

## A Comparative Study on the Effectiveness of Trisodium Phosphate, Citric Acid and Ionizing Radiations in Control of *Salmonella* Enteritidis, *Escherichia coli* O157:H7 and *Staphylococcus aureus* in Beef and Chicken

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**Abstract.** A comparative study on the effectiveness of trisodium phosphate (TSP), citric acid (CA) and gamma radiation in the control of *E. coli* O157:H7, *Salmonella* Enteritidis and *Staphylococcus aureus* in beef and chicken meat was undertaken. In beef meat samples inoculated with *E. coli* O157:H7 and treated with 10% solution of commercial grade CA, extra pure CA and TSP for 10 min, the bacterial count was reduced 2.2, 2.3 and 4.9 log, respectively. Similarly when chicken meat samples were inoculated with *S. Enteritidis* and treated 10% solution of extra pure CA and TSP for 10 min, the initial count was reduced by 3.5 and 5.2 log, respectively. In beef and chicken meat samples, inoculated with *E. coli* O157:H7, *S. Enteritidis*, and *Staphylococcus aureus* and subjected to gamma radiations, an absorbed dose of 2 kGy was needed to eliminate the *E. coli* O157:H7. 3 kGy was adequate to eliminate the *S. Enteritidis*, and 4 kGy was found adequate to eliminate the *S. aureus*.

**Keywords:** trisodium phosphate, citric acid, gamma radiation, beef, chicken, *Salmonella* Enteritidis, *E. coli* O157:H7, *Staphylococcus aureus*

### Introduction

*Escherichia coli*, *Salmonella* spp. and *Staphylococcus aureus* are found naturally in the environment and intestinal tracts of many warm-blooded animals including chickens, turkeys and cows. During slaughtering and processing, raw meat and poultry can be contaminated with *E. coli* O157:H7, *S. Enteritidis* and *S. aureus*. Most of the food-borne diseases, all over the world, are related to meat and poultry. It is recognized that food of animal origin is a rich medium for microbial growth, especially fresh meat and poultry. In addition, processed meats have pH values, which favour the growth of most of the organisms. Commercially, several techniques are available for the elimination of pathogens from red meat and poultry. Among such techniques are the use of citric acid (CA), trisodium phosphate (TSP) and ionizing radiations which are the focus of this investigation. Saudi Arabia has approved application of radiations to foods on commercial scale and import and export of irradiated foods. Ministry of Commerce and Industry has also approved the use of CA, TSP and irradiation techniques for treatment of *Salmonella* in poultry.

*E. coli* O157:H7 infection causes severe bloody diarrhoea and abdominal cramps. The infection is associated with consumption of undercooked or raw hamburger (ground beef), unpasteurized fruit juices, cheese, curds and raw milk. *E. coli* O157:H7 is a rod-shaped, motile bacterium, gram negative, facultatively anaerobic pathogen. *S. Enteritidis* outbreaks

continue to occur in the U.S. The CDC estimates that 75% of these outbreaks are associated with the consumption of raw or inadequately cooked grade A whole shell eggs. Person infected with *S. Enteritidis* bacterium usually has fever, abdominal cramps, and diarrhoea beginning 12 to 72 h after consuming contaminated food or beverage. The illness usually lasts 4 to 7 days and most persons recover without antibiotic treatment.

Molins (1991) reported that trisodium phosphate plays a major role in poultry and meat processing due to its antimicrobial activity, water holding capacity and lipid oxidation property. EL-Shenawy and Marth (1991) noted that a combination of two preservatives, potassium sorbate and lactic or acetic acid inactivated *Listeria monocytogenes* more effectively than potassium sorbate by itself. Palumbo and Williams (1994) reported that artificial combination of frankfurters with *L. monocytogenes* followed by a 2 min dip in 1% lactic, acetic, and tartaric or citric acids resulted in a 1-2 log kill of the bacteria. Greer and Dilts (1995) reported that effects of organic acids are not always positive in terms of food safety. *Listeriae* (*Listeria monocytogenes* and *Listeria* spp.) when exposed to organic acids, may survive and repair themselves during storage at low temperatures and can multiply if other barriers are not present.

Paul *et al.* (1990), extended the shelf life of lamb meat under refrigerated conditions up to 7 days using low dose of radiations (below 10 kGy). Hitoshi *et al.* (1993) irradiated shrimps

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for disinfection and reported that irradiation neither deteriorates the quality nor leaves any residue and does not thaw the fish during the process. They also reported that 3-5 kGy irradiations can destroy aerobic bacteria, *Vibrio* spp., *Aeromonas hydrophila*, *L. monocytogenes* and *Salmonella typhimurium* in shrimps. The World Health Organization (1999) refers the food irradiation, as one of the most significant contributions to public health made by food science and technology for the reduction and elimination of pathogens in food. Irradiation effectively controls common food borne pathogens and treating of packaged food minimizes the possibility of cross contamination prior to use. Lewis *et al.* (2002) demonstrated that even a small dose of 1.8 kGy of radiations by electron beam can effectively eliminate *Salmonella* from poultry breast fillets without significantly altering product quality. Olson (1998) reported that radiations destroy bacteria at a known rate and since it is used on packaged product, no contamination can occur until the package is opened.

This investigation compares three techniques TSP, CA and gamma irradiation to identify the most effective technique among them. In addition, the present study also investigates the effectiveness of these three techniques for the treatment of *E. coli* O157:H7, *S. Enteritidis* and *S. aureus* in beef and chicken meat.

## Materials and Methods

*E. coli* O157:H7, *S. Enteritidis* and *S. aureus* were used in this study. Commercial grade citric acid was obtained from Daliyan F. T. Z. Wenda International Trade Co. Ltd. China, whereas citric acid (extra pure) and trisodium phosphate (extra pure) were the products of Scharlau Chemie Co., Spain.

