2.2 RED MEAT PROCESSING

2.2.1 Kaess and Weidemann (1968) – Beef Muscle Slices

Summary: The effects of continuous ozone treatment at 0.3EC on the growth of psychrophilic meat spoilage organisms, grown on muscle slices with equilibrium relative humidity (EH) of 99.3, 98.5 and 98.0% were investigated. Ozone concentrations ranged from 0.13 to 5.0 mg/m^3 in air and the microorganisms included pigmented and non-pigmented *Pseudomonas*, the yeast *Candida scottii*, and the molds *Thamnidium* and *Penicillium*. A treatment at EH 99.3% with air containing carbon dioxide (11%) and ozone (0.6 mg/m^3) was included.

Small but significant inhibitory effects on non-pigmented *Pseudomonas* species and on the yeast *Candida scottii* were obtained with ozone concentrations $\exists$ 2 mg/m^3 (EH 99.3%). A concentration of 0.6 mg/m^3 was not always significantly effective with non-pigmented *Pseudomonas* species, but significant decreases were recorded for the slower growing pigmented *Pseudomonas* species.

With ozone concentrations $\exists$0.6 mg/m^3, the population density of 10^8 cells/cm^2, at which bacterial colonies first become manifest in air (slime point), was increased to about 10^9/cm^2. Lowering the EH or introducing 11% carbon dioxide into the storage further delayed the appearance of the "slime point" of the bacterial population owing to ozone treatment.

In the presence of ozone, the lag phase of *Thamnidium* and *Penicillium* was longer but the growth rate was about the same as that of the controls. No aerial mycelium appeared with ozone concentrations $\exists$0.6 mg/m^3.

The color of the treated muscle surface did not differ from that of controls when the ozone concentration of the storage atmosphere was # 0.6 mg/m^3.

In the present experiments a continuous ozone concentration was decreased in steps to find the effect on growth of microorganisms at which color changes of chilled meat remained negligible during storage.

Methods

Sample Preparation: Meat samples were obtained from fresh semitendinosus muscles under sterile conditions. Circular discs with a diameter of 7.6 cm were cut with a cutting cylinder from samples 0.12 cm. thick, sliced from the muscle perpendicular to the fiber direction, with a mechanical cutter. The slices fitted sterile, stainless steel sample holders which exposed a meat area of 35 cm^2/side to the ozone.

Equilibrium relative humidity (EH) of 98.3 and 98.0% over the slices was obtained by exposing them, in a duct, to an air stream flowing at a rate of 3.5 m/sec at 2EC and 80% RH, to reduce the moisture content to the values for a definite EH.

Apparatus: A continuous stream of air (30 L/h) with EH of 99.3, 98.5 or 98.0% and a constant temperature of 0.3EC was drawn through the containers. The ozone concentrations ranged from

0.15 to 5 mg/m^3. In two experiments the effect of a concentration of 0.6 mg/m^3 ozone in a mixture of air with 11% CO_2 was tested. The EH was maintained by passing the dry air through distilled water in a gas wash bottle and then through a glass coil (20 ft long, 0.5 inch inner diameter) filled with sulfuric acid by submerging it in a desiccator with the solution having the concentration to establish the EH.

Microbiological Procedures: Diluted cultures of typical meat spoilage, non-pigmented (Nos. 1482, 131, 39 and A2) and pigmented (Nos. 221, 91 and 41) *Pseudomonas* species, grown for 3 days in nutrient broth at 23EC, were sprayed on the meat samples in the holders. Suspensions of the yeast *Candida scottii*, grown in Wickerham's Y.M. broth, were blended in the Buehler homogenizer for 1 min, diluted in 0.5% peptone water and filtered before spraying. Samples were taken with sterile cutting cylinders from each of the two slices used for an experiment and the population determined by the poured plate method. Bacteria were plated on nutrient agar and yeasts on potato dextrose agar. Incubation was for 5 days at 20EC.

Spores were washed off slope cultures of *Thamnidium* or *Penicillium* species with a small quantity of physiological saline, to which a drop of non-ionic detergent had been added as wetting agent. This suspension of spores was used to inoculate meat samples in the center with the ground end of a sterile glass rod.

Histological Examination: Pieces of about 1 cm^2 were cut from meat slices and fixed at room temperature by placing the side free of organisms on top of several layers of filter paper, the lower layers of which were submerged in 10% formalin solution. With this method losses of microbial colonies were avoided. After fixation the samples were dehydrated in an alcohol series, embedded in paraffin and sectioned at a thickness of 10 Φ.

Sections of samples with bacterial colonies were stained with Gram stain; sections with mold were overstained with haematoxylin and cosin, and both examined with the light microscope.

Statistical Analysis: The significance of the effect of ozone on bacterial growth was tested by examining the difference between the logarithms of counts of treated and control samples at the middle of the time range and the extent to which the difference remained significant. when averaged over increasing ranges of time. The significance of differences was given in all cases for a probability level of 5%.

Results

Effects of ozone on growth of bacteria: At 0.3EC and 99.3% EH, ozone concentrations of 0.15-5 mg/m^3 delayed moderately the growth of non-pigmented *Pseudomonas* species (Figure 2.2.01). In the prese4nce of ozone, the lag phase was increased but the growth rate was not affected. Differences in population density between *Pseudomonas* 131, 1482 and A_2 exposed to ozone concentrations 5 or 2 mg/m^3 and controls, as shown in Figure 2.2.01 were significant, except for organisms 1482, for which treatment (5 mg/m^3) was significant only towards the end of the storage time. With the low concentration of 0.6 mg/m^3, differences between treated and untreated samples were secured for organism 1482 but not for 131. The bactericidal effect practically disappeared with an ozone concentration of 0.15 mg/m^3.

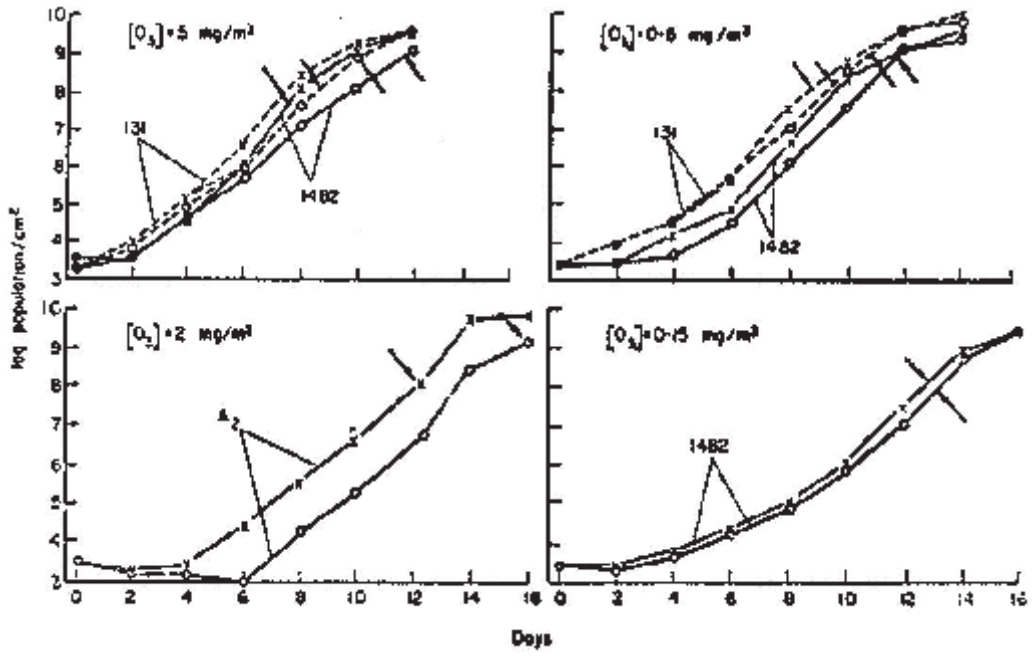

Figure 2.2.01. Logarithm-time growth curves for non-pigmented *Pseudomonas* species 131, 1482 and A_2. Organisms growing on slices of *Musculus semitendinosus* in air or in air-ozone mixtures at 0.3EC and 99.3% EH. Ozone concentrations of 0.15-5 mg/m^3. The appearance of slime point is indicated by arrows. " = Ozone; X = air (Kaess and Weidemann, 1968).

The retardation of growth of pigmented *Pseudomonas* species was significant, mainly due to an extended lag phase. In some cases the variance between ranges or within ranges of growth curves was significant, caused probably by the variation in composition of the muscle (connective tissue, fat content) and also by the variation of ozone absorption within and between slices.

The time at which bacterial colonies first became manifest in air at a population density of 10^8 organisms/cm^2 was delayed until the population increased to approximately 10^9/cm^2 (Figures 2.2.01 and 2.2.02) with concentrations of ozone $\exists$ 0.6 mg/m^3.

Histological examination showed that at the time when colonies of bacteria growing in air had merged into a film, the surface of muscle exposed to an ozone concentration of 5 mg/m^3 was free of organisms and colonies were situated predominantly in crevices of the tissue and openings of vessels, where they were protected from the direct effects of ozone. At lower concentrations of ozone, individual colonies developed leaving sterile areas of muscle between them. The distinct dark layer, observed on the muscle surface after storage for 12 days at an ozone concentration of 5 mg/m^3 was absent on tissue stored at lower concentrations.

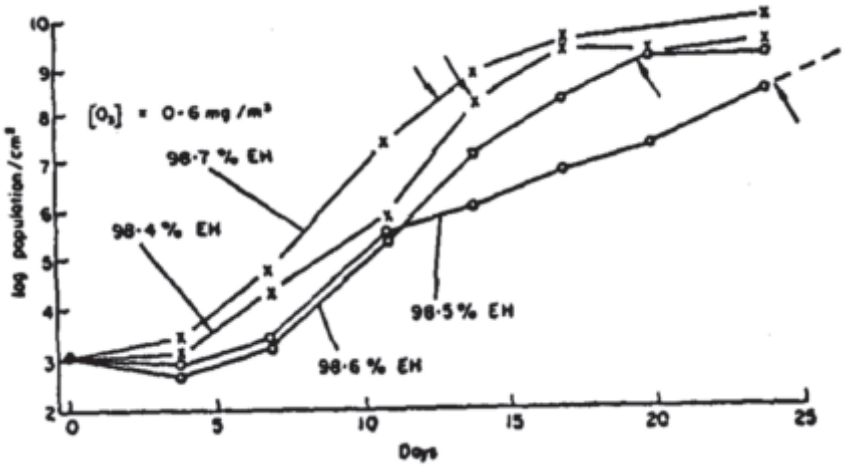

Figure 2.2.02. Logarithm-time growth curves for *Pseudomonas* 1482 growing on slices of *M. semitendinosus* with an EH of approximately 98.5% (exact value indicated on curve) in air and air-ozone mixture of 98.5% EH at 0.3EC. Each curve represents growth on an individual slice. Slime point is indicated by arrows. " = Ozone; X = air (Kaess and Weidemann, 1968).

Influence of Equilibrium Relative Humidity: Figure 2.2.02 shows the maximum inhibitory effect of ozone (0.6 mg/m^3) obtained with the non-pigmented *Pseudomonas* 1482 at an EH of 98.5%. The retarding effects of the ozone were significant, but differences were smaller and insignificant in another experiment. The slime point again was delayed until the population increased to 10^9 cells/cm^2. With an EH of 98.0% (moisture content 150% dry weight), growth of *Pseudomonas* 1482 in air was prevented. In a mixture of air and ozone (0.6 mg/m^3) the population declined slowly and approximately linearly (0.035 log population/day).

Bacterial Growth in Air-Carbon Dioxide-Ozone Mixture: Experiments with *Pseudomonas* species exposed to an atmosphere with 11% CO_2 at 0.3EC confirmed earlier results of Scott (1938) which showed that the growth rate of bacteria could be reduced by 50-60% by addition of 10% CO_2 to the air. *Pseudomonas* 131 was the most sensitive to CO_2 of the species tested. With this markedly reduced growth rate, the effect of adding 0.6 mg/m^3 ozone to the air-carbon dioxide mixture was significant only at the end of the experiment for *Pseudomonas* 1482 and there was no increased effect for *Pseudomonas* 131. Colonies were visible at a cell density of 10^8/cm^2 when growing in air with 11% CO_2, but the slime point appeared at a density of about 10^9/cm^2 in an atmosphere with 11% CO_2 and 0.6 mg/m^3 ozone.

Effect of Ozone on the Growth of Yeasts: The growth of *Candida scottii* on muscle slices kept at 0.3EC and 99.3% EH was retarded significantly in air with 5 mg/m^3 and 2 mg/m^3 ozone, but the effect was insignificant in air with 0.6 mg/m^3 ozone. Instead of the moist appearance (slime point) of control samples, a dry dull surface was apparent on treated samples.

