

Study On Effect Of Acetic Acid Spray On Escherichia Coli Load And Meat Ph At An Export Abattoir, Modjo, Ethiopia.

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ABSTRACT

The study was conducted to determine the effect of 2.5% acetic acid spray on *E. coli* load in goat carcasses slaughtered in an export abattoir in Modjo, Ethiopia. A total of 144 sample's swabs were taken from 24 carcasses. 48 swabs were from front leg and hind leg areas before acetic acid spray, immediately after acetic acid spray and after 24 hrs of chilling at 2±1°C. Following incubation of on media at 37 °C for 48 hrs, *E. coli* CFU/cm² was counted. The log mean of *E. coli* count before acetic acids spray, immediately after spray and after chilling were 2.53Log₁₀ CUF/cm², 1.35Log₁₀ CUF/cm² and 1.97Log₁₀ CUF/cm², respectively. The number of *E. coli* counts before acetic acid spray was higher in samples from front leg than hind leg area. The means of *E. coli* counts before and after acetic acid spray showed significant difference. Relatively lower pH were measured in sprayed chilled

carcasses (with mean pH=5.77) than non-sprayed chilled carcasses (Mean pH=5.98). Therefore, the authors recommend that 2.5 % acetic acid spray with appropriate sanitation procedures, implementation of food safety management systems/hazard analysis and critical control points can reduce *E.coli* load, lowers the pH and improve the darkness of carcasses.

INTRODUCTION

Gradual increase in world population and change in lifestyles has resulted in demands for quality oriented foods of animal origin. Meanwhile, the number of incidences of food poisoning cases is increasing throughout the world (Goksoy et al., 2000). The percentage of the population suffering from food borne diseases each year has been reported to be up to 30%. It is estimated that 76 million illnesses with 325,000 hospitalizations and 5,000 deaths occur each year in the US (Scoti and Stevenson, 2006). According to a recent estimation, food-borne illnesses cost the U.S. \$152 billion in health-

related expenses each year. Worldwide, *Campylobacter*, *Salmonella* and Shigatoxin-producing *E. coli* (STEC) are among the most important bacterial food-borne pathogens (Loretz, 2010).

Food animals are naturally contaminated with a variety of potential pathogens. Meat processors have been applying many microbiological control methods during the slaughter and processing of the meat. Even if the existing approaches to food safety management system has given safe food supply in some countries, estimates of the morbidity due to food borne illness clearly showed that the existing approaches still inadequate. Thus, reducing the primal surface contamination and avoiding or limiting the microbial growth helps to extend the shelf life of meat. Several intervention strategies have been developed to reduce the level of bacteria on carcass surfaces such as washing and sanitizing with chilled water, hot water, chlorinated water, food grade acids and salts, alone and in combination. Topical spray washes with lactic or acetic acid solutions are widely employed in the meat industry as a post harvest intervention to reduce meat bacterial load. Organic acids are legally allowed as a surface (including meat) decontaminant in the USA; the US Department of Agriculture permits the use of lactic acid for pre-evisceration rinsing of carcasses (Smulders, 1987).

The color of fresh meat during retail display is of prime importance in consumer acceptability and it is claimed that the problem of early darkening of carcasses of highland animals exists for both sheep and goats, without a noticeable difference in magnitude (Abebe et al., 2010). Recently, customers claimed the problem of early darkening of carcasses of highland sheep and goats. To avoid such problems some export abattoirs came to a decision to use acetic acid as treatment and request the Ministry of Agriculture for an approval. Starting from 2011, Ministry of Agriculture of Ethiopia has approved abattoirs to use acetic acid spray on carcasses. So, some export abattoirs in Ethiopia are

using 2%-3% acetic acid with the intention of improving early darkening of the carcass. However, the effect of acetic acid spray has not been assessed in none of the export abattoirs. Therefore, this study was conducted with the objectives of determining the effect of 2.5 % acetic acid spray on *E. coli* load, the combined effect of acetic acid spray and chilling on *E. coli* load and the effect of acetic acids spray on pH and color of goat carcass.

MATERIALS AND METHODS

Study area and animals

The study was conducted in an export abattoir located in Modjo town, Ethiopia, from October 2011 to April 2012. The abattoir slaughters 500-1500 sheep and goats daily depending on the demand from customers, availability of supply of animals and air cargo space. The study goats are Ethiopian indigenous goat types sourced from lowland and mid highland areas of Ethiopia including Borena, Awash, Metahara, Arbaminch, Jinka, Miesso, Bable, Bati (Wollo). These goats were kept under traditional extensive management condition and these goats were apparently healthy at the time of slaughter.