**Collection of meat samples.** Beef and chicken meat samples were purchased from local market. Five replicates of each of the two samples were used in this study. Beef and chicken meat samples were collected in plastic bags from the local retail stores, and brought to the laboratory within 15-20 min of purchase. In the laboratory, meat samples were packed in polyethylene bags and refrigerated at low temperature of 0-1 °C.

**Enumeration of microorganisms associated with beef and chicken meat samples.** Meat samples were blended in an electric blender. Ten grams of blended sample, in five replicates of both beef and chicken samples were kept separately in pre-sterilized polyethylene bags containing 90 ml sterile distilled water, mixed well and diluted serially in 10-fold dilution. From different dilutions, 0.1 ml was inoculated on different non-selective and selective solid agar plates, separately, for the enumeration of total bacteria (nutrient agar), and total coliform (MacConkey agar). All the agar plates were incubated at 37 °C for 48-96 h.

**Treatment of beef and chicken samples with commercial grade citric acid, extra pure citric acid and trisodium phosphate.** Ten grams each of the meat samples (beef and chicken) in five replicas were treated/agitated with 10% commercial citric acid, 10% extra pure citric acid and 10% extra pure trisodium phosphate solutions, separately, for 10 min and then the reaction was completed by washing with sterile distilled water. The enumeration of the survived microorganisms was performed following the methods described above.

**Treatment of beef samples inoculated with *Escherichia coli* O157:H7 with commercial grade citric acid, extra pure citric acid and trisodium phosphate.** A total of 30 beef samples (10 g each) were inoculated by injection with 24 h cultured pathogenic *E. coli* O157:H7 (10<sup>6</sup> CFU/ml) and kept for 2 h at 37 °C. Treatment of the samples was made as follows: Five samples were dipped in 5% solution of commercial grade citric acid and five, in 10% solution of commercial grade citric acid) for 5, 10, 15 and 20 min. Five samples were treated with 5% solution of extra pure citric acid and five with 10% solution of extra pure citric acid for 5, 10, 15 and 20 min. Five samples were treated with 5% solution of trisodium phosphate and five, with 10% solution of trisodium phosphate for 5, 10, 15 and 20 min. The treated samples were then washed with sterile distilled water and then placed in 90 ml sterile saline solution (0.85% NaCl), separately macerated and shaken well for a few minutes. They were then diluted serially using sterile saline solution.

**Treatment of chicken samples inoculated with *Salmonella* Enteritidis with extra pure citric acid and trisodium phosphate.** Ten grams of chicken meat samples (in twenty replicas) were inoculated aseptically with 24 h cultured pathogenic *S. Enteritidis* (10<sup>7</sup> CFU/ml) and kept for 2 h at 37 °C. Five samples were then treated with 5% solution of extra pure citric acid and the other five treated with 10% solution of the same for 5, 10, 15 and 20 min. Five samples were then treated with 5% solution of trisodium phosphate and other five, with 10% solution of the same for 5, 10, 15 and 20 min. The treated samples were then washed with sterile distilled water to stop the reaction and then put into 90 ml sterile saline solution, separately macerated, and shaken well for few minutes and then diluted serially using sterile saline solution.

**Treatment of meat samples with gamma radiations.** For irradiation, 5 g of ground chicken and beef samples, each in five replicas, were packed in pre-sterilized polyethylene bags, and kept in frozen condition overnight. Samples were later placed in beakers and irradiated at -2.5 °C with radiation doses of 2.5, 5.0, 7.5 and 10 kGy per hour, using gamma cell - 220, (MDS Nordion, International Inc., Canada).

**Treatment of beef inoculated with *E. coli* 0157:H7, *Salmonella* Enteritidis and *S. aureus* with gamma radiations.** 5 g each of ground chicken meat and beef samples in five replicas were packed in pre-sterilized polyethylene bags and sterilized using high radiation dose (10 kGy per hour). The sterilized samples were later inoculated with 0.5 ml of broth culture of the bacterial pathogens (pre-incubated for 24 h at 37 °C) and incubated at 37 °C for 2 h for attachment to the meat surface. The samples were placed in a beaker and kept overnight in frozen condition. The samples inoculated with *E. coli* 0157:H7 and *S. Enteritidis* were irradiated at -2.5 °C with doses of 1, 2, 3, and 4 kGy per hour whereas the samples inoculated with *S. aureus* were irradiated at the same temperature with doses of 1, 2, 3, 4, and 5 kGy per hour using the same irradiator.

## Results and Discussion

**Quantitative determination of microorganisms.** Beef and chicken meat samples were subjected to microbiological investigations. The highest and lowest counts on MacConkey agar for the beef were  $4.0 \times 10^5$  CFU/g and  $8.7 \times 10^3$  CFU/g, respectively. Also, the highest count and lowest count for chicken meat, were  $1.8 \times 10^2$  CFU/g and  $1 \times 10^2$  CFU/g, respectively. Bacterial counts increased on further incubation. The chicken meat samples were less contaminated than the beef samples as the highest and lowest counts of total bacteria were less in the chicken than in the beef; the chicken samples were also free from coliforms. Similar results were reported by Aziz *et al.* (2002). They reported maximum initial bacterial count of  $4.9 \times 10^6$  CFU/g in beef samples. The population of psychrotrophic bacteria in chicken meat was found to be 3.9 log CFU/g (Hwang and Beuchat, 1995). According to Varteltzis *et al.* (1997), aerobic microorganisms count on raw chicken meat was 4.7 log CFU/cm<sup>2</sup>. Fung *et al.* (1980) classified meat quality (based on the total bacterial count on the meat surface) into four categories. Meat is considered to have low count when viable cell count on the meat surface ranges between 0-2 log CFU/cm<sup>2</sup>; intermediate when in the range of 3-4 log CFU/cm<sup>2</sup>; high in the range of 5-6 log CFU/cm<sup>2</sup>; and very high when 7 or over log CFU/cm<sup>2</sup>.