Effect of Ozone on the Growth of Molds: The lag phase of *Thamnidium* and of *Penicillium* was increased by about two days over that of the controls (Figure 2.2.03) when the molds developed on muscle stored in air with ozone concentrations of 0.16-5.0 mg/m^3. The growth rate of

Thamnidium was slightly but significantly smaller than controls with ozone concentrations of 5 and 1.9 mg/m^3, but not with smaller concentrations. *Penicillium* had a tendency to grow at a slightly higher rate in ozone treated muscle than in controls.

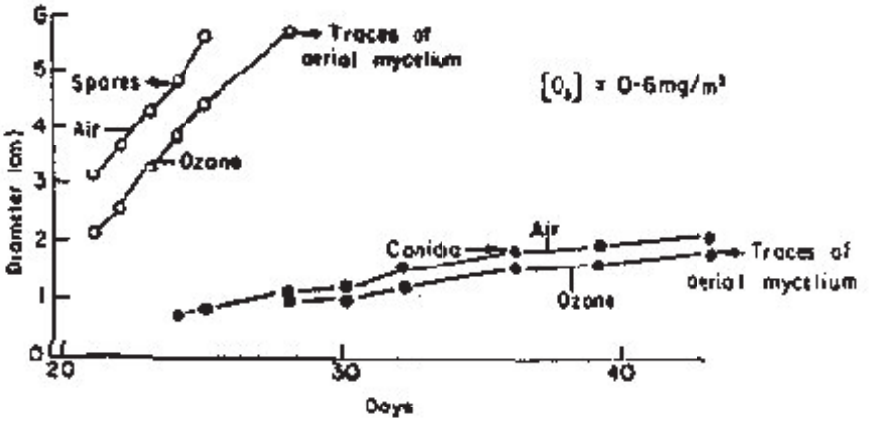

Figure 2.2.03. Increase with time of colony diameter of *Thamnidium* (") and *Penicillium* (!) growing on slices of *M. semitendinosus* in air and air with 0.6 mg/m^3 ozone at 0.3EC and 99.3% EH (Kaess and Weidemann, 1968).

Normal aerial mycelium did not develop on mold growing in air with ozone at concentrations ∃ 0.6 mg/m^3. At a concentration of 0.6 mg/m^3, traces of mycelium were just visible on the muscle slices at the end of the experiment. At an ozone concentration of 0.16 mg/m^3 thickened sterile aerial hyphae of *Thamnidium* grew to a length of 1-2 mm (controls about 10 mm). *Penicillium* hyphae had a length of a fraction of 1 mm.

Histological examination showed that *Penicillium* exposed to 1.9 mg/m^3 ozone developed freely between and within muscle fibers. Aerial mycelium with conidia appeared on the control sample. Similar results were obtained with *Thamnidium* but short, single, thickened aerial hyphae showed up on the muscle surface in an atmosphere with 1.9 mg/m^2 ozone.

Effects of Ozone on Color of Meat: The meat pigments myoglobin and hemoglobin were quickly oxidized to brown heme compounds in an ozone concentration of 2 mg/m^3 or more, but at a level of 0.6 mg/m^3 there was no difference in color between samples and controls when the storage life was terminated at population densities of 10^9 and 10^8 cells/cm^2, respectively. Short times between slaughter and the beginning of cooling and treatment, and also high pH values (close to 6) tended to prolong storage times for the onset of discoloration.

Discussion: The need to prevent discoloration of the muscle surface sets low limits to the permissible concentration or a continuous ozone treatment. The absence of a linear relationship between the duration of treatment to produce a brown discoloration, and the reciprocal ozone

concentration excludes using the Bunsen-Roscoe law for the calculation of the concentration-time relationship of discoloration.

The effect on growth of microorganisms of ozone at concentrations which did not noticeably cause discoloration of the muscle varied with organisms. The reduction of growth was greatest with naturally slow growing pigmented *Pseudomonas* species, but only small when the growth rates of rapidly growing *Pseudomonas* 1482 and 131 were artificially reduced by decreasing the EH of the muscle or by using an atmosphere with 11% CO_2. The retarding effect of ozone(0.6 mg/m^3) at an EH of 98.5% on growth of *Pseudomonas* was of the same order as that obtained with control samples whose moisture content (% dry weight) was lowered by 20%, i.e., at an EH of 98.3%. The increased inhibition of bacterial growth due to ozone (up to 10 mg/m^3, 3 hr/day) with decreasing EH of muscle, was not observed with a continuously applied low ozone concentration (0.6 mg/m^3).

Ozone was mainly effective on organisms directly exposed to concentrations ∃ 0.6 mg/m^3. Bacterial growth appeared to be strongly reduced in the distinct dark layer on muscle exposed to an ozone concentration of 5 mg/m^3. This inhibition probably was due to oxidation of tissue close to the surface. As a consequence of this, bacteria started growth in crevices under the surface, formed isolated colonies, and the appearance of the slime point -was delayed. At ozone concentrations ∃ 0.6 mg/m^3 of *Thamnidium* and *Penicillium* developed mycelium almost entirely in the substrate.

Optimal conditions for muscle stored at 0.3EC were obtained by applying a continuous ozone concentration of 0.6 mg/m^3.

2.2.2 Greer and Jones (1989) – Beef Carcasses

Abstract: A study was undertaken, using experimental slaughter and dressing conditions, to determine the effects of ozone (0.03 ppm) upon beef carcass shrinkage, carcass characteristics, muscle quality and total mesophilic and psychrotrophic bacteria. Paired sides were either continuously ozonated using a commercial ozone generator or subjected to conventional air chilling under identical conditions of humidity (95%) and temperature (1.6EC) for up to nine days of aging. After aging, control and ozone-treated sides were processed and bacterial growth and retail case life determined for steaks on simulated, retail display. Cooler shrinkage over nine days was significantly higher (10.6 g/kg) in ozone-treated compared to control sides and this difference between treatments increased (14.9 g/kg) following trimming of discolored and dry muscle tissue. Although ozone prevented bacterial growth on carcass surfaces, it did not affect the retail case life (odor, appearance) or reduce bacterial growth on retail steaks. Ozone had the deleterious effects of dramatically increasing shrinkage weight losses and trim losses and darkening lean muscle color.

Materials and Methods

Treatments: Beef carcass aging was conducted in identical meat coolers (11.5 m long, 3.2 m wide, 5.1 m high) with cooling units operating at 7.47 x 10^{10} joules/h and 266 kL/min. Under these conditions, air velocity was 0.5 m/s, cooler temperature was 1.6 + 0.2EC and the temperature measured at the carcass surface (after 24h of chilling) was 2.6 ∀ 0. 1EC. Relative

humidity was monitored using a continuous recording hygrothermograph and found to be identical in both control and ozonated coolers (95%).

An Ozone Air Sterilizer was installed. On the day of installation and following 6 d of carcass aging, cooler levels of ozone were determined by drawing air into Gastec Detector Tubes using a piston type volumetric pump. Thirty, 100 mL air samples were taken at each sampling interval at several locations throughout the cooler. Ozone concentration remained constant at 0.03 ppm. An identical cooler, in the absence of ozone. served as control.

After slaughter, left and right sides of 10 beef carcasses from crossbred beef animals (average live weight = 450 kg) were chilled for 24 h at 10EC before randomly assigning alternate sides to the ozone or control treatments. This approach was taken to obtain more realistic bacterial levels on the carcass surface. Carcass quality measurements and bacterial samples were taken before and after 9 d of carcass aging at 1.6EC.

Case Life Studies: Following carcass aging for 9 d, six rib-eye steaks were fabricated from each carcass side subjected to each treatment. To minimize the effects of contamination during cutting and to standardize the procedure for both treatments, steaks were processed on cutting tables rinsed with pressurized 80EC water and sanitized with Foam-Eze immediately prior to processing carcasses. Cutting knives were cleaned before cutting each rib by immersion in 80EC water for 30 s. Carcasses subjected to each treatment were segregated and processed separately to avoid cross-contamination during processing.

Steaks were wrapped in an oxygen-permeable (2185 cc/m^2/24 h) polyvinyl chloride film and randomly placed in a horizontal retail case under laboratory-simulated retail conditions (50 steaks/treatment). The remaining 10 steaks/treatment were immediately examined to determine initial bacterial densities, appearance and odor. On days 2, 4, 6, 8 and 10 of retail display, an experienced five-member sensory panel for odor and appearance evaluated 10 steaks from each treatment. The same 10 steaks (at each sampling time) were examined for bacterial content as described below.

Bacterial Analyses: Sides were sampled immediately before and immediately following aging by aseptically excising 10 cm^2 areas at three locations (chuck, plate, hip). Following homogenization (Stomacher 400) and decimal dilution (0.1% peptone - water), 0.1 mL amounts were surface plated in duplicate on plate count agar (Difco). One set of plates was incubated at 35EC for 48 h to enumerate mesophilic bacteria and a duplicate set at 7EC for 7 d to enumerate psychrotrophic bacteria. Bacterial numbers on steaks during retail display were determined in a similar fashion following the excision of 10 cm^2 of tissue from the surface of each steak. At each sampling time, the same steaks were used for both bacterial analyses and visual evaluation.

Statistical Analyses: All shrinkage, meat quality, bacterial counts and shelf life data were analyzed by analysis of variance. The effects of ozone on shrinkage weight losses and meat quality was performed using a one-way analysis of variance. Differences in bacterial numbers were evaluated by two-way analysis of variance. Classification variables included number of days of retail display at the time of sampling, aging treatment and appropriate interaction. Differences in retail case life were evaluated in an identical fashion. The significance of

differences between means were determined using the Student's 't' test. Results were considered significant if the calculated probability was less than 0.05.

Carcass Contamination: The data in Tables 2.2.01 (psychrotrophs) and 2.2.02 (mesophiles) compare the effects of ozone and control treatments upon quantitative changes in bacterial densities after 9 d of carcass aging. Results were similar for either bacterial group. Prior to the aging treatment, there were no significant ($P > 0.1$) differences in the numbers of bacteria on carcasses. After 9 d of carcass aging, however, bacterial numbers on control carcass sides were about 10-fold greater than those on ozone-treated carcasses ($P < 0.05$). This difference was found since bacterial populations increased significantly ($P < 0.02$) on control carcasses during 10 d of aging but remained essentially the same on ozone-treated carcasses ($P > 0.1$).

Table 2.2.01. Effects of ozone on psychrotrophic bacteria on beef carcasses [1]
(Greer and Jones, 1989)

Sample time	Log bacteria/cm^2		
	Control	Ozone	P
Before aging	2.87	2.81	0.892
After 9 days aging	4.03	2.83	0.011
P	0.013	0.957	
[1] Least square means of ten sides. The standard error was 0.32.			

Bacterial Growth on Steaks: The effects of ozone treatment of carcasses on the growth of psychrotrophs (Figure 2.2.04) on retail rib-eye steaks was compared to untreated control samples for up to 10 d of retail display. Since similar results were obtained for the mesophilic bacterial population, the growth of psychrotrophic bacteria, only, was chosen for the purposes of illustration. Bacterial numbers on steaks derived from control or ozone-treated carcasses were not significantly different ($P > 0.1$) on days 0, 2 or 4 of retail display. However, bacterial densities when compared to control samples were significantly ($P < 0.01$) greater on steaks derived from ozone-treated carcasses after 6, 8 and 10 days of retail display.

Table 2.2.02. Effects of ozone on mesophilic bacteria on beef carcasses [1]
(Greer and Jones, 1989)

Sample time	Log bacteria/cm^2		
	Control	Ozone	P
Before aging	2.95	2.90	0.912
After 9 days aging	3.95	3.10	0.043
P	0.018	0.621	
[1] Least square means of ten sides. The standard error was 0.29.			

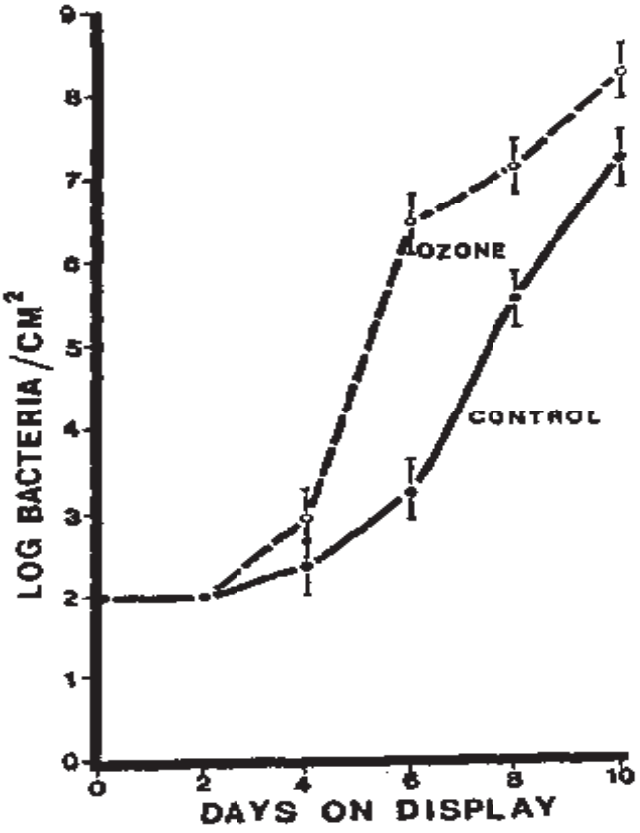

Figure 2.2.04. Effect of ozone on the growth of psychrotrophic bacteria during the retail display of beef rib-eye steaks. Each point represents the least squares mean (Λ SE) for 10 steaks (Greer and Jones, 1989).