Study Design

A total of 24 goat carcasses were selected randomly from a standard commercial slaughtering procedure. A total of 144 swabbed samples were taken from each carcasses' front and hind legs before acetic acid spray, after 30 minutes of acetic acid spray and after chilling 24 hrs at $2\pm 1^{\circ}\text{C}$ of the sprayed carcass. 2.5% acetic acid solution spray was done for 10 seconds using low-pressure hand held sprayers. Swabbing at the time of sampling was done at the area of 50 cm² that are delineated by sterile aluminum template (10 mm X 5mm). The pH of the carcasses was determined with a hand HANNA pH meter and repeated twice: 15 min after dressing before spraying with 2.5% acetic acid solution and just after chilling the carcass at $2\pm 1^{\circ}\text{C}$ for 24 hours. Carcass pH measurements were done for non-sprayed non-chilled carcass, non-sprayed chilled carcass and sprayed chilled

carcass. After the treatment, carcass color changes were monitored subjectively after 24 hrs chilling at $2\pm 1^{\circ}\text{C}$.

Bacteriological sample processing

A swab was first soaked in 10 ml of peptone water in a test tube and rubbed first horizontally and then vertically several times on the sampling site within the metal template. The swab was then put into sterile test tube filled with 10 ml of 0.1% sterile peptone water and transported using an insulated ice box at 4°C . For culturing, after thorough agitation of the sample, starting from the higher concentration rate, 1 ml was poured to sterilized petridishes previously filled with 15 ml sterilized MacConkey agar. Mixing was done by moving the petridishes in a circular motion and was left on a table until solidified. The inoculated media were incubated at 37°C for 48 h according to Quinn et al.,(2002). Preparation of decimal dilutions, identifications and enumerations of *E. coli* was done following the method described in Feng et al. (2002) and HPB (Health Product and Food Branches) Methods (2001). Indole, Methylred, Voges-proskauer reaction and Citrate utilization tests (IMVICTest) were done on presumptive *E. coli* subcultured on Broth agar to differentiate of *E. coli*, from other related species. Interpretation of biochemical test results was accomplished according to (Peng et al., 2001; Prescott, 2002).

Determining E coli load

Following the IMVIC test to confirm the grown colony, the total count of *E. coli* was determined according to US Bacteriological Analytical Manual (Feng et al., 2001;

ISO 17604:2005). After 48 hrs of incubation, colonies were counted visually. The numbers of colonies grown and counted were then multiplied by the level of dilution. Finally; the total number of *E. coli* per ml of sample culture was obtained. *E. coli* counts were converted to \log_{10} CFU/cm² before data analysis in order to normalize the data.

Data were encoded into Microsoft Excel. The database was transferred to SPSS 15.0 for windows version. After normalizing the data by using the \log_{10} , and descriptive statistics. The means of sample specific *E. coli* counts were compared using t-test at 95% confidence interval.

In conclusion, spraying of goat carcasses with 2.5% acetic acid significantly reduced *E. coli* count. This indicates the effectiveness of acetic acid (2.5%) as decontaminant. In addition, acetic acid spray spraying of goat carcass with acetic acid reduces the pH and darkness. Therefore, meat export abattoirs can improve the safety and the quality of meat and meat by products using such organic acids. All Export abattoirs should implement Food Safety Management System/Hazard Analysis and Critical control Points incorporating acetic acid spray as a safe microbial decontaminant. The decontaminant effect of acetic acid and other organic acids with various concentrations, temperature, chilling duration and other factors affecting its efficacy should be further validated.

RESULTS

Mean of total E. coli

The log mean of *E. coli* count before acetic

Table 1: Total *E.coli* count for different treatments.

Sample Type	Sample size	Mean Log10CFU/cm ²	95% CI for mean Log10CFU/cm ²	SD	Min	Max
TECBS	48	2.53	2.15-2.91	1.31	0.00	4.38
TEAS	48	1.35	1.04-1.65	1.06	0.00	3.12
TECASC	48	1.97	1.64-2.30	1.12	-0.04	3.87

TECBS= Total *E. coli* count before spray, TECAS =Total *E. coli* after spray, TECASC= Total *E. coli* after spray and chilling.

Table 2: *E.coli* count for different treatments of front leg and hind leg.

