INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2006/08–1–1–4
http://www.fspublishers.org

Incidence of *Bacillus cereus* in Some Meat Products and the Effect of Gamma Radiation on Its Toxin(s)

MERVAT A.M. ABOSTATE, DALIA A. ZAHRAN† AND HALA N. EL.HIFNAWI[1]‡
Departments of Radiation Microbiology, †Health Radiation Research, and ‡Drug Radiation Research, National Center for Radiation Research and Technology, Atomic Energy Authority, P.O. Box 29, Nasr City, Cairo, Egypt
[1]Corresponding author's e-mail: elhefinawi2005@yahoo.com

ABSTRACT

Forty-five samples of meat luncheon, chicken luncheon and minced chicken (15 sample for each) were collected from Cairo and Giza supermarkets. The mean values of total bacterial and total bacilli count contaminating meat luncheon were 1.02 $x10^7$ and 1.66 x 10^5 CFU/g, respectively. The mean values for chicken luncheon were 1.33 x 10^7 and 7.53 $x10^4$ CFU/g, respectively. However these values for minced chicken were 5×10^6 and 1.4×10^4 CFU/g, respectively. The incidence of *Bacillus cereus* in meat luncheon, chicken luncheon and minced chicken was 60% 53.3% and 60%, respectively. Toxicity test revealed that 2 out of 9, 2 out of 8 and 1 out of 9 isolates from meat luncheon, chicken luncheon and minced chicken gave mice lethal toxin. D10 value of the two highly toxigenic strains of *Bacillus cereus* (no 6 & no 24) after exposure to gamma radiation was 2.2 and 1.9 kGy, respectively. Gamma irradiation had no effect on *Bacillus cereus* toxin lethality till 2 mega. 1 mega gamma radiation may activate *Bacillus cereus* toxin lethality.

Key Words: *Bacillus cereus*; Toxin; Meat products; Gamma radiation

INTRODUCTION

Bacillus cereus is a sporeforming, gram positive, aerobic, rods bacteria. It has been long known as ubiquitous organism found in air, soil and water (Claus & Berkeley, 1986). *Bacillus cereus* is the aetiologic agent of two distinct types of food poisining characterized either by diarrhea and abdominal pain or by nausea and vomiting after ingestion of contaminated foods (Thayer & Boyd, 1994; Nelms *et al.*, 1997).

Hemolytic activity, mouse lethal toxin (MLT), lecithinase (LC) vascular permeability (Vp) activity and factor responsible for fluid accumulation in ligated intestinal loops of rabbits and mice have been found in culture filtrates of *Bacillus cereus* isolated from food poisoning cases (Shinagawa *et al.*, 1991). Abostate (1996) showed the powerful role of hemolysin in the toxicity and virulence of *Bacillus cereus* toxin(s).

Microorganisms control in meat products is the major concern in the preparation of high quality foods (Jo *et al.*, 2004). The hygienic state of animals prior, during and after slaughter can be critical to the finished product quality (Satin, 2002). During slaughtering process the meat is exposed to many sources of *Bacillus cereus* contamination (Lawrie, 1998). The incidence of *Bacillus cereus* is higher in cooked and processed (ground beef) meat than in raw meat samples (Nortje *et al.*, 1999; Mosupye & Von Holg, 2000).

Irradiation is known to be the best method for the control of potentially pathogenic microorganisms in meat without affecting its physical state (Gants, 1998). Food irradiation is generally defined as the process in which foods are exposed to certain forms of ionizing energy from radioactive sources mainly gamma rays. Cobalt-60 is a highly penetrating source of ionizing radiation used in food either fresh or after processing and packaging. Irradiated foods are not radioactive (Satin, 2002).

Because of the effectiveness of irradiation in controlling common food-borne pathogens, in treating packaged food, and thereby minimizing the possibility of cross-contamination prior to consumer use, most food safety officials and scientists view irradiation as an effective critical point in Hazard Analysis and Critical Control Points (HACCP) established for meat and poultry processing (Satin, 2002).