The data in Figure 2.2.04 would suggest that differences in bacterial numbers were not due to differences in bacterial growth rates, but rather due to a slightly extended lag phase for bacterial growth on control steaks.

Conclusions: The current study constitutes the first known report of the effects of ozone during the aging of carcass beef. The results showed that although ozonization prevented bacterial growth on carcass surfaces during 9 d of cooler aging, it did not improve the bacterial condition, nor the keeping quality of retail beef steaks derived from ozone-treated carcasses. The inhibition of bacterial growth on carcass surfaces cannot be unequivocally attributed to ozone. That is, evaporative losses from ozone-treated carcasses were significantly greater than those of untreated control carcasses and surface dessication is known to limit bacterial growth. The increased moisture losses from ozone-treated carcasses may have been due to the deterioration of surface tissues (Kaess and Weidemann, 1968a) although this contention remains speculative.

Surprisingly, bacteria were able to initiate growth more rapidly on retail steaks derived from ozone-treated carcasses. Relative to this, the lag phase of bacterial growth was somewhat shorter and consequently, bacterial densities were substantially greater on steaks from ozone-treated carcasses when compared to untreated control samples (after 6, 8 and 10 d of retail display).

Although this result may have been artifactual, it is conceivable that ozone selected for a bacterial population with the potential to initiate growth more rapidly at muscle surfaces.

The current study used ozone at concentrations of 0.03 ppm which is considerably less than the 3.88 ppm found to be effective in delaying poultry spoilage (Yang and Chen, 1979). Contrarily, it has been reported that even 500 ppm ozone had no effect on the microflora of refrigerated beef (Yang and Chen, 1979).

Ozonization of carcasses for up to 9 d of aging did not improve the keeping quality or bacterial condition of retail steaks. Ozone treatment did, however, significantly prevent the growth of organisms on carcass surfaces. This latter benefit undoubtedly would be offset by the substantial shrink and trim losses promoted by ozone.

2.2.3 Mitsuda et al. (1990) – Ozone + CO_2 with Beef

Abstract: The synergistic effect of ozone and carbon dioxide gases on the sterilization of food was investigated. Raw beef and an agar plate of *Escherichia coli* were sterilized with a mixture of ozone and carbon dioxide gases in polyvinyl chloride film bags and stored. In both direct sterilization and the storage tests, the survival percentages for the mixed gases of ozone and carbon dioxide were lower than for those of the individual gases.

The reasons for this synergistic effect were considered to be that the bactericidal effect of ozone gas was retained during the storage period by the quenching effect of carbon dioxide gas to the chain reaction of ozone degradation, and by the bacteriostatic effect of carbon dioxide gas. The mixture of ozone and carbon dioxide gases sterilized both the surface and the inside of the food and the agar plate at the same time.

Materials and Methods: Raw beef marbled with fat was purchased from a local market, sliced to a thickness of 0.7-1.0 cm (about 20 g), and each slice packed in a bag made of polyvinyl chloride. The organisms (a representative strain which behaved biochemically as a typical member of *Escherichia coli*) were cultured in it medium comprising a standard agar for bacterial growth at 37EC for 48 hours.

Preparation of the Mixed Gas: The ozone gas was generated using commercial grade oxygen as the gas supply. The concentration of ozone gas generated in this apparatus was estimated to be 20-40 $g/m^{3.}$ Mixtures of ozone and carbon dioxide gases were prepared with a gas mixing tool by regulating the pressure of the gases to ratios of 3:1, 2:1, 1:1, 1:2 and 1 kg/cm^2 ozone (oxygen): 3 kg/cm^2 carbon dioxide.

Sterilization and Storage of Foods: In the food sterilization process, a bag of 20 cm x 1:1 cm, which had been made of a polyvinyl chloride film having no air-permeability, was used. After opening, each bag which contained the food was 80% sealed. The food in each bag had been exposed for 5 minutes to ozone gas, carbon dioxide gas or their mixture. Then the bag was completely sealed. Microbiological tests were carried out just after the 5-minute sterilization, and later after storage for 7 or 14 days in a refrigerator.

Microbiological Test for Sterilized Foods: Agar plate samples were prepared in accordance with a method of mixing and dilution. Twenty grams of sliced raw beef meat were homogenized

with 100 mL of physiological saline in an homogenizer. One mL of liquid was mixed with 10 mL of agar culture medium and then incubated at 37EC for 48 h. Each test was carried out in triplicate.

Sterilization of Bacterial Cells in the Agar Plate: To sterilize bacterial cells of *Escherichia coli*, plates containing bacterial cells were prepared by mixing one mL of properly diluted cells, which had been precultured in a brain heart infusion broth and 15 mL of agar culture medium in a glass Petri plate of 9 cm diameter. The sterilizing method was the same as in the case of the food described above. After opening, each 20 x 13 cm bag was 80% sealed. The plate in each bag had been exposed for 5 minutes to ozone gas, carbon dioxide gas or their mixture. Just after the 5-minute sterilization, plates were taken out of the bags and then incubated at 37EC for 48 h. For the storage tests, the bags were completely sealed after the 5-minute sterilization and the plates were stored in the same gas at 37EC for 48 hrs. Calculations of the amount of bacteria in these plates were carried out after 48 hrs.

Results and Discussion

Sterilization of Raw Beef: As shown in Table 2.2.03, a large number of bacteria remained in the raw beef even after 5 minutes of sterilization. The lethal threshold concentration for the bacterial cells was reported to be 0.1-0.2 mg/liter when the bacterial cells were bubbling with ozone in a test solution. A low concentration of ozone was reported to be ineffective when organic matter was present, because the organic matter interferes with the action of the ozone on the bacterial cells. Ozone also was reported to kill all bacteria immediately at a critical concentration known as the "all-or-nothing" response." So these results indicate that the organic matter present in the beef samples exerted an ozone demand and prevented the full utilization of the applied dose as a disinfectant.

Table 2.2.03. Survival of bacteria in different gases and gas mixtures just after exposure of sliced beef meat at room temperatures for 5 min at 5EC for 7 days (Mitsuda et al., 1990)

Kind of gas and mixing ratio	**Number of bacteria (survival %)**			
	just after exposure		**after storage**	
Control	1.4 x 10^3	100%	40 x 10^3	100%
Ozone gas	0.9	64	8.0	20
CO_2 gas	1.3	93	30	75
Mixed gas (O_3:CO_2, v/v)				
3:1	0.5	36	8.0	20
2:1	0.6	43	4.0	10
1:1	0.6	43	5.2	13
1:2	1.0	71	6.4	16
1:3	1.2	86	7.1	18

After storing the samples in the same gases in bags at 5EC for 7 days, the number of bacteria increased, although the increases varied with the kind of gas and the mixing ratio of ozone and

carbon dioxide. Not only pure carbon dioxide gas but also pure ozone gas did not reduce the growth of bacteria. However, the retarding effect of the gas mixture was observed. The most effective mixing ratios of ozone and carbon dioxide gas were 2:1, 1:1, 1:2, and 1:3.

Sterilization of Bacterial Cells in an Agar Plate: To find the mechanism of this synergistic effect, a sterilization test for bacterial cells in an agar plate with ozone gas, carbon dioxide gas and a mixture of these gases was carried out. In the usual way, the numbers of the surviving bacterial cells were measured after ozone was bubbled through the bacterial suspension. In this study, however, the sterilization test was carried out on the bacterial cells located in the agar plate.

As shown in Table 2.2.04, the sterilization effect after 5 minutes was restricted, because the bacterial cells that were located on the surface of the agar plate were included as a part of the cells on the plate. Both in the five minutes of sterilization and the storage of the agar plate, the sterilization effect of some samples under the gas mixture was larger than that of samples under ozone and carbon dioxide gas in the single use. Desirable results were obtained when the mixing ratio of the ozone and carbon dioxide was 1:3 and 1:1.

Table 2.2.04. Survival of *Escherichia coli* in different gases and gas mixtures just after exposure at room temperature for 5 min and at 37EC for 48 hours (Mitsuda et al., 1990)

Kind of gas and mixing ratio	**Number of bacteria (survival %)**			
	just after exposure		**after storage**	
Control	440	100%	422	100%
Ozone gas	32	7	86	20
CO_2 gas	163	37	311	74
Mixed gas				
(O_3:CO_2, v/v)	56	13	125	30
3:1	55	13	122	29
2:1	300	68	0	0
1:1	310	70	124	29
1:2	5	1	166	39
1:3				

In addition, the number of bacteria after exposure to carbon dioxide was greater than that for ozone, but the size of the colonies of bacteria was smaller for carbon dioxide. Furthermore, the colonies that resulted after exposure to ozone gas were found inside the samples.

After the investigation on the mechanism of the interaction between carbon dioxide gas and protein, carbon dioxide binding sites in protein in the gas-solid phase system were revealed to be ,-amino, ∀-amino and guanidiurn groups. On the findings of these characteristics of carbon dioxide, the reasons for this synergistic effect observed in this study were considered as follows:

1. Ozone was reported to be bactericidal rather than bacteriostatic and carbon dioxide gas was known to be bacteriostatic. The synergistic effect could not be assumed to be caused by the

simple summation of the bactericidal effect of ozone gas, and the bacteriostatic effect of carbon dioxide gas, because the optimal conditions for the synergistic effect were obtained in a restricted ratio of both gases.

2. The bactericidal effect of ozone gas was assumed to be retained during these storage periods by the quenching effect of carbon dioxide gas to the chain reaction of ozone degradation. Decrease of the bacterial number for the storage period was assumed to be caused by the high solubility and the bacteriostatic effect of carbon dioxide gas to the bacterial cells which were located not only on the surface but also inside the agar plate.

2.2.4 Gorman et al. (1995b) – Beef Brisket Fat

Abstract: Various chemical solutions (5% hydrogen peroxide, 0.5% ozone, 12% trisodium phosphate, 2% acetic acid, and 0.3% commercial sanitizer), water (16 to 74EC spray-washing interventions, and hand-trimming/spray-washing treatments were compared for their ability to remove fecal material and to reduce bacterial contamination on beef brisket fat samples in a model spray-washing cabinet. The samples were inoculated with 2.5 cm^2 of a bovine fecal paste inoculated with *Escherichia coli* (ATCC 11370). Hand-trimming followed by spray-washing with plain water (16 to 74EC when it came in contact with the sample; 20.68 bar pressure; for 36 or 12 s corresponding to chain speeds of 100 or 300 carcasses per h) lowered ($P < 0.05$) microbiological counts, compared to the inoculated control, by 1.41 to 2.50 log colony-forming units (CFU)/cm^2. Additionally, spraying with chemical solutions (16EC; 1.38 bar, 12 or 36 s), before or after spray-washing with plain water (20.68 bar) of 16EC (36 s), 35EC (12 s) or 74EC (12 s) reduced bacterial counts by 1.34 to 2.87, 1.18 to 2.86, or 0.96 to 3.42 log CFU/cm^2 , respectively. Reduction in counts was influenced by water temperature (16 to 74EC, type of chemical solution, and sequence of spray application. **Under the conditions of this study, hydrogen peroxide and ozonated water were more effective ($P < 0.05$) than trisodium phosphate, acetic acid, and a commercial sanitizer when applied after first washing with plain water.** Trisodium phosphate maintained its activity when used before washing with water. In general, water of 74EC caused reductions ($P < 0.05$) exceeding 3.0 log CFU/cm^2, which were higher than those achieved by trimming and spray washing. No spreading of bacteria in areas immediately adjacent to the inoculation site was detected following spray washing.