**Treatment with citric acid (commercial).** When beef and chicken meat samples were separately treated with 10% solution of commercial citric acid (CA) for 10 min, no bacterial growth was found after 24 h incubation but when the incubation period was increased up to 76 h, growth was found to be  $9 \times 10^4$  CFU/g and  $9.3 \times 10^4$  CFU/g for beef and chicken meat samples, respectively. It may be due to the recovery of the injured bacterial cells during incubation period. Shapiro and Holder (1960) found that citric acid was less effective than tartaric acid in preventing growth of microorganism on salad

vegetables. Treatment of salad vegetables with concentration of 1500 ppm citric acid had no effect on microbial growth. Citric acid is used to inhibit yeast growth such as *S. cerevisiae*. The activities of citric acid depends on pH concentration (Doores, 1990).

**Treatment with citric acid (extra pure) and trisodium phosphate (extra pure).** When beef samples were treated with 10% CA (extra pure) and 10% TSP (extra pure) separately, for 10 min, no bacterial growth was found for viable cell counts and coliform counts after 24 h incubation. However, when the incubation period was increased up to 76 h, growth was found and total counts reached  $1.6 \times 10^2$  CFU/g and  $1.2 \times 10^1$  CFU/g, respectively, for 10% CA (extra pure) and 10% TSP (extra pure). The growth after 76 h incubation could be due to the recovery of the injured cells during incubation time. The residual activity of the acids suppressed the growth of the bacteria. Dorsa *et al.* (1998) washed the meat samples with organic acids and found similar results. A small number of microorganisms survived and grew on agar medium after prolongation of the incubation period.

When the chicken meat samples were treated with 10% CA (extra pure) and 10% TSP (extra pure) concentrations, separately for 10 min, no growth was found for either viable cell count or coliform count after 24 h of incubation. However, as the incubation hours were increased up to 76 h, the growth was found only for total count which was  $1.2 \times 10^2$  CFU/g and  $1.2 \times 10^1$  CFU/g, respectively for CA and TSP treatments. Meat may be contaminated with microbes during slaughtering and packaging into retail cuts of meat. Solutions of organic acid and TSP are used for removing or washing away the microbes and/or inhibiting their growth during refrigerated storage. However, large number of microbes persist in injured or in good condition and can grow on the meat stored under refrigeration. The growth after 76 h may be due to the recovery of the injured cells during incubation time. Similar results were reported by Dorsa *et al.* (1997). They treated the meat samples with acid and TSP solutions and the treatment suppressed the bacterial growth but after more incubation hours, the injured bacteria grew on the solid plate medium.

**Effect of citric acid (commercial and extra pure) on pathogenic *E. coli* inoculated into red meat samples.** Fig. 1 shows the effect of commercial grade citric acid on *E. coli* O157:H7 inoculated into beef samples. When beef samples in raw condition were inoculated with freshly cultured *E. coli*, treated with two concentrated solutions of commercial grade citric acid (5%, and 10%) for 5, 10, 15 and 20 min and were cultured on selective Sorbitol MacConkey agar medium for 24-48 h, it was found that the initial count of  $6.3 \times 10^6$  CFU/g decreased by 2.1 log and 3.2 log CFU/g after keeping and agitating in

5% and 10% solution of commercial CA for 5 min whereas after 10 min the count decreased by 3.2 log and 2.2 log CFU/g, respectively. In 15 min, 1.2 log and 2.1 log CFU/g reduction and in 20 min 1.3 log CFU/g reduction, was observed. The results indicated that the treatment of beef with 10% solution of commercial grade citric acid for 5 min resulted in more reduction of bacterial count than other treatments. Also, treatment of beef with 5% solution of commercial grade citric acid for 10 min yielded more reduction than other treatments.

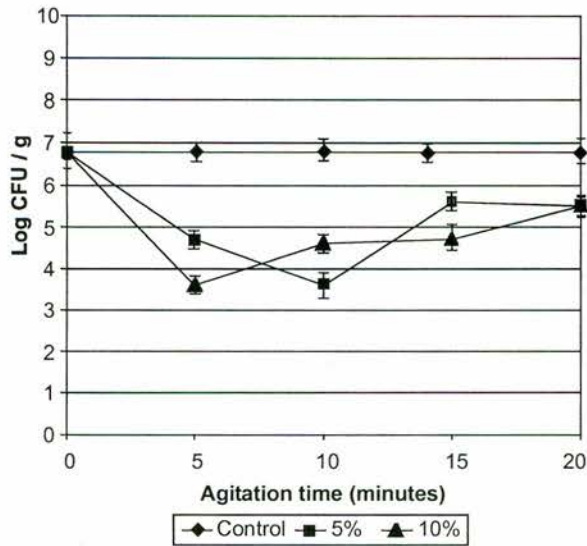


Fig. 1. Effect of commercial grade citric acid on *E. coli* O157:H7 inoculated in ground beef.

Fig. 2 shows the effects of extra pure citric acid on *E. coli* O157:H7 inoculated in beef. The initial count was  $6.3 \times 10^6$  CFU/g and small numbers of bacteria decreased after being kept and agitated in 5% and 10% concentrations of extra pure CA for 5 min, whereas after 10 min the reduction was 1.2 log and 2.3 log CFU/g, respectively. In 15 min, 3.1 and 3.4 log CFU/g reduction occurred in 5% and 10% concentrations, respectively, and in 20 min, 4 log reduction for both treatments was observed. With the increase of time, more number of *E. coli* O157:H7 decreased. Podolak *et al.* (1996) used organic acid on beef samples for inhibition of pathogens and reported that the injured bacteria could grow on selective agar medium after additional incubation.