Recently, the US Center for Disease Control (CDC) estimated that if half of the ground beef, pork, poultry and processed luncheon meats in the US were irradiated, there would be over 880,000 fewer cases of food-borne illness, 8500 fewer hospitalization 6660 fewer catastrophic illness, and 352 lives saved every year (Anonymous, 2003).

WHO recommended removing any dosage limit so that it would be possible to achieve commercial sterility as in canning (Anonymous, 1999). High dose irradiated foods are particularly suitable for immunocompromised people who often require a sterile diet. The radiation resistance of a specific organism may vary according to the environment in which it is irrradiated (Tallentire, 1980).

D10 values of bacteria in food are affected by a number of factors, such as water activity, composition, irradiation temperature, presence of oxygen. In addition, some of the constituents of complex food system, such as

proteins, are thought to compete with the cells for the interaction with radiolytic free radicals, thereby reducing the net effect of radiation damage and making the organisms sometimes more radiation resistant (Urbain, 1986).

The objective of this study was to evaluate the microbial load *(Bacillus cereus)* and its ability to produce toxin(s) in some meat products together with the effect of gamma rays on *Bacillus cereus* count and toxin(s).

MATERIALS AND METHODS

Sampling. Forty-five samples of meat luncheon, chicken luncheon and minced chicken (15 samples each) were bought from different supermarkets in Cairo and Giza, to determine their microbial load (total bacterial count, bacilli count & *Bacillus cereus* count).

Microbiological analysis. Twenty-five grams of each sample was homogenized in 225 mL sterile physiological saline solution (0.85% Nacl) in 500 mL conical flask using a Stomacher model 400 (Seward laboratory london) for 1-2 min, then decimal dilutions were prepared. Total bacterial counts were enumerated on plate count agar (PCA) medium using pour plate technique. Total bacilli were counted on Luria broth (L.B) medium (tryptone 10 g, yeast extract 5 g & Nacl 5 g per Liter) and *Bacillus cereus* on Mossel's *Bacillus cereus* selective agar (MYP) medium (Mossel *et al.*, 1967) by spread method on prepoured plates.

Production of toxins. According to Shinagawa *et al.* (1991), *Bacillus cereus* strain 6 and 24 were inoculated on Brain heart Infusion Agar slants and incubated at 32°C for 14-16 h. Then from each fresh slant inoculate 20 mL Brain Heart Infusion Broth (BHIB) in 100 mL conical flasks for 16 h at 32 °C on a shaking water bath at 150 cycles/m. One ml from the 16 h culture of each strain was inoculated into 100 ml BHIB containing 1% glucose (BHIG) in 500 mL conical flasks for 16 h at 32°C with continuous shaking at 150 cycles/m. Bacterial cells were removed by centrifugation at 8000 rpm for 20 m at 4°C, followed by filteration of the supernatants through disposable millipore (0.45 µ) filters. The resulting filterate or culture free-cells were used as crude toxins.

Toxicity test. Thirty-five isolated strains of *Bacillus cereus* and one standard *Bacillus cereus* strain (ATCC 11778) were used to produce *Bacillus cereus* crude toxins. 0.5 mL of each crude toxin was injected intravenous in the tail vein of each mouse (18-20 g). Three mice were used for each strain.

Determination of D10 values. Thirty-six samples of meat and chicken luncheon 10 g each were heat sealed in polyethylene bags. The samples were sterilized using 25 kGy by accelerated electrons (current, 2.1 m A & speed 1.6 m/ min) in National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt. Sterility test was confirmed by plate count. Two *Bacillus Cereus* strains (strain no 6 for meat luncheon & strain no 24 for chicken luncheom) were used for artificial inoculation. The strains were maintained on nutrient agar (oxoid). The cultures were propagated for 16-18 h (stationary phase cells) in 100 mL flask containing 50 mL nutrient broth (oxoid), which was agitated on rotary shaker at 150 rpm and incubated at 35°C. Then the cultures were serially diluted in sterile saline (0.85% NaCl) for standardization by pour plate assay in duplicate using nutrient agar plates incubated for 18 h at 35°C.