Materials and Methods

Inoculum Preparation: A pure culture of a streptomycin-resistant *Escherichia coli* strain (ATCC 11370) was prepared at a certified laboratory by incubation for 24 h at 37EC in nutrient broth containing 625 Φg of dihydrostreptomycin/mL. A portion (100 mL) of the culture, diluted to 10^8 colony-forming units (CFU)/mL, was mixed aseptically with 300 g of fresh bovine feces (collected each day of experimentation from the holding pens of a large commercial beef slaughter facility) in a sterile stomacher bag by hand massaging for two min. To obtain the desired paste consistency, sterile water was added to the inoculated fecal material to achieve a ratio of 3 parts added liquid to 1 part fecal material. The inoculum then was transported from the laboratory in coolers with ice packs to a commercial slaughter facility (within a distance of one mile) for inoculation of samples on the same day.

Sample Handling and Decontaminating Treatments: Hot (< 15 min postmortem) adipose tissue pieces, cut from the brisket area of beef carcasses prior to any routine carcass washing or trimming, were transported to a room adjacent to the slaughter area using clean plastic trays. Before testing, each piece was cut aseptically with a sterile knife blade into a 10 by 10 cm square portion, which was inoculated with a plastic inoculating loop used to transfer the inoculated fecal paste to its center. The total inoculation area was 2.5 cm^2 and was obtained by using a 0.625-cm^2 inoculating loop and transferring inoculum to the adipose tissue for four times. Each analytical sample unit consisted of three (10 by 10 cm) pieces of adipose tissue (from three beef carcasses) which, after inoculation, were held for 15 min at room temperature (20EC) to allow for attachment of fecal material and bacteria. The three pieces of adipose tissue then were treated by hand-trimming (using a sterile knife blade and forceps to remove all visible fecal contamination) or by spray-washing under specified conditions.

Spray washing was done in a model, conveyorized, two-chamber, spray-wash cabinet, especially designed and built for these studies. The first washing chamber contained one 0.3125-cm (MEG 2150) oscillating nozzle, with oscillation set at 80 rpm to cover the entire length of the sample being washed. Only water was administered in this chamber, and the variables studied included slaughter chain speed (100 or 300 carcasses per h, corresponding to 36 or 12 s of exposure time), spray-washing pressure (2.76, 6.89, or 20.68 bar), and water temperature (16, 35, 66, or 74 ∀ 5EC). In the second chamber the sanitizing agents were applied at 16EC, 1.38 bar, and for 12 or 36 s. The hot water and chemical solutions were applied in two sequences, one following and the second preceding the plain water spray-washing treatment.

Visual Evaluation and Sampling: The treatment samples were evaluated visually by 2 or 3 people for any remaining fecal material. Visual scores were based on a 5-point scale in which 0 indicated no visible fecal material contamination, 1 indicated sparse evidence of fecal material, 3 indicated presence of fecal material, and 5 indicated obvious, dense fecal material contamination (detected on the unwashed inoculated samples). The samples were also evaluated visually before and after hand-trimming and/or spray-washing.

Following hand-trimming and/or spray-washing or before any treatment for the controls -- as appropriate -- tissue samples were taken, aseptically, from each piece of adipose tissue, using sterile cork borers (3.175-cm diameter), and a sterile scalpel and forceps. The samples were taken from the center (at the site of inoculation) of all three pieces of adipose tissue for each sampling unit and placed (all three samples) in a single sterile stomacher bag for subsequent microbiological analysis; this was designated as sample A. Two additional similar samples were taken, aseptically, from positions above and below the center of the brisket adipose tissue pieces and placed into another single sterile stomacher bag for subsequent microbial analysis, this was designated as sample B and consisted of six individual pieces of fat. The B sample was taken to determine the effect of spray-washing in causing translocation of microorganisms (from the center site to surrounding sites) through splashing or run-off and contamination of areas adjacent to the inoculation site. The A and B samples then were placed in coolers with ice packs for transportation to the laboratory (within a distance of one mile) for subsequent microbiological analysis; all testing was initiated within 2 h of hand-trimming or spray-washing.

Microbiological Analyses: Samples were diluted to a 10-1 dilution with sterile phosphate buffer (pH 7.0, KH_2PO_4) and then stomached using a model 400 stomacher for 2 min. Plating

was done on nutrient agar without or with 625 Φg dihydrostreptomycin/mL with a spiral plating system. The inoculated plates were incubated at 37EC for 48 h, then colonies were counted using a model 800 processor with a model 500 laser colony counter. The results were expressed and recorded in CFU/cm^2 of surface adipose tissue.

Statistical Analysis: A one-way completely randomized design was used in the study, which involved six replicates for each treatment, and least-square means (LSM) were calculated from the six replications. The LSM were separated using the least significant difference (LSD) procedure with the alpha level set at 0.05.

Results and Discussion

Table 2.2.05 and 2.2.06 summarize visual evaluation scores for fecal material contamination and microbiological counts for the site of inoculation (A) for the various treatment methods employed, including ozone (OZ 0.5% in water). The uninoculated samples contained 5.42 and 4.28 log CFU/cm^2 of total plate and streptomycin-resistant counts (data not shown), respectively, and these counts increased, after application of the inoculated fecal paste, to 6.66 and 6.26 log CFU/cm^2, respectively. The microbiological counts for the areas adjacent to the site of inoculation (B) are presented in Tables 2.2.07 and 2.2.08. These counts were 4.89 and 4.23 log CFU/cm^2 for total plate and streptomycin-resistant counts, respectively, in the uninoculated samples (data not shown), and 4.92 and 4.43 log CFU/cm^2 , respectively, in the inoculated samples. Because trends in the changes of microbiological counts after application of the spray-washing or hand-trimming treatments were similar between total plate and streptomycin-resistant bacterial counts, only the total plate counts are discussed in the remainder of this paper.

Reduction in Microbiological Counts: Trimming alone or trimming followed by a single spray-washing treatment of plain water (16 to 74EC; 20.68 bar; 12 or 36 s) significantly ($P < 0.05$) reduced the microbiological counts compared to the inoculated control. This indicates that the required trimming under the zero tolerance directive of FSIS-USDA reduces microbiological contamination after carcasses are contaminated with fecal material. A significant amount of contamination remained on the samples, however, even after trimming or trimming/spray-washing treatments. This study included evaluation of water temperatures (16, 35, 66, or 74EC), spraying pressures (2.76, 6.89, or 20.68 bar) and chain speeds (12 or 36 s), in an attempt to provide information that could be useful to meat packing plants operating under different conditions. Approximate reductions in bacterial counts achieved by a single spray-washing (no trimming) treatment of plain water were in the range of 1 to 2 log CFU/cm^2 compared to the inoculated/untrimmed/unwashed control. The higher pressures (i.e., 20.68 bar) generally were more effective. It is interesting to note that these washing (no trimming) treatments were as effective ($P > 0.05$) (at the higher pressure) as the trimming and washing combinations discussed earlier.

A large number of treatments in this study involved use of the two spray-washing chambers of the cabinet: one operating with plain water and the second with cold (16EC water alone or in combination with chemical interventions (including ozone). The plain water treatment was examined at 16EC, 20.68 bar, and 36 s, as well as at 35EC or 74EC, 20.68 bar, and 12 s (Tables 2.2.05 and 2.2.06). The chemical interventions were applied in the second chamber as solutions in 16EC water at 1.38 bar and for 12 s (300 carcasses per h). The total experiment was

performed in two sequences; in the first sequence, the plain water treatments were followed by the chemical interventions (Table 2.2.05), and in the second sequence, the chemical interventions were followed by the plain water treatments (Table 2.2.06).

Table 2.2.05. Visual fecal contamination scores and microbiological counts of beef brisket adipose tissue samples artificially inoculated and hand-trimmed or spray-washed under specific treatment conditions (sample location A, inoculation site) (Gorman et al., 1995b)

Treatments							
First Wash				Microbiological counts		Visual evaluation[a]	
Solution temperature (°C)	Pressure (bar)	Exposure time (s)		Total plate [log CFU/cm² (SD)]	Streptomycin-resistant [log CFU/cm² (SD)]	Before treatment [score (SD)]	After treatment [score (SD)]
Inoculated/Untrimmed/Unwashed				6.66 (0.35)^{a}	6.26 (0.58)^{a}	5.00 (0.00)	5.00 (0.00)^{a}
Inoculated/Trimmed/Unwashed				4.16 (1.16)^{ef}	4.24 (1.04)^{defg}	5.00 (0.00)	0.35 (0.41)^{bc}
Inoculated/Trimmed/Washed (16°C water, 20.68, 36 s)				5.05 (0.59)^{bcd}	4.35 (0.37)^{cdefg}	5.00 (0.00)	0.10 (0.22)^{bc}
Inoculated/Trimmed/Washed (35°C water, 20.68, 12 s)				4.34 (0.89)^{de}	3.97 (0.67)^{fg}	5.00 (0.00)	1.00 (1.83)^{b}
Inoculated/Trimmed/Washed (66°C water, 20.68, 12 s)				4.78 (0.51)^{cde}	4.08 (0.33)^{efg}	5.00 (0.00)	0.33 (0.41)^{bc}
Inoculated/Trimmed/Washed (74°C water, 20.68, 12 s)				5.22 (0.22)^{bc}	4.85 (0.34)^{bcd}	5.00 (0.00)	0.14 (0.24)^{bc}
Water	16	20.68	36	4.60 (0.44)^{cde}	3.87 (0.18)^{g}	5.00 (0.00)	0.25 (0.27)^{bc}
Water	35	2.76	12	5.17 (0.26)^{bc}	4.72 (0.54)^{bcde}	5.00 (0.00)	0.83 (0.41)^{bc}
Water	35	6.89	12	4.66 (0.51)^{cde}	4.01 (0.82)^{efg}	5.00 (0.00)	0.83 (0.26)^{bc}
Water	35	20.68	12	5.19 (0.16)^{bc}	4.63 (0.63)^{bcdef}	5.00 (0.00)	0.42 (0.49)^{bc}
Water	66	2.76	12	5.81 (0.24)^{b}	5.09 (0.63)^{bc}	5.00 (0.00)	0.17 (0.41)^{bc}
Water	66	6.89	12	5.19 (1.33)^{bc}	4.94 (1.07)^{bc}	5.00 (0.00)	0.00 (0.00)^{c}
Water	74	2.76	12	5.62 (0.21)^{b}	5.32 (0.20)^{b}	5.00 (0.00)	0.00 (0.00)^{c}
Water	74	6.89	12	5.68 (0.10)^{b}	5.16 (0.53)^{b}	5.00 (0.00)	0.00 (0.00)^{c}
Water	74	20.68	12	4.74 (0.79)^{cd}	4.05 (0.41)^{efg}	5.00 (0.00)	0.17 (0.26)^{bc}

[a–g] Means in the same column with different superscript letters are significantly different ($P < 0.05$).

[a] Score of 0, no visible fecal material contamination; score of 1, sparse evidence of fecal material; score of 3, presence of fecal material; score of 5, obvious, dense fecal material contamination.

When the first spray-washing action involved use of plain water at 16, 35, or 74EC followed by additional plain water at 16EC, the reductions in total plate counts were 1.35, 1.24, and 3.37 log CFU/cm^2, respectively (Table 2.2.05). ***When the first spray-washing treatments of 16 or 35EC plain water were followed by chemical solution interventions, the most effective sanitizing agents were hydrogen peroxide (5%) and ozonated water (0.5%), achieving reductions in total plate counts of 2.60 to 2.87 and 2.72 to 2.86 log CFU/cm^2, respectively.*** The least effective chemical agents were the commercial sanitizer (0.3%) and acetic acid (2%), achieving reductions of 1.43 to 1.94 and 2.01 to 2.02 log CFU/cm^2, respectively, while trisodium phosphate (12%) reduced the contamination by 2.26 to 2.30 log CFU/cm^2. When the first spray-washing treatment involved use of 74EC plain water, reductions in total plate counts achieved by any of the treatments that followed in the second chamber of the spray-washing cabinet, including the 16EC plain water, were 3.06 to 3.42 log CFU/cm^2 (Table 2.2.05). Thus, when water of 74EC was employed, reductions in contamination exceeded 3.0 log CFU/cm^2, irrespective of the presence or absence of chemical sanitizers. ***When the spray-washing water temperature was 16 or 35EC, a subsequent treatment with chemical solutions increased reductions in total plate counts on beef brisket fat, with hydrogen peroxide and ozonated water being the most effective treatments.***

Table 2.2.06. Visual fecal contamination scores and microbiological counts of beef brisket adipose samples artificially inoculated and hand trimmed or sanitized then spray-washed first under specific treatment conditions (sample location A, inoculation site) (Gorman et al., 1995b)

