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Effect of Ionizing Radiations on *Escherichia coli* and *Pseudomonas lundensis* in Minced Beef and Chicken

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Abstract. *Escherichia coli* and *Pseudomonas lundensis* in minced beef and chicken were subjected to radiation doses of 0.5, 1.0, 1.4, 1.9, 2.3 and 2.9 kGy and 0.3, 0.6, 0.8, 1.0 and 1.4 kGy, respectively, for bacterial inactivation. *E. coli* count on minced beef was reduced by 6 log cycles after a radiation dose of 1.4 kGy, with a D_{10} value of 0.23, whereas, *P. lundensis* count in minced chicken was reduced by 5 log cycles after a radiation dose of 1.0 kGy, with a D_{10} value of 0.18. *E. coli* and *P. lundensis* were sensitive to radiation treatments and relatively low doses of radiation reduced their numbers significantly.

Keywords: *E. coli*, *P. lundensis*, gamma rays, irradiation, meat shelf life

Introduction

Many food poisoning outbreaks have been related to the consumption of inadequately cooked poultry and beef contaminated with harmful pathogenic bacteria (Doyle, 1991). Microbial safety and shelf life of meat depend on minimizing the initial level of microbial contamination and prolonging the lag phase during microbial growth. The total counts on chicken carcasses were found to be log 3.3/cm^2 before chilling, which increased to 3.81/cm^2 after cutting and to log 4.08/cm^2 after packaging (May, 1962). *Pseudomonas* spp. are the predominant bacteria on raw poultry and beef and are responsible for spoilage of the food (Jay, 1992). Gill (1983) found that *Pseudomonas alkaligenes, Acinetobacter* and *Moraxella* spp. are particularly responsible for the spoilage of beef, whereas other species play relatively minor role in the process. *P. lundensis* is usually involved in spoilage of milk, cheese, meat and fish (Gennari and Dragotto, 1992). Off-odours were detected on poultry surface when the total count on the surface reached to log 7-7.5/cm^2 and slime was noticed when the counts increased to log 7.5-8.0/cm^2 (Ayres, 1960).

Many researches indicated that irradiation treatment significantly reduced both food-borne pathogens and food spoilage bacteria (Satin, 2002). Ionizing radiation is a safe and effective process to inhibit growth of pathogenic bacteria such as that of *E. coli* O157:H7 in meat and meat products. It can eliminate pathogenic bacteria and reduce microbial loads in the meat, resulting in enhanced microbial safety and shelf life of poultry and meat to a great extent (Urbain, 1986). Treating poultry and ground beef with low doses of radiation can kill 99.9% of *Salmonella* population in poultry and reduce a higher percentage of *E. coil* O157:H7 in ground beef (Olson, 1998). US Food and Drug Administration (FDA) approved the use of radiation treatment to eliminate *Salmonella* in poultry and *E. coli* O157:H7 in ground beef. The Codex Alimentarius Commission evaluated the safety and effectiveness of radiation as food preservative and adapted the Codex general standards for irradiated foods (Patterson and Loaharanu, 2000).

The D-value dose required to destroy 90% of the viable organisms for gram-negative bacteria is between 0.29-240 Gy, and for gram positive bacteria is between 180-890 Gy (Farkas, 2006). Gram negative psychrotrophic microorganisms are susceptible to radiation process (Monk *et al.*, 1995). Rod shaped bacteria are generally more sensitive to radiation than cocci.

Sensitivity of bacteria to radiation differs among microorganisms due to the difference in their chemical and physical structures and their ability to recover from radiation injury. Treatment of poultry with 1 to 2.5 kGy dose does not affect its sensory, nutritional and technical qualities. Kiss and Farkas (1972) found that the shelf life of chilled poultry, treated with 1 to 2.5 kGy radiation dose, increased three folds. A radiation dose of 1 kGy is adequate to destroy all *Vibrio vulnificus* in oysters (Mallett *et al.*, 1991). Off flavours were detected when poultry was exposed to over 2.5 kGy radiation (Sudarmadji and Urbain, 1972). Hence the objective of this investigation was to study the effects of ionizing radiation on the *E. coli* and *P. lundensis* in minced beef and chicken, respectively and to determine the D_{10} values for these organisms.

Materials and Methods

Meat preparation. Beef was cut into small pieces, approximately one inch wide, using aseptic technique. Then, the beef

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was ground to mince in sterile stomacher bags. Minced beef was divided into 42 portions (10 ± 0.2 g) and put into sterile stomacher bags (177 mm x 304 mm). Minced beef was squashed into a thin layer in the bag (towards the bottom of the bag). The air in the stomacher bag was removed and the bag was sealed. Fourteen samples were prepared for each trial. Minced chicken was divided into 36 portions (10 ± 0.2 g) and put into sterile stomacher bags (177 mm x 304 mm). Twelve chicken samples were prepared for each treatment. The minced chicken and beef were sterilised by radiation at 20 kGy. The radiation treatment was carried out at Queen's University of Belfast.