Fig. 3 shows the effects of extra pure citric acid on *E. coli* O157:H7 inoculated in autoclaved beef. The initial count of the *E. coli* O157:H7 inoculated into autoclaved samples was about  $6.3 \times 10^6$  CFU/g. The initial total count was reduced by 1.4 log to 1.6 log CFU/g after keeping and agitating for 5 min in 5% and 10% concentrations of extra pure CA, whereas after 10

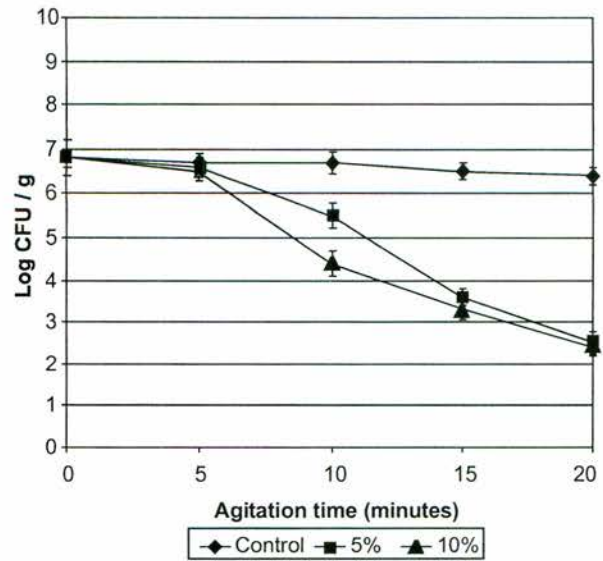


Fig. 2. Effect of extra pure citric acid on *E. coli* O157:H7 inoculated in ground beef.

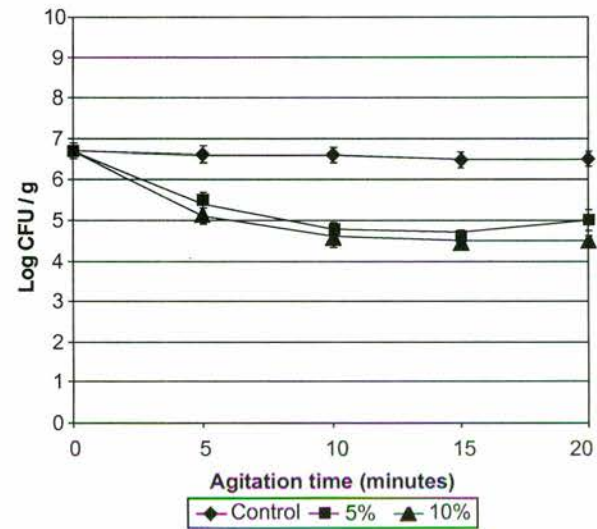


Fig. 3. Effect of extra pure citric acid on *E. coli* O157:H7 inoculated in autoclaved ground beef.

min the reduction was 2.1 log for both of the concentrations. In 15 min, 2.1 log CFU/g reduction was observed for both concentrations, and in 20 min, reduction was 1.2 log and 2.2 log CFU/g, respectively. Agitating for 10 min in 5% and 10% concentrations yielded more reduction than other treatments.

**Effect of trisodium phosphate (extra pure) on pathogenic *E. coli* inoculated in beef samples.** Fig. 4 shows the effect of extra pure trisodium phosphate on *E. coli* O157:H7 inoculated in beef samples. The initial count was  $6.3 \times 10^6$  CFU/g which

was reduced by 2 log CFU/g after keeping and agitating for 5, 10, 15 and 20 min, respectively in 5% concentration of TSP. The reduction of *E. coli* O157:H7 inoculated in beef by dipping and agitation in 10% of TSP concentration for 5, 10, 15, and 20 min was 3.8, 4.9, 4.0 and 3.4 log CFU/g, respectively. Agitating for 10 min, in 10% concentrations resulted in more reduction than other treatments. Similar results are also reported by Morris *et al.* (1997) and Kim *et al.* (1994). They used 10% trisodium phosphate to reduce *E. coli* O157:H7 and *S. typhimurium* on beef and pork surface and got 4.0 log CFU/g reduction in 10 min. Similar results have been reported by Yoon and Oscar (2002). They inoculated *S. typhimurium* in sterile ground chicken breast patties and washed with phosphate solution for 10 min and obtained similar results.

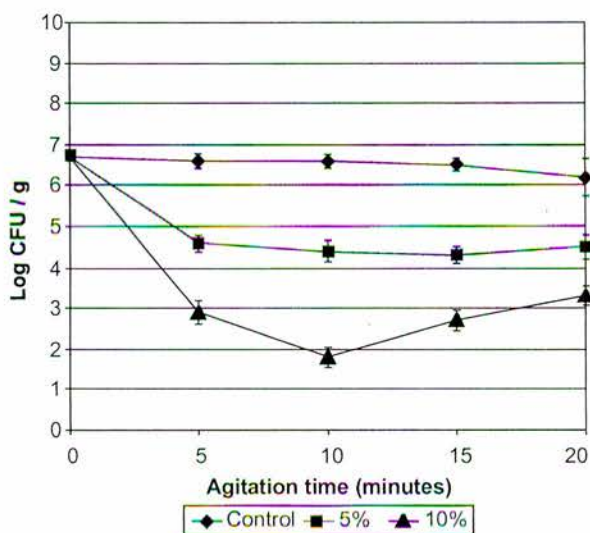


Fig. 4. Effect of extra pure trisodium phosphate on *E. coli* O157:H7 inoculated in ground beef samples.