One ml of the standardized inoculum was surface inoculated in each sterile sample and left overnight at 4°C. Inoculated samples were then exposed to 2, 4, 6, 8 and 10 kGy radiation doses of gammarays (Co-60) in NCRRT (3 samples for each dose) while the control samples (0 kGy) were left unirradiated. The dose rate was 4.3 kGy/h. Serial dilutions from each sample were made and assayed for CFU by standard pour plate technique using nutrient agar (oxoid). Petri plates were incubated at 35°C for 24 h before counting. The average number was calculated for each sample. The log number of survivors was plotted against the absorbed radiation dose in kGy. Linear regression was applied using Excel program to produce the best fitting line for each treatment, from which the D 10 values were calculated as the reciprocal of the absolute value of the regression line (Lopez-Gonzalez, 1999).

Effect of radiation on *Bacillus cereus* toxin. 5 mL of crude toxin (6 or 24) in screw capped test tubes were exposed to 1 mega or 2 mega (Co-60). Three tubes were used for each dose or for the un-irradiated control. Then 0.5 mL from each tube was injected in mice.

RESULTS AND DISCUSSION

Sampling. Results shown in Tables I, II and III revealed total bacterial and total bacilli counts contaminating 45 samples of meat luncheon, chicken luncheon, minced chicken (15 samples each), respectively. The mean values were 1.02×10^7 and 1.66×10^5 for Table I, 1.33×10^7 and 7.35×10^4 for Table II and 5×10^6 1.4×10^4 for Table III. The total bacterial count is considered an index of quality, which gives an idea about the hygienic measures during processing and helps in the determination of the keeping quality of the product (Aberle *et al.*, 2001). High total bacterial count might be attributed to the contamination of the product from different sources or un-satisfactory processing as well as it may be due to un-suitable condition during storage (ICMSF, 1980; Cox *et al.*, 1998; Gill & Jones, 1999; Zahran, 2004).

The incidence of *Bacillus cereus* (Table IV) in meat luncheon, chicken luncheon and minced chicken was 60%, 53.3% and 60%, respectively. *Bacillus cereus* is one of the potential spoilage bacteria associated with red meat (Nel *et al.*, 2004). In a study done by Mosupye and Von Holg (2000), *Bacillus cereus* was predominont in both raw and prepared food stuffs. They also mentioned that the presence of *Bacillus cereus* at high levels, indicate a potential risk of producing toxins.

Table I. Bacterial count contaminating meat luncheon

Count (CFU/ml)	Minimum	Maximum	Mean
Total bacteria	8.0×16^5	3.2×10^7	1.02×10^7
Total bacilli	4.0×10^2	2.5×10^5	1.66×10^5

Table II. Bacterial count contaminating chicken luncheon

Count (CFU/ml)	Minimum	Maximum	Mean
Total bacteria	8.0×16^5	3.1×10^7	1.33×10^7
Total bacilli	2.0×10^2	1.2×10^5	7.53×10^4

Table III. Bacterial count contaminating minced chicken

Count (CFU/ml)	Minimum	Maximum	Mean
Total bacteria	7.5×16^6	4.0×10^7	5×10^6
Total bacilli	1.2×10^4	6.4×10^7	1.4×10^4

Table IV. Incidence of *Bacillus cereus* in the examined samples

Samples	Positive samples		Negative samples	
	No	%	No	%
Meat luncheon	9	60	6	40
Chicken luncheon	8	53.3	7	46.6
Minced chicken	9	60	6	40

Toxicity test. The toxigenicity of the tested strains from all positive samples indicated that five of them (isolated *Bacillus cereus* strains no 2, 6, 24, 28 & 38) were lethal to mice. Comparing the lethality of the five strains with that *Bacillus cereus* ATCC 11778, revealed that strains 6 and 24 were the greatest strains in toxicity. One of the three mice used for each strain died immediately after injection, another one died after one hour while the third one died overnight, mean-while the three mice of the standard strain ATCC 11778 died overnight.

