The high incidence of B. cereus was recorded in minced meat samples (65%) followed by sausage(40%), beef burger(35%) and luncheon(35%). The use of nisin 100g/ton in combination with potassium sorbate 2000g/ton resulted in decrease count of B. cereus from 7.30 log cfu/g to < 2.00 log cfu/g. This combination have synergistic action and cause bactericidal effect on B. cereus while the use of sodium phosphate alone resulted in mild decrease in B. cereus count from 7.30 log cfu/g to4.52 log cfu/g.

Keywords: Bacillus cereus; Antimicrobial effect; Meat products; Food preservatives.

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1. INTRODUCTION

Ready to eat meat products minced meat , beef burger and sausage and luncheon are highly demoned due to their high biological value , reasonable price , agreeable tast and easy during serving (Soliman,1999).

Meat products are considered excellent media for the growth of many microorganisms including *Bacillus cereus*. On the other hand, meat products constitute public health hazard where bacteria are responsible for unfavorable changes or pathogenic microorganisms can lead to infection and intoxication (Kozareva et al., 1982).

Bacillus cereus food poisoning is a major concern worldwide. This bacterium is an aerobic spore-former commonly found in soil. It can be isolated from raw meat, processed foods and vegetables and entered into the food chain either through contaminated food or water. Food poisoning from the past outbreaks include boiled and fried rice, vegetables, cooked meats, soups, and raw vegetable sprouts (FDA, 2012).

Certain strains of *Bacillus cereus* are capable of producing a heat- labile diarrheal enterotoxin and/or a heat- stable emetic enterotoxin, as well as other toxins leading to human gastroenteritis after ingestion of food containing preformed enterotoxins rather than a result of colonization or infection of host (Granum 1994).

Meat preservation became necessary for transporting meat for long distances without

spoiling or changes in texture, color and nutritional value (Nychas et al., 2008).

Traditional methods of meat preservation such as drying, smoking, brining, fermentation, refrigeration and canning have been replaced by new preservation techniques such as chemical, bio preservatives and non-thermal techniques (Zhou et al., 2010).

Nisin is a preservative that is used to inhibit the germination and outgrowth of spores. Antimicrobials which inhibit the growth of *B. cereus* include benzoate, sorbates and ethylene diamine tetraacetic acid (Jenson and Moir 2003).

Sorbic acid (2, 4-hexadienoic) and its salts are widely used throughout the world as meat preservatives for inhibiting bacteria and fungi. A concentration of 0.3% sorbates in food is high enough to inhibit the microorganisms. The sorbic acid has an inhibitory mechanism via depression of internal pH (Feiner, 2006). Phosphates have the ability to retard the microbial growth because they bind heavy metal ions (Cassen, 1994).

Voris and Stumbo (1965) mentioned that 650 compounds were tested as food preservatives and nisin was the only one reached commercial development due to the following:

-Its toxicity is acceptable by recognized authorities.
-It had not any deleterious effect on the organoleptic properties of food.
-It is stable enough along the shelf life of food.

The Canadian Food and Drug Act recorded that the allowable limit of potassium sorbate is 1000 ppm. and limited the amount of total added phosphate in meat and poultry to 0.5% (DJC, 2009).

The purpose of the current study was planned out to determine the incidence of *B.cereus* in different types of meat products such as minced meat, beef burger ,sausage and luncheon to study the effect of some preservatives on *B.cereus*.

2. MATERIALS AND METHODS

2.1. Collection of samples:

80 random samples of ready to eat meat products minced meat, Beef burger, sausage and luncheon 20 of each were collected from different shops, supermarkets and street vendors in Gharbia governorat. Each sample was subjected to bacteriological examination for presence of B.cereus.

2.2. Preparation of samples:

It was applied according to ICMSF (1974).

2.3. Isolation and Enumeration of B.cereus (Harrigan and Mc Cane 1976)

From each previously prepared dilution, 0.1 ml was seeded into the surface of the polymyxin-pyrovate – egg yolk-mannitol – bromothymol blue agar (PEMBA).

The inoculum was spread over the entire surface of the agar with a sterile bentted glass rod and the plates were inverted and incubated at 37C° for 24 hours then examined for typical colonies of *bacillus cereus* which were turquoise to peacock blue color, about 5mm in diameter and surrounded by a zone of egg yolk precipitation of the same colour . The plates were re-incubated for further 24 hours in order to detect all *B.cereus* colonies. *Bacillus cereus* count / g of the examined sample were calculated (the number of such colonies were multiplied by the reciprocal of the dilution that the countable plate represents) and recorded.

Suspected colonies were picked up and subculture on nutrient agar slopes and incubated at 37C° for 24 hours, then refrigerated at 40 C° for further microbiological examination (Cruickshank

et al. 1975) and biochemical identifications (Holbook and Anderson.1980).