**Effect of citric acid (extra pure) and trisodium phosphate (extra pure) on pathogenic *Salmonella* Enteritidis inoculated in chicken meat.** Fig. 5 shows the effect of extra pure citric acid on *S. Enteritidis* inoculated in chicken meat. The initial count of *S. Enteritidis* was  $10^7$  CFU/g which decreased by 2.9 log CFU/g after keeping and agitation in 5% and 10% CA for 5 min, whereas after 10 min, the decrease was 3.0 and 3.5 log CFU/g, respectively, after 15 min, 3.2 and 4.6 log CFU/g, respectively, and after 20 min, 4.3 and 4.9 log CFU/g, respectively. With the increase of time, count of *S. Enteritidis* was further reduced. Similar results were also reported by Podolak *et al.* (1996). They stated that raw beef inoculated with pathogens, when treated with chemical for a maximum of 10 min, had bacterial number reduced by 3.0 log CFU/g.

Fig. 6 shows the effect of extra pure trisodium phosphate on *S. Enteritidis* inoculated in chicken meat. Raw chicken meat

samples were treated with different concentrations of extra pure TSP (5% and 10%) for 5, 10, 15, and 20 min and were cultured on selective Brilliant Green Agar medium for 24-48 h. The initial count was  $3.1 \times 10^7$  CFU/g. After standing and agitation for 5, 10, 15, and 20 min at 5% concentration, the number was reduced by 1.9, 4.1, 4.6 and 4.8 log CFU/g, respectively. The reduction of *S. Enteritidis* in chicken meat after dipping and agitation in 10% of TSP for 5, 10, 15, and 20 min was 4.9, 5.2, 5.5 and 6.2 log CFU/g, respectively. Similar results were also reported by Morris *et al.* (1997) and Kim *et al.* (1994). They treated the chicken meat samples with 5% and 10% solutions for 10 min and reported 4.5 and 5.4 log CFU/g reduction, respectively.

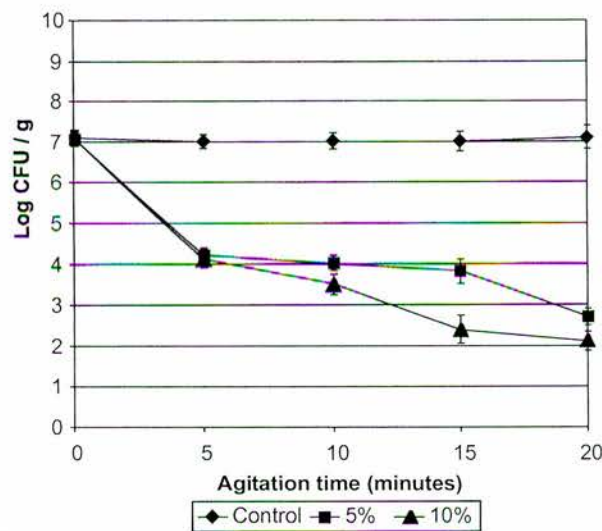


Fig. 5. Effect of extra pure citric acid on *Salmonella* Enteritidis inoculated in chicken samples.

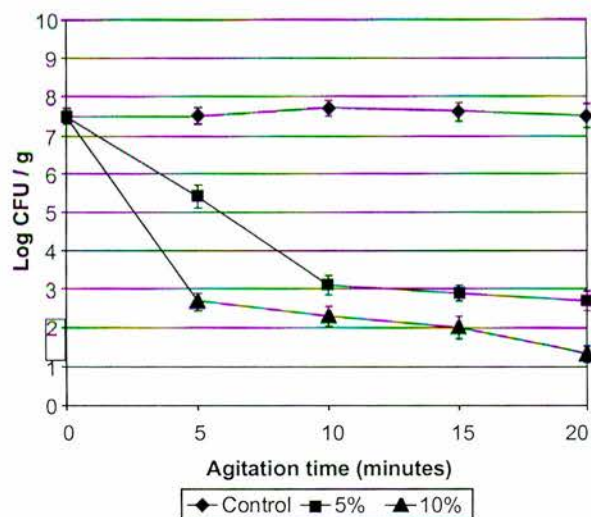


Fig. 6. Effect of extra pure trisodium phosphate on *Salmonella* Enteritidis inoculated in chicken samples.

**Effect of gamma radiations on microbes associated with beef and chicken meat.** The inactivation curves of total counts in beef and chicken samples are shown in Fig. 7. The initial total bacterial count of beef and chicken samples was  $7.9 \times 10^3$  and  $8 \times 10^3$  CFU/g, respectively. For both samples, total counts were reduced by 1.2 and 2.7 log after applying an absorbed dose of 2.5 and 5 kGy, respectively. The remaining microorganisms tended to have high resistance to radiation and the reduction was 3.8 log CFU/g when the meat was irradiated at an absorbed dose of 7.5 kGy. The total counts in chicken and beef were totally eliminated when they were treated at 10 kGy. The higher resistance tendency towards radiation may be due to the presence of gram positive and spore forming bacteria (Jay, 1994).

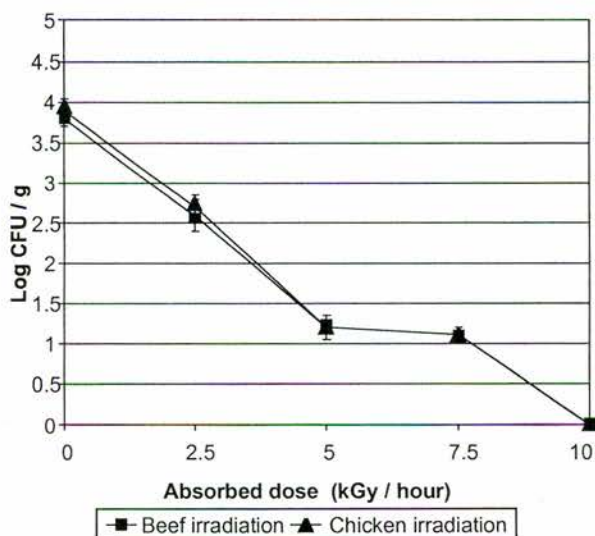


Fig. 7. Effect of radiations on beef and chicken microflora.