Treatments								Microbiological counts		Visual evaluation[x]	
First wash				Second wash							
	Solution temp. (°C)	Pressure (bar)	Exposure time (s)		Solution temp. (°C)	Pressure (bar)	Exposure time (s)	Total plate [log CFU/cm² (SD)]	Streptomycin-resistant [log CFU/cm² (SD)]	Before treatment [score (SD)]	After treatment [score (SD)]
Inoculated/Untrimmed/Unwashed								6.66 (0.35)[a]	6.26 (0.58)[a]	5.00 (0.00)	5.00 (0.00)[a]
Inoculated/Trimmed/Unwashed								4.16 (1.16)[cdefgh]	4.24 (1.04)[defghi]	5.00 (0.00)	0.35 (0.41)[hi]
Inoculated/Trimmed/Washed (16°C water, 20.68, 100 carcasses/h)								5.05 (0.59)[bcd]	4.35 (0.37)[defgh]	5.00 (0.00)	0.10 (0.22)[i]
Inoculated/Trimmed/Washed (35°C water, 20.68, 300 carcasses/h)								4.34 (0.89)[defg]	3.97 (0.67)[efghi]	5.00 (0.00)	1.00 (1.83)[b]
Inoculated/Trimmed/Washed (66°C water, 20.68, 300 carcasses/h)								4.78 (0.51)[bcdef]	4.08 (0.33)[defghi]	5.00 (0.00)	0.33 (0.41)[hi]
Inoculated/Trimmed/Washed (74°C water, 20.68, 300 carcasses/h)								5.22 (0.22)[bc]	4.85 (0.34)[bcdef]	5.00 (0.00)	0.14 (0.24)[i]
Water	16	20.68	36	Water	16	1.38	36	5.31 (0.87)[bc]	4.98 (0.70)[bcd]	5.00 (0.00)	0.00 (0.00)[i]
Water	16	20.68	36	TSP[w] (12%)	16	1.38	36	4.36 (0.53)[defg]	3.72 (0.44)[fghi]	5.00 (0.00)	0.25 (0.42)[hi]
Water	16	20.68	36	AA (2%)	16	1.38	36	4.64 (0.43)[cdef]	4.40 (0.45)[defg]	5.00 (0.00)	0.50 (0.45)[hi]
Water	16	20.68	36	HP (5%)	16	1.38	36	3.79 (0.85)[gh]	3.87 (0.62)[efghi]	5.00 (0.00)	0.17 (0.41)[i]
Water	16	20.68	36	OZ (0.5%)	16	1.38	36	3.94 (0.70)[fgh]	3.74 (0.42)[fghi]	5.00 (0.00)	0.17 (0.26)[i]
Water	16	20.68	36	CS (0.3%)	16	1.38	36	4.72 (0.71)[bcde]	4.58 (0.51)[cdef]	5.00 (0.00)	0.25 (0.42)[hi]
Water	35	20.68	12	Water	16	1.38	12	5.42 (0.93)[b]	5.38 (0.27)[b]	5.00 (0.00)	0.00 (0.00)[i]
Water	35	20.68	12	TSP (12%)	16	1.38	12	4.40(1.03)[defg]	3.44(0.33)[ij]	5.00 (0.00)	0.17(0.26)[i]
Water	35	20.68	12	AA (2%)	16	1.38	12	4.65 (0.42)[cdef]	4.34 (0.57)[defgh]	5.00 (0.00)	1.08 (0.58)[b]
Water	35	20.68	12	HP (5%)	16	1.38	12	4.06 (0.49)[defgh]	4.19 (0.94)[defgh]	5.00 (0.00)	0.00 (0.00)[i]
Water	35	20.68	12	OZ (0.5%)	16	1.38	12	3.80 (0.49)[gh]	3.70 (0.54)[ghij]	5.00 (0.00)	0.08 (0.20)[i]
Water	35	20.68	12	CS (0.3%)	16	1.38	12	5.23 (0.74)[bc]	5.03 (0.58)[bc]	5.00 (0.00)	0.08 (0.20)[i]
Water	74	20.68	12	Water	16	1.38	12	3.29 (0.12)[j]	3.24 (0.00)[j]	5.00 (0.00)	0.08 (0.20)[i]
Water	74	20.68	12	TSP (12%)	16	1.38	12	3.60 (0.34)[hij]	3.48 (0.38)[hij]	5.00 (0.00)	0.25 (0.42)[hi]
Water	74	20.68	12	AA (2%)	16	1.38	12	3.24 (0.00)[j]	3.24 (0.00)[j]	5.00 (0.00)	0.25 (0.42)[hi]
Water	74	20.68	12	HP (5%)	16	1.38	12	3.44 (0.36)[ij]	3.24 (0.00)[j]	5.00 (0.00)	0.08 (0.20)[i]
Water	74	20.68	12	OZ (0.5%)	16	1.38	12	3.49 (0.32)[ij]	3.24 (0.00)[j]	5.00 (0.00)	0.17 (0.26)[i]
Water	74	20.68	12	CS (0.3%)	16	1.38	12	3.29 (0.12)[j]	3.24 (0.00)[j]	5.00 (0.00)	0.17 (0.26)[i]

[x] Score of 0, no visible fecal material contamination; score of 1, sparse evidence of fecal material; score of 3, presence of fecal material; score of 5, obvious, dense fecal material contamination.

[a-j] Means in the same column with different superscript letters are significantly different ($P < 0.05$).

[w] Abbreviations: SD, standard deviation; TSP, trisodium phosphate; AA, acetic acid; HP, hydrogen peroxid; OZ, ozonated water; CS, commercial sanitizer.

When the sequence of the application of the interventions was reversed (application of the sanitizing agents followed by plain water spray-washing), the effectiveness of the sanitizers was different, with trisodium phosphate (12%) being the most effective treatment (Table 2.2.06). When the chemical intervention preceded the water treatments of 16 or 35EC, reductions in levels of total plate counts achieved by water alone, trisodium phosphate (12%), acetic acid (2%), ozonated water (0.5%), hydrogen peroxide (5%), and the commercial sanitizer (0.3%) were 2.13 to 2.80, 2.30 to 2.33, 1.33 to 2.14, 1.36 to 1.40, 1.18 to 1.34, and 1.21 to 1.42 log CFU/cm^2, respectively. When the spray-washing treatment that followed application of the chemical sanitizer involved water at 74EC, the only agent that maintained its activity and caused reductions in total plate counts exceeding 3 log CFU/cm^2 was trisodium phosphate (12%). The loss of activity of chemicals when followed by plain-water spray-washing possibly was due to physical removal or dilution of the sanitizing agents, while trisodium phosphate would appear to act by a different mechanism in decontaminating beef adipose tissue. Thus, the sequence of application would be important if chemical interventions were to be used, but chemical interventions would not be necessary if hot water (74EC) was the intervention chosen for carcass decontamination.

In addition, the variation in counts among samples spray-washed with 74EC water, as measured by the standard deviations shown in Tables 2.2.05 to 2.2.06 generally was lower than that of hand-trimming and spray-washing treatments at lower temperatures. Furthermore, hot-water spray washing resulted in greater reductions in bacterial counts than combinations of hand trimming and washing. Thus, these data indicate that spray washing with hot water is an effective means of reducing microbial counts on the external fat surfaces of beef. The reduction in total bacterial counts by use of hot water (74EC), which exceeded 3.00 log CFU/cm^2, compared favorably with the reduction of 1.44 to 2.32 log CFU/cm^2 achieved by the presently used treatments of hand-trimming followed by spray-washing (Tables 2.2.05 to 2.2.06). ***The data also indicated that chemical treatments such as hydrogen peroxide and ozonated water may be worth further testing for their potential application in packing-plant operations that are unable to supply hot water.***

Effect of Spray-Washing on Spreading of Contamination: Bacterial numbers in the areas of beef brisket samples immediately adjacent to the inoculation site were always lower than counts on the inoculation site, both before and after application of spray-washing or chemical interventions (Tables 2.2.05 to 2.2.08). The results demonstrate that there was no spreading of the bacteria in the inoculum onto areas immediately adjacent to the inoculation site through the spray-washing action, or that the spray-washing treatments diluted bacterial contamination, resulting in reduced counts. Comparing the counts from the areas adjacent to the inoculation site (location B), which were recovered after application of specific treatments, with the original control counts, the counts were lower ($P < 0.05$) for those samples washed with 74EC water (Tables 2.2.07 to 2.2.08). Thus, spray-washing under the conditions of this study either diluted the contamination or did not move or spread the bacteria to areas adjacent to the site of artificial contamination (location A). Use of spray-washing pressures of less than 20.7 bar should not have resulted in bacteria being embedded into the tissue.

Reductions in Visual Fecal Evaluation Scores: All treatments achieved virtual elimination of the visible fecal material contaminants that had been placed on the samples before processing (Tables 2.2.05 to 2.2.06). The change in scores from before treatment (score of 5) to after treatment was significantly ($P < 0.05$) lower and in many instances the decrease was between 4.17 and 5.00, suggesting that these treatments produced very clean sample surfaces (from a physical contaminant standpoint). Therefore, the treatments tested not only reduced bacterial counts, but also cleaned the surfaces of the fat.

Table 2.2.07. Microbiological counts of beef brisket adipose tissue samples obtained at locations (B) adjacent to the inoculation site before and after hand-trimming or sanitizing and spray-washing under specific treatment conditions (Gorman et al., 1995b)

Treatments								Microbiological counts	
First wash				Second wash					
	Solution temperature (°C)	Pressure (bar)	Exposure time (s)		Solution temperature (°C)	Pressure (bar)	Exposure time (s)	Total plate [log CFU/cm² (SD)]	Streptomycin-resistant [log CFU/cm² (SD)]
Inoculated/Untrimmed/Unwashed								4.92 (0.94)^{ab}	4.43 (0.93)^{bcd}
Inoculated/Trimmed/Unwashed								4.90 (0.76)^{ab}	3.80 (0.79)^{efg}
Inoculated/Trimmed/Washed (16°C water, 20.68, 36 s)								4.56 (0.58)^{bcd}	3.90 (0.55)^{cdefg}
Inoculated/Trimmed/Washed (35°C water, 20.68, 12 s)								3.85 (0.85)^{defg}	3.66 (0.82)^{efgh}
Inoculated/Trimmed/Washed (66°C water, 20.68, 12 s)								4.89 (0.35)^{ab}	3.94 (0.10)^{cdefg}
Inoculated/Trimmed/Washed (74°C water, 20.68, 12 s)								4.87 (0.22)^{ab}	4.31 (0.71)^{bcdef}
Water	16	20.68	36	Water	16	1.38	36	4.93 (1.33)^{ab}	5.06 (0.76)^{a}
Water	16	20.68	36	TSP[a](12%)	16	1.38	36	4.28 (0.76)^{bcdefg}	3.69 (0.86)^{efgh}
Water	16	20.68	36	AA (2%)	16	1.38	36	4.47 (0.55)^{bcd}	4.25 (0.53)^{bcdefg}
Water	16	20.68	36	HP (5%)	16	1.38	36	3.26 (0.28)^{fgh}	3.01 (0.20)^{hi}
Water	16	20.68	36	OZ (0.5%)	16	1.38	36	3.33 (0.40)^{fgh}	2.93 (0.00)^{i}
Water	16	20.68	36	CS (0.3%)	16	1.38	36	3.95 (0.82)^{cdefg}	4.61 (0.63)^{abc}
Water	35	20.68	12	Water	16	1.38	12	5.53 (0.38)^{a}	4.84 (0.86)^{ab}
Water	35	20.68	12	TSP (12%)	16	1.38	12	5.00 (0.76)^{ab}	4.08 (0.92)^{cdefg}
Water	35	20.68	12	AA (2%)	16	1.38	12	4.31 (0.32)^{bcd}	3.84 (0.52)^{defg}
Water	35	20.68	12	HP (5%)	16	1.38	12	2.93 (0.00)^{i}	3.06 (0.32)^{hi}
Water	35	20.68	12	OZ (0.5%)	16	1.38	12	3.45 (0.42)^{efgh}	2.98 (0.13)^{hi}
Water	35	20.68	12	CS (0.3%)	16	1.38	12	4.57 (1.04)^{bc}	4.17 (0.87)^{bcdefg}
Water	74	20.68	12	Water	16	1.38	12	3.48 (0.44)^{efgh}	3.23 (0.48)^{ghi}
Water	74	20.68	12	TSP (12%)	16	1.38	12	3.76 (0.89)^{defg}	3.57 (1.06)^{efghi}
Water	74	20.68	12	AA (2%)	16	1.38	12	2.93 (0.00)^{i}	3.05 (0.29)^{hi}
Water	74	20.68	12	HP (5%)	16	1.38	12	3.11 (0.28)^{gh}	2.93 (0.00)^{i}
Water	74	20.68	12	OZ (0.5%)	16	1.38	12	3.51 (0.50)^{defgh}	2.93 (0.00)^{i}
Water	74	20.68	12	CS (0.3%)	16	1.38	12	2.98 (0.13)^{i}	2.93 (0.00)^{i}

[a-i] Means in the same column with different superscript letters are significantly different ($P < 0.05$).
[a] TSP, trisodium phosphate; AA, acetic acid; HP, hydrogen peroxide; OZ, ozonated water; CS, commercial sanitizer.

Conclusions

The results of this study indicated that application of hot water (74EC at the surface of the sample) in beef spray-washing processes resulted in reductions of microbiological counts of the order of 3.0 log CFU/cm^2 compared to the combination of hand-trimming and spray-washing with colder (< 35EQ water, which achieved reductions in total plate counts of 1.44 to 2.32 log CFU/cm^2. Furthermore, spray-washing with hot water resulted in less variability in bacterial counts obtained after treatment compared to hand-trimming and/or spray-washing with water of lower temperatures. This greater variability in bacterial counts for hand-trimming treatments indicated the potential for cross-contamination during the process.

Table 2.2.08. Microbiological counts of beef brisket adipose tissue samples obtained at locations (B) adjacent to the inoculation site before and after hand-trimming or sanitizing then spray-washing under specific treatment conditions (Gorman et al., 1995b)

Treatments									
First wash				Second wash				Microbiological counts	
	Solution temperature (°C)	Pressure (bar)	Exposure time (s)		Solution temperature (°C)	Pressure (bar)	Exposure time (s)	Total plate [log CFU/cm² (SD)]	Streptomycin-resistant [log CFU/cm² (SD)]
Inoculated/Untrimmed/Unwashed								4.92 (0.94)^{abc}	4.43 (0.93)^{bcd}
Inoculated/Trimmed/Unwashed								4.90 (0.76)^{abc}	3.80 (0.79)^{efgh}
Inoculated/Trimmed/Washed (16°C water, 20.68, 36 s)								4.56 (0.58)^{bcd}	3.90 (0.55)^{defgh}
Inoculated/Trimmed/Washed (35°C water, 20.68, 12 s)								3.85 (0.85)^{efg}	3.66 (0.82)^{fgh}
Inoculated/Trimmed/Washed (66°C water, 20.68, 12 s)								4.89 (0.35)^{abc}	3.94 (0.10)^{defgh}
Inoculated/Trimmed/Washed (74°C water, 20.68, 12 s)								4.87 (0.22)^{abc}	4.31 (0.71)^{bcdef}
Water	16	1.38	36	Water	16	20.68	36	3.72 (0.49)^{efg}	3.44 (0.68)^{gh}
TSP[a] (12%)	16	1.38	36	Water	16	20.68	36	3.24 (0.30)^{gh}	2.93 (0.00)^{i}
AA (2%)	16	1.38	36	Water	16	20.68	36	4.14 (0.29)^{cde}	4.17 (0.31)^{cdef}
HP (5%)	16	1.38	36	Water	16	20.68	36	5.20 (0.27)^{ab}	4.86 (0.18)^{ab}
OZ (0.5%)	16	1.38	36	Water	16	20.68	36	4.83 (0.27)^{abc}	4.69 (0.41)^{abc}
CS (0.3%)	16	1.38	36	Water	16	20.68	36	4.96 (0.43)^{abc}	4.65 (0.87)^{abc}
Water	16	1.38	12	Water	35	20.68	12	3.76 (0.75)^{efg}	3.41 (0.55)^{gh}
TSP (12%)	16	1.38	12	Water	35	20.68	12	3.63 (0.46)^{efg}	2.93 (0.00)^{i}
AA (2%)	16	1.38	12	Water	35	20.68	12	5.07 (0.28)^{abc}	5.06 (0.31)^{a}
HP (5%)	16	1.38	12	Water	35	20.68	12	4.97 (0.40)^{abc}	4.75 (0.39)^{abc}
OZ (0.5%)	16	1.38	12	Water	35	20.68	12	4.52 (0.82)^{cd}	4.10 (0.35)^{cdef}
CS (0.3%)	16	1.38	12	Water	35	20.68	12	5.21 (0.12)^{ab}	5.00 (0.17)^{a}
Water	16	1.38	12	Water	74	20.68	12	3.41 (0.53)^{fgh}	3.30 (0.49)^{gh}
TSP (12%)	16	1.38	12	Water	74	20.68	12	2.93 (0.00)^{h}	2.93 (0.00)^{i}
AA (2%)	16	1.38	12	Water	74	20.68	12	4.93 (0.29)^{abc}	4.83 (0.24)^{ab}
HP (5%)	16	1.38	12	Water	74	20.68	12	5.01 (0.08)^{abc}	4.42 (0.34)^{bcd}
OZ (0.5%)	16	1.38	12	Water	74	20.68	12	4.64 (0.40)^{bcd}	3.97 (0.61)^{defg}
CS (0.3%)	16	1.38	12	Water	74	20.68	12	5.33 (0.50)^{a}	4.11 (0.30)^{cdef}

[a–i] Means in the same column with different superscript letters are significantly different ($P < 0.05$).
[a] TSP, trisodium phosphate; AA, acetic acid; HP, hydrogen peroxide; OZ, ozonated water; CS, commercial sanitizer.

Chemical interventions in the form of spray-washing solutions, such as hydrogen peroxide, ozonated water, and trisodium phosphate, increased reduction of microbial counts when following spraying with water of lower temperatures (e.g., 35EC, but their activity was overshadowed when their application followed spraying with hot (74EC water. The sequence of application for the chemical interventions was important since hydrogen peroxide and ozonated water lost their activity when their application preceded spraying with plain water, while trisodium phosphate maintained the same activity both when preceded or followed by plain water spray-washing. This finding suggests differences in mechanisms of decontamination among the interventions tested. Spray-washing treatments caused no spreading of the bacterial inoculum onto areas immediately adjacent to the inoculation site or diluted the contamination resulting in lower counts after spray washing. Trimming and spray-washing treatments achieved extensive removal of visible fecal material contaminants from the samples. Based on the conditions and results of this study, the use of elevated temperature (e.g., 74EC water may be the most useful treatment in the decontamination and the removal of fecal material from beef carcasses. As an alternative to hot water, the chemical interventions should be investigated for their potential application with water sprays of lower temperature (16 to 35EC).

2.2.5 Reagan et al. (1996) – Beef Carcasses

Abstract: A study to compare procedures and interventions for removing physical and bacterial contamination from beef carcasses was conducted in six carcass conversion operations that were representative of modern, high-volume plants and located in five different states. Treatment procedures included trimming, washing, and the current industry practice of trimming followed by washing. In addition, hot (74 to 87.8EC at the pipe) water washing and rinsing with ozone (0.3 to 2.3 ppm) or hydrogen peroxide (5%) were applied as intervention treatments. Beef carcasses were deliberately contaminated with bovine fecal material at >4.0 log colony-forming units (CFU)/cm^2 in order to be better able to observe the decontaminating effects of the treatments. Samples (10 by 15 cm, 0.3 to 0.5 cm thick) for microbiological testing were excised as controls or after application of each procedure or intervention and analyzed for aerobic mesophilic plate counts, *Escherichia coli* Biotype I counts, and presence or absence of *Listeria spp*., *Salmonella spp*., and *Escherichia coli* O157:117. Average reductions in aerobic plate counts were 1.85 and 2.00 log CFU/cm^2 for the treatments of trimming-washing and hot water washing, respectively. ***Hydrogen peroxide and ozone reduced aerobic plate counts by 1.14 and 1.30 log CFU/cm^2, respectively.*** In general, trimming and washing of beef carcasses consistently resulted in low bacterial populations and scores for visible contamination. However, the data also indicated that hot- (74 to 87.8EC at the pipe) water washing was an effective intervention that reduced bacterial and fecal contamination in a consistent manner.

Materials and Methods

Selection of Beef Carcasses: Six beef-slaughtering operations were selected as being representative of modern, high-volume plants. The plants were geographically dispersed, being located in five states, and were operated by four different companies. Four of the plants processed predominantly fed steers and heifers, while the other two processed mostly nonfed cows. The carcasses used for testing were randomly selected at 5- to 10-min intervals from the carcasses being processed (from 100 to 400 head per h, depending on the particular plant), and deliberately contaminated with fecal material obtained from the external surface of the hide of each carcass. The carcasses were contaminated by manual digitation on the inside round at the "high-rimmer" area of processing, immediately after the hide was opened, to create an area of contamination approximately 1.9 cm in diameter (ca. 2.84 cm^2). Testing was performed during the months of June and July 1994, and it was spread over 3 days in each plant with 8 carcasses of each treatment tested on a given day.

Intervention Treatments: Four primary treatments were evaluated in each of the six packing plants: inoculated, not treated control (CNT); trimmed only (T); washed only (W); and the combination of trimmed and washed (TW). The control (CNT) carcasses were neither trimmed nor washed in the area of contamination (before sampling), while the trimmed and washed (TW) carcasses were subjected to the current industry practice of trimming to remove all visible contamination and then washing using automated spray-washers before entrance of the carcasses into the chiller. Carcasses were identified with tags for specific treatments and the areas to be trimmed and sampled were circled with purple, edible ink. Carcasses were subjected to standard trimming practices, but were sampled prior to final washing. Knife-trimming (to remove visible contamination) was performed by plant personnel to meet USDA-FSIS zero-tolerance standards for removing fecal and other visible material The plant personnel trimming carcasses were

instructed to routinely immerse the knife (approximately 15 cm in length) and the hook in hot (82EC) water prior to touching a new carcass surface. Trimming varied among individuals, but generally involved placing the hook above the contaminated area and, in one motion downward, removing the contaminated portion.

The W carcasses were not trimmed in the marked area of contamination, but they were processed through the standard automated spray-washer before sampling. The approximate length of the cabinets was 4 and 11 m for two of the Cary cabinets, while the length of the spray was approximately 3 and 7 m, respectively. In all of the washers, the angle of spray was 25E, but three also had bars at 0E. All washers had four type #2510 nozzles, while three also had bar-type four-hole nozzles. The total water output ranged from a low of 605 to a high of 2,683 liters/min. The water temperatures during normal washing ranged between 28 and 42EC; the pressures between 410 and 2,758 kPa and the spray-washing times between 18 and 39 s.

The experimental intervention treatments of a hot- (74 to 87.8EC at the pipe) water final wash with no trimming (HW), no trimming but carcass rinsing with ozonated (0.3 to 2.3 ppm) water (OZ) after final washing, and no trimming but carcass rinsing with hydrogen peroxide (5% solution) after final washing (PER) were evaluated at two of three plants (two fed steer and heifer plants and one nonfed cow plant). Because of differences in facilities and equipment between the three plants, there was some variation in the level and extent of application of these intervention treatments. The specific conditions-where available or accessible-for each intervention in each plant are given in Table 2.2.09.

Sampling: In practice, the CNT and T carcasses were sampled on the final trimming rail of the slaughtering chain before washing, while the W and TW carcasses were sampled immediately after washing. Each sample, consisting of a 10 by 15 cm area (150 cm^2) and approximately 0.3 to 0.5 cm thick, was aseptically excised from the original contaminated area of the inside round using standardized sterile templates. The broad area of the inside round of carcasses to be used in the study was marked with purple edible ink. In addition, test carcasses were appropriately tagged. After excision, the samples were immediately chilled on ice, and shipped with "blue ice" packets in insulated containers by overnight air express to the analytical laboratory (Chicago, IL). The temperature of the samples was determined on arrival at the analytical laboratory, and samples were inspected for any obvious signs of temperature abuse. The samples were considered to have been properly maintained during shipment if they were received by the laboratory within 24 h, the sample temperature was below 5EC, and the "blue ice" packets were still frozen.

Table 2.2.09. Description of hot-water and chemical-intervention treatments evaluated for decontamination of beef carcasses (Reagan et al., 1996)

Treatment	Plant 1	Plant 2	Plant 3
Hot Water (HW)			
Water Temp. EC at pipe	NP [a]	74	87.8
Wash duration (s) [b]		18	11
Pressure (kPa)		2,413	1,310
Ozonated water (OZ)			
Concentration (ppm)	0.3-0.9	2.3	NP
Rinse duration (s)	3	13	NP
Pressure (kPa)	138	138	NP
Hydrogen Peroxide (PER)			
Concentration (%, vol/vol)	5.0	5.0	NP
Rinse duration (s)	3	13	NP
Pressure (kPa)	138	138	NP

[a] Not performed.

[b] The wash cabinet in plant 3 provided a continuous wash of the entire carcass; the cabinet in plant 2 provided for a three-stage, sequential wash, beginning at the hind legs and moving down the carcass. The result of this design is that, although the total wash was 22 seconds, each portion of the carcass received a wash for a period equivalent to approximately 33% of that time.

Microbiological Analyses: Samples were weighed and homogenized or stomached for 2 min in 200 mL of Butterfield's phosphate buffer. A Waring blender was used to homogenize samples from plant 1, while a Stomacher 400 was used to homogenize samples from plants 2 through 6. Homogenates were analyzed for *Salmonella spp*., *Listeria spp*., *Escherichia coli* O157:H7, aerobic plate counts, and *E. coli* counts. Analysis for each of the three pathogens used portions of 25 mL from the stomached samples (20% of the blended samples) and determined presence or absence of the pathogen in a sample. The lactose preenrichment method was used for *Salmonella spp*. A two-step broth enrichment procedure was used for *Listeria spp*., with the second broth being incubated for 40 to 48 h and then streaked for isolation onto modified Oxford medium and lithium chloride phenylethanol moxalactain agar for isolation of the organism. *Escherichia coli* O157:H7 was isolated and identified by the procedure of Okrend et al. Aerobic plate counts and *E. coli* Biotype I most probable numbers were determined according to standard procedures. Colony-forming units per gram were converted to colony-forming units per cm^2 by multiplying the count by the sample weight and dividing by the fascia surface area of the sample. The sensitivities of the pathogen detection methods were 0.05 and 0.03 organisms per cm^2 for stomached and blended samples, respectively.

The lowest numerical visual score (0.16) obtained after application of the decontamination treatments was that for the trimmed and washed (TW) samples (Table 2.2.10). This score was significantly ($P < 0.05$) lower than those obtained with any other treatment, indicating that the currently applied decontamination treatment resulted in carcasses with the least visible

contamination. Trimmed (T), hot water (HW), ozone (OZ) and peroxide (PER) treatments resulted in visual scores which were higher than those for the TW treatment, but were lower ($P < 0.05$) than that achieved with washing (W) only. Conventional washing without trimming was the least effective treatment in removing visible contamination from the carcasses.

Table 2.2.10. Visual scores for cleanliness of intentionally contaminated carcasses before and after application of decontamination treatments (Reagan et al., 1996)

Treatment [a]	Mean visual score [b]	No. carcasses treated	SD of mean
Before treatment			
Control (CNT)	3.51A	144	0.65
Trimmed (T)	3.47A	144	0.50
Washed (W)	3.39A	144	0.50
Trimmed and washed (TW)	3.42A	144	0.49
Hot-water washed (HW)	3.33A	48	0.52
Hydrogen peroxide (PER)	3.47A	47	0.50
Ozone (OZ)	3.52A	48	0.55
After treatment			
Control (CNT)	3.44A	144	0.70
Trimmed (T)	0.47D	144	0.88
Washed (W)	1.14B	144	0.78
Trimmed and washed (TW)	0.16E	144	0.35
Hot-water washed (HW)	0.54D	48	0.46
Hydrogen peroxide (PER)	0.85C	47	0.68
Ozone (OZ)	0.66CD	48	0.55

[a] See text and Table 2.2.09 for a description of treatments.

[b] Means within a scoring time followed by different letters are different ($P < 0.05$).

Washing only, or application of ozone (OZ) and hydrogen peroxide (PER) rinses, resulted in average reductions in bacterial populations of approximately 1 log unit (Table 2.2.11). Although the application of the two chemical interventions, OZ and PER, resulted in lower ($P < 0.05$) average microbiological populations than the control samples, the means were not significantly ($P > 0.05$) different from those achieved with conventional washing or trimming. It was interesting to note the relatively large populations of bacteria remaining on the surface after the treatments, even though the scores for visible contamination on carcasses treated by several of these interventions were quite low.

Table 2.2.11. Populations of aerobic bacteria and *Escherichia coli* Biotype I on beef carcasses which were intentionally contaminated and then decontaminated with specific intervention treatments (Reagan et al., 1996)

Bacteria counted (treatment [a])	Mean counts [b]	No. carcasses treated	SD of mean
Aerobic plate count			
Control (CNT)	4.20A	142	1.32
Trimmed (T)	2.88C	142	1.10
Washed (W)	3.24B	144	1.15
Trimmed and washed (TW)	2.35D	144	0.99
Hot-water washed (HW)	2.20D	46	0.69
Hydrogen peroxide (PER)	3.06BC	48	1.09
Ozone (OZ)	2.90BC	48	1.04
***E. coli* Biotype I**			
Control (CNT)	2.23A	142	1.22
Trimmed (T)	0.62C	142	0.69
Washed (W)	1.19B	144	0.99
Trimmed and washed (TW)	0.56C	144	0.59
Hot-water washed (HW)	0.41C	48	0.28
Hydrogen peroxide (PER)	1.25B	47	0.80
Ozone (OZ)	1.09B	48	0.90

[a] See text and Table 2.2.09 for a description of treatments.

[b] Aerobic plate counts, CFU/cm^2; *E. coli,* MPN/cm^2. Means followed by different letters are different ($P < 0.05$).

The mean populations of *E. coli* Biotype I followed the same general trend as did the populations of aerobic bacteria (Table 2.2.11), with the lowest populations obtained with the TW, HW, and T treatments. As with the aerobic plate counts, the HW treatment resulted in the least carcass-to-carcass variation, as was indicated by the lower standard deviation. Although the trimming-only (T) treatment resulted in statistically ($P < 0.05$) lower populations of aerobic bacteria than the W treatment and higher than the TW treatment, T was not significantly ($P > 0.05$) different from TW for *E. coli* counts. Use of these treatments (T, TW, HW) resulted in an average reduction in populations of approximately 1.7 log units when compared to the control. As was the case with the aerobic bacteria, the W, OZ, and PER treatments resulted in significant ($P < 0.05$) reductions in *E. coli* counts compared to the control.

All of the processing treatments -- trimming, washing, and trimming and washing -- significantly ($P < 0.05$) reduced the incidence of *Listeria spp.* and *Salmonella spp*. on the carcasses (Table 2.2.12). Trimming and washing resulted in the lowest incidence of these two bacteria of potential public health significance, although the individual treatments could not be statistically differentiated.

Table 2.2.12. Incidence of bacteria of public health significance on samples from beef carcasses which had been intentionally contaminated and then decontaminated with specific intervention treatments (Reagan et al., 1996)

Treatment [b]	Number of samples (positive/total) [a]		
	Listeria spp.	*Salmonella spp.*	*E. coli* O157:H7 [c]
Control (CNT)	61/142	43/142	1/142
Trimmed (T)	35/140*	11/142*	3/142
Washed (W)	39/143*	13/144*	1/144
Trimmed and Washed (TW)	18/143*	2/144*	2/144
Hot-water washed (HW)	15/45	1/46*	0/46
Hydrogen peroxide (PER)	16/47	15/47	0/47
Ozone (OZ)	11/48*	19/48	0/48

[a] Treatment values within a genus marked with asterisks (*) are significantly ($P < 0.05$) different from the control treatment value.

[b] See text and Table 2.2.09 for a description of treatments.

[c] *E. coli* O157:H7: insufficient number of positive samples to determine treatment differences.

The current industry practice of trimming and washing reduced the incidence of *Listeria spp*. from 43.7% to 12.6%, and reduced the incidence of *Salmonella spp.* from 30.3% to 1.4%. Although the initial incidence levels of these two bacteria seem high, these carcasses were deliberately contaminated to obtain sufficiently high counts for statistical analysis and are not typical of the average cattle being processed. Hot water washing also significantly ($P < 0.05$) reduced the incidence of *Salmonella spp*. when compared to that of the control, but the reduction of *Listeria spp.* incidence was not significant ($P > 0.05$). PER and OZ treatments also reduced the incidence of pathogens and their effect should have included any residual activity, since sample analysis was conducted on the day after treatment. The total number of samples that were positive for *E. coli* O157:H7 was insufficient to compare treatments and/or to differentiate for their ability to reduce the level of *E. coli* O157:H7 contamination. However, the data suggested that none of the treatments could be relied upon to completely eliminate that pathogen (*E. coli* O157:H7) from the carcasses.

Conclusions: Ozone and hydrogen peroxide treatments, as applied in this study, had only minor effects and were approximately equivalent to conventional washing in reducing bacterial populations on beef.

2.2.6 Gorman et al. (1997) – Beef Washing and Storage

Abstract: Spray-washing reduced aerobic plate counts (APC) by 0. 88 to 2.83 log colony-forming units (CFU)/cm^2, with hot water (74EC) being the most effective treatment. Counts exceeded 6 log CFU/cm^2 in 1-3, 7-11, 11-16, 16-23 and 23-29 days of storage for unwashed, washed with hydrogen peroxide, washed with 35EC water or ozonated water or trimmed/washed with 35EC water, washed with commercial sanitizer, and washed with trisodium phosphate,

respectively. Samples washed with acetic acid or water of 74EC reached only 4.31 and 4.36 log CFU/cm^2, respectively, at 29 days of storage. Increases in the concentration of thiobarbituric acid reactive-substances (TBARS) were slowest in samples washed with trisodium phosphate. Spray washing with 2% acetic acid or 74EC water were the most effective treatments for reducing microbial growth, followed by trisodium phosphate which also reduced lipid oxidation during storage of beef.

Materials and Methods

Inoculum Preparation: A pure culture of streptomycin-resistant *Escherichia coli* (ATCC 11370) was prepared in nutrient broth (containing 625 mg of dihydrostreptomycin/mL at 35EC for 24 h. A dilution of the Culture (10^8 CFU/g) then was mixed with fresh bovine feces (randomly collected each day of experimentation from the holding pens of a large commercial beef slaughter facility) in a sterile Whirl-Pak bag by hand massaging for 2 min. The objective of this procedure was to obtain a high and consistent inoculum, and a desired fecal paste consistency, which was achieved by addition of sterile water to the inoculated fecal material to reach a ratio of three parts of total added liquid, to one part of fecal material. The inoculum then was transported from the laboratory, in coolers with ice packs, to a commercial slaughtering/dressing facility for inoculation of samples and subsequent spray-washing within the same day.

Sanitizing Agents: Sanitizing agents tested by spraying them on beef samples included 2% acetic acid, 12% trisodiurn phosphate, 5% hydrogen peroxide (30% stock solution), 0.5% ozonated water, and 0.3 % of a commercial sanitizer (RPM Acid Sanitizer) consisting of decanoic acid 3%, nonanoic, acid 3%, phosphoric acid 8.5%, sulfuric acid 9.5%, propionic acid 10% and inert ingredients 66%.

The sanitizing agent solutions were prepared by mixing tap water with the specified chemical within 24 h of use and holding them in closed containers in a cold room (4EC) until used. The solutions of water plus chemical sanitizers were sprayed on the samples at 16EC, 1.38 bar and at exposure times of 12 s (equivalent to 300 carcasses/h), after the samples were spray-washed with water (35EC) at 20.68 bar for 12s.

Sample Handling and Decontaminating Treatments: A sample of adipose tissue (approximately 1.25 cm thick) from the outside surface of the chuck of beef carcasses was removed using a sterile knife-blade, prior to any routine carcass washing or trimming (< 15 min postmortem), and was transported to a room adjacent to the slaughtering/dressing area of the packing plant using plastic trays cleaned with an alcohol spray. Each piece of adipose tissue was cut aseptically with a knife blade to form a 10 cm x 10 cm portion and inoculated by use of sterile plastic inoculating loops. Inoculating loops were immersed into the fecal paste and the inoculum was transferred to the center of each square of adipose tissue to achieve a 2.5 cm^2 area with contamination on the side with intact fascia. The 2.5 cm^2 area was obtained using a 0.625 cm^2 inoculating loop and transferring inoculum to the adipose tissue for the appropriate number of times (n = 4).

After inoculation, each piece of adipose tissue was held for 15 min at room temperature (20EC) to allow for attachment of fecal material and bacteria. The pieces of adipose tissue then were

treated by hand trimming or spray washing at the specified conditions. Spray washing was done in a specially designed, test-size, conveyorized, model spray washing cabinet with one 0.3125 cm (MEG 2150) diameter oscillating nozzle; the nozzle oscillation was set at 80 rpm and the oscillation pattern covered the entire length of the piece of adipose tissue being washed at a distance of 12 cm. The cabinet was custom-made for these studies and designed to simulate slaughter production-speed as well as the wash-action of a final carcass wash, spray-washing cabinet. In addition to rinsing, with the different sanitizing agents, samples that had been spray washed with water, other samples were spray-washed with water at 35EC or 74EC for 12 s (300 carcasses/h) at 20.68 bar. Additional samples were trimmed with a sterile knife and forceps, cutting vertically to remove all visible fecal contamination, and the samples subsequently were spray washed (35EC, 20.68 bar, 12 s). The knife and forceps were decontaminated between samples using alcohol and a flame.

Packaging and Visual Evaluation: Following the spray-washing treatment, each piece of adipose tissue was placed onto a 10 cm x 10 cm Styrofoam tray and overwrapped with polyvinyl chloride film. The samples then were placed in coolers containing ice packs; and, after all treatments were completed, the samples were stored in a cold room (4EC) under 350 lux of continuous, cool white fluorescent illumination. Duplicate samples were randomly obtained for each of the treatments on days 1, 3, 7, 11, 16, 23 and 29, and evaluated for total populations of aerobic microorganisms and for content of thiobarbituric acid-reactive substances (TBARS).

Analyses: Total aerobic plate counts (APC) were determined by aseptically cutting a 3 cm by 3 cm square from the center of each piece of adipose tissue, approximately 1.25 cm thick, and transferring the tissue into a sterile stomacher bag containing sterile 0.1% peptone water (Difco). The samples then were macerated for 2 min using a model 400 stomacher. Duplicate samples were serially diluted with sterile 0.1% peptone water and spread-plated on tryptic soy agar (Difco). The inoculated plates were incubated for 24 h at 35EC. The colonies then were counted, recorded and results were expressed as log CFU/cm^2.

Statistical Analysis: A completely randomized design was used in this study with dependent variables of total aerobic counts, thiobarbituric acid-reactive substances, and visual evaluation scores for the center area and the surrounding areas. Independent variables consisted of treatment, replication, day, and all possible interactions of the above. The study was replicated twice and duplicate samples were analyzed each time. The data were analyzed by analysis of variance using the General Linear Models procedure of SAS. Least squares means (LSM) were calculated with n=4 (except ozone, n=2). The LSM were separated using the Least Significant Difference procedure. The alpha level was set at 0.05 throughout the study.

Results And Discussion: The treatment-by-day interaction was not significant ($P > 0.05$) for APC; however, differences ($P<0.05$) were noted for the individual effects of treatments and days of storage. Inoculation increased contamination by 1.82 log CFU/cm^2, while treatments (during their application) reduced microbiological population levels compared to the inoculated/unwashed control, by 0.88 to 2.83 log CFU/cm^2, with the most effective treatment being hot (74EC) water (Table 2.2.13).

The 74EC plain water spray-washing treatment and the acetic acid treatment were the most effective ($P > 0. 05$) in reducing microbial growth during sample storage. The maximum APC

reached by these treatments during the 29 days were 4.36 and 4.31 CFU/cm^2, respectively (Table 2.2.13). Times at which samples exceeded 6 log CFU/cm^2 were 7-11 days for hydrogen peroxide; 11-16 days for samples treated with 35EC water, ozonated water, or trimmed and washed with 35EC water; 16-23 days for samples treated with commercial sanitizer; and, 23-29 days for samples treated with trisodiurn phosphate. In general, the most effective decontamination treatments in terms of inhibiting microbial growth during product storage were hot (74EC) water and acetic acid (2%), followed by trisodium phosphate (12%). The other treatments had a lesser effect on rate of microbial growth, which was similar to that on uninoculated/unwashed samples.

The TBARS values for samples from all treatments gradually increased during the 29-day storage period with the F-test being significant ($P < 0.05$) for the treatment-by-day interaction (Table 2.2.14). There was no difference ($P > 0.05$) among samples from different treatments, in the TBARS values on sampling days 1, 3 and 7. Samples treated with trisodium phosphate (12%) reached a maximum value of 3.94 mg malonaldehyde/kg of wet tissue on day-29 of storage, whereas the uninoculated/-unwashed samples reached a level of 4.27 mg/kg of wet tissue on day-23. Samples of spray-washed adipose tissue from the other treatments sustained more rapid increases in TBARS during storage than did samples treated with trisodium phosphate.

Table 2.2.13. Effects of spray-washing treatments on microbial growth (log CFU/cm^2) on samples of beef adipose tissue during 29 days of aerobic storage (4EC) (Gorman et al., 1997)

Day	Uninoculated/ Unwashed	Inoculated/ Unwashed	Inoculated/ Trimmed/ Washed (35C)	Water Wash (35C)	Water Wash (74C)	Acetic Acid (2%)	Ozonated Water (0.5%)	Hydrogen Peroxide (5%)	Trisodium Phosphate (12%)	Commercial Sanitizer (0.3%)[y]
1	2.97(0.80)^{abD}	4.79(2.02)^{aB}	3.91(0.80)^{abC}	3.70(0.74)^{abB}	1.96(0.25)^{bB}	3.50(0.72)^{abAB}	3.30(0.04)^{abB}	2.93(0.51)^{abB}	3.10(0.51)^{abC}	2.92(0.32)^{abD}
3	4.02(1.72)^{bCD}	6.86(0.34)^{aA}	2.52(1.14)^{bC}	3.95(0.40)^{bB}	2.47(0.81)^{bB}	2.38(0.84)^{bAB}	4.52(0.22)^{abB}	3.54(0.65)^{bB}	2.51(1.31)^{bC}	3.57(0.72)^{bD}
7	4.80(2.62)^{abCD}	7.16(0.97)^{aA}	3.91(1.60)^{bcC}	3.70(0.53)^{bcB}	2.26(0.93)^{cB}	2.31(0.86)^{cB}	5.00(0.64)^{abAB}	3.80(1.49)^{bcB}	4.13(1.68)^{bcBC}	4.45(1.58)^{bCD}
11	4.40(2.11)^{abCD}	7.67(0.90)^{aA}	4.28(1.23)^{abBC}	4.49(1.66)^{bcAB}	4.35(2.08)^{cAB}	3.53(0.75)^{cAB}	5.43(0.43)^{abcAB}	6.40(1.06)^{abA}	4.44(1.76)^{bcBC}	4.72(2.00)^{bcBCD}
16	5.90(2.29)^{abABC}	6.98(1.36)^{aA}	6.11(2.68)^{abAB}	6.07(0.55)^{abA}	3.14(1.86)^{cAB}	4.24(2.32)^{bcAB}	7.59(1.20)^{aA}	6.07(0.54)^{abA}	3.80(1.52)^{bcBC}	5.90(1.73)^{abABC}
23	6.85(2.35)^{abA}	8.74(0.46)^{aA}	7.66(2.31)^{abA}	6.23(1.60)^{bcdA}	4.61(2.94)^{cbA}	3.48(1.36)^{cAB}	7.68(1.14)^{abA}	6.67(0.93)^{bcA}	5.24(1.20)^{abcAB}	6.82(0.63)^{abcA}
29	6.31(1.45)^{bcdAB}	9.11(0.34)^{aA}	8.02(0.45)^{abA}	5.87(1.26)^{bcdAB}	4.36(1.80)^{cdAB}	4.31(1.43)^{dA}	---[z]	6.83(1.54)^{abA}	7.04(1.10)^{abA}	6.59(1.49)^{bcAB}

[a-e] Means (standard deviations) in the same row with unlike superscripts are different at $P<0.05$

[A-D] Means (standard deviations) in the same column with unlike superscripts are different at $P<0.05$

[y] Commercial sanitizer consists of 3% decanoic acid, 3% nonanoic acid, 8.5% phosphoric acid, 9.5% sulfuric acid, 10% propionic acid, and 66% inert ingredients

[z] Data were not collected

Table 2.2.14. Effect of spray-washing treatments on thiobarbituric acid reactive substances (mg of malonaldehyde/kg of wet tissue) in samples of beef adipose tissue during 29 days of aerobic storage (4EC) (Gorman et al., 1997)

Day	Uninoculated/ Unwashed	Inoculated/ Unwashed	Inoculated/ Trimmed/ Washed (35C)	Water Wash (35C)	Water Wash (74C)	Acetic Acid (2%)	Ozonated Water (0.5%)	Hydrogen Peroxide (5%)	Trisodium Phosphate (12%)	Commercial Sanitizer (0.3%)[y]
1	0.33(0.03)^{D}	0.36(0.10)^{C}	0.36(0.04)^{D}	0.41(0.11)^{C}	0.47(0.03)D	0.59(0.15)^{D}	0.42(0.15)^{B}	0.64(0.31)^{D}	0.39(0.12)^{C}	0.39(0.13)^{C}
3	0.53(0.04)^{D}	0.52(0.09)^{C}	0.72(0.08)^{CD}	0.59(0.08)^{C}	0.74(0.32)^{D}	0.82(0.13)^{D}	0.82(0.16)^{B}	0.75(0.04)^{D}	0.51(0.09)^{C}	0.58(0.10)^{C}
7	0.88(0.11)^{CD}	1.05(0.23)^{C}	1.53(0.72)^{CD}	0.94(0.16)^{C}	1.23(0.43)^{CD}	1.50(0.19)^{CD}	1.33(0.30)^{B}	1.33(0.15)^{D}	1.03(0.45)^{BC}	1.30(0.23)^{C}
11	1.95(0.67)^{abC}	1.28(0.38)^{bC}	1.69(0.48)^{abC}	1.63(0.42)^{abBC}	2.47(0.79)^{abC}	2.69(0.49)^{aC}	2.21(0.53)^{abB}	2.64(0.58)^{aC}	1.84(0.75)^{abB}	2.74(0.69)^{aB}
16	3.33(1.79)^{bcB}	1.46(0.23)^{dC}	3.35(1.18)^{bcB}	2.62(1.14)^{cdB}	4.26(0.19)^{abB}	4.13(0.77)^{abB}	2.02(0.17)^{cdB}	5.31(0.80)^{aB}	2.09(0.33)^{cdB}	2.78(1.22)^{aB}
23	4.27(3.17)^{abcB}	3.01(1.50)^{cB}	4.69(1.90)^{abA}	5.76(1.33)^{aA}	4.91(1.05)^{abB}	5.68(1.00)^{aA}	5.77(0.11)^{aA}	4.91(0.72)^{abB}	3.79(0.74)^{bcA}	5.36(2.18)^{aA}
29	6.82(3.03)^{abcA}	5.28(0.18)^{cdA}	6.12(0.92)^{bcA}	1.96(0.12)^{eBC}	7.80(0.47)^{aA}	6.51(0.55)^{abcA}	—[z]	7.11(1.17)^{abA}	3.94(0.35)^{dA}	6.14(1.51)^{bcA}

$^{a-e}$ Means (standard deviations) in the same row with unlike superscripts are different at P<0.05
$^{A-D}$ Means (standard deviations) in the same column with unlike superscripts are different at P<0.05
^{w} Results on days 1, 3, and 7 are not significantly different among samples in any treatment; therefore, they do not have superscripts
^{y} Commercial sanitizer consists of 3% decanoic acid, 3% nonanoic acid, 8.5% phosphoric acid, 9.5% sulfuric acid, 10% propionic acid, and 66% inert ingredients
^{z} Data were not collected

Based on these data, spray-washing with hot water or acetic acid not only reduced initial contamination, but also increased the microbial shelf life of beef adipose tissue stored in an aerobic environment. Other spray washing treatments reduced the rate of microbial growth only to those levels inherent to uninoculated/unwashed samples. Among control and spray washed samples, those treated with trisodium phosphate had the lowest level of malonaldehyde per kg of wet tissue.

SUMMARY OF RED MEAT PROCESSING SECTION

Antimicrobial effects of ozone on beef carcasses were reported in six major investigations in 1968,1989, 1990, 1995, 1996, and 1997.

Gaseous ozone (0.6 mg/m^3) in air and ozone in 11% CO_2 at 98% Equilibrium Relative Humidity slightly delayed surface growth of *Pseudomonas, Candida, Penicillium*, and *Thamnidium* on stored carcasses, but sub-surface growth in crevices was not inhibited. Optimal conditions for muscle stored at 0.3EC were obtained by applying a continuous ozone concentration of 0.6 mg/m^3.

Ozonated water (0.3 to 2.3 mg/L ozone at 35EC) rinse and hydrogen peroxide (5%, 35EC) rinse reduced surface microbial counts (*Salmonella, Listeria, E. coli*, and APC) about the same as hot (74 to 87.8EC) water rinse, i.e., on the order of 3 log CFU/cm^2, when applied for 12 seconds. When the first spray washing treatments of 16 or 35EC plain water were followed chemical solution interventions, the most effective sanitizing agents were hydrogen peroxide (5%) and

ozonated water (0.5 mg/L), achieving reductions in total plate counts of 2.60 to 2.87, and 2.72 to 2.86 CFU/cm^2, respectively.

TBA values were similar after storage for 28 days at 4EC for carcasses washed with water, acetic acids, ozone water, and peroxide water. The data indicated little or no consistent effect of the chemical interventions on development of oxidative by-products.

Petitioners Comment

Based on these six investigations, treatment of beef carcasses using high pressure (68 bar) spraying with hot (74EC) 5% hydrogen peroxide for 12 seconds followed by low pressure (20.68 bar) cool ozone water (0.5 mg/L at 16EC) for 12 seconds is likely to produce best surface count reduction and modest shelf life extension. The impact of microbial population selection needs further evaluation, particularly hot water (87.6EC) that will select for heat resistant survivors, such as spore formers, e.g., *Clostridium botulinum* in subsurface crevices. Regrowth of such survivors could increase the risk of pathogenic microorganisms in the final product.