Type of sample	No of sample	Mean log ₁₀ CFU/cm ²	SD	Min.	Max.
ECBSF	24	2.84	0.87	1.97	3.71
ECBSH	24	2.25	1.60	0.53	3.82
ECASF	24	1.57	1.00	0.57	2.57
ECASH	24	1.13	1.10	0.03	2.33
ECASCF	24	2.06	1.04	1.03	3.09
ECASCH	24	1.89	1.20	1.69	3.09

ECBSF= *E. coli* count before spray of front leg area, ECBSH= *E. coli* count before spray hind leg area, ECASF= *E. coli* count after spray front leg area, ECASH= *E. coli* count after spray hind leg area, ECASCF= *E. coli* count after spray and chilling Front leg area, ECASCH=*E. coli* count after spray and chilling hind leg area.

acids (2.5%) spray, 30 minutes after application of acetic acids and after 24 hrs chilling at 2±1°C were 2.53 Log₁₀ CFU/cm², 1.35 Log₁₀ CFU/cm² and 1.97Log₁₀ CFU/cm² respectively. The respective maximum values were 4.38 log₁₀ CFU/cm², 3.12 Log₁₀ CFU/cm² and 3.87Log₁₀ CFU/cm² (Table 1). The log mean of *E. coli* count before acetic acid spray for samples from front leg and hind leg areas were 2.84Log₁₀CFU/cm² and 2.25Log₁₀CFU/cm² respectively (Table 2).

Comparison of the means of E.coli count before and after acetic acid spray

Paired t-test statistical analysis for mean of *E. coli* counts before and after acetic acid spray and between the two sampling sites showed significant difference (p<0.05) (Table 3).

The combined effect of acetic acid spray and chilling on Escherichia coli load

The log₁₀CFU/cm² mean of *E. coli* count of carcasses sprayed acetic acid and chilled at

2±1°C were high compared with the log₁₀C-FU/cm² mean of carcasses immediately after spray. The paired t-test on the difference in *E. coli* count between the two treatments were statistically significant (p<0.05) (Table 3).

Effect of acetic acid spray on pH and colour

The mean pH value of goat carcasses at 15 minutes after slaughter was found to be 6.38. Relatively lower pH were obtained in sprayed chilled carcasses than in non-sprayed chilled carcasses (Table 4). The mean pH of non-sprayed chilled carcass and sprayed chilled carcasses were significantly different (p<0.05). Non-sprayed chilled and sprayed chilled carcasses were visually and subjectively monitored for color changes. According to subjective observation, sprayed goat carcasses after chilling showed less darkness.

Table 3: Paired comparison of the mean difference of in total *E. coli* count before and after acetic acid spray.

Type of sample	Sample size	*Mean of difference	SD	95%.CI for the mean	t-test	P-value
ECBS	48	2.53	1.31	2.15-2.91	13.39	0.00
ECAS	48	1.35	1.07	1.04-1.66	8.77	0.00
ECASC	48	1.97	1.12	1.65-2.30	12.19	0.00

*=Log₁₀CFU/cm² value , ECBS = *E. coli* count before spray, ECAS= *E. coli* count after spray and ECASC = *E. coli* count after spray and chilling, SD= Standard deviation of the mean difference, CI= Confidence level of the mean difference.

Table 4. Paired comparison of the mean of pH value before and after acetic acid spray after chilling.

Type of sample	Sample size	Mean of difference	SD	95% CI for the mean	t-test	P value
PHNSNC	24	6.38	0.40	6.20-6.56	73.78	
PHNSCC	24	5.97	0.10	5.98 -6.04	306.60	0.23
PHSCC	24	5.77	0.00	5.73-5.81	196.00	0.01

PHNSNC = pH for non-sprayed & non-chilled carcass, PHNSCC = pH for non-sprayed chilled carcass, PHSCC = pH for sprayed chilled carcasses, SD = Standard deviation, CI = Confidence level of the mean difference.

DISCUSSION

In the current study, spraying of goat carcasses with 2.5% acetic acid significantly reduced total *E. coli* count by 1.18 log₁₀ CFU/cm² compared with carcasses before spray with acid. High total *E. coli* count indicates poor hygienic practice in the slaughter house as it is indicator of fecal contamination. The goat carcass *E. coli* count found in this study (2.2 Log₁₀ CFU/cm² -2.9 Log₁₀ CFU/cm²) was comparable with previous work done in Ethiopia reported by Mengistu (2007) as mean value *E. coli* count ranging from 2.4 Log₁₀ CFU/cm² -2.9 Log₁₀ CFU/cm² at different abattoir. Similarly, Assegid (2008) reported *E. coli* mean value ranging from 1.7 Log₁₀ CFU/cm² - 2.8 Log₁₀ CFU/cm².

The number of *E. coli* count before acetic acid spray on front leg area was high as the Log₁₀ CFU/cm² mean of *E. coli* count before 2.5% acetic acid spray from samples of front leg area and hind leg area were 2.8 and 2.3 Log₁₀ CFU/cm² respectively. The differences in *E. coli* count between these two sampling sites were statistically significant (p<0.05). The lower part of the goat carcass (front leg area) is more exposed for various contaminants as water used to wash fecal materials is spilled up from the floor. The rump region of the carcass was the most contaminated area with fecal organisms than the other sites, usually associated with the skinning process and the presence of more fecal and dirt matter prior to slaughter (Gill et al., 1996). Contamination of carcass may be occurred from the gut, skin, equipment, personnel and splashes of water from the floor during cleaning and slaughtering pro-

cess (McEvoy et al., 2003; Assegid, 2008).