**Effect of gamma radiations on pathogenic *E. coli* O157:H7 inoculated in meat samples.** Fig. 8 shows the effect of irradiation on the *E. coli* O157:H7 inoculated in ground beef and chicken meat samples. Beef and chicken meat inoculated with *E. coli* O157:H7 were irradiated with doses of 1, 2, 3, and 4 kGy. The initial count of *E. coli* O157:H7 in the beef samples was  $1.9 \times 10^7$  CFU/g which decreased to  $1.9 \times 10^4$  CFU/g after an absorbed dose of 1 kGy. There was no growth of *E. coli* O157:H7 in the selective media after an absorbed dose of 2 kGy. The initial count of *E. coli* O157:H7 in chicken was  $3.1 \times 10^7$  CFU/g which decreased to  $1.2 \times 10^4$  CFU/g after an absorbed dose of 1 kGy. There was no growth of *E. coli* O157:H7 in the selective media after application of an absorbed dose of 2 kGy. *E. coli* O157:H7 inoculated in chicken and beef samples was more sensitive to radiation treatment.

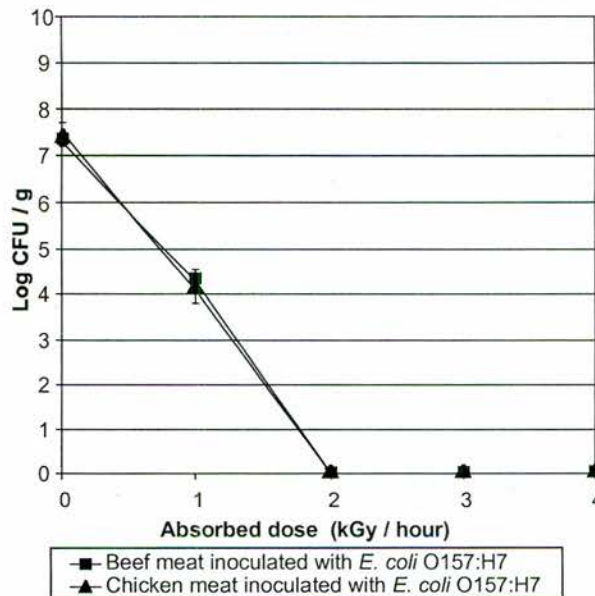


Fig. 8. Effect of radiations on the *E. coli* O157:H7 growth in ground beef and chicken samples.

**Effect of gamma radiations on pathogenic *Salmonella* Enteritidis inoculated in beef and chicken samples.** Fig. 9 shows the effects of radiation on *S. Enteritidis* in ground beef and chicken meat samples. *S. Enteritidis* was inoculated in beef and chicken meats, separately and subjected to gamma radiation. The initial count of *S. Enteritidis* in the chicken meat was  $6.3 \times 10^7$  CFU/g which decreased to  $5 \times 10^5$  CFU/g and  $2.5 \times 10^2$  CFU/g after an absorbed dose of 1 and 2 kGy, respectively.

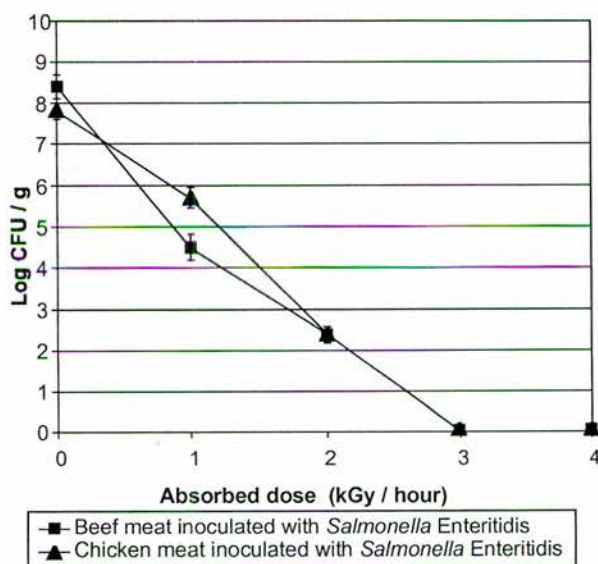


Fig. 9. Effect of radiations on the *Salmonella* Enteritidis growth in ground beef and chicken meat samples.

There was no growth of *S. Enteritidis* in the selective media after an absorbed dose of 3 kGy. The average initial count of *S. Enteritidis* in beef was  $2.5 \times 10^8$  CFU/g and decreased to  $3.1 \times 10^4$  CFU/g and  $2.5 \times 10^2$  CFU/g after an absorbed dose of 1 and 2 kGy, respectively. There was no growth of *S. Enteritidis* in the selective media after applying an absorbed dose of 3 kGy. *S. Enteritidis* inoculated in chicken and red meat samples was also sensitive to radiations. Similar results have been reported by Mossel (1977). He applied radiation doses for elimination of bacterial pathogens to the inoculated chicken and beef samples and reported that 3 kGy dose was adequate to eliminate totally *Salmonella* from the samples.

