

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

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ABSTRACT

Received at: 22/4/2015

Accepted: 15/6/2015

This study was carried out to evaluate the prevalence of *Bacillus cereus* in four types of meat products represented by minced meat, beef burger, sausage and luncheon (20 of each) collected from different shops and supermarkets in Gharbia Governorate. The high incidence of *B.cereus* was recorded in minced meat samples (65%) followed by sausage (40%), beef burger (35%) and luncheon (35%). Minced meat samples were irradiated at 10 kGy to ensure sterilization then being examined for studying the antimicrobial effect of some preservatives on isolated *B.cereus* from the different samples. The use of nisin 100g/ton in combination with potassium sorbate 2000g/ton resulted in decreased count of *B.cereus* by three log cfu/g. This combination have synergistic action and cause bactericidal and bacteriostatic effect on *B.cereus*.

Key Words: *Bacillus cereus*; nisin; potassium sorbate; meat products; food preservatives.

INTRODUCTION

Meat products such as minced meat, beef burger, sausage and luncheon are highly demined due to their high biological value, reasonable price, and agreeable taste 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). *B.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 (Food and Drug Administration, 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).

Today, mankind depends on food additives; in fact, the industrialized world would not have been possible without them, USDA (2014). In order to ensure that the food reaches its destination in good conditions, special requirements are needed, mainly to prevent contamination and spoilage (Lerner *et al.*, 2011). The food additive is a substance not normally consumed as a food by itself and not normally used as a typical ingredient of the food, whether or not it has nutritive value, the intentional addition of which to food for a technological (including organoleptic) purpose in the manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food (Carocho *et al.*, 2014). 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).

Voris and Stumbo (1965) mentioned that 650 compounds were tested as food preservatives. Antimicrobials which inhibit the growth of *B. cereus* include benzoate, sorbates and ethylene diamine tetraacetic acid (Jenson and Moir 2003). Generally, preservatives like nisin and potassium sorbate were

approved by FAO (2012) after extensive testing and were safe.

Nisin is a preservative and antibacterial agent that is used to inhibit the germination and outgrowth of spores; it alters cell properties in bacteria to render it harmless. Nisin preparation is considered as safe for human consumption, FDA (2014); it may slow or stop squamous cell head and neck cancers; the Food and Drug Administration and the World Health Organization approved nisin as safe for human consumption decades ago (Joo *et al.*, 2012).

Sorbic acid (2, 4-hexadienoic) and its salts are widely used throughout the world as meat preservative 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). Potassium sorbate is slightly acidic in nature with a pH of about 6.5, when mixed in water it forms a weak acids solution of sorbic acid. The primary use of potassium sorbate is to increase the shelf life of various commercial products without causing any alteration in the taste, smell or color of the food (DJC, 2009). It is widely used in the packaging of canned fruits, vegetables, dairy products and meat products (beef and fish). The Canadian Food and Drug Act recorded that the allowable limit of potassium sorbate is 1000 ppm. Potassium sorbate, also known as E202, which serves as a preservative in a wide range of foods (Cassen, 1994 and Mamur *et al.*, 2010).

Ionizing radiation typically is produced by Gamma radiators or electron accelerators and can kill microorganisms without increasing the temperature of irradiated material. Irradiation damages microbial cell components, including DNA and the cytoplasmic membrane. Irradiation sensitivity of foodborne microorganisms is affected by intrinsic and extrinsic factors. Microorganisms that exhibit increased radiation resistance seem to have efficient mechanisms for repairing damaged DNA (Mendonça and Daraba, 2014).

Aim of the work: 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 as well as studying the effect of some preservatives on *B.cereus*.

MATERIALS and METHODS

1. Collection of samples: A total of 80 random samples of meat products; minced meat, beef burger, sausage and luncheon (20 of each) were collected from different shops, supermarkets and street vendors in Gharbia Governorate. Each sample was subjected

to bacteriological examination for presence of *B.cereus*.