2.4. Antimicrobial effect of chemical preservatives on B. cereus isolated from some meat products:

The effect of addition of some chemical preservatives (nisin, potassium sorbate, sodium phosphate and combination of nisin, potassium sorbate) was studied in irradiated minced meat in a dose of 5.6 KGY(National Center for Radiation Research and Technology, Naser City, Cairo).

2.4.1. Preparation of B. cereus strain:

B. cereus strains were grown on *Bacillus cereus* selective agar medium for 24 hrs. at 37 °C. Pure colonies were grown in nutrient broth at 37 °C for 24 hrs. and streaked on Bacillus cereus selective agar medium for 24 hrs. at 37 °C. One colony was transferred to another Bacillus cereus selective agar medium and incubated at 37 °C for 24 hr. Culture was transferred to nutrient broth and incubated at 37 °C for 24 hr. A cell suspension to an approximate concentration of 8.87 log cfu /ml was obtained depending upon the opacity of the culture (Baker and Breach 1980).The produced suspension was used for experimental inoculation.

2.4.2. Preparation of sample:

Meat was mixed aseptically, manually with growth of *Bacillus cereus* in nutrient broth at 37 °C for 24 hr to reach possible maximum *Bacillus cereus* count /g (Agata et al., 2002). Ten grams from the mixture was cultured and counted. *Bacillus cereus* count /g was 7.30 log cfu/g. then each part was classified into 8 groups A, B, C, D, E, F, G, H. Groups A, B, C, D, E, F and G were inoculated *B. cereus* suspention, while group H was considered as control negative (not inoculated with test strain). Group A was treated by (0.025%) 100g/ton nisin , group B was treated by (0.05%%) 200 g/ton nisin , group C was treated by (0.075%) 300 g/ton nisin, group D was treated by 1000g/ton potassium sorbate. group E was treated by 2000g/ton, potassium sorbate. Group F was treated with1000 g/ton phosphate. Group G was treated by combination of 100 g/ton nisin and 2000 g/ton potassium sorbet, while group H leaved without any treatment (considered as control positive). Then all inoculated and non-inoculated groups were stored in plastic bags at 4 °C in refrigerator, and examined bacteriologically at 1st day and after 7th day. All groups were removed aseptically from bags. 10gm of each sample was homogenate with 90 ml of buffered peptone water 0.1 %, then one ml from each homogenate was transferred into a tube containing 9 ml peptone water, then tenfold serial dilution were obtained till 10^{-7}**.**

3. RESULTS

From table (1), *B.cereus* was isolated from 13 samples of minced meat with an incidence of 65%, 7 samples from 20 Beef burger samples with an incidence of 35%. In addition, *B.cereus* was isolated from 8 samples of sausage out of 20 samples with an incidence of 40% and 7 samples out of 20 Lunchan samples with an incidence of 35%. From the result obtained in table (2), the minmum, the maximum and mean values of *B.cereus* in examined samples were 2.69 , 4.60 log cfu /g and 4.22 log cfu /g. for Beef burger; 3.95 log cfu /g, 5.87 log cfu /g and 5.08 log cfu /g for minced meat; 3.30 log cfu /g, 5.09 log cfu /g and 4.68 log cfu /g for sausage and 2.84 log cfu /g, 4.30 log cfu /g and 3.90 log cfu /g for luncheon, respectively. Concerning the results obtained in table (3&4) the use of nisin 100g/ton in compination with pot. Sorbate 2000 g/ ton on irradiated and artificially inoculated raw minced meat showed that decrease the count of *B.cereus* from 7.00 log cfu /g to <2.00 log cfu /g , use of nisin 300g/ton decrease the count of *B.cereus* to 2.68 log cfu /g, use of nisin 200g/ton decrease the count of *B.cereus* to 2.86 log cfu /g , use

of nisin 100g/ton decrease the count of *B.cereus* to 3.22 log cfu /g, use of pot. Sorbate 1000g/ton decrease the count of *B.cereus* to 4.39 log cfu /g, while use of pot. Sorbate 2000g/ton decrease the count of *B.cereus* to 3.07 log cfu /g and use of sodium phosphate 1000g/ton decrease the count of *B.cereus* to 5.58 log cfu /g.

Table (1): Incidence of Bacillus cereus in the examined meat product samples (n=20).

Samples	Positive sample		Negative sample	
	No.	%	No.	%
Minced meat	13	65	7	35
Beef burger	7	35	13	65
sausage	8	40	12	60
luncheon	7	35	13	65
Total (80)	35	43.75	45	56.25

Table (2): Statistical analytical results of Bacillus cereus Count (log cfu /g) of examined meat product samples (n=20).