Culture details. *E. coli* NCTC 10538 is present in the intestinal tract of most of the warm blooded animals. It is used as an indicator of faecal contamination. *P. lundensis* is a bacterial species isolated from meat. It is gram negative, rod shaped, motile, oxidase and catalase positive, is capable of using glycerol as source of carbon and grows at low temperatures (Jay, 1992).

Culture preparation. Tryptic soy broth (TSB) was inoculated by taking a loopful of culture from triple sugar iron agar slope (TSI). The broth was incubated for 18 h at 37 °C to reach stationary phase. After that a decimal dilution series was prepared up to 10^{-4} from the stationary phase culture. One ml of the 10^{-4} dilution was transferred to 10 ml of TSB and incubated at 37 °C for 18 h.

Inoculation of minced beef and chicken. Sterile scissors were used to open each stomacher bag containing pre-sterilised minced chicken (10 ± 0.2 g). Each stomacher bag containing the pre-sterilised chicken sample, was inoculated with 1 ml of bacterial suspension (*P. lundensis*). The bag was resealed. Sterile scissors were also used to open the stomacher bag containing the pre-sterilised mince beef. Bags containing minced beef were also inoculated with 1 ml of the bacterial suspension (*E. coli* NCTC 10538). Heat sealers were used to reseal the bags. The inoculated samples were stored overnight at 4 °C before irradiation.

Radiation processs. Minced beef and chicken samples were radiated using Cobalt 60 source (Gammabeam 650, Nordion International Inc., Canada) at a dose rate of 1.2 kGy/h for radiation resistance tests and 10 kGy/h for sterilisation. The temperature was kept at 4 °C during the radiation processs. The Gammachrome YR (PMMA) Dosimeter (United Kingdom Atomic Energy Authority, Harwell) was used in the radiation resistance tests to confirm the dose received. The change in absorbance at 530 nm was measured spectrophotometrically and the corresponding doses were obtained from a calibration graph provided by the National Physics Laboratory, Teddington, UK.

Radiation treatment. Minced chicken samples, inoculated with *P. lundensis*, were treated with 0.3, 0.6, 0.8, 1.0 and 1.4 kGy radiation doses, whereas, 0.5, 1.0, 1.4, 1.9, 2.3 and 2.9 kGy radiation doses were used to treat minced beef samples, inoculated with *E. coli* NCTC 10538. The experiment was performed thrice.

Microbiological Analysis. Viable cell counts for *P. lundensis* and *E. coli* in minced chicken and beef were analyzed by using TSI and MacConkey agar. Maximum recovery diluent (90 ml) was poured in stomacher bag containing 10 g of minced chicken or beef and homogenized for 2 min. Decimal dilution series was prepared and spread plated (0.1 ml) in duplicate on the TSI for *P. lundensis* count and on MacConkey agar for *E. coli*. Plates were incubated at 44 °C for 48 h for *E. coli* and at 30 °C for 48 h for aerobic total count.

Results and Discussion

Effect of ionizing radiation on *Escherichia coli.* At the beginning of the experiment, *E. coli* count on minced beef (control) was log 7.9 CFU/g. After the minced beef was radiated at 0.5, 1.0 and 1.4 kGy, the *E. coli* counts decreased to log 5.3, 3.7 and 1.8 CFU/g, respectively. However, when minced beef was radiated at 1.9 kGy, there was no viable *E. coli* growth in the plates. The results showed that *E. coli* was sensitive to radiation treatment. According to Niemand *et al.* (1983) the chilled beef, which was radiated at a dose of 2.5 kGy, had their aerobic and anaerobic bacterial counts reduced by 4-5 log. Vacuum-packaged beef sirloin, which was radiated by a dose of 2 kGy, had a shelf life of 10 weeks at 4 °C as compared to the untreated samples which had a shelf-life of only 4 weeks at 0 °C (Niemand *et al.*, 1981). Psychrotrophic aerobic bacteria were reduced by 3 logs when meat was irradiated at 2.5 kGy (Lefebvre *et al.*, 1992). The coliforms and *E. coli* O157:H7 counts were undetectable after treatment with 1.0 and 1.5 kGy, and stored for 15 and 30 days, respectively, at -18 °C, (Halkman, 2004).

Fig. 1 shows the survival curve of the *E. coil* in minced beef and the D_{10} value after treatment with radiation. The r-square (R^2) of the regression line was 0.99. D-value was obtained as the reciprocal of the slope of the line. The results also indicated that D_{10} value for *E. coil* in minced beef was 0.23 kGy at 4 °C. The D_{10} value for *E. coli* O157: H7 was 0.241 at 4 °C in beef (low fat) and 0.251 kGy in ground beef (high fat) (Clavero *et al.*, 1994). According to Halkman (2004), the D_{10} value for coliforms and *E. coli* O157: H7 was 0.293 and 0.245 kGy at 18-20 °C in minced beef, respectively.