2. Preparation of samples: It was applied according to ICMSF (1974).

3. Isolation and Enumeration of *B.cereus* (Harrigan and McCane, 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 bent glass rod and the plates were inverted and incubated at 37°C for 24 hours then examined for typical colonies of *B.cereus* which were turquoise to peacock blue color, about 5mm in diameter and surrounded by a zone of egg yolk precipitation of the same color. The plates were re-incubated for further 24 hours in order to detect all *B.cereus* colonies. *B.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 37 °C for 24 hours, then refrigerated at 40°C for further microbiological examination (Cruickshank *et al.*, 1975) and biochemical identifications (Holbook and Anderson, 1980).

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, and combination of nisin, potassium sorbate) was studied on irradiated minced meat.

- **Irradiation of minced meat samples:** the samples were subjected to 10 kGy of gamma radiation using the Indian Co-60 gamma cell type 4000A at the NCRRT (the National Center for Radiation Research and Technology in Cairo, Egypt). The gamma-cell gave a dose rate of 2.6 kGy/h at the time of experiment.

- **Preparation of *B. cereus* strain:** *B. cereus* strains were grown on *B. cereus* selective agar medium for 24 hr at 37 °C. Pure colonies were grown in nutrient broth at 37 °C for 24 hr and streaked on *B. cereus* selective agar medium for 24 hr at 37 °C. One colony was transferred to another *B. 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.

- **Preparation of sample:** Meat was mixed aseptically, manually with growth of *B.cereus* in nutrient broth at 37 °C for 24 hr to reach possible maximum *B.cereus* count /g (Agata *et al.*, 2002). Ten grams from the mixture was cultured and counted. *B.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* suspension, 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 G was treated by combination of 100 g/ton nisin and 2000 g/ton potassium sorbate, 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. Ten gm 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 10⁷.

5- Statistical analysis

Whole experiment was conducted 2 times and the results were presented as means, standard deviation and the least significant difference test $p \leq 0.05$ (Draper and Smith, 1998). All statistical procedures were computed using the Microsoft Excel 2007 in order to compare the mean values of the investigated parameters. The mean values obtained from chemical analysis of irradiated samples were compared with non-irradiated samples using the SPSS14 (2006) software. (Petrie and Watson, 1999) Differences were considered to be statistically significant with values of $P < 0.05$.

RESULTS

Table 1: Incidence of *Bacillus cereus* in the examined meat product samples.

Samples (n=20)	Positive sample		Negative sample	
	No.	%	No.	%
Minced meat (20)	13	65	7	35
Beef burger(20)	7	35	13	65
Sausage (20)	8	40	12	60
Luncheon (20)	7	35	13	65
Total (80)	35	43.75	45	56.25

Table 2: Statistical analytical results of *B.cereus* Count (log cfu /g) of examined meat product samples.

Samples(n =20)	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 Nisin and Pot. sorbate on the survival of *B.cereus* inoculated into irradiated minced meat after 24 hrs. (n=5).

Preservatives	Min.	Max.	Mean± S.D.
Nisin100g/ ton	4.48	4.95	4.704 ± .173 ^b
Nisin200g/ ton	2.60	3.30	2.965 ± .279 ^d
Nisin300g/ ton	2.30	3.23	2.846 ± .389 ^d
Pot.sorbat1000g/ ton	5.45	5.78	5.632 ± .139 ^a
Pot.sorbat2000g/ ton	3.08	3.65	3.361± .217 ^c
Nisin100g/ton+ Pot. sorbat2000g/ton	2.30	3.15	2.826± .384 ^d

S.D = Standard Deviation of mean

a-b-c-d different letters within the same column differ significantly at P < 0.05

Data are expressed as mean log colony-forming units per gram.