Samples	Min.	Max.	Mean ± S.D.
Minced meat	3.95	5.87	5.08 ± 2.38^{a}
Beef burger	2.69	4.60	4.22 ± 1.48^{ab}
sausage	3.30	5.09	4.68 ± 1.83^{ab}
luncheon	2.84	4.30	3.90 ± 0.72^{b}

S. D = Standard Deviation of mean a-b different letters within the same column differ significantly at $P < 0.05$ Data are expressed as mean log colony-forming units per gram.

Table (3): Antimicrobial effect of preservatives on the survival of *Bacillus cereus* inoculated into irradiated minced meat after 24 hrs. (n=5).

Preservatives	Min.	Max.	Mean± S.D.
Nisin100g/ tons	4.48	4.95	$4.704 \pm .173^{b}$
Nisin200g/ tons	2.60	3.30	$2.965 \pm .279^{d}$
Nisin300g/ tons	2.30	3.23	$2.846 \pm .389^{d}$
Pot.sorbat1000g/ tons	5.45	5.78	$5.632 \pm .139^{a}$
Pot.sorbat2000g/ tons	3.08	3.65	$3.361 \pm .217^{c}$
Na.Phosphat1000g/ tons	5.41	5.70	$5.583 \pm .107^{a}$
Nisin100g/tons+ Pot.sorbat2000g/tons	2.30	3.15	$2.826 \pm .384^{d}$

S.D = Standard Deviation of mean. a-b-c-d different letters within the same column differ significantly at $P < 0.05$

Table (4): Antimicrobial effect of preservatives on the survival of *Bacillus cereus* inoculated into irradiated minced meat after 7 days (n=5).

Preservatives	Min.	Max.	Mean± S.D.
Nisin100g/ton	3.00	3.53	3.22 ± .207 [b]
Nisin200g/ton	2.30	3.30	2.866 ± .383 [bc]
Nisin300g/ton	2.30	3.11	2.684 ± .365 [c]
Pot.sorbat1000g/ton	4.08	4.75	4.391 ± .264 [a]
Pot.sorbat2000g/ton	2.85	3.30	3.0761± .183 [b]
Na.Phosphat1000g/ ton	4.30	4.75	4.52± .167 [a]
Nisin100g/ ton + Pot.sorbat2000g/ ton	-	-	2.00±- [d]<
Control -ve	Deteriorated after four days		
Control +ve	Deteriorated after two days		

S.D. = Standard Deviation of mean. a-b-c-d different letters within the same column differ significantly at $P < 0.05$. Control negative (-ve) irradiated minced meat storage at 4^0C, Control positive(-ve) irradiated minced meat inoculated with log7.00/g of *Bacillus cereus* storage at 4^0C. Data are expressed as mean log colony-forming units per gram.

4. DISCUSSION

Meat additives are considered the main source of *B.cereus* contamination in meat products. Improper handling of meat products after cooking allow the spore of *B.cereus* to germinate and resulting vegetative cells multiply and lead to food poisoning (Torky, 2004).

Concerning the minced meat samples , it was found that out of 20 examined samples, *B.cereus* was isolated from 13 samples with an incidence of 65% as summarized in table(1).

It is evident from the result achieved in table (2) that the minimum *B.cereus* count was 3.95log cfu/g and the maximum was 5.87log cfu/g with a mean value of 5.08±2.38 log cfu/g.

The obtained results were nearly similar to that recorded by El-Sayed et al. (1999) and El- Ghamry (2004) who found that the incidence of B.cereus in minced meat was 58%and 55%, respectively. On the other hand, comparatively lower results of 35% B.cereus in minced meat were reported by Hafez et al. (1990) and Torky (1995) who found that the mean value was 1.6x103±0.7x103 cfu/g.

The presence of *B.cereus* with high percentage in minced meat may be attributed to the storage in room temperature, high content of curing salts and spices in addition to cross contamination between raw and cooked products, besides all of the problems of fluctuation of temperature during cooking (Torky 1995).

The results given in table (1) reflected the presence of *B.cereus* in 7 samples from 20 Beef burger samples with an incidence of 35%.

Table (2) revealed that the minimum *B.cereus* count in Beef burger was 2.69 log cfu /g and the maximum was 4.60 log cfu/g with a mean value of 4.22±1.48 log cfu /g.

Approximately similar findings were recorded by Torky (1995) who found that the incidence of B.cereus in Beef burger was 40%. The obtained results were nearly similar to those reported by El-sherif et al. (1991) who found an average *B.cereus* count of 3x104cfu/g. While, the higher incidence of

48% and 65% were recorded by Ahmed (1991) and Heikal et al. (2006) respectively. On the other hand lower counts were recorded by Lacona et al. (1995) who found that the count of *B. cereus* was 10^2/g.

In table (1), the results showed that from 20 of sausage samples, 8 samples were positive with an incidence of 40%.