Thyer et al. (1992) inoculated chicken wings with *Salmonella typhimurium* followed by irradiation with 1.8 and 2.7 kGy and reported that 2.7 kGy was adequate to eliminate *S. typhimurium*. Broiler chicken's breast and leg when inoculated with *Salmonella* followed by irradiation with 2 kGy, became free from *Salmonella* (Klinger et al., 1986). Kiss and Farkas, 1972, found that *Salmonella* were eliminated from chicken surface following irradiation with 2-5 kGy. In addition, Lamuka et al. (1991) found that *Salmonella* was eliminated from mechanically deboned chicken meat by the application of 2.6 kGy. Jay (1994) reported a dose of 2.5 kGy to be sufficient to destroy *Salmonella* in refrigerated and frozen chicken carcasses. *Salmonella* was eliminated from fresh chicken meat and chicken meat at  $-40^\circ\text{C}$  by treatment with 2 kGy (Kamat et al. 1991). ICGFI (1991) recommended that radiation doses of 1-2.5 kGy for refrigerated poultry and over 3 kGy for frozen poultry are sufficient to control pathogens.

**Effect of gamma radiations on pathogenic *Staphylococcus aureus* inoculated in beef and chicken samples.** Fig. 10 presents the effect of radiations on the *S. aureus* in ground beef and chicken samples. *S. aureus* was inoculated in beef and chicken meats, separately and subjected to gamma radiation. The initial count of *S. aureus* in beef was  $2.9 \times 10^8$  CFU/g which decreased to  $3.9 \times 10^5$  and  $3.1 \times 10^3$  CFU/g after applying absorbed doses of 1 and 2 kGy, respectively. There was no growth of *S. aureus* in the selective media after an absorbed dose of 3 kGy. The initial count of *S. aureus* in chicken was  $1.4 \times 10^8$  CFU/g which decreased to  $8.1 \times 10^5$  CFU/g,  $2.6 \times 10^4$  CFU/g and  $1.3 \times 10^2$  CFU/g after an absorbed dose of 1, 2 and 3 kGy, respectively. There was no growth of *S. aureus* in the selective media after an absorbed dose of 4 kGy. The *S. aureus* is gram positive coccus and its compositional structure may have survived more adverse environment. The presence of pathogenic *S. aureus* in food is a risk for human health. Contamination of the food by this pathogen may take place during mishandling, cutting, processing and distribution of the food

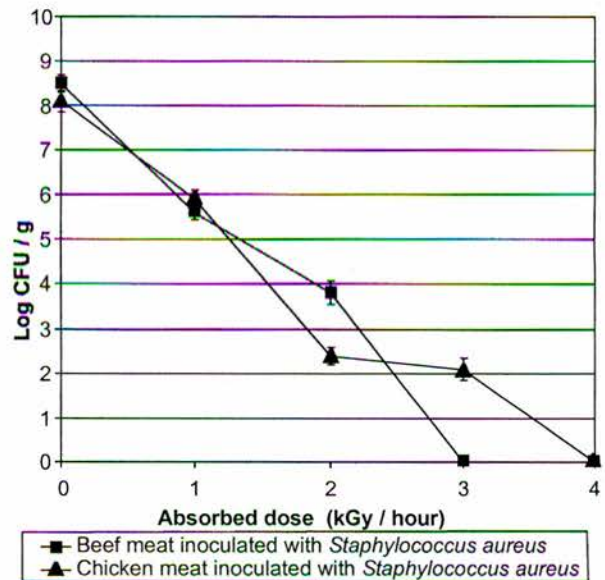


Fig. 10. Effect of radiations on *Staphylococcus aureus* growth in ground beef and chicken samples.

samples. With favorable environment, the organism can increase in appropriate number ( $10^8$ ). Similar results were reported by Gonzalez et al. (1981). They irradiated some of the fishery products with gamma radiations for the elimination of pathogenic microbes and reported that after an absorbed dose of 4 kGy, no pathogenic *S. aureus* could grow on selective agar medium. Mechanically deboned chicken meat were inoculated with  $10^4$  CFU/g of *S. aureus* and irradiated with gamma radiation 0, 0.75, 1.5, 2.25, and 3 kGy; the results indicated that the viable cell count was detected in the samples irradiated with 0 and 0.75 kGy (Thyer and Boyd, 1992).

## Conclusion

The following conclusion can be drawn from the present work. Commercial citric acid, extra pure citric acid, and trisodium phosphate did not completely eliminate *S. Enteritidis* in chicken and *E. coli* O157:H7 in beef. TSP is more effective than CA for reducing *S. Enteritidis* in chicken and *E. coli* O157:H7 in beef. Use of 10% concentration of both extra pure CA and TSP for 10 min is best for reducing inoculated pathogen in meat samples among the investigated concentrations. Application of low radiation dose (3 kGy) is sufficient for total elimination of pathogenic *E. coli* O157:H7 and *S. Enteritidis* from the beef and chicken samples. A radiation dose of 4 kGy is sufficient for total elimination of pathogenic *S. aureus* from beef and chicken samples. Among the investigated treatments, radiation is the most effective for total elimination of *S. Enteritidis* in chicken and *E. coli* O157:H7 in beef.