The failure in many cases to isolate *Bacillus cereus* from the blood or spleen of dead animals from cereobacillus disease suggested that the illness was toxemia (Burdon *et al.*, 1967) Wong *et al.* (1988) found that 100% of 183 isolates gave positive hemolysin activity and 3 of 11 selected isolates showing strong haemolysin activity, killed adult mice. Garcia-Arribas *et al.* (1988) found that 24 out of 39 *Bacillus cereus* strains gave positive mice lethal test. All tested strains possessed phospholipase activity.

The pathogenicity and toxigenicity, in mice of tested strains indicated the difference among the strains. This difference might be attributed to the difference in virulence of strains or to the immunity of the mice Goepfert *et al.* (1972) indicated that when the generalized infection dose occurred, it was possible that the host was in a weakened condition and un-able to defend itself against the invading cells Burdon *et al.* (1967) showed that the illness produced by virulent *Bacillus cereus* culture might be called "cereobacillus disease".

Determination of D10 values. Survivor curves were done for the two highly toxigenic strains of *Bacillus cereus* (6 & 24) in meat luncheon and chicken luncheon, respectively after exposure to different gamma irradiation doses (Fig. I & Fig. II). The D10 values (dose required to inactivate 90% of a microbial population) for strain no 6 and no 24 was 2.2 and 1.9 kGy, respectively. These results are in agreement with Abd El-Hady (1993) who found that the D10 values of 3 strains of *Bacillus cereus* were 2.3, 2.2 and 2.0 kGy, while Abostate (1991) found that the D10 value was 1.5 kGy. On the other hand, the D10 value of *Bacillus cereus* in marinated beef ribs was 0.66 ±0.01 kGy (Jo *et al.*, 2004), in roast beef meal components was 0.126-0.288 kGy (Grant *et al.*, 1993), while in semi-dried seafood products was 0.64 kGy (Chawla *et al.*, 2003).

Thayer *et al.* (1995) reported that reduced water content or increased Nacl levels may result in the survival levels of the food-borne pathogens of irradiated meat to be greater than expected. Although Bhide *et al.* (2001) reported that gamma irradiation is not very effective against gram positive spore forming bacteria. Like *Bacillus cereus* and *Clostridium* spp. Shay *et al.* (1988) required a 2-5 kGy dose of irradiation to destroy the vegetative bacteria of *Bacillus cereus* 2 kGy gamma irradiation dose decreased growth and toxin production by *Bacillus cereus* in roast beef and gravy at abuse temperatures of 15 and 22°C (Grant *et al.*, 1993).

Thayer and Boyd (1994) stated that controlling stationary phase cells and endospores may be more important for food safety than controlling logarithmic phase cells because of the high radiation resistance of the

Fig.1. Dose response curve of highly toxigenic *Bacillus cereus* isolated from meat luncheon

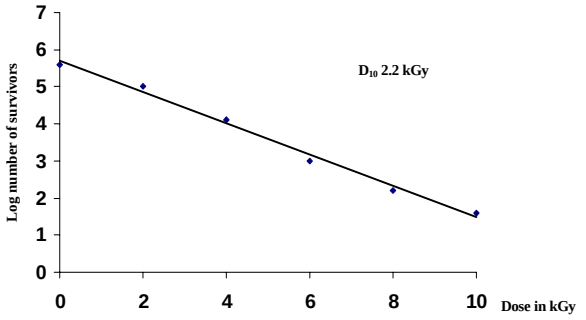
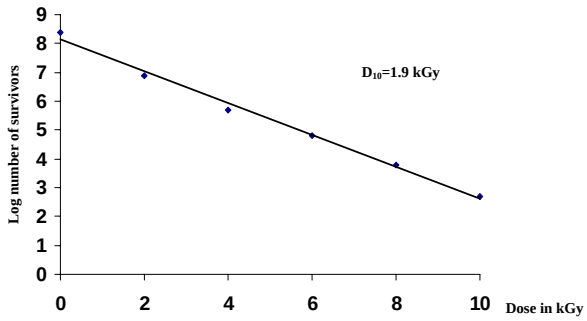


Fig. 2. Dose response curve of highly toxigenic *Bacillus cereus* isolated from chicken luncheon



endospore and because the stationary phase would probably be reached in most abused foods.