Effect of ionizing radiation on *Pseudomonas lundensis.* No viable cells of *P. lundensis* were detected in the minced chicken when irradiated with 1.4 kGy. Therefore, the results indicate

that the 1.4 kGy is effective in deactivating and controlling the growth of *P. lundensis* in minced chicken. *P. lundensis* count in the untreated minced chicken was log 7.1 CFU/g. Fig. 2 shows the significantly lower counts of *P. lundensis* in minced chicken, treated with radiation. After treatment with 0.3, 0.6, 0.8 and 1.0 kGy, *P. lundensis* counts decreased to 5.8, 4.2, 2.7, 1.8 log CFU/g, respectively.

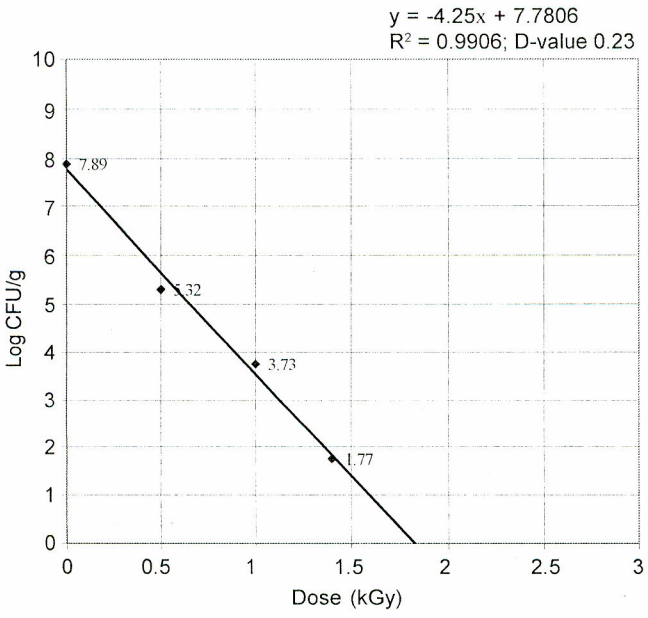

Fig 1. Population of *E. coli* NCTC 10538 in minced beef and D-values after treatment with radiation.

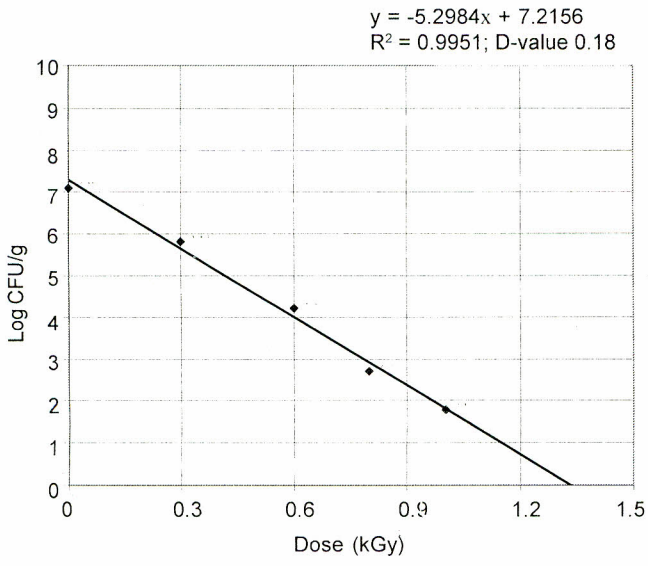

Fig. 2. Population of *Pseudomonas lundensis* in minced chicken and D-values after treatment with radiation.

Aziz *et al.* (2002) found that *Pseudomonas*, coliform including faecal coliform and coagulase-positive Staphyllococci were eliminated when poultry meat was treated with 3 kGy radiation. *Pseudomonas* sp. is very sensitive to radiation processes (Jay, 1992). *Salmonella* in American filet decreased to log 2 when treated with 1 kGy of radiation, whereas *Campylobacter jejuni* and *Yersinia enterocolitica* were reduced to more than 4 log (Kampelmacher, 1984) after the radiation treatment. The radiation can eliminate a wide range of gram negative bacteria which are responsible for the spoilage of meat and meat products.

The results indicated that D_{10} value for *P. lundensis* in minced chicken was 0.18 kGy at 4 °C. The r-square (R^2) of the regression line was 0.99. D_{10} value for *P. fluorescens* in beef (low fat) was 0.13 at 5 °C (Maxcy and Tiwari, 1973). However, D_{10} value for *P. putida* was 0.08 at 4 °C (Patterson, 1988).

Conclusion

The results of this study have indicated that ionizing radiation would be a very effective procedure to control pathogenic and non-pathogenic bacteria in meat and meat products. A low dose of radiation is enough to extend shelf life of meat and meat products. The benefit of the use of low dose for treatment of meat is that it will not change the meat characteristics but will enhance the shelf life considerably. Also, the meat quality and safety will be improved and meat losses due to spoilage will be reduced.

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