Table 4: Antimicrobial effect of Nisin and Pot. sorbate on the survival of *B.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	2.86 ± .383 ^{bc}
Nisin300g/ton	2.30	3.11	2.68 ± .365 ^c
Pot.sorbat1000g/ton	4.08	4.75	4.39 ± .264 ^a
Pot.sorbat2000g/ton	2.85	3.30	3.07± .183 ^b
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 °C

Data are expressed as mean log colony-forming units per gram.

Control positive (+ve) irradiated minced meat inoculated with log 7.00/g of *B.cereus* storage at 4 °C.

DISCUION

Meat products are considered the main source of *B.cereus* contamination in meat; improper handling of meat products after cooking allow the spores of *B.cereus* to germinate and result in vegetative cells that multiply and lead to food poisoning (Torky, 2004 and FDA, 2012). 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%. 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%. The results showed that from 20 of sausage samples, 8 samples were positive with an incidence of 40%. The given results reflected the presence of *B.cereus* in 7 samples out of 20 luncheon samples with an incidence of 35%. 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%. While, the higher incidence of 70% 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 obtained results were nearly similar to that recorded by El-Sayed *et al.* (1999) and El-Ghamry (2004); they 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). Torky (1995) found that the

incidence of *B.cereus* in beef burger was 40%. While, the higher incidence of 48% and 65% were recorded by Ahmed (1991) and Heikal *et al.* (2006) respectively. The obtained results were lower than the results reported by Khalil (1997); they found that the incidence of *B.cereus* in luncheon was 50%. 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, equipments and knives also considered as source of contamination of meat during processing (El-Mossalami *et al.*, 1994).

From the result obtained in Table (2), the minimum, 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. It is evident from the result achieved that the minimum *B.cereus* count was 3.95 log cfu/g and the maximum was 5.87 log cfu/g with a mean value of 5.08 ± 2.38 log cfu/g. 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 and Torkey (1995) who found that the mean value was $1.6 \times 10^3 \pm 0.7 \times 10^3$ cfu/g. The obtained results were nearly similar to those reported by El-Sherif *et al.* (1991); they found an average *B.cereus* count of 3×10^4 cfu/g. Heikal *et al.* (2006) recorded a mean value of $8.79 \times 10^4 \pm 5.09 \times 10^4$ /g.

On the other hand lower counts were recorded by Lacona *et al.* (1995); they found that the count of *B. cereus* was 10^2 /g. 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 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. Eid *et al.* (2008) found that the mean value was $33.8 \times 10^4 \pm 1.84 \times 10^4$ cfu /g. The results proved that some types of cooked products were possible to mishandling and temperature which lead to growth of *B. cereus* and toxin production as recorded by Smith *et al.* (2004).

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 (Torkey, Amal, 2004).

Concerning the results obtained in table (3); the use of nisin 100g/ton in combination with pot. sorbate 2000 g/ ton on irradiated and artificially inoculated raw minced meat showed decreased in the count of

B.cereus more than the use of Nisin 100g/ton alone or pot. sorbate 2000 g/ ton alone. The use of Nisin 300g/ton decreased the count of *B.cereus* to 2.68 log cfu /g, the use of Nisin 200g/ton decrease the count of *B.cereus* to 2.86 log cfu /g, the use of Nisin 100g/ton decreased the count of *B.cereus* to 3.22 log cfu /g, the use of pot. sorbate 1000g/ton decreased the count of *B.cereus* to 4.39 log cfu /while use of pot. sorbate 2000g/ton decrease the count of *B.cereus* to 3.07 log cfu /g. Table (3) revealed that the addition of 3 concentrations of nisin (100, 200 and 300 g/ton) reduced log the count of inoculated *B. cereus* by 3-5 log cycles; similar results were recorded by Roberts and Hoover (1996) found that *B.cereus* initial count was reduced by three log cycles when Nisin concentration was 1.0 I.U. / ml. The addition of pot. Sorbate (1000 and 2000 g/ton) reduced log *B.cereus* by 2 and 4 log cycles respectively. Feiner (2006) mentioned that a concentration of 0.3% sorbate was enough to inhibit *B.cereus*. The best result obtained by adding nisin 100g/ton in combination with potassium sorbate 2000 g/ ton. as they have synergistic action (bacteriostatic and bactericidal).