Relatively low number of total *E. coli* count were obtained from carcasses sprayed with acetic acids (mean 1.35 Log₁₀ CFU/cm²) than carcasses before sprayed (mean 2.53 Log₁₀ CFU/cm²). Similarly, it is indicated that decontamination with organic acid solution reduces the number and prevalence of food borne pathogens and microbial load of meat (Huftman, 2002). The reduction in total *E. coli* count in acetic acids spray carcasses indicates the effectiveness of acetic acid spray as decontaminant.

A variety of organic acids applied as a spray or dips for decontamination purpose have been studied extensively and appear to constitute an effective bactericidal or bacteriostatic surface treatment which also effectively prevents the attachment microorganisms (Hardin et al., 1995; Bolder, 1997; Pipek et al., 200). Moreover, this study demonstrated the effectiveness of lactic acid (2%) on sheep carcass by spraying after 30 minutes of acetic acid application and after 24 hrs chilling at 2+10C showed a 2.06 Log₁₀ CFU/cm² *E. coli* reduction. The antimicrobial effect of the organic acids is due to reduction of pH below the growth range and metabolic inhibition by the undissociated molecules (Beyaz and Tayar, 2010). Acetic acid has been shown to be effective against *E. coli* O157:H7 by reducing the pathogen by 0.1 Log₁₀ CFU/cm² to 4.67 Log₁₀ CFU/cm² (Joseph et al., 2006) and 2% acetic acid reduce load of *E. coli* by 1.6 Log (Ransom et al., 2003). Thus, bacterial load reduction after acid washing has been suggested to result from several factors, including the

immediate decontamination of bacteria from meat surfaces; the bactericidal combination of acid concentration and application temperature and from residual inhibitory effect that may initially be bactericidal due to lowered pH on the meat surface for a short time following an acid wash (Carpenter et al., 2011).

In this study, *E.coli* count was found to be higher in goat carcasses before spray with 2.5% acetic acid, followed by chilling (combined effect of acetic acid spray and chilling) compared with the *E.coli* count of carcasses that have been sprayed with 2.5% acetic acid before chilling. Compared with the immediate effect of acetic acid spray on *E.coli* count, the antibacterial activity of the combined effect of spraying and chilling was low. This increase in *E.Coli* was statistically significant ($P<0.05$). This increase may be due to cross contamination from workers hand, facilities and apron during chilling. According to Spescha et al. (2006) the antibacterial activity of air chilling on red meat carcasses is mainly based on the surface desiccation achieved by high air velocity. However, chilling of beef carcass may increase, decrease or no changes in microbial contamination depending on temperature, air speed, humidity, carcass spacing and duration (Arthur et al., 2004; Corantin et al., 2005; Kinsella et al., 2006).

The mean pH value of goat carcasses at 15 minutes after slaughter in this study was 6.38. Relatively lower pH was obtained in acetic acid sprayed chilled carcass (mean pH= 5.77) compared with non-sprayed chilled carcass (mean pH= 5.98). Twenty four hours after slaughter the pH value of the meat gradually decreased from 7.0 to between 5.0 and 6.0 due muscle cells disintegration. Abebe et al. (2010) reported that the pH of goat carcass at 15 minutes and 24 hrs post slaughter chilling were recorded 6.54 and 5.83 respectively. This implies that spray of acetic acid and chilling are capable of reducing the pH of the carcass surface by remarkable magnitude, making it difficult for microbes to survive.

Goat carcasses that were chilled after spray acetic acid showed less darkness than non sprayed chilled carcass after 24 hrs. Darkening of carcasses of highland animals exists for both sheep and goats, without a noticeable difference in magnitude (Abebe et al., 2010). According to Stivarius et al. (2001), the main cause of fresh meat discoloration is accumulation of metamyoglobin at the lean surface. This metamyoglobin production is high at pH values above 5.8 but acetic acid spray in this study drops the mean pH which may decrease formation of metamyoglobin and decrease discoloration. Acceptable concentration of Organic acids like acetic acids are approved for use, as several intervention strategies have been developed by food industries to reduce the level of bacteria on food including meat such as washing and sanitizing with chilled, hot and chlorinated water, food grade acids and salts, alone and in combination.

Acceptable concentration of Organic acids like acetic acids are approved for use, as several intervention strategies have been developed by food industries to reduce the level of bacteria on food including meat such as washing and sanitizing with chilled, hot and chlorinated water, food grade acids and salts, alone and in combination. The authors recommend that 2.5 % acetic acid spray with appropriate sanitation procedures, implementation of food safety management systems/hazard analysis and critical control points can reduce *E.coli* load, lowers the pH and improve the darkness of carcasses.

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