Effect of radiation of *Bacillus cereus* toxin. After exposing crude toxin of both isolated strains no 6 or no 24 to 1 mega of gamma radiation, two of the three mice used for each strain were died immediately after injection, while the third one died within one hour. However the un-irradiated control of each strain died within 16 h. Mean-while, the two crude toxins exposed to 2 mega when injected in mice, one died immediately after injection, while the two others died with-in 14-16 h. The results indicated that exposure to 1 mega gamma radiation did not affect the toxicity of *Bacillus cereus* toxin, but it may activate its toxicity Kamat *et al.* (1987) reported a similar result that gamma irradiation had no effect on *Bacillus cereus* toxin lethality.

Food irradiation can not be used to destroy microbial toxins nor viruses and spores be killed at the low doses used to kill vegetative pathogens (below 10 kGy). That is why irradiation treatments below 10 kGy are regarded similarly to heat pasteurization. However, irradiation is not a stand-alone process that can guarantee safe food. It must be integrated as part of an overall good manufacturing practice program (Satin, 2002).

REFERENCES

Abd El-Hady, H.N., 1993. Resistance induced in some isolates of bacteria due to repeated exposure to gamma irradiation. *M.Sc. Thesis*, Faculty Science, Ain Shams University, Cairo, Egypt

Aberle, E.D., J. Forrest, D.E. Gerrard and E.W. Mills, 2001. *Principles of Meat Science* (4th ed). Hunt Publishing Co., Kendall, USA

Abo-State, M.A.M., 1991. Control of *Bacillus cereus* isolated from certain foods. *M.Sc. Thesis*, Faculty Science, Cairo University Cairo, Egypt

Abo-State, M.A.M., 1996. Study of genetic back-ground and effect of radiation on toxin production by *Bacillus cereus. Ph.D Thesis*, Faculty Science, Cairo University, Giza Egypt

Anonymous, 1999. *High dose irradiation: wholesomeness of food irradiated with doses above 10 kGy report of joint FAO/IAEA/WHO study group*, Geneva World Health Organization

Anonymous, 2003. Irradiation enhances food safety and quality. *Food Prot. Trends*, 23: 573–4

Bhide, M.R., A.M. Paturkar, A.T. Sherikar and V.S. Waskar, 2001. Presensitization of micro-organisms by acid treatments to low dose gamma irradiation with special reference to *Bacilus cereus*. *Meat Sci.*, 58: 253–8

Burdon, K.L., J.S. Davis and R.D. Wende, 1967. Experimental infection of mice with *Bacillus cereus*: Studies of pathogenesis and pathogenic changes. *J. Infect. Dis.*, 117: 307–16

Chawla, S.P., D.H. Jo, C. Kim, J.W. Lee and M.W. Byun, 2003. Effect of gamma irradiation on survival of pathogens in kwanegi, a korean traditional semi-dried seafoof products. *J. Food Prot*., 66: 2093–6

Claus, D. and R.C.W. Berkeley, 1986. Genus *Bacillus*. *In*: Sneath, P.H.H, N.S. Mair, M.E. Sarpe and J.C. Holt (eds.), *Bergey's Manual of Systematic Bacteriology*, Vol. II, 9th Edition. pp: 1105–39. Williams and Wilkins, Baltimore

Cox, N.A., S.M. Russell and J.S. Bailey, 1998. The microbiology of stored poultry. *In*: Davies, A and R. Board (eds.), *Microbiology of Meat and Poultry*, pp: 266–87. Blackie Academic and Professional, London

Gants, R. 1998. Irradiation: weighing the risks and benefits. *Meat Poul.*, 5: 34–42

Garcia Arribas, M.L., C.J. Plaza, M.C. Rosa and M.A. Mosso, 1988. Characterization of *Bacillus cereus* strains isolated from drugs and evaluation of their toxins. *J. Appl. Bacteriol.*, 64: 257–64

Gill, C.O. and T. Jones, 1999. The microbiological effects of breaking operations on hanging beef carcass sides. *Food Res. Int.*, 32: 453–59

Goepfert, J.M., W.M. Spira and H.U. Kim, 1972. *Bacillus cereus* food poisoning organism. *J. Milk Food Technol*., 35: 213–27