Table (4) show that after 7 days; control negative (-ve) non-irradiated minced meat stored 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 *B. cereus* stored at 4 °C deteriorated after two days due to multiplication of microorganisms. Aouadhia *et al.* (2015) found that the minimum inhibitory concentrations of nisin and potassium sorbate were 5×10^3 IU/ml and 2%; they also found that nisin and potassium sorbate inhibited vegetative cell growth of *B. sporothermodurans*. Ávila *et al.* (2014) reported that nisin (0.05–12.5 µg/ml) was able to inhibit the growth of vegetative cells and spores of food born microorganisms. Arqués *et al.* (2004) proved that the antimicrobial activity of reuterin individually or in combination with nisin (100 IU/ml) against different food-borne Gram-positive and Gram-negative pathogens was increased.

CONCLUSION

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 (FDA's HACCP Programs 2009) and Good Manufacturing Practices emphasis to protect the consumers against food borne illness and production of maximum safety to consumers. It is recommended according to the results of this study to use nisin 100g/ton in combination with potassium sorbate 2000 g/ ton as they have synergistic action (bacteriostatic and bactericidal).

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تأثير بعض المواد الحافظة على ميكروب الباسلس سيرس المعزول من بعض منتجات اللحوم

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أجريت هذه الدراسة على أربع أنواع من منتجات اللحوم (اللحوم المفرومة -البيف بيرجر- السجق واللاتشون) تم تجميعها من العديد من السوبر ماركت من محافظة الغربية وتم فحص جميع العينات للكشف عن مدى تواجد ميكروب الباسلس سيرس والذي يعتبر احد الميكروبات المسببة لمرض التسمم الغذائي في الانسان ويمثل خطورة كبيرة علي صحة المستهلك وقد تمت دراسة تأثير المواد الحافظة بالتركيزات الأتية: النيسين بتركيز ١٠٠ و٢٠٠ و٣٠٠ جرام/الطن - البوتاسيوم سوربيت بتركيز ١٠٠٠ و٢٠٠٠ جرام/الطن- الفوسفات بتركيز ١٠٠٠ جرام/الطن - الجمع بين النيسين بتركيز ١٠٠ جرام/الطن والبوتاسيوم سوربيت بتركيز ٢٠٠٠ جرام/الطن. وقد تم تعريض العينات قبل حقنها بالميكروب محل الدراسة الى جرعة ١٠ كيلوجراي من أشعة جاما للتأكد من تعقيمها. وأوضحت نتائج الدراسة تواجد ميكروب الباسلس سيرس في جميع انواع العينات بنسب مختلفه وكانت أعلى نسبة عزل الميكروب من عينات اللحم المفرومة حيث كان متوسط العد الكلي ٥.٠٨ لوج خليه/جرام ويليهما السجق ٤.٦٨ لوج خليه/جرام ثم البيف بيرجر ٤.٢٢ لوج خليه/جرام وأخيرا اللاتشون ٣.٩٠ لوج خليه/جرام. وبدراسة تأثير المواد الحافظة علي ميكروب الباسلس سيرس المحقون في اللحم المفروم المعقم بالإشعاع بعد مرور يوم ثم بعد مرور سبعة أيام من بدء التجربة وجد أن أفضل هذه المواد هو الذي جمع بين النيسين بتركيز ١٠٠ جرام/الطن والبوتاسيوم سوربيت بتركيز ٢٠٠٠ جرام/الطن حيث أن لهم تأثير مميث علي الباسلس سيرس. هذا وقد تمت مناقشة الاجراءت الصحيه الواجب اتباعها لمنع تلوث منتجات اللحوم بهذا الميكروب للحد من خطورتها علي الصحة العامه.