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Acetic acid as an intervention strategy to decontaminate beef carcasses in mexican commercial slaughterhouse

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Abstract

Beef can be contaminated during the slaughter process, thus other methods, besides the traditional water washing, must be adopted to preserve meat safety. The objective of this study was to evaluate the effect of 2% acetic acid interventions on the reduction of indicator bacteria on beef carcasses at a commercial slaughterhouse in Mexico. Reduction was measured by the count of mesophilic aerobic bacteria (TPC), total coliform (TC), and fecal coliform (FC) (log CFU/ cm²). Among the different interventions tested, treatments combining acetic acid solution sprayed following carcass water washing had greater microbial reduction level. Acetic acid solution sprayed at low pressure and longer time (10-30 psi/ 60 s) reached higher TPC, TC, and FC reductions than that obtained under high pressure/ shorter time (1,700 psi/ 15 s; $P < 0.05$). Exposure time significantly affected microbial reduction on carcasses. Acetic acid solution sprayed after carcass washing can be successfully used to control sources of indicator bacteria on beef carcasses under commercial conditions.

Keywords: bovine carcass; acid treatment; microbial load.

1 Introduction

Beef may be the vehicle of foodborne diseases as a result of deficient sanitary conditions during animal slaughter (HERNÁNDEZ et al., 2007; LORETZ; STEPHAN; ZWEIFEL, 2011). The possibilities of eliminating pathogenic microorganisms from meat have received considerable attention in the last decades (SOFOS et al., 1999). Intervention strategies and their effects on microorganism levels have had an impact on industry economics and also on public health matters (BOLDER, 1997).

Bovine carcasses can be contaminated during the slaughter process through the contact with the animal's skin and hair, limbs, blood, stomach, gut contents, bile and other excretions, facilities, equipment, and hands and worker's clothes (RAHKIO; KORKEALA, 1997; SAMMARCO et al., 1997; SIRAGUSA et al., 1998; SMULDERS; GREER, 1998; DELAZARI et al., 1998; STOPFORTH et al., 2007; SOFOS, 2008). Carcass washing, chilling, storage (ESCUTIA, 1996; SIRAGUSA et al., 1998; McEVOY et al., 2004), and processing (BAIRD et al., 2006; KOOHARAIE et al., 2007) can also contribute to the reduction of the final microbial load on beef.

Only a small fraction of the microbial flora is eliminated by the carcass washing procedure commonly practiced at slaughterhouses (BOUTTIER et al., 1996; BOLTON; DOHERTY; SHERIDAN, 2001), thus the preservation of meat must be guaranteed by other methods to maintain its intrinsic quality and safety (FERNANDES et al., 1998).

Many chemical compounds have been shown to reduce bacteria populations. Ransom et al. (2003) reported that chemical compounds are able to reduce the incidence of pathogens and other bacteria counts upon beef carcasses or their cuts by 1 to 3 logs (TINNEY et al., 1997; RANSOM et al., 2003). Organic acids such as acetic, citric, and lactic acid are widely used in the U.S. and Canada for carcass decontamination and are included among the different strategies for carcass (GORMAN et al., 1995; TINNEY et al., 1997; CASTILLO et al., 1998; BACON et al., 2000; LORETZ; STEPHAN; ZWEIFEL, 2011) and meat decontamination under controlled conditions at the laboratory (DORSA; CUTTER; SIRAGUSA, 1998). It is known that acetic acid inhibits mainly yeasts and bacteria as *Bacillus* spp., *Clostridium* spp., *Pseudomonas* spp., *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* sp. and *Staphylococcus aureus* (DAVIDSON; TAYLOR, 2007); it also inhibits mesophilic enteric bacteria, which are more sensitive to organic acids than the pathogenic bacteria species (SOFOS et al., 1999). However, only little data on the effect of acid solutions in microbial reduction of carcasses at slaughter plants under commercial conditions is available (ALGINO; INGHAM; ZHU, 2007; LORETZ; STEPHAN; ZWEIFEL, 2011). Therefore, the aim of this study was to evaluate the reduction in bacterial counts on beef carcass surfaces using 2% acetic acid solution at different spraying pressure and time as a carcass decontamination strategy in a commercial slaughterhouse in Mexico.

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2 Materials and methods

2.1 Experimental design

Carcasses were randomly selected during the bovine slaughter process at a commercial type slaughterhouse in the State of Mexico, Mexico, and gathered into five groups for treatment application as described below. The samples were collected during a period of four different working days at the slaughterhouse, hence the number of carcasses in treatments varied according to the company's slaughter schedule on each day of sample collection. However, all the treatments were performed on the sampling days.