Grant, I.R. C.R. Mixon and M.F. Patterson, 1993. Effect of low dose irradiation on growth and toxin production by *Staph. aureus* and *Bacillus cereus* in roast beef and gravy. *Int. J. Food Microbiol*., 18: 25–36

ICMSF, 1980. *Microbial Ecology of Foods*, Vol I factors affecting life and death of microorganisms. Academic Press, New York

Jo, C., N.Y. Lee, H.J. Kang, D.H. Shin and M.W. Byun, 2004. Inactivation of food-borne pathogens in marinated beef rib by ionizing radiation. *Food Microbiol.*, 21: 543–8

Kamat, A.S., S.P. Nene, D.P. Nerkar and G.B. Nadkarni, 1987. The nature of toxins produced by *Bacillus cereus* BIS-59. *J. Food Safety*, 8: 71–81

Lawrie, R.A., 1998. *Lawrie's Meat Science (6th ed.)*. Wood-head Publishing Limited, Cambridge

Lopez-Gonzalez, V., S.M. Peter, E.B Robert and A.M. Elsa, 1999. Influence of various commercial packaging conditions on survival of *Escherichia coli* 0157: H7 to irradiation by electron beam versus gamma rays. *J. Food Prot.*, 62: 10–5

Mossel, D.A.A., M.J. Koopman and E. Jongerius, 1967. Enumeration of *Bacillus cereus* in foods. *Appl. Microbiol*., 15: 650–3

Mosupye, F.H. and A. Von Holg, 2000. Microbiological hazard identification and exposure assessment of street food vending in Johannesburg, South Africa. *Int. J. Food Microbiol*., 61: 137–45

Nel, S., J.F.R. Lues, E.M. Buys and P. Venter, 2004. Bacterial populations associated with meat from the deboning room of a high throughput red meat abattoir. *Meat Sci.*, 66: 667–74

Nelms, P.K., O. Larson and D. Barnes–Josiah, 1997. Time to *Bacillus cereus* about hot chocolate. *Public Health Rep*., 112: 240–4

Nortje, G.L., S.M. Vorster, R.P. Greebe and P.L. Steyn, 1999. Occurrence of *Bacillus cereus* and *Yersinia enterocolitica* in South African retail meats. *Food Microbiol.*, 16: 213–7

Satin, M., 2002. Use of irradiation for microbial decontamination of meat: situation and perspectives. *Meat Sci.*, 62: 277–83

Shay, B.Y., A.F. Egan and P.A. Wills, 1988. The use of irradiation for extending the storage life of fresh and processed meat. *Food Technol.*, (Australia), 40: 310–3

Shinagawa, K., K. Ichikawa, N. Matsusaka and S. Sugii, 1991. Purification and some properties of *Bacillus cereus* mouse lethal toxin. *J. Vet. Med. Sci*., 53: 469–74

Tallentire, A., 1980. The spectrum of microbial radiation sensitivity. *Radiat. Phys. Chem.*, 15: 83–9

Thayer, D.W. and G. Boyd, 1994. Control of enterotoxic *Bacillus cereus* on poultry or red meats and in beef gravy by gamma irradiation. *J. Food Prot.*, 57: 758–64

Thayer, D.W., G. Boyd, J.B. Fox and L. Lakritz, 1995. Effects of Nacl, sucrose and water content on the survival of salmonella typhimurium on irradiated pork and chicken. *J. Food Prot*., 58: 490–6

Urbain, W.M., 1986. Biological effects of ionizing radiation. *In*: *Food irradiation. Food Science and Technology Series*, pp: 83–117. Academic Press, London

Wong, H., M. Chang and J. Fan, 1988. Incidence and characterization of *Bacillus cereus* isolates contaminating dairy products. *Appl. Enviorn. Microbiol.*, 54: 699–702

Zahran, D.A., 2004. Using gamma irradiation as an option for controlling bacteria contaminating some foods of animal origin. *Ph. D., Thesis*, Faculty Veterinary Medicine, Zagazig University (Banha Branch), Banha, Egypt

(Received 01 September 2005; Accepted 20 December 2005)