## References

- Aziz, H.N., Mahrous, S.R., Youssef, B.M. 2002. Effect of gamma-ray and microwave treatment on the shelf-life of beef products stored at 5 °C. *Food Control* **13**: 437-444.
- Doores, S. 1990. pH control agent and acidulants. In: *Food Additives*, A. L. Brannen, P. M. Davidson, S. Salminen, (eds.), pp. 477-510, Marcel Dekker, Inc., New York, USA.
- Dorsa, W.J., Cutter, C.N., Siragusa, G.R. 1998. Long-term bacterial profile of refrigerated ground beef made from carcass tissue, experimentally contaminated with pathogens and spoilage bacteria after hot water, alkaline, or organic acid washes. *J. Food Prot.* **61**: 1615-1622.
- Dorsa, W.J., Cutter, C.N., Siragusa, G.R. 1997. Effects of steam-vacuuming and hot water spray wash on the microflora of refrigerated beef carcass surface tissue inoculated with *Escherichia coli* O157:H7, *Listeria innocua*, and *Clostridium sporogenes*. *J. Food Prot.* **60**: 114-119.
- EL-Shenawy, M.A., Marth, E.H. 1991. Organic acids enhance the antilisterial activity of potassium sorbate. *J. Food Prot.* **54**: 593-597.
- Fung, D.Y.C., Kastner, C.L., Hunt, M.C., Dickman, M.E., Kropf, D.H. 1980. Mesophilic and psychrotrophic bacteria population on hot-boned and conventionally processed beef. *J. Food Prot.* **43**: 547-550.
- Gonzalez, O.N., Sanchez, F.C., Vista, J.B., Ronquillo, R.P. 1981. Elimination of risk resulting from pathogenic microorganisms in some Phillippine fishery products by gamma irradiation. *Phillip. J. Food. Sci.* **110**: 45-53.
- Greer, G.G., Dilts, B.D. 1995. Lactic acid inhibition of the growth of spoilage bacteria and cold tolerant pathogens on pork. *Int. J. Food Microbiol.* **25**: 141-151.
- Hwang, C., Beuchat, L.R. 1995. Efficacy of lactic acid/sodium benzoate wash solution in reducing bacterial contamination of raw chicken. *Int. J. Food Microbiol.* **27**: 91-98.
- ICGFI. 1991. Code of food irradiation practice for prepackaged meat and poultry (to control pathogens and/or extend shelf-life), International Consultative Group on Food Irradiation. *ICGFI Document No. 4*, Vienna, Austria.
- Ito, H., Rashid, H.O., Sangthong, N., Adulyatham, P., Rattagood, P., Ishigaki, I. 1993. Effect of gamma irradiation on frozen shrimps for decontamination of pathogenic bacteria. *Radiat. Phys. Chem.* **42**: 279-282.
- Jay, J.M. 1994. *Modern Food Microbiology*, 4<sup>th</sup> edition, Chapman & Hall, New York, USA.
- Kamat, A.S., Alur, M.D., Nerkar, D.P., Nair, P.M. 1991. Hygienization of Indian chicken meat by ionization radiation. *J. Food Safety* **12**: 59-71.
- Tasi, S. 1994. Reduction of *Salmonella* on post-chill chicken carcasses by trisodium phosphate ( $\text{Na}_3\text{PO}_4$ ) treatment. *J. Food Safety* **14**: 9-17.
- Kiss, I., Farkas, J. 1972. Raduriation of whole eviscerated chicken carcass. *ACTA Aliment.* **1**: 73-86.
- Klinger, I., Fuchs, V., Basker, D., Juven, B.J., Lapidot, M., Eisenberg, E. 1986. Irradiation of broiler chicken meat. *Israel J. Vet. Med.* **42**: 181-192.
- Lamuka, P.O., Chawan, G.B., Sunki, G.R., Rao, D.R., Shackelford, L.A. 1991. Effect of lyophilized thermophilus whey and irradiation on bacteriological quality of mechanically deboned chicken meat. Paper presented at *IFT91, Annual Meeting*, Dallas, TX, USA.
- Lewis, S.J., Velasques, A., Cuppett, S.L., McKee, S.R. 2002. Effect of electron beam irradiation on poultry meat safety and quality. *J. Poultry Sci.* **81**: 896-903.
- Molins, R.A. 1991. *Phosphates in Food*, pp. 272, 1<sup>st</sup> edition, CRC Press, Boca Raton, FL, USA.
- Morris, C.A., Lucia, L.M., Savell, J.W., Acuff, G.R. 1997. Trisodium phosphate treatment of pork carcasses. *J. Food Sci.* **62**: 402-403.
- Mossel, D.A.A. 1977. The elimination of enteric bacterial pathogens from food and feed of animal origin by gamma irradiation with particular reference to *Salmonella* radication. *J. Food Quality* **1**: 85-104.
- Olson, D.G. 1998. Irradiation of food. *Food Technol.* **52**: 56-62.
- Palumbo, S.A., Williams A.C. 1994. Control of *Listeria monocytogenes* on the surface of frankfurters by acid treatments. *J. Food Microbiol.* **11**: 293-300.
- Paul, P., Venugopal, V., Nair, P.M. 1990. Shelf-life enhancement of lamb meat under refrigeration by gamma irradiation. *J. Food Sci.* **55**: 865-866.
- Podolak, R.K., Zayas, J.F., Kastner, C.L., Fung, D.Y.C. 1996. Inhibition of *Listeria monocytogenes* and *Escherichia coli* O157:H7 on beef by application of organic acids. *J. Food Prot.* **59**: 370-373.
- Shapiro, J.E., Holder, I.A. 1960. Effect of antibiotic and chemical dips on the microflora of packaged salad mix. *Appl. Microbiol.* **8**: 341-345.
- Thayer, D.W., Boyd, G. 1992. Gamma ray processing to destroy *Staphylococcus aureus* in mechanically deboned chicken meat. *J. Food Sci.* **57**: 848-851.
- Thayer, D.W., Dickerson, C.Y., Rao, D.R., Boyd, G., Chawan, C.B. 1992. Destruction of *Salmonella typhimurium* on chicken wings by gamma radiation. *J. Food Sci.* **57**: 586-589.
- Varteltzis, K., Soutos, N., Koidis, P., Ambrosiadis, J., Genigeorgis, C. 1997. Antimicrobial effects of sodium triphosphate against bacteria attached to the surface of chicken carcasses. *Food Microbiol. Technol.* **20**: 665-669.



- World Health Organization. 1999. High-dose irradiation: wholesomeness of food irradiated with doses above 10 kGy. *Report of Joint FAO/IAEA/WHO study Group, Geneva. Technical Report Series, 980.*
- Yoon, K.S., Oscar, T.P. 2002. Survival of *Salmonella typhimurium* on sterile ground chicken breast patties after washing with salt and phosphates and during refrigerated and frozen storage. *J. Food Sci.* **67**: 772-775.