From the results achieved in table (2) the minimum *B.cereus* count in sausage was 3.30 log cfu/g and the maximum was 5.09 log cfu/g with a mean value of 4.68±1.83 log cfu/g.

The obtained findings proved to be similar to those reported by Torky (1995) who found that the incidence of *B.cereus* in sausage was 40% with count range from 102 t0 105/g. While, the higher incidence of 70% with a mean value of 8.79 x 104±5.09x104/g was recorded by Heikal et al.(2006). On the other hand, comparatively lower results of 28%,30% *B.cereus* in sausage were reported by El-Sayed et al. (1999) and Eid et al. (2008).

The results given in table (1) reflected the presence of *B.cereus* in 7 samples out of 20 Luncheon samples with an incidence of 35%.

It is evident from the result achieved in table (2) that the minimum *B.cereus* count was 2.84 log cfu/g and the maximum was 4.30 log cfu /g with a mean value of 3.90±0.72 log cfu /g.

The obtained results were lower than the results reported by Khalil (1997) who found that the incidence of *B.cereus* in luncheon was 50%, and Eid et al. (2008) who found that the mean value was$33.8x10^4$ $\pm 1.84x10^4$ cfu /g.

Meat additives are considered the main source of *B. cereus* contamination in meat products. Improper handling of meat products after cooking allow the spore of *B.cereus* to germinate and resulting vegetative cells multiply and lead to food poisoning (Torky, 2004).

The obtained results revealed that the meat products contained high *B.cereus* count and this may be attributed to contamination of flesh used for manufacture, mincing machine, grinders, equipment and knives also considered as source of contamination of meat during processing (El-Mossalami et al., 1994).

The best result obtained by using nisin 100g/ton in combination with potassium sorbate 2000 g/ ton. As they have synergistic action (bacteriostatic and bactericidal).

Control negative (-ve) irradiated minced meat storage at 4°C deteriorated after four days as a result of growth of different microorganisms. Control positive (-ve) irradiated minced meat inoculated with log 7.00/g of Bacillus cereus storage at 4°C deteriorated after two days due to multiplication of microorganisms.

Some types of cooked products are possible to mishandling and temperature, which lead to growth of *B. cereus* and toxin production (Smith et al., 2004).

The microbiological examination of food stuffs plays an important role in assuring the safety and quality of food. Even though the implantation of Hazard Analysis and Critical Control Point (HACCP) system and G.M.Ps. (Good Manufacturing Practices) emphasis to protect the consumers against food borne illness and production of maximum safety to consumers.

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التأثيرات المضاده للبكتريا لبعض المواد الحافظه على ميكروب الباسلس سيرس المعزول من بعض منتجات اللحوم.

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الملخص العربي

اجريت هذه الدراسة على اربعة انواع من منتجات اللحوم (اللحوم المفرومة –البيف بيرجر _ السجق –اللانشون) تم تجميعها من العديد من السوبر ماركت من محافظة الغربية وتم فحص جميع العينات للكشف عن مدى تواجد ميكروب الباسلس سيرس ودراسة تأثير المواد الحافظة (النيسين بتركيز 100 و200 و300 جرام/الطن _ البوتاسيوم سوربات بتركيز 1000 و2000جرام/الطن _ الفوسفات بتركيز 1000 جرام/الطن – الجمع بين النيسين بتركيز 100 جرام/الطن والبوتاسيوم سوربات بتركيز 2000 جرام/الطن) واوضحت نتائج الدراسة تواجد ميكروب الباسلس سيرس فى جميع انواع العينات بنسب مختلفة وكانت اعلى نسبه عزل الميكروب من عينات اللحم المفرومة حيث كان متوسط العد الكلي 5.08 لوج خليه/جرام و يليها السجق 4.68 لوج خليه/جرام ثم البيف بيرجر 4.22 لوج خليه/جرام واخيرا اللانشون 3.90 لوج خليه/جرام. وبدراسة تأثير المواد الحافظة علي ميكروب الباسلس سيرس بعد مرور يوم ثم بعد مرور سبعة ايام من بدء التجربة وجد ان افضل هذه المواد هو الذي جمع بين النيسين بتركيز 100جرام/الطن والبوتاسيوم سوربات بتركيز 2000 جرام/الطن حيث انهم لهم تأثير مميت علي الباسلس سيرس والذي يعتبر احد الميكروبات المسببة لمرض التسمم الغذائي في الانسان ويمثل خطورة كبيرة علي صحة المستهلك. هذا وقد تمت مناقشة الإجراءات الصحية الواجب اتباعها لمنع تلوث منتجات اللحوم بهذا الميكروب للحد من خطورتها على الصحة العامة.

(مجلة بنها للعلوم الطبية البيطرية: عدد 26(1):75-83, مارس 2014)