- Non-treated group: this group was composed of 28 carcasses that were sampled to establish the initial bacteria load. Carcasses were sampled immediately after the evisceration step;
- Treatment 1: this group was composed of 45 carcasses washed twice with water from the network system at 2,100 psi for 120-180 s. The samples were taken after the washing step commonly performed during the bovine slaughter process;
- Treatment 2: composed of 18 carcasses sprayed with a 2% acetic acid solution at 10-30 psi for 60 s, applied immediately after the evisceration step;
- Treatment 3: composed of 34 carcasses washed with water from the network system at 2,100 psi for 120-180 s and immediately sprayed with a 2% acetic acid solution at low pressure of 10-30 psi for 60 s;
- Treatment 4: composed of 25 carcasses washed with water from the network system at 2,100 psi for 120-180 s and immediately sprayed with a 2% acetic acid solution at high pressure of 1,700 psi for 15 s.

Each carcass in Treatments 2, 3, and 4 was sprayed with 1,000 mL of 2% acetic acid solution prepared from 99% glacial acetic acid (Sigma-Aldrich, MO, USA) at 22 ± 3 °C.

2.2 Sampling protocol

The sampling method was applied according to the European Union recommendations for bovine carcass sampling for microbiological analysis (EUROPEAN..., 2001). An area of 100 cm² of each half-carcass was sampled by thoroughly rubbing the surface of the brisket and flank zones with sterile

swabs. When different strategies were applied, the sampling was performed five to eight minutes after each treatment.

Immediately after sample collection, the swabs from the same carcass were pooled together in plastic bags containing 100 mL of 1% sterile peptone water at 2-4 °C and transported to the laboratory for the microbiological analysis within three hours after sampling (EUROPEAN..., 2005).

2.3 Microbiological methods

Counts of aerobic mesophilic (TPC), total coliform (TC), and fecal coliform (FC) were performed in the samples according to the NOM-092 and NOM-113/ SSA1-1994 of the Mexican Official Standards for total plate count and coliform count in food (MÉXICO, 1995a, b). The results were expressed as logarithmic colony forming units (log CFU) per cm².

2.4 Statistical analysis

Data were analyzed by comparison of means by analysis of variance through ANOVA using SPSS 10.0 software program for windows. When significant differences were found among the treatment means ($P < 0.05$), the Tukey's test was used to compare means.

3 Results and discussion

As shown in Table 1, non-treated carcasses showed contamination of aerobic mesophilic, total coliform, and fecal coliform at levels of 3.28 log CFU/cm², 1.93 log CFU/cm² and 1.80 log CFU/cm², respectively. All intervention treatments, Treatments 1 to 4, were effective to reduce total and fecal coliform counts on bovine carcasses in comparison to non-treated carcasses ($P < 0.05$). In fact, washing the carcasses caused a slight increase in TPC ($P < 0.05$) in relation to the non-treated carcasses. Despite the fact that water washing is routinely used in meat processing and proved to be effective in removing visible contaminants, soil, hair, or other debris (LORETZ; STEPHAN; ZWEIFEL, 2011), washing carcasses with cold and warm water not only showed hardly any reduction, but it also tended to spread bacteria on the carcass surface (BELL, 1997; JERICO; BRADLEY; KOZUB, 1995). Ariyapitipun, Azlin and Clarke (1999) compared different intervention strategies on top round beef and found that water washes did not significantly reduce mesophilic bacteria, *Enterobacteriaceae*, and even *Pseudomonas* count on the meat samples.

Table 1. Total plate count, total coliform, and fecal coliform counts on the bovine carcasses surface before and after treatments.

Treatments	Carcasses (n)	Total plate count (log CFU/ cm ²)	Total coliform (log CFU/ cm ²)	Fecal coliform (log CFU/ cm ²)
Non treated	28	3.28 ^b	1.93 ^a	1.80 ^a
Treatment 1	45	3.90 ^a	1.00 ^b	0.77 ^c
Treatment 2	18	3.40 ^{ab}	1.36 ^b	1.30 ^b
Treatment 3	34	2.48 ^c	0.39 ^c	0.40 ^d
Treatment 4	25	3.17 ^b	0.63 ^c	0.52 ^{cd}

Treat. 1 = Water washing; Treat. 2 = Acetic acid, 10-30 psi/ 60 s; Treat. 3 = Water washing + acetic acid, 10-30 psi/ 60 s; Treat. 4 = Water washing + acetic acid, 1700 psi/ 15 s. Means with different letters in the same column are significantly different ($P < 0.05$).

The acetic acid spraying of the carcasses after evisceration and before the washing step (Treatment 2) showed no differences ($P > 0.05$) in aerobic mesophilic and total coliform count from the simple washing treatment (Treatment 1).

On the other hand, evidence of the effectiveness of the combination of water washing plus 2% acetic acid spraying (Treatments 3 and 4) is shown in this study. Both treatments 3 and 4 significantly reduced ($P < 0.05$) the contamination of the carcasses by aerobic mesophilic bacteria and coliforms although T3 (lower pressure/ longer time) showed higher level of bacterial reduction than Treatment 4 ($P < 0.05$). The logarithmic reduction in total plate count, total coliform, and fecal coliform on beef carcasses in Treatment 3 were 0.80, 1.54, and 1.40 log CFU/ cm², respectively, whereas the logarithmic reduction for the same indicator bacteria in Treatment 4 were 0.11, 1.30, and 1.28 log CFU/ cm², respectively.

According to Hugas and Tsigarida (2008), acetic acid washes work better when used in combination with other interventions than alone. The use of high pressure (1,700 psi) to spray acetic acid on carcass surface might have spread some bacteria on them and hence led to lower bacterial reduction. Furthermore, it suggests that although acetic acid concentration and temperature were similar, the exposure time played an important role in the final results. On the other hand, Bacon et al. (2000) found that higher levels of microorganisms on some carcasses after spraying acetic acid can be attributed to different microbial content between animals, individual effects of removing guts, carcass washing, or another source of contamination at slaughterhouses.

Microbial reductions with acetic acid interventions on beef carcasses have been reported in other studies. Gorman et al. (1995) found average reductions in aerobic bacteria and *E. coli* of 2.0 log CFU/ cm² and 1.9 log CFU/ cm², respectively, on beef carcasses treated twice with acetic acid plus water washing at two different temperatures, first washing at 14 °C and second washing at 35 °C. Bacon et al. (2000) found a total plate count reduction of 5.5 log CFU/ cm² on samples obtained after the steaming of pre-eviscerated carcasses and subsequently washing plus spraying 1.5 % acetic acid at 20 °C. Delazari et al. (1998) found logarithmic reduction lower than 0.3 log UFC/ cm² in the number of *E. coli* O157:H7 after 1.5% acetic acid spraying at 20 °C on previously contaminated beef carcasses. Tinney et al. (1997) reported total plate count bacteria reduction of 0.20 log CFU/ cm² and coliforms reduction of 0.41 log CFU/ cm² after spraying 2% acetic acid solution on beef carcasses.

Despite these previous reports, the consensus findings on acid interventions are that topical application of 1 to 3% organic acids to meat surfaces produces less than 2 log reduction in

pathogen numbers (CARPENTER; SMITH; BROADBENT, 2011). Besides, most of the studies cited were conducted under controlled conditions and were not conducted in commercial facilities. The use of interventions at slaughter plants reduced the bacterial loads on carcasses to some extent, but the reductions were clearly lower than those obtained under laboratory conditions. Acetic acid or lactic acid treatment mainly yielded bacterial reductions below two orders of magnitude on carcasses (LORETZ; STEPHAN; ZWEIFEL, 2011).

Algino, Ingham and Zhu (2007) reported that under commercial conditions, 2.5 % acetic acid solution spraying at the end of slaughter reduced naturally occurring *E. coli* and total coliform on carcasses by 0.71 log CFU/ cm² and 0.81 CFU/ cm², respectively. The same authors also reported aerobic bacteria reduction higher than 0.5 log CFU/ cm² in 50.81% of the carcasses. In the present study, fecal coliform reduction on carcasses treated with 2% acetic acid and water washing (T3 and T4) ranged from 1.28 to 1.40 log CFU/ cm², total coliform ranged from 1.30 to 1.50, and aerobic bacteria from 0.11 to 0.80 log CFU/ cm².

A positive point of organic acid decontaminate interventions is that the antimicrobial action of acids goes beyond their spraying on carcasses. Their action after spraying may be of particular importance for the control of pathogens because rapid proliferation of pathogens can take place in decontaminated carcasses. Acid washing with acetic acid imparts residual inhibition of pathogens from a short-term bactericidal effect for about 2 days after washing. (CARPENTER; SMITH; BROADBENT, 2011).

According to the European criteria for beef carcass contamination (EUROPEAN..., 2005), *Enterobacteriaceae* count lower than 1.5 log CFU/ cm² and aerobic bacteria count lower than 3.5 log CFU/ cm² are considered satisfactory. All samples (100%) in the treatment interventions (T1, T2, T3 and T4) showed average coliform count (TC and FC) lower than that of the standard (Table 1). Regarding the count of aerobic bacteria, only the samples in the water washing treatment (T1) had higher average count than that of the microbiological standard.

Concerning the ranges of microbiological counts on beef carcasses treated with acetic acid plus water washing, 100% of sample in Treatment 3 and 92% of samples in Treatment 4 presented total coliform count lower than 2.0 log CFU/ cm² (Table 2). For fecal coliform, 100% of samples in Treatment 3 and 96% in Treatment 4 showed counts lower than 2.0 log CFU/ cm² (Table 3). For aerobic bacteria, 100% of samples in Treatment 3 and 72% in Treatment 4 presented total plate count lower than

Table 2. Frequency distribution for total coliform levels on beef carcasses before and after intervention treatments.

Intervention treatments	Carcasses (n)	Carcasses (%) with total coliform levels at indicated log CFU/ cm ²				
		Non detected	0.1-1.0	1.1-2.0	2.1-3.0	> 3.0
Non treated	28	0.00	21.42	32.14	25.00	21.42
Treatment 1	45	0.00	68.88	20.00	8.88	2.22
Treatment 2	18	0.00	50.00	38.88	0.00	11.11
Treatment 3	34	29.41	64.70	5.88	0.00	0.00
Treatment 4	25	56.00	12.00	24.00	8.00	0.00

Treat. 1 = Water washing; Treat. 2 = Acetic acid, 10-30 psi/ 60 s; Treat. 3 = Water washing + acetic acid, 10-30 psi/ 60 s; Treat. 4 = Water washing + acetic acid, 1700 psi/ 15 s.

Table 3. Frequency distribution for fecal coliform levels on beef carcasses before and after intervention treatments.

Intervention treatments	Carcasses (n)	Carcasses (%) with fecal coliform levels at indicated log CFU/ cm ²				
		Non detected	0.1-1.0	1.1-2.0	2.1-3.0	> 3.0
Non treated	28	0.00	21.42	42.85	25.00	16.66
Treatment 1	45	31.11	46.66	15.55	6.66	0.00
Treatment 2	18	0.00	50.00	33.33	11.11	5.55
Treatment 3	34	29.41	64.70	5.88	0.00	0.00
Treatment 4	25	52.00	20.00	24.00	4.00	0.00

Treat. 1 = Water washing; Treat. 2 = Acetic acid, 10-30 psi/ 60 s; Treat. 3 = Water washing + acetic acid, 10-30 psi/ 60 s; Treat. 4 = Water washing + acetic acid, 1700 psi/ 15 s.

Table 4. Frequency distribution for aerobic plate count levels on beef carcasses before and after intervention treatments.

Intervention treatments	Carcasses (n)	Carcasses (%) with aerobic bacteria levels at indicated log CFU/ cm ²				
		Non detected	0.1-2.0	2.1-3.0	3.1-4.0	> 4.0
Non treated	28	21.42	0.00	0.00	42.85	32.14
Treatment 1	45	0.00	0.00	0.00	86.66	13.33
Treatment 2	18	0.00	0.00	0.00	100.00	0.00
Treatment 3	34	0.00	20.58	64.70	14.70	0.00
Treatment 4	25	0.00	24.00	24.00	24.00	28.00

Treat. 1 = Water washing; Treat. 2 = Acetic acid, 10-30 psi/ 60 s; Treat. 3 = Water washing + acetic acid, 10-30 psi/ 60 s; Treat. 4 = Water washing + acetic acid, 1700 psi/ 15 s.

4.0 log CFU/ cm² (Table 4). However, none of the samples of both treatments showed aerobic count higher than 5.0 log CFU/ cm².

Enterobacteriaceae count ranging from 1.5 to 2.5 log CFU/ cm² and aerobic bacteria count ranging from 3.5 to 5.0 log CFU/ cm² are considered acceptable by the European microbiological criteria (EUROPEAN..., 2005).

4 Conclusions

The large bacterial reductions observed in the present study indicate that spraying a 2% acetic acid solution after carcass washing can be used successfully to reduce populations of indicator microorganisms and thus control a major source of pathogenic bacteria on beef carcasses under commercial conditions. Additionally, it is a cheap strategy, and it does not imply that the facility should be renovated.

It is important to mention that Good Manufacturing Practices at slaughterhouses must be followed and decontamination treatments always must be considered part of an integral food safety system (LORETZ; STEPHAN; ZWEIFEL, 2011), and they should not be used to hide possible malpractice.

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