Effect of Lactic and Acetic Acid Spray on the Microbiological Quality and pH of Goat Carcass

By

Biruk Getachew

A Thesis Submitted to Centre for Food Science and Nutrition, College of Natural Sciences in Partial Fulfilment of the Requirement for the Degree of Masters of Science in Food Science and Nutrition.

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DECLARATION

I, the under signed, declare that this thesis is my original work and that all the sources of materials used for the thesis have been correctly acknowledged.

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List of Acronyms and Abbreviations

ANOVA: Analysis of Variance

cfu/ cm^2 : Colony Forming Units per Square Centimetre

CSA: Central Statistics Agency

^0C : Degree centigrade

E. coli: Escherichia coli

EMDTI: Ethiopian Meat and Dairy Technology Institute

ES: Ethiopian Standard

FAO: Food and Agricultural Organization

g: gram

HACCP: Hazard Analysis Critical Control Point

hr(s): hour(s)

ISO: International organization for standards

Kg: Kilogram

Km: Kilometre

L. monocytogenes: Listeria monocytogenes

Log: logarithm

LS: Lauryl sulphate

LSD: Least Significant Difference

$\overline{x}$: mean

min: minute

ml: millilitre

mm: millimetre

MPN: Most Probable Number

MRD: Maximum Recovery Dilution

SEM: Standard Error Mean

SD: Standard Deviation

TPC: Total Plate Count

USDA- FSIS: United States Department of Agriculture Food Safety and Inspection System

(v/v) : Volume by Volume

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First I thank God for everything, my sincere gratitude go to my advisors Negussie Retta (Professor) and Abiy Zegeye (Dr.) for their guidance and encouragement throughout my research.

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Abstract

Experiments were performed to investigate the effects of 2% lactic acid, 2.5% acetic acid and 1% lactic acid on *E.coli,* Total Plate Count and pH change of goat carcasses slaughtered in MJ export abattoir located in Modjo town. The effects of the organic acids before spray, 30min after spray and 24hrs after sprayed- chilled carcass were determined. Eighty-four carcasses were used for *E.coli* and Total Plate Count. Swab Samples were collected from carcass surface area of $100cm^2$ at four locations (brisket, lateral thorax, flank and midloin). While For pH measurements forty- eight carcasses were used and a total of ninety- six samples were taken at 30 minute and 24hours at the four locations. Application of 2 % lactic acid, 2.5% acetic acid and 1% lactic acid improved carcass pH and resulted in a significant reduction in *E.coli* count and TPC of goat carcass for export as compared to untreated goat carcass. By considering the overall performance of 2% lactic acid, 2.5% acetic acid and 1% lactic acid it was concluded that application of 2% lactic acid would improve microbial and pH quality of goat carcass.

Keywords: Acetic Acid, *E.coli*, Lactic Acid, pH, Total Plate Count

1. Introduction

1.1. Background

Livestock is an important economic resource and an essential source of livelihood for approximately 80 percent of Ethiopia's rural population. Ethiopia has an estimated national herd of 53.99 million heads of cattle, 25.5 million sheep and a goat population estimated at 24.06 million (CSA, 2013).

In live goat, the muscle meat is virtually sterile. However, other parts of the animal such as skin, hooves and intestine contain enormous numbers of bacteria. Depending on the slaughter hygiene, these bacteria find their way to contaminate the carcass. Most bacteria reach the carcass via human contact, tools, contact with equipment or through water and air. Therefore, the focus of meat plant internal hygiene measure is mainly on bacteria (Gunter and Peter, 2007).

Microbiological sampling and testing of carcasses has been introduced to verify that procedures based on Hazard Analysis and Critical Control Point (HACCP) function correctly in slaughter houses. Microbiological analysis of carcass surfaces has become an important source of information in developing and implementing HACCP systems for slaughtering and dressing operations (Capita *et al.*, 2004).

Organic acids have been used in the meat industry as a topical treatment for reduction of pathogenic microorganisms. United States Department of Agriculture Food Safety Inspection System USDA-FSIS Directive 7120.1 regarding safe and suitable ingredients used in the production of meat and poultry products states organic acid concentrations up to 2.5% (v/v) are approved for pre-chill carcass washes, with up to 5% (v/v) lactic acid approved for spray application to carcasses prior to fabrication (FSIS, 2010).

The potential use of organic acids as shelf life extenders and antimicrobial action both immediate decontamination effects and inhibitory effects over time has been widely studied, focusing above all on the inhibition of pathogens such as *Listeria monocytogenes, E. coli and Salmonella* (Koohmaraie *et al.,* 2005; Devrin and Mustafa, 2010; Winkler and Harris, 2009).

Organic acids like lactic and acetic have been reported to decrease populations of *E. coli* when sprayed on sheep carcass or used as a wash (Dubal *et al.,* 2004). The application of 2 % of lactic acid to the sheep carcass immediately after slaughter would increase shelf-life, reduce the pathogenic microorganisms and therefore will aid public health (Devrin and Mustafa, 2010). While 2.5 % acetic acid spray with appropriate sanitation procedures, lowers the pH and improve the darkness of carcasses (Amsalu *et al.,* 2013).

1.2. Statement of the Problem

Ethiopian meat export is mainly in form of chilled goat/sheep carcass and offal's. Out of this around 90% is from goat (EMDTI, 2012). Under Ethiopian meat export abattoirs condition, despite the application of acetic acid, other potential organic acids like lactic acid were not well studied. Currently some export abattoirs are using 2.5% acetic acid for carcass wash but still there are complaints from customers on the microbial quality and darkness of carcasses exported.

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 (Amsalu *et al.*,2013).

Specifically, decontamination effects of lactic acid on goat carcass under Ethiopian export abattoirs condition were not studied. So far no comparative studies were conducted for implementation of HACCP in the export abattoirs on the efficacy of lactic acid spray in

reduction of microbial load against the performance of acetic acid on export goat carcass. Therefore, it is imperative to research and observe its effect on microbial load and pH change of export goat carcass.

Alternative to the current application rate of 2.5 % acetic acid by Ethiopian export abattoirs, no research findings were provided an empirical data to answer which organic acid (lactic or acetic) perform better on goat carcass under the Ethiopian export abattoir working condition.

This study, therefore, was designed to provide information in relation to microbial quality and pH changes on goat carcass by comparing the traditional application of 2.5% acetic acid against two different concentrations of lactic acid (1% and 2%).

1.3. Objective

1.3.1. General Objective

- To determine the extent of microbial quality and pH changes of goat carcass with spray of 2.5% acetic acid, 2% lactic acid and 1% lactic acid.

1.3.2. Specific Objectives

- To investigate the extent of goat carcass decontamination with spray of different level of acetic acid and lactic acid on TPC and *E. coli*

- To examine the extent of goat carcass pH change at different carcass locations with spray of different level of acetic acid and lactic acid on TPC and *E. coli*

2. Literature Review

2.1. Microbial Quality of Goat Carcass

Microbiologic testing would allow individual plants to benchmark their hygienic standard and evaluate the efficacy antimicrobial interventions (Terrance *et al.,* 2004). Microbiological risk assessment can be considered as a tool that can be used in the management of the risks posed by food borne pathogens, including the elaboration of standards for food in international trade (WHO, 2009).

The presence of bacteria in meat should receive particular attention, because their presence indicates public health hazard (Mboto *et al.,* 2012). Total plat count is a good indicator for the overall bacterial load of meat and meat products. Critical hygienic dimensions are reached when the total number of bacteria on fresh carcass is between 10000 and 100000 cfu per gram. In slaughtering and handling process the principle is to keep bacterial counts as low as possible thorough adequate hygienic measures. Total plat count numbers exceeding 100,000 cfu per gram (10^5 per cm^2) are not acceptable and alarm signals that meat hygiene along the slaughter and handling chain must be urgently improved (Gunter and Peter, 2007).

Table 1. Recommended Microbiological Criteria for Goat Carcass

Microbial analysis	Microbiological condition		
	Good microbiological standard	Critical microbiological condition	Not Acceptable
Total plate count per cm^2	$<10^4$	$>10^4$ and $<10^5$	$>10^5$
Enterobacteriaceae per cm^2	$<10^2$	$>10^2$ and $<10^3$	$>10^3$

Source: (Gunter and Peter, 2007)

Total plat count does not give total picture on the nature of the microorganism whether the bacteria are harmful or harmless. Therefore, practicable microbiological standards should be there, in addition to the Total plat count and always include the number of hygienically sensitive microorganisms, which can be used as an indicator for specific hygienic risks. This can be done using selective bacteria culture media. Hence, indicator bacteria most commonly used are the group of *Enterobacteriaceae* (James *et al.*, 2005).

Escherichia coli, also known as *E. coli*, is used as the preferred indicator to detect and measure contamination in the assessment of food safety. *E.coli* constitute about one percent of the normal gut microbial population (FAO, 2012).

There are many vectors that can be used to transfer *E. coli* into meat products. The feces of the animal can be transferred on the hides and carcass, the equipment can be contaminated, personnel might not use proper hygienic practices, airborne contamination, and rodents, insects, and other animals are all potential sources (Bonardi *et al.*, 2001).

Some strains can be an opportunistic pathogen causing a number of infections such as Gram-negative sepsis, urinary tract infections, pneumonia in immune suppressed patients, and meningitis in neonates. Its common occurrence and survival characteristics led to the adoption of *E.coli* as an indicator of faecal contamination. This usage has been transferred to foods where greater circumspection is required in interpreting the significance of positive results (Adams and Moss, 2005).

Serologically over 200 O serotype strains of *Escherichia* have been recognized because the flagellar proteins are less heterogeneous than the carbohydrate side chains that make up the O groups, considerably fewer H antigenic types exist. Based on disease syndromes, characteristics and effect on certain cell cultures serological groupings five virulence groups of *E.coli*: *Enteroaggregative, Enterohemorrhagic, Enteroinvasive, Enteropathogenic* and *Enterotoxigenic* are recognized (James *et al.*, 2005).

For enumeration of *Escherichia coli* in foods, food ingredients and water the Most Probable Number (MPN) method is applicable. The procedure involves a multiple tube fermentation technique where three or more decimal dilutions of the sample are inoculated into tubes of broth medium and incubated at a specific temperature and for a specific time. The method is progressive and based on the number of tubes indicating the presence or absence of *E.coli* can be estimated from a standard statistical MPN table. The method has been shown to produce satisfactory results with naturally-contaminated foods and water for the detection of *E. coli* (Ciira, 2003).

Factors contributing to the persistence of *E. coli* in food systems include inadequate control of processing parameters. Processed food can be contaminated by raw materials, unsanitary water treatment and handling, as well as by cross-contamination. The bacteria can continue to grow in food, unless relevant process parameters are controlled. Examples of contaminated foods with *E.coli* include: raw/under-processed meat, unpasteurised dairy products, unpasteurised fruit juices and raw vegetables. Effective prevention and control of contamination in abattoirs requires the application of good hygiene practices, the application of Hazard Analysis and Critical Control Point (HACCP)-based management practices and risk-based meat inspection system (FAO, 2012).

2.2. Changes of pH in Goat Carcass

Upon slaughter of a well-rested meat animal, the usual 1% glycogen is converted to lactic acid, which directly causes a lowering in pH. Some foods are characterized by inherent acidity; others owe their acidity or pH to the actions of certain microorganisms. Regardless of the source of acidity, the effect on keeping quality appears to be the same (James *et al.*, 2005).

A key determinant of meat quality is pH. The ultimate pH is determined at 24 hours post-slaughter whole carcass, using a pH meter inserted in deep muscle. Good quality meat usually has a pH of 5.4–5.8. Immediately post-mortem, a small amount of muscle specific carbohydrate, glycogen is broken down to lactic acid within the first 12 hours. This biochemical process serves as an important function in establishing acidity in meat (Gunter and Peter, 2007). pH value determines environmental microbial balance. Low pH has a bacteriostatic effect on pathogzenic microorganisms. Accordingly, meats with pH values above 6 are generally considered as unsuitable for storage because of the favourable development of proteolytic microorganisms. The muscle of a live animal has a pH of 7.1 (Ameha, 2008).

2.3. Organic Acids and pH

2.3.1. Lactic Acid

Lactic acid, also known as milk acid is a carboxylic acid with the chemical formula $C_3H_6O_3$. It has a hydroxyl group adjacent to the carboxyl group. In solution, it can lose a proton from the carboxyl group, producing the lactate ion $CH_3CH\ (OH)\ COO^-$. As shown in table 2 compared to acetic acid, its pKa is 1 unit less, meaning lactic acid deprotonates ten times as easily as acetic acid does. In industry, lactic acid fermentation is performed by lactic acid

bacteria such as (*Lactobacillus, Leuconostoc, Lactococcus* and others) which convert glucose and sucrose to lactic acid (Bibek, 2004; Mohamed *et al.,* 2008).

Lactic acid is often used for surface decontamination as it is a natural component of meat produced during post-mortal glycolysis and thus it is not considered as typical additive (Peter *et al.,* 2004).

2.3.2. Acetic Acid

The preservative activity of vinegar has been known from ancient times. The acid has a two-fold importance: as a preservative and as a seasoning agent (enhance flavor). It is more active against yeasts and bacteria than against moulds. Acetic acid is an organic compound and simplest carboxylic acid with the chemical formula, CH_3COOH usually sold as glacial (95% acetic acid). The concentrated form is corrosive to the skin and lungs, but the typical dilution (5%) is considered non-toxic and non-irritating. Acetic acid is typically applied by spraying, misting or immersing an item in a diluted solution. It is a colourless liquid and the main component of vinegar and has a distinctive sour taste and pungent smell. Acetic acid is generally bacteriostatic at 0.2% but bactericidal above 0.3%, and more effective against gram-negative bacteria, this effect is pH dependent. It is considered as a food additive and approved for usage by many countries (Belitz *et al.,* 2009; Bibek 2004).

The 2% Acetic acid solution sprayed after carcass washing can be successfully used to control sources of indicator bacteria on beef carcass under commercial conditions (Carranza *et al.,* 2013). Acetic acid can significantly decrease the survival time for the *E. coli* strains (Breidt *et al.,* 2004).

2.3.3. How pH Affects Microorganisms

Microorganisms that are important in food spoilage tend to maintain an internal cytoplasmic pH of 6.5 to 7.0 in acidophiles and 7.5 to 8.0 in neutrophiles. The internal pH is tightly regulated. For nutrient transport and energy synthesis, the microorganisms also maintain a transmembrane pH gradient. When lactic and acetic acid are added to the environment (carcasses), depending on i) pH of the food, ii) pKa of the acid and iii) temperature, some of the organic acid molecules dissociate whereas others remain undissociated as shown in table 2. At the pH of most foods (pH 5 to 8), the organic acid molecules remain generally dissociated; as a result, hydrogen ion concentration [H+] in the environment increases, which interferes with the transmembrane proton gradient of microbial cells. To overcome this, the cells transport protons through the proton pump, which causes depletion in energy and a decrease in cytoplasmic pH. The structures on the cell surface, outer membrane or cell wall, inner membrane or cytoplasmic membrane, and periplasmic space are also exposed to hydrogen ion concentration [H+]. This can adversely affect the ionic bonds of the macromolecules and thus interfere with the nutrient transport and energy generation, and in turn interfere with microbial growth (Bibek, 2004; James *et al.*, 2005).

Table 2. Influence of pH on the Amount (%) of Dissociated Ions of Acetic and Lactic acids

Acid type	Acid pKa	% Dissociated at pH		
		4	5	6
Acetic	4.8	15.5	65.1	94.9
Lactic	3.8	60.8	93.9	99.3

Source: (Bibek, 2004)

2.4. Antimicrobial Effect of Lactic and Acetic Acid on Meat Surface

Organic acids can be used to improve the microbiological quality (Wan, 2007). When using organic acids as decontaminating agent it is very important to specify that initial bacterial load of carcases, the decontamination technique applied and the characteristics of acids used

are essential parameters to be taken into account in the process of reducing microorganism (Mehmet *et al.,* 2012). Organic acids have a clear and significant benefit against acid-intolerant species such as *E.coli*, *Salmonella* and others (Dibner and Buttin, 2002).

The microbial quality and safety of meat products that reach to consumers depends on extent of exposure to contamination, access of microorganisms to products (live animal, carcasses, fresh meat) and decontamination methods of product (Koutsoumanis *et al.*,2006).

In a recent study goat carcass decontaminated with 2.5% acetic acid and the obtained log mean value of *E. coli* count immediately after spray and after chilling were 1.35 log cfu/cm^2 and 1.97 log cfu/cm^2, respectively. Relatively lower pH was measured in sprayed chilled carcasses with mean pH=5.77 than non sprayed chilled carcasses mean pH=5.98 (Amsalu *et al.,* 2013).

Devrin and Mustafa, (2010) have observed sheep carcass decontaminated with spraying lactic acid solution at two different concentrations (1% and 2%) on microbiological quality of carcass spraying after slaughter and one day cold storage. Thirty minutes after spraying the total viable count (TVC), the number of *coliforms* and *Eschericia coli,* a total reduction of 1.57, 2.69 and 2.06 log cfu/cm^2 respectively was obtained for the 1% lactic acid application. While for 2% lactic acid applied the reduction rate for microorganisms were TVC 1.77 log cfu/cm^2, *coliforms* 2.98 log cfu/cm^2 and *Eschericia coli* 2.23 log cfu/cm^2 obtained.

In an attempt to manage beef carcass contamination, spray wash treatments utilizing three concentrations (1%, 1.5% and 2%) of acetic, lactic, propionic and formic acids were performed. For acetic acid spray log reductions of *E.coli* at 1 % and 2% were 1 log cfu/gm, 1.3log cfu/gm respectively. Surface pH range at 1 % was 4.86 – 5.49 while for 2% it was 4.7 – 5.4. Relative to unsprayed beef carcass lactic acid sprayed log reductions of *E. coli* at 1 %

and 2% were 1.08log cfu/gm, 1.4log cfu/gm respectively. Surface pH Range at 1 % was 4.7 – 5.35 and at 2% was 4.47 – 5.22 (Raftari *et al.*, 2009).

In another experiment, commercial lactic acid spray cabinet that applied 2% lactic acid to preevisceration carcasses reduced aerobic plate counts by 1.6 log cfu/100 cm^2 and *Enterobacteriaceae* counts by 1.0 log cfu/100 cm^2. Lactic acid treatments reduced *E.coli* O157:H7 prevalence by 35% (Joseph *et al.*, 2006). In a similar research beef carcasses were inoculated and treated pre-chilled with hot water wash (35°C/95°F) followed by a lactic acid spray (2%, 55°C/131°F). Lactic acid treatments resulted in a 5 log reduction of *E.coli* (Castillo, 2001).

Mehmet *et al.*,(2012) have conducted that by comparing the inhibitory effects of various decontamination agents (1% Lactic Acid, 2% Lactic Acid, 2% Acetic Acid, 0.1% Acidified Sodium Chloride, 0.1% Sodium Acetate and 0.1% Cetylpridinium Chlorine) on experimentally contaminated raw beef samples with *Listeria monocytogenes* stored at +4°C for five days and after treatment reported log reductions were 6.52 cfu/cm^2, 5.29 cfu/cm^2, 6.52 cfu/cm^2, 7.64 cfu/cm^2, 6.42 cfu/ cm^2 and 6.30 cfu/cm^2 respectively. The highest level of bacterial inhibition was determined in meat samples treated with 2% lactic acid while the lowest level was in samples treated with 0.1% acidified sodium chloride.

The use of 0.4% concentration of lactic acid as a wash solution on chicken carcass effectively eliminated the growth rate of *Salmonella* and *E.coli* at day 0 and day 6 with or without refrigeration than when washed with distilled water. Lawal (2010), has suggested that commercially purchased chicken carcass should wash with (0.4%) lactic acid and then stored under refrigeration for at least six days to totally inhibit the presence of microbes and prevent rate of recontamination before consumption.

According to Gill and Newton, (1982) the inhibitory effect of the lactic acid in meat on gram-negative psychrotrophs appears to be due mainly to the decrease in pH. Psychrotrophs isolated from a meatworks included a large number of strains which would not grow on meat at normal pH at chilled temperatures.

Treatment of broiler carcasses with pre-chill water containing acetic or lactic acid can help to decontaminate and to increase the shelf life of carcasses without altering the colour and appearance of the skin (Kamil *et al.*, 2001).

3. Materials and Methods

3.1 Study Site

Experiments were carried out from October 2013- March 2014 at MJ export abattoir located 75 Km South East of Addis Ababa in Modjo town, Oromia Regional State, Ethiopia. The original name of the study site was coded with new name (MJ). MJ abattoir is amongst the leaders in the Ethiopian meat export business, with slaughtering capacity of 2,500 heads of goat/ sheep per day. Usually, MJ export abattoir slaughters 800 to 1400 heads of goat/sheep daily depending on demands of destination market. In addition to the export abattoir laboratory facility, microbial analyses were carried out in Food Microbiology Laboratory of Centre for Food Science and Nutrition, Addis Ababa University Science Faculty.

3.2. Experimental Units

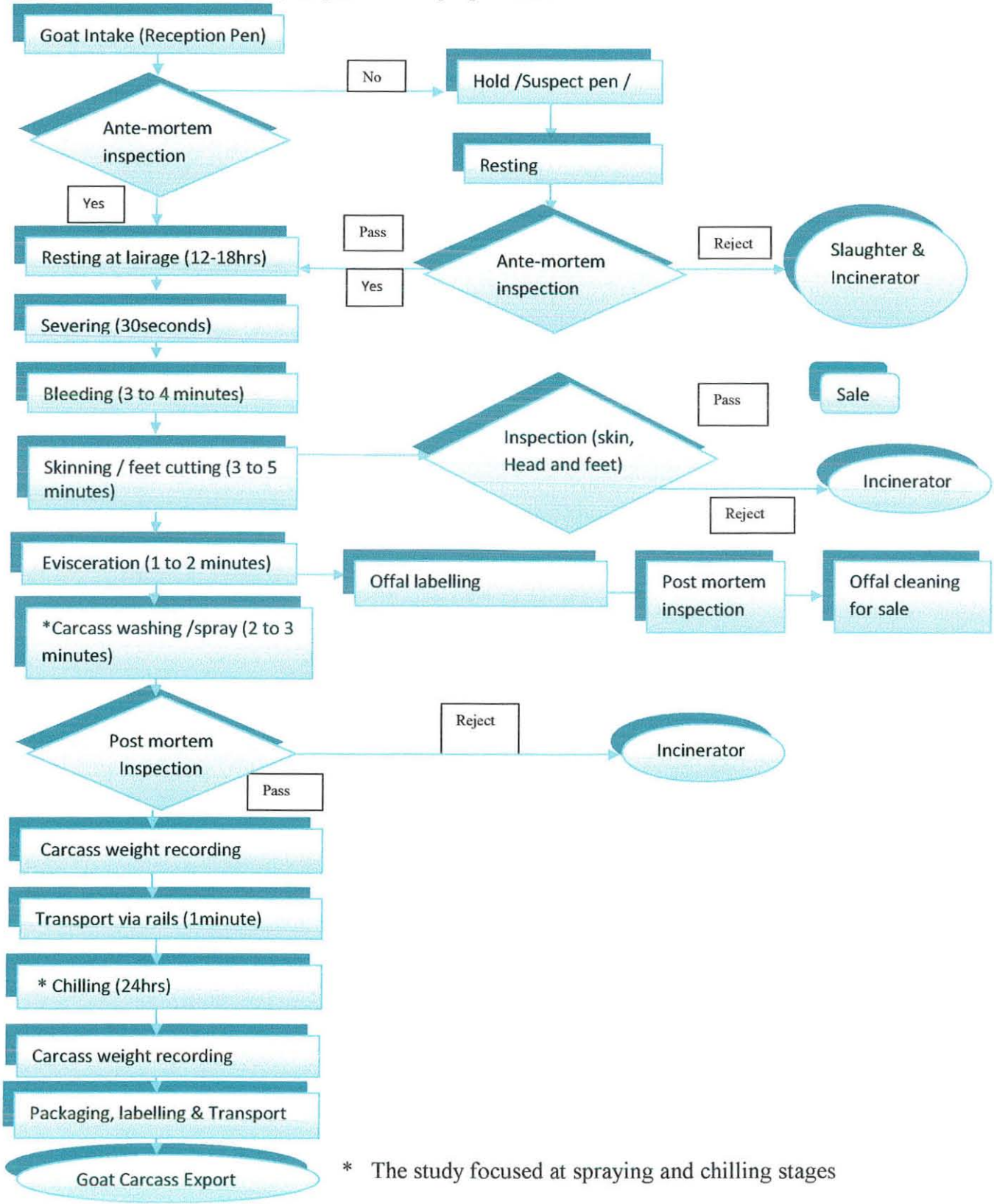
Experimental units were Ethiopian indigenous goat types from lowland and mid highland areas of Ethiopia including Borena, Awash, Metahara, Arbaminch, Jinka, Miesso, Bable, Bati (Wollo). These goats were reared under traditional extensive management condition. Eighty-four male goat carcasses were used for the experiments, which were bought by the export abattoir. Average dressing percentage of carcasses was 39% with weight range of 8 to 12 Kg.

3.3. Management/Handling of the Experimental Units

Minimizing stress before slaughter was important for economic reasons related to meat quality as well as for animal welfare. Goats were handled carefully at all times with minimal use of force. During the pre-slaughter period 12–18 hours in the lairage, goats were kept under conditions which prevent any further contamination of feet, hides, fleeces or skins. The goats were provided ample drinking water during their stay in the lairage as this served to lower bacterial load in the intestine and facilitated the removal of skin. The goats were held without feed prior to slaughter. Withholding feed results in greater ease of evisceration and

minimizes the migration of ingested bacteria from the gastrointestinal tract into the blood stream. Goat carcass preparation steps are summarised in Figure 1.

Figure 1. Flow chart summary of goat carcass preparation



* The study focused at spraying and chilling stages

3.4. Treatment

In order to investigate treatments before and after effects of lactic and acetic acid spray on the microbiological quality and pH change of the carcass, the experiments were done on the following treatments: 2.5% Acetic Acid, 2% Lactic Acid, 1% Lactic Acid and Control Group.

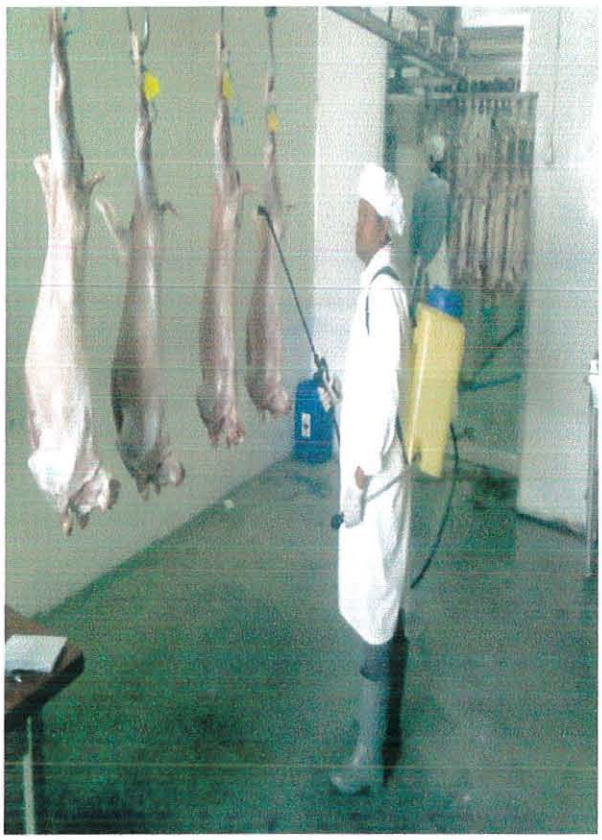


Figure 2. Acids Spray of Treatments of Carcasses

Spraying of treatments was done with Titan knapsack sprayer working capacity of 16 litres, equipped with a long telescopic wand 135 Centimetres and with 0.3 bar pressure flow rate. The time of spraying for single carcass was 20 seconds and spraying distance from carcass was 1.5 meter. Spraying procedure rule was one carcass at a time, starting from hind leg to the tip of fore leg. Hence, topical carcass sides were covered with the anticipated treatment. Lactic acid treatments (2% and 1%) used were obtained from Neway Chemicals Store. While the 2.5% acetic acid was obtained from MJ export abattoir.

3.5. Method of the Research

3.5.1. Microbiological

Goat carcasses were drenched with 2.5% acetic acid, 2% lactic acid and 1% lactic acid. Samples were taken before spraying, 30min after spraying and 24hours after spraying and chilling at 2^0C chilled carcass. A total of 84 carcasses were selected randomly from standard export abattoir slaughter procedure. Eighty-four swabbed samples were collected per treatments. Surface area for cotton swab per sampling site was 100cm^2: (25 cm^2 brisket, 25 cm^2 lateral thorax, 25 cm^2 flank and 25 cm^2 mid loin).

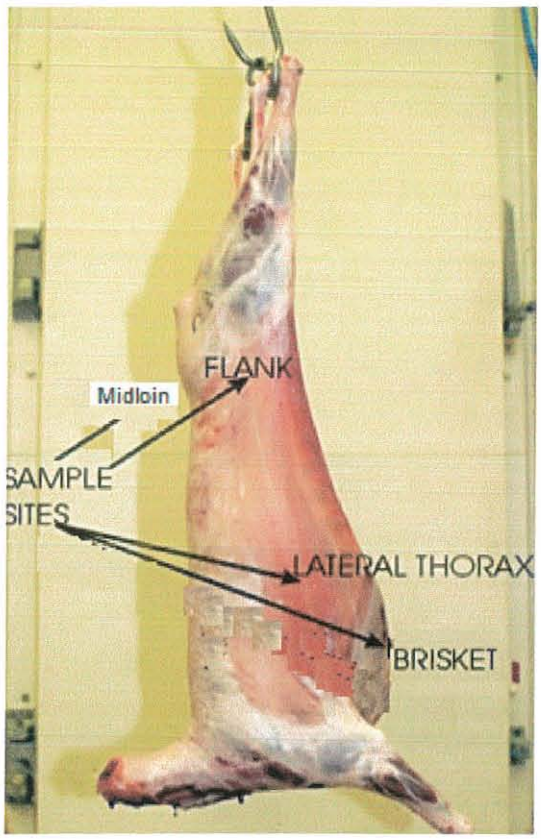


Figure 3. Carcass Sampling Locations

Serial dilutions were done in sterile pepton water with 0.1% concentration. Left side counterpart of carcass was used as control for each treatment and mean log cfu/cm^2 for *E.coli* and TPC were determined.

3.5.1.1. Total Plate Count

The analysis for TPC was carried out using:

- ISO 4833:2003 Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of microorganisms. Colony-count technique at 30 °C.

A. Method principle

The basic principles of TPC under aseptic condition sample preparation, inoculum mixing with Plate Count Agar (PCA), solidification process, incubation of plates, counting of colonies with colony counter.

B. Procedure:

1. Sample Preparation

For single microbial sample total surface area for cotton swabbing was 100 cm^2 collected from the following sampling sites; 25 cm^2 each from brisket, lateral thorax, flank and midloin. Aluminium template of 25 cm^2 was used to determine the surface area. Swabs then put into sterile tubes containing 10ml of pepton water, placed in insulated box container with ice bag and taken to the laboratory. Swabs were subsequently mixed well with vortex mixer.

2. Dilutions and Dilution Factors

From original dilution 1ml was transferred with pipette into a tube containing 9ml of pepton water and mixed by shaking. From the 10^{-1} dilution transferred with the same pipette 1ml to the tube 10^{-2} containing 9ml of the diluents. Repeated until the required numbers of dilutions were made, the dilutions were 10^{-2} and 10^{-3}.

3. Plating

On appropriately marked duplicate dishes 1ml of each final serial dilutions (10^{-2} and 10^{-3}) were poured into 90mm petri dishes, then mixed with 15-20ml of molten PCA, which was kept at 45 ^{0}C and allowed to solidify. Further more negative control tests were prepared.

4. Incubation - Dishes were inverted and incubated at 30 ^{o}C for 48 h to 72 h.

5. Counting the colonies.

Following incubation, number of colonies including pinpoint size was counted. Normal plates with range of 25-250 colonies per dilution colonies were counted and recorded.

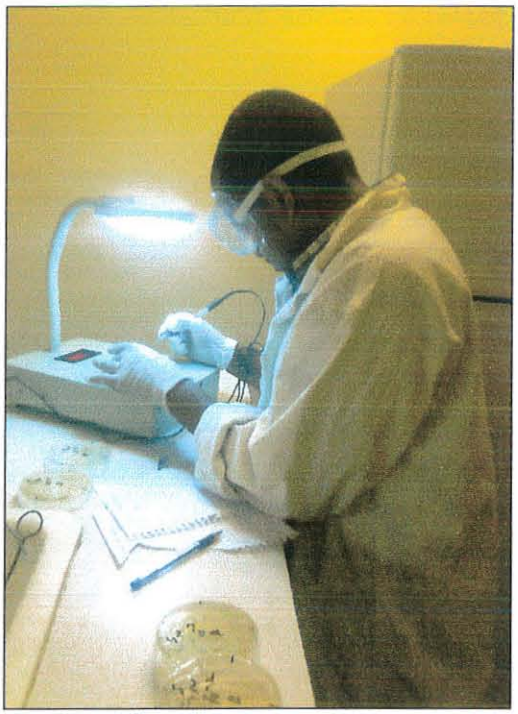


Figure 4. Colony Counting

6. Calculating cfu per cm^2: Use the two dilutions, as n_1 and n_2 to calculate the results.

$$N = \frac{\sum C}{(n_1 + 0.1n_2)\, d}$$

Where:

N= is the number of cfu per cm^2

C = is the sum of colonies on four plates counted (2dilutions with duplicate for each)

n_1= is the number of plates counted at first dilution.

n_2= is the number of plates counted at second dilution,

d = is the dilution factor corresponding to the first dilution

Σ= sum

0.1= constant

The result converted to log by rounding to two significant digits and expressed as a number between 1.0and 9.9 multiplied by 10^X where X is the appropriate power of 10.

Table 3. Example for calculating (log_{10}) carcass per single treatment

$\sum C$	n_1	n_2	D	N=Colony count (cfu/cm^2)	log cfu/cm^2
99	2	2	10^{-2}	4500	3.65
120	2	2	10^{-2}	5500	3.74
130	2	2	10^{-2}	5900	3.77
124	2	2	10^{-2}	5600	3.75

3.5.1.2. *E. coli* count

The analysis was carried out using the following ISO procedure:

- ES- ISO (Ethiopian Standard- International Organization for Standardization). 7251: 2012. (2012). Microbiology of food and animal feeding stuffs - Horizontal method for detection and enumeration of presumptive *Escherichia coli*- Most Probable Number technique.

A. Method principle

E.coli from swab samples enriched using selective enrichment media. When incubated at 44 ^{0}C in the presence of lactose produces gaseous emission while they also convert tryptophan to Indole.

B. Procedure

Initial suspensions were incubated in test tubes containing durham tubes with 9ml to 10ml selective enrichment medium (Lauryl Sulfate Broth) at 37 °C. The tubes were examined for gas production after 24hr and 48hr. If the tubes had given rise to opacity, cloudiness or gaseous emission, an aliquot was subcultured to tubes containing 10ml *E.coli* broth (EC broth) at 44 °C up to 48 hr. Further subcultured into tubes containing 5ml pepton water and incubated at 44 °C up to 48hr. Finally tubes were inspected for indole production with addition of 0.5 ml of inodole reagent and examined after 1minute, presence of red color ring in the tubes indicated presence of *E.coli*. Enumerations were done with Most Probable Number (MPN) according to ES ISO 7251:2012.

3.5.2. Carcass pH

The pH of the carcasses was determined with pH meter (intelligent model: YK-2001PH, IEC1010). Total of 96 pH measurements were taken from four carcass locations (brisket, lateral thorax, flank and midloin) three samples per each locations for each treatments after 30minutes spray and 24 hours sprayed-chilled carcass.

3.5.3. Ethical Clearance

Ethical clearance was obtained from Minister of Agriculture (Animal and Plant Health Regulatory Directorate) and MJ export abattoir.

3.6. Statistical Analysis

The statistical analysis were carried out by using Paired t-test (μD) /Within-subjects t-test and one way ANOVA with IBM SPSS statistics program version 20 software. The paired t- test was used to investigate each treatments effect on the microbial quality (TPC and *E. coli*) of goat carcass before spray, 30 minute after spray and 24 hours after spray and chilling. While the one way ANOVA was used to evaluate the efficacy against treatments. Furthermore, it was used to analyze the extent of goat carcass pH change at 30 minutes after spray and 24 hours after spray and chilling.

4. Result and Discussion

4.1. Results

4.1.1. Microbial Quality

Mean log cfu/cm^2 goat carcass decontamination results of 2.5% acetic acid, 2% lactic acid and 1% lactic acid in accordance with *E. coli* count and TPC for all treatments are presented in the following sections.

4.1.1.1. Application of 2.5% Acetic Acid on *E.coli* count and TPC

Application of 2.5% acetic acid resulted in highest reduction with 0.78 log cfu /cm^2 of *E.coli* when 24 hours sprayed chilled carcass compared with the control group (Table 5). The next higher 0.605 log cfu/cm^2 reduction was obtained from the difference between sprayed 30 minute and 24 hours sprayed chilled carcass (Table 6). The smallest result 0.41 log cfu/cm^2 was obtained from the difference between sprayed 30 minute carcass and control group (Table 4).

Whereas, TPC highest 0.29 log cfu/cm^2 reduction was obtained from the difference between sprayed 30 minute and control group (Table 4). The next better 0.25 log cfu/cm^2 reduction was obtained from comparison of 24 hours sprayed chilled carcass with its control group (Table 5). The least reduction 0.01 log cfu/cm^2 was obtained from the difference of sprayed 30 minute with 24 hours sprayed chilled carcass (Table 6).

As shown in table 4, the application of 2.5 % acetic acid on carcass 30 minute after spray was exhibited quite different log cfu /cm^2 *E.coli* count than the non-sprayed carcass or control 30 minute group. The *E.coli* count for the control 30 minute group was 1.36 ± .050 log cfu /cm^2 while the treated or sprayed carcass 30 minutes group was found to be 0.96±.057 log cfu/cm^2. The obtained t-test result showed significant difference ($p<0.05$).

Table 4. Microorganisms (log cfu/cm^2) examined at the 30 minute control (n=21) and 2.5% acetic acid sprayed carcass after 30 minute (n=21).

Microbial quality type	Control (30minute) X± SEM	sprayed (30 minute) X ± SEM	Mean difference ± SEM	95% Confidence Interval of the Difference		t-test	P-value
				Lower	Upper		
E.coli	1.36±.050	0.96±.057	0.40762±.085	0.23004	0.58520	4.788	0.000*
TPC	3.91±.008	3.61±.007	0.29524±.011	0.27252	0.31796	27.103	0.000*

* Significant at $p<0.05$

Likewise for TPC, 30 minute after application of 2.5 % acetic acid on carcass showed different log mean TPC count than control group. The TPC for the non-sprayed or control group was 3.91 ±.008 log cfu /cm^2 while the treated 30 minutes group was found to be 3.61 ± .007 log cfu/cm^2 and significant difference ($p<0.05$) was observed (Table 4).

It is evident from table 5 that the log mean of *E.coli* count after 2.5% acetic acid sprayed chilled carcass or treated 24 hours was .35± .024 log cfu/cm^2 and for non-sprayed carcass or control 24 hours groups was found 1.13 ±.035 log cfu/cm^2. Comparison of the means of *E.coli* count for control 24 hours and sprayed chilled carcass or treated 24 hours was confirmed significant difference ($p<0.05$). While log mean of TPC after spray 2.5% acetic acid sprayed chilled carcass or treated 24 hours was 3.60 ±.011 log cfu/cm^2 and for 24 hours control groups was found 3.85 ± .012 log cfu/cm^2.

Comparison of the means of TPC count for control 24 hours and treated 24 hours confirmed significant difference ($p<0.05$).

Table 5. Microorganisms (log cfu/cm^2) examined on the control 24 hours group (n=21) and 2.5% acetic acid sprayed chilled carcass after 24 hours (n=21).

Microbial quality type	Control (24hours) X± SEM	Sprayed (24hours) X ± SEM	Mean difference ± SEM	95% Confidence Interval of the Difference		t-test	P-value
				Lower	Upper		
E.coli	1.13±.035	0.35±.024	0.78000 ±.040	0.69609	0.86391	19.391	0.000*
TPC	3.85 ±.012	3.60 ±.011	0.2471 ±.016	0.21400	0.28009	15.596	0.000*

* Significant at p<0.05

The difference between carcasses sprayed with 2.5% acetic acid after 30 minutes spray with carcass sprayed chilled carcass or treated 24hours. Log mean of *E.coli* count after 30 minutes spray of 2.5% acetic acid was 0.96 ±.057 log cfu/cm^2 and sprayed chilled carcass or treated 24 hours was 0.35 ±.024 log cfu/cm^2. The difference between the two groups were statistically significant at p<0.05 (Table 6).

Table 6. Microorganisms (log cfu/cm^2) examined on the sprayed 30 minute (n=21) and 2.5% acetic acid sprayed chilled carcass after 24 hours (n=21).

Microbial quality type	Sprayed (30minutes) X± SEM	Sprayed (24hours) X ± SEM	Mean difference ± SEM	95% Confidence Interval of the Difference		t-test	P-value
				Lower	Upper		
E.coli	0.96 ±.057	0.35±.024	0.6057± .05426	0.49253	0.7189	11.163	0.000*
TPC	3.61±.007	3.60 ±.011	.01000±.01067	-0.0123	0.0323	0.937	0.360

* Significant at p<0.05

Unlike the *E.coli* result table 6 shows, non significant difference ($p>0.05$) for TPC examined on the log means of 2.5% acetic acid sprayed carcass 30 minutes and carcass sprayed chilled or treated 24 hours. Application of 2.5 % acetic acid on carcass 30 minute after spray was 3.61±.007 log cfu/cm^2 while the treated 24 hours group was 3.60 ±.011 log cfu/cm^2 (Table 6).

4.1.1.2. Application of 2% lactic Acid on *E.coli* count and TPC

The 2 % lactic acid spray resulted highest reduction 0.86 log cfu /cm^2 of *E.coli* when 24 hours sprayed chilled carcass compared with the control group (Table 8) . Furthermore, 0.609 log cfu/cm^2 reduction from the difference between sprayed 30 minute and 24 hours sprayed chilled carcass was obtained (Table 9). The least result 0.51 log cfu/cm^2 was obtained from the difference between sprayed 30 minute carcass and control group (Table 7).

But, TPC highest 0.31 log cfu/cm^2 reduction was obtained from the difference between sprayed 30 minute and control