Trials to control *Bacillus cereus* spoilage of luncheon in two processing meat plants

Dalia F. Khater [1] and Wael F. El Taras[2]

Food Hygiene Dept., Animal Health Research Institute[1] and Department of Hygiene and Preventive Medicine (Zoonoses) Faculty of Veterinary Medicine Kafrelsheikh University [2]

Abstract

Spoilage of luncheon meat produced by two large processing meat plants accompanied with huge economic losses was assessed after a few storage days of production. The principal spoilage bacteria isolated from luncheon meat stored at room temperature have been identified as strains of *Bacillus cereus*. Bacteriological examination revealed that the mean values of total *B. cereus* counts were $7.7 \times 10^6 \pm 2.9 \times 10^6$ for plant I and $1.5 \times 10^8 \pm 6.6 \times 10^7$ CFU/gm for plant II. *Bacillus cereus* were isolated in this study from different raw materials as milk powder (skimmed & full cream), why milk, corn starch, potato starch, flour, Lora paper for plant I and Soya bean (powdered & crushed) for plant II. The contamination levels were ranged from 10^3 to 10^6. The addition of lactate1% or nisin 0.025% either alone or in combination to luncheon meat formula can restrict the potential for *B. cereus* growth. Moreover, the addition of nisin 0.125% was sufficient to prevent the growth of *B. cereus* in luncheon meat product. The study concluded that *B. cereus* may be one of a significant etiological agent of food spoilage, especially among meat products in Egypt.

Introduction

Spoilage is commonly manifested as "off" odors and flavors caused by presence of volatile compounds produced as a result of bacterial metabolism. Certain microorganisms cause deterioration of food quality by altering the sensory characteristics of foods. One of the primary concern in this regard are the spore-forming pathogens that have relatively short lag times and the ability to grow rapidly and/or that may normally be present in large numbers, organisms that possess such characteristics include *Bacillus cereus* **(16).**

Bacillus cereus is one of the few sporeforming, aerobic bacteria recognized as a bacterial pathogen. It is widespread in soil, milk, meat and poultry surfaces, cereals, starches, herbs and spices. The spore-forming ability of *Bacillus* spp. promotes the survival of members of this genus during food processing treatments, and the spores may then germinate if the food is left at room temperature or even at refrigeration temperatures **(5).** The presence of large numbers of *Bacillus cereus* (greater than10^5

organisms/g) in food is indicative of active growth and proliferation of the organism and is consistent with a potential hazard to health **(18; 43).**

Bacillus cereus, is a well-known food-poisoning organism that may cause human illness due to the fact that it produces either heat-stable emetic toxin (cereulide) usually found in fried and cooked rice and pasta or heat-sensitive diarrheal enterotoxins (HBL, NHE, and BcET) most frequently found in milk and meat products **(20; 41).**

In addition, *Bacillus* strains are widespread in meat and meat products **(3; 4; 12; 13; 38; 44)**. The literature is replete with examples of outbreaks of foodborne illness that have resulted from *B. cereus* **(19; 22; 23; 24; 48)**. However, few previous papers reported the presence of *B. cereus* in luncheon meat **(2; 11; 33)**.

During this study, spoilage of luncheon meat in two large processing meat plants accompanied with huge economic losses was assessed. Clear bulging of plastic casing and gas formation indicated spoilage after a few storage days of production. However, 3 months of the expected shelf-lives were still remaining. Therefore, the aim of this work was to identify the main microbial food spoilage process associated with luncheon production and the control strategies available to impede spoilage at various stages of processing and storage.

Materials and methods

1.Collection of luncheon sampling Twenty samples of luncheon meat (5 kg) showing physical signs of apparent spoilage (clear bulging of plastic casing and gas formation) were collected from two large processing meat plants (ten samples from each plant) in Egypt, then the plastic casing was removed under aseptic condition and the samples were examined according to **E.O.S.Q.C. (14)**.

2. Bacteriological analyses

Sample processing A 10 grams of luncheon meat was aseptically weighed into 90 ml of 0.9% NaCl (w/v) and 0.1% (w/v) peptone water in a sterile plastic bag, and then blended in a Stomacher (Seward Medical, London, UK) for 30 seconds, ten-fold serial dilutions were used for bacteriological examination.

Bacterial counts The spread plate technique was used to prepare duplicate plates for determination of aerobic plate counts (APC) and anaerobic plate counts (AnPC) **(26)**. After incubation, duplicate agar plates between 30 and 300 colonies were counted, and then mean counts were calculated.

Isolation and enumeration of Bacillus cereus *Bacillus cereus* was isolated and enumerated according to the method recommended by **Holbrook & Anderson (27),** 0.1 ml of diluted sample was plated in duplicate on *Bacillus cereus* Selective Agar, polymyxin pyruvate egg yolk manitol bromothymol blue agar (PEMBA). Plates were incubated at 37°C for 24 h plus an additional 24 h at room temperature to facilitate the development of turquiose to peacock blue colonies suspected to be *Bacillus cereus.*

Identification of Bacillus cereus All colonies that were rough in texture, turquiose to peacock blue in color, surrounded by greyish zones of egg yolk precipitate and mannitol negative were picked from the plates. Biochemical tests were carried out as described by **Harmon (25)**. Motility, hemolytic activity on trypticase soy sheep blood agar and rhizoid growth on nutrient agar were determined.

3. Examination of raw materials Samples from different raw materials used in production of the luncheon meat, were collected from two processing meat plants and examined for isolation and enumeration of *Bacillus cereus.* Moreover, other samples were obtained from different stages of luncheon processing for determination of APC, AnPC and BcC.

4. Control of *Bacillus cereus* in luncheon meat The following antimicrobial treatments were applied to restrict the growth of *Bacillus cereus* in luncheon meat product. For plant, I lactat1% alone or in combination with nisin 0.025% (100 g Nisaplin " a commercial nisin preparation contains 2.5% active nisin"/ton), were used during processing of luncheon meat, and for plant II nisin 0.025% or nisin 0.125% was applied. After treatments, 10 samples from each treatment were collected. Moreover, 10 samples without any treatment (control) were also collected from each plant. The collected samples were stored for 2 weeks at room temperature, then examined for determination of APC, AnPC and BcC.

The mean values were analyzed statistically (ANOVA, 95% confidence) to determine significant differences between the antimicrobials. The means

of corresponding count types were analyzed for significant differences between the plants.

Results

(Table 1): Mean values of aerobic, anaerobic plate and *Bacillus cereus* (CFU/g) counts of spoiled luncheon meat samples (N=10).

Plant	Aerobic Plate Count			Anaerobic Plate Count			*Bacillus Cereus* Count		
	Min	Max	mean±SE	Min	Max	mean±SE	Min	Max	mean±SE
Plant I	$7.5x10^7$	$4.7x10^9$	$1.08x10^9 \pm 5x10^8$	$5x10^6$	$5.3x10^8$	$1.6x10^8 \pm 6.5x10^7$	$2x10^5$	$3.3x10^7$	$7.7x10^6 \pm 2.9x10^6$
Plant II	$7.5x10^6$	$7.1x10^9$	$2.3x10^9 \pm 8x10^8$	$1.6x10^6$	$2.7x10^8$	$7x10^7 \pm 2.7x10^7$	$4.3x10^6$	$4.7x10^8$	$1.5x10^8 \pm 6.6x10^7$

(Table 2): Isolation and enumeration of *bacillus cereus* (CFU/g) on raw materials used in processing of luncheon meat.

Raw material	Plant I	Plant II
Meat	NI	NI
Water	NI	NI
Milk powder (full cream)	$2.5x10^6$	*
Milk powder (skimmed)	$2x10^5$	*
Why milk	$3.7x10^5$	*
Corn starch	$9.9x10^5$	NI
Potato starch	$6.5x10^6$	*
Soya bean (crushed)	*	$7.8x10^6$
Soya bean (powdered)	NI	$4.9x10^5$
Spices (liquid)	*	NI
Spices (powdered)	NI	*
Flour	$3x10^3$	NI
Black pepper	NI	NI
White pepper	NI	NI
Lora paper	$3.5x10^4$	*

NI = *Bacillus cereus* not isolated

* This material not used by the plant

(Table 3): Aerobic, anerobic plate and *Bacillus cereus* count (CFU/g) of samples collected from different processing stages of luncheon meat product.

Processing stage	Plant I			Plant II		
	APC	AnPC	BcC	APC	AnPC	BcC
Chopping	$4x10^4$	$2.7x10^4$	NI	$7.4x10^4$	$6.9x10^4$	NI
Grinding	$5.3x10^4$	$3.9x10^4$	NI	$8.5x10^4$	$9.7x10^4$	NI
Blending	$3.2x10^5$	$1.1x10^5$	$9.3 x10^3$	$3.8x10^5$	$3.5 x10^4$	$8.5 x10^3$
Emulsification	$4.3x10^5$	$4.1x10^5$	$1 x10^4$	$7.1 x10^5$	$5.5 x10^4$	$9 x10^3$
Casing	$4.6x10^5$	$4.3x10^5$	$4 x10^4$	$1.5 x10^5$	$2.8 x10^5$	$9.6 x10^3$
Cooking	$3.1x10^3$	$2.2x10^3$	$1.1 x10^3$	$5.6 x10^3$	$9.6 x10^3$	$3.2 x10^3$

NI = *Bacillus cereus* not isolated

APC= aerobic plate count, **AnPC**= anerobic plate count, **BcC**= *Bacillus cereus* count

(Table 4): Mean populations (log CFU/g) of aerobic, anerobic plate and *Bacillus cereus* counts on luncheon meat samples (N=10), formulated with and without antimicrobials, two weeks after using antimicrobial substances, followed by packaging and storage at room temp.

Plant	Treatment	APC		AnPC		BcC	
Plant I	No treatment (control)	8.6771	A*	7.8708	A	6.6315	B
	Lactate 1%	4.2771	B	3.2666	B	2.8236	C
	Lactate 1% + Nisin 0.025%	2.7487	D	2.6653	C	1.9042	D
Plant II	No treatment (control)	8.8902	A	7.4735	A	7.6046	A
	Nisin 0.025%	3.6902	C	3.2785	B	2.8484	C
	Nisin 0.125%	2.5877	D	1.9700	D	0.0000	E

*Means with the same letter are not significantly different

APC= aerobic plate count, **AnPC**= anerobic plate count, **BcC**= *Bacillus cereus* count

The collected spoiled luncheon meat samples revealed clear bulging of plastic casing and gas formation. Very offensive odour was detected on cutting. Moreover, softening and discolouration of the surface together with liquefaction of some areas of the product were also observed. These changes were noticed after a few of storage (5-20 days) after production.

Bacteriological analyses Examination of the apparent spoiled luncheon meat samples revealed high aerobic plate count which ranged from $7.5x10^7$ to $4.7 x10^9$ (CFU/g) for plant I and from $7.5 x10^6$ to $7.1 x10^9$ (CFU/g) for plant II. Moreover, Anaerobic plate count ranged from $5 x10^6$ to $5.3 x10^8$

(CFU/g) for plant I and from1.6 x10^6 to 2.7 x10^8 (CFU/g) for plant II (table 1). Isolation and enumeration of the *Bacillus cereus* count revealed high incidence, from 2 x10^5 to 3.3 x10^7 (CFU/g) for plant I and from 4.3 x10^6 to 4.7 x10^8 (CFU/g) for plant II.

Sources of *Bacillus cereus* *Bacillus cereus* was detected in different raw materials. Milk powder (skimmed & full cream), why milk, corn starch, potato starch, for plant I and Soya bean (powdered & crushed) for plant II were recorded to be contaminated with high counts (10^5 to 10^6 CFU/g) of *Bacillus cereus* (table 2), while flour and Lora paper for plant II were less contaminated (10^3 to 10^4 CFU/g). In addition, *Bacillus cereus* was also detected in samples collected from different processing stages of luncheon meat product (table 3).

Control of *Bacillus cereus* Addition of Lactate 1% to the formula of luncheon meat in plant I and nisin 0.025 % in plant II significantly decrease *Bacillus cereus* count from log 6 to log 2 in plant I and from log 7 to log 2 in plant II. Combining lactate 1% and nisin 0.025 % to the formula of luncheon meat resulted in an increased reduction in the viable count of *Bacillus cereus* (log1) in plant I. Moreover, increasing the level of nisin in the formula of luncheon meat to 0.125% in plant II completely inhibit the growth of *Bacillus cereus* (table 4).

Discussion

The presence of food spoilage organisms and pathogens in foods is a major concern to the food processing industry, government regulatory agencies and food consumers. Foodborne pathogens have been responsible for several food poisoning outbreaks, some of which have resulted in serious illness and death. In addition, the presence of pathogenic organisms in foods has led to numerous product recalls, product losses, and considerable negative publicity to the food industry.

Bacteriological analysis of apparent spoiled luncheon meat revealed high number of both total aerobic and anaerobic bacterial count indicating either mixed bacterial contamination or facultative anaerobic microorganisms that

grow well in both aerobic and anaerobic condition. The mean values of total aerobic and anaerobic bacterial count were $1.08 \times 10^9 \pm 5 \times 10^8$ and $1.6 \times 10^8 \pm 6.5 \times 10^7$ for plant I and $2.3 \times 10^9 \pm 8$. X 10^8 and $7 \times 10^7 \pm 2.7 \times 10^7$ CFU/gm for plant II respectively (table 1). Previous investigations (**2; 19; 42; 45; 46**) recorded that the mean values of total aerobic bacterial count of unspoiled luncheon samples collected from Egyptian markets were 1.02×10^7; 1.72×10^6; 4.56×10^5; 1.3×10^5; 9.5×10^5, respectively. Meanwhile, our findings are not similar to those reported by **El-Bab and Sayed (11)** and **Torky (46)** who mentioned that the mean values of anaerobic bacterial count of beef luncheon collected from markets in different governorates were 0.37×10^2 and1.3×10^3 CFU/gm, respectively.

The results of this study indicate that the principal spoilage bacteria isolated from luncheon meat stored at room temperature have been identified as strains of *Bacillus cereus*. *Bacillus cereus* was responsible for those degradative changes (surface softening and discolouration, gas production and eventual product liquefaction) regarded as overt product spoilage. Bacteriological examination revealed that the mean values of total *Bacillus cereus* counts were $7.7 \times 10^6 \pm 2.9 \times 10^6$ for plant I and $1.5 \times 10^8 \pm 6.6 \times 10^7$ CFU/gm for plant II, respectively. From the epidemiological point of view, *Bacillus cereus* was previously detected in luncheon meat (**2; 33**), the contamination levels were generally > 10^5/g.

The preliminary results reported in this paper are reassuring with regard to the potential risk due to the presence of *Bacillus cereus* in heat treated meat products. It should be noted that *Bacillus cereus* are usually counted after thermal process. In this way, only spores are counted, and therefore, the total amount of *Bacillus* cells is unknown and their potential risk could be underestimated. Some information is available about the ability of *Bacillus* spores to germinate during sausage ripening and storage (**4; 21; 28**); therefore, it is essential that control measure should be directed at preventing the germination and outgrowth of spores. Further studies are needed to understand which processing parameters can stimulate or repress spore germination and cell replication.

On the other hand, outbreaks of *B. cereus* are not common, due to the high number of organisms that are necessary to cause infection, but the resistance of its toxins to heat treatment makes it a threat nonetheless in both

raw and cooked foods **(40)**. The diagnosis of *Bacillus cereus* food poisoning can be confirmed by the isolation of greater than or equal to 10^5 *B. cereus* organisms per gram from epidemiologically- implicated food. Underreporting of such outbreaks is likely because illness associated with *B. cereus* is usually self-limiting and not severe. In addition, findings of a recent survey about culture practices for outbreaks of apparent foodborne illness indicate that 20% of public health laboratories do not make *Bacillus cereus* testing routinely available **(32)**. However, the product studied here was in such an advanced stage of spoilage that its consumption would be very improbable.

The high counts of *Bacillus cereus* strains may be due to the different sources of contamination. *Bacillus cereus* were detected in this study in different raw materials as milk powder (skimmed & full cream), why milk, corn starch, potato starch, flour, Lora paper for plant I and Soya bean (powdered & crushed) for plant II respectively. The contamination levels were ranged from 10^3 to 10^6 (table 2). The distribution of thermophilic aerobic sporeforming bacteria in food ingredients is of interest to the food microbiologist because of their potential importance as spoilage organisms in food. The presence of spores of thermophilic bacteria in spices, milk, flour, starch and Soya bean has been recognized as a potential source of spoilage organisms for sometime **(15; 30; 31; 34; 44; 47)**. Since *B. cereus* could derive from different sources and their presence cannot be ruled out, highly effective control measures should be applied for *Bacillus* strains when new ingredients are adopted.

In the present study, our target was to control *Bacillus cereus* spoilage of luncheon meat. Several ways of inhibiting foodborne pathogens in food products using bacteriocins have been reported **(29)**. We investigated the use of two bacteriocins active against *Bacillus cereus*. The effectiveness of lactate and a commercial nisin preparation, which contains 2.5% active nisin, were tested (table 3). Our data showed that the addition of lactate1% or nisin 0.025% (100 g/ton) either alone or in combination to luncheon meat formula can restrict the potential for *Bacillus cereus* growth. Moreover, the addition of nisin 0.125% (500 g/ton) was sufficient to prevent the growth of *Bacillus cereus* in luncheon meat product. Our results substantially agreed with those obtained by **Ronner et.al (39)** who found that nisin was the most

widely effective against the strains of *Bacillus cereus*. Our results proved that increasing the level or kinds of preservative factors is effective in decreasing the number of colony forming units of food borne pathogens.

As demonstrated previously **(6; 7)** and as recorded in this study, nisin treatments reduced bacterial populations greater than 99%, for the tested organisms. The growth of organisms on non-selective media following nisin treatments (0.125%) can be attributed to the presence of nisin-resistant contaminants. Despite differences associated with the various parameters (tissue type, day, organism) examined in this study, nisin treatments were effective for reducing populations of *Bacillus cereus* in fresh luncheon. Nisin treatments may not only improve the shelf life of meat products by inhibiting the growth of spoilage bacteria such as *Bacillus cereus*, but may also enhance the microbiological safety of products by reducing levels of pathogenic bacteria.

The bactericidal mechanism of nisin involves pore formation in the cytoplasmic membrane, leading to an efflux of amino acids, potassium, inorganic phosphate, and a partial efflux of intracellular ATP **(1; 35; 36)**. Both the vegetative cells and the spores of bacilli were reported to be sensitive to nisin **(10; 37)**. Spores of a sensitive strain were claimed to be more sensitive to nisin than the vegetative cells **(9)**. Nisin possibly inhibits the spores during the early stages of germination **(37)**. The inhibitory effect of nisin alone against different bacilli has been shown in laboratory media and in foods **(8)**.

The results of this study concluded that ***B. cereus*** could be a significant etiological agent of food spoilage, especially among meat products in Egypt. Contamination of meat products *with B. cereus* could derive from different sources particularly the ingredients. In order to prevent spoilage of luncheon meat with *Bacillus cereus*, good manufacturing practices should be maintained. Moreover, the application of low doses of nisin in combination with lactate or high doses of nisin alone could restrict the growth of *Bacillus cereus* in luncheon meat product.

References

1. **Abee, T. (1995)** Spore-forming bacteriocins of Gram-positive bacteria and self-protection mechanisms of producer organisms, FEMS Microbiol. Lett. 129, 1-10.
2. **Abostate, M. A. M., Zahran, D. A. and El-Hifnawi, H. N. (2006)** Incidence of *Bacillus cereus* in some meat products and the effect of gamma radiation on its toxin(s). International Journal of Agriculture and Biology, 8 (1), 1-4.
3. **Asplund, K.; Nurmi, E., Hill, P., and Hirn, J. (1988)** The inhibition of the growth of Bacillus cereus in liver sausage. Int. J. Food Microbiol. 7:349-352.
4. **Bell, R.G. and De Lacy, K.M. (1983)** A note on the identity and properties of the spoilage microflora of chub-packed luncheon meat stored at ambient temperature. Can Sep; 29(9):1220-3.
5. **Christiansson, A., J. Bertilsson, and B. Svensson (1999)** *Bacillus cereus* spores in raw milk: factors affecting the contamination of milk during the grazing period. J. Dairy Sci. 82:305-314.
6. **Cutter, C. N. and Siragusa, G. R. (1994)** Decontamination of beef carcass tissue with nisin using a pilot scale model carcass washer. Food Microbiol. 11, 481–489.
7. **Cutter C. N. and Siragusa, G. R. (1996)** Reductions of Listeria innocua and Brochothrix thermosphacta on beef following nisin spray treatments and vacuum packaging. Food Microbiology, 13, 23–33
8. **Delves-Broughton, J. (2005)**: Nisin as a food preservative. Food Australia 57 (12), 524-527
9. **Delves-Broughton, J., Blackburn, P., Evans, R.J. and Hugenholz, J., (1996)** Applications of the bacteriocin, nisin, Antonie van Leeuwenhoek 69, 193-202.
10. **De Vuyst L. and Vandamme, E.J. (1994)**. Nisin, a lantibiotic produced by *Lactococcus lactis* subsp. *lactis*: properties, biosynthesis, fermentation and applications, In: De Vuyst, L., Vandamme, E.J. (Eds.), Bacteriocins of lactic acid bacteria: microbiology, genetics and applications, Blackie Academic & Professional, London, pp 151-221.
11. **El-Bab, G. F. A. F. and Sayed, E. M., (2005)** Some bacterial and chemical investigations on chicken luncheon and beef luncheon. Veterinary Medical Journal Giza, 53(3), 855-862.
12. **Encinas, J. P., Sanz-Gomez, J., García-López, M. L., García-Armesto, M. R., and Otero, A. (1996)**. Evaluation of different systems for the identification of *Bacillus* strains isolated from Spanish fermented sausages. Meat Sci. 42:127-131.
13. **Encinas, J. P.; Sanz, J. J.; García-Lopez, M. L.; and Otero, A. (1999).** Behaviour of *Listeria* spp. in naturally contaminated chorizo (Spanish fermented sausage). Int. J. Food Microbiol. **46**:167-171.
14. **E.O.S.Q.C. [Egyptian organization for standrization and quality control] (2005)** Egyptian standers for requirements of luncheon, No 1114.

15. **Fang, T. J., Chen, C. Y. and Kuo, W. Y., (1999)** Microbiological quality and incidence of *Staphylococcus aureus* and *Bacillus cereus* in vegetarian food products, Food Microbiol. 16, 385-391.

16. **[FDA] Food and Drug Administration of the U.S. Department of Health and Human Services (2001)** December 31, Evaluation and Definition of Potentially Hazardous Foods. Chapter 3: Factors that Influence Microbial Growth.

17. **Gab-Alla, H.M. (1990)** Sanitary status of some meat and poultry products marketed Sharkia governorate. M.V.Sc. thesis, Fac.Vet. Med., Zagazig University.

18. **Gilbert, R.J.; de Louvois, J.; Donovan, T.; Little, C.; Nye, K.; Ribeiro, C.D., Richards, J.; Roberts, D and Bolton, F.J. (2000):** Guidelines for the microbiological quality of some ready-to-eat foods sampled at the point of sale of the PHLS Advisory Committee for Food and Dairy Products Commun Dis Public Health 3 (3), 163-167.

19. **Granum, P. E. and Baird-Parker, T. C. (2000)** *Bacillus* species in Lund, B. M., Baird- Parker, T. C. and Gould, G. W. (EDs), The microbiological safety and quality of food, Aspen Publishers, pp. 1029-1056.

20. **Granum, P. E., and Lund, T. (1997)** *Bacillus cereus* and its food poisoning toxins. FEMS Microbiol. Lett. 157, 223-228

21. **Grohs, B. M., and Kunz, B. (1999)** Antimicrobial effect of spices on sausage spoiling microorganisms using a model medium for sausage type frankfurter. Adv. Food Sci. 21, 128–135.

22. **Haeghbaert, S., Le Querrec, F., Bouvet, P., Gallay, A., Espié, E. and Vaillant, V. (2002a)** Les toxi-infections alimentaires collectives en France en 2001. Bulletin Epidémiologique Hebdomadaire 50, 249-253.

23. **Haeghbaert, S.; Le Querrec, F.; Gallay, A.; Bouvet, P.; Gomez, M. and Vaillant, V. (2002b)** Les toxi-infections alimentaires collectives en France, en 1999 et 2000. Bulletin Epidémiologique Hebdomadaire 23, 105-109.

24. **Haeghbaert, S., Le Querrec, F., Vaillant, V., Delarocque-Astagneau, E. and Bouvet, P. (2001)** Les toxi-infections alimentaires collectives en France en 1998. Bulletin Epidémiologique Hebdomadaire 15, 65-70.

25. **Harmon, S.M. (1982)** New method for differentiating members of the Bacillus cereus group: collaborative study. J. Assoc. Anal Chem. 65, 1133-1139.

26. **Harrigan, F.W., and McCance, M.E. (1974)** Laboratory methods in microbiology. Academic Press, London.

27. **Holbrook, R. and Anderson, J.M. (1980)** An improved selective and diagnostic medium for the isolation and enumeration of Bacillus cereus in foods. Can. J. Microbial. 26, 753-759.

28. **Houben, J. H., and Krol, B. (1989)** Effect of citric acid, citrate and slight aw decreases on the bacteriological stability of Hague liver sausage. Meat Sci. 24:163–176.

29. **Hutkins, R.W.; Berry, E. D. and Liewen, M.B. (1993)** Composition and method for inhibiting pathogens and spoilage organisms in foods. US Patent, 5186962, 2-16.

30. **ICMSF (International Commission on Microbiological Specification for Foods (2005)** Microbioal Ecology of Food Commodities, 2 Nd edition, Kluwer Academic / Plenum Publishers, New York.

31. **Iurlina, M. O.; Saiz, A. I.; Fuselli, S. R. and Fritz, R. (2006)** Prevalence of Bacillus spp. in different food products collected in Argentina. LWT - Food Science and Technology, 39 (2), 105-110.

32. **Kenneth Todar (2006)** Todar's Online Textbook of Bacteriology. University of Wisconsin-Madison Department of Bacteriology, Bacillus cereus Food Poisoning.

33. **Khalil, N. G. (1997)** Incidence of Bacillus cereus in some food stuffs with the special reference to its production of thermonuclease enzyme in Assiut city. Assiut Veterinary Medical Journal, 75, 55-63.

34. **McKee, L. H. (1995)** Microbial contamination of spices and herbs: a review. Lebensmittel-Wissenschaft & Technologie, 28 (1), 1-11.

35. **Moll, G.N.; Roberts, G.C.K.; Konings, W.N. and Driessen, A.J.M. (1996)** Mechanism of lantibioticinduced pore-formation, Antonie van Leeuwenhoek 69, 185-191.

36. **Montville, T.J. and Chen, Y. (1998)** Mechanistic action of pediocin and nisin: recent progress and unresolved questions, Appl. Microbiol. Biotechnol. 50, 511-519.

37. **Ray, B. (1992)** Nisin of Lactococcus lactis ssp. lactis as a food biopreservative. In: Food biopreservatives of microbial origin (eds B. Ray and M.A. Daeschel) pp. 207–264. Boca Raton, Florida, America.

38. **Rodel, W. and Lucke, F. K. (1990)** Effect of redox potential on Bacillus subtilis and Bacillus licheniformis in broth and in pasteurized sausage mixtures. Int. J. Food Microbiol. 10:291-301.

39. **Ronner, A.b.; Degnan, A.J.; Johnson, M.E.; Luchansky, J.B. and Lee Wong, A.C. (1999):** growth and biocontrol of enterotoxigenic *Bacillus cereus* in infant formula and processed cheese prepared with milk powder. Annual Report, pp 23

40. **Rusul, G. and Yaacob, N. H. (1995):** Prevalence of *Bacillus cereus* in selected foods and detection of enterotoxin using TECRA-VIA and BCET-RPLA International Journal of Food Microbiology 25 (1995) 131-139

41. **Ryan, K.J. and Ray, C.G. (editors) (2004).** *Sherris Medical Microbiology*, 4th ed., McGraw Hill. ISBN 0-8385-8529-9

42. **Shaltout, F.A. and Ibrahim, H.M. (1997)** Quality evaluation of locally produced luncheon and Alexandrian sausage. Benha, Vet. Med. J., 8 (2), 279-289.

43. **Slabyj, B.; Bushway, A. and Hazen, R. (2003):** Microbiological Quality and Safety of Food. University of Maine Orono, ME 04473

44. **Te Giffel, M. C.; Beumer, R. R.; Leijendekkers, S. and Rombouts, F. M. (1996)** Incidence of Bacillus cereus and Bacillus subtilis in foods in the Netherlands. Food Microbiology, 13 (1), 53-58.

45. **Tolba, K.S. (1994)** Micro Flora in locally processed frozen meat.Vet. Med. J.Giza. 42 (2) 99.

46. **Torky, A.A. (2004):** Trials for inhibition of some food poisoning microorganisms in meat products PH.D.SC thesis, Fac.Vet. Med., Cairo University.

47. **van Netten, P., van de Moosdjik, A., van Hoensel, P., Mossel, D. A. A. and Perales, I. (1990)** Psychrotrophic strains of *Bacillus cereus* producing enterotoxin. Journal of Applied Bacteriology 69, 73-79.

48. **WHO (2000)** Surveillance Programme for Control of Foodborne Infection and Intoxications in Europe, 8 th report 1993-1998 and 1999-2000.

محاولات للسيطرة على فساد الانشون بالباسيلس سيرس فى إثنين من مصانع تصنيع اللحوم

داليا فتحى خاطر[1], وائل فوزى التراس[2]

[1]قسم صحة الأغذية, معمل طنطا بمعهد بحوث صحة الحيوان, [2] قسم الأمراض المشتركة بكلية طب بيطرى كفر الشيخ

الملخص العربى

أجريت هذه الدراسة على فساد لانشون اللحم بعد أيام قليلة من إنتاجه مما أدى إلى خسارة إقتصادية فادحة فى مصنعين من مصانع تصنيع اللحوم بمصر.حيث تم عزل و تصنيف ميكروب الباسليس سيرس كسبب لفساد اللانشون. أفاد الفحص البكتريولوجى أن متوسط العد الكلى لميكروب الباسليس سيرس كان 7و7 ×10^{6} ± 2,9×10^{6}خلية بالجرام بالنسبة للمصنع الأول, 5و1×10^{8} ± 6,6×10^{7}خلية بالجرام للمصنع الثانى . وقد تم تحديد مصادر تلوث اللانشون بميكروب الباسليس سيرس فى المواد الخام المستخدمة فى التصنيع وشملت بودرة اللبن, شرش اللبن, نشا الذرة, نشا البطاطس, الدقيق, ورق لورا بالنسبة للمصنع الأول وفول الصويا (البودرة والمجروش) بالنسبة للمصنع الثانى وقد تراوح معدل التلوث فى المواد الخام بين 10^{3} إلى 10^{6} وأثبتت الدراسة أن إضافة اللاكتات 1% أو النيسين 0,025% على حده أو مخلوطهما إلى مكونات تصنيع الانشون قد حد من خطورة ونمو الميكروب المسبب للفساد , كما أثبتت الدراسة أن إضافة النيسين 0,125% كان كافيا لمنع نمو ميكروب الباسليس سيرس فى منتج اللانشون وقد خلصت الدراسة إلى أن ميكروب الباسليس سيرس من الأسباب المهمة لفساد الانشون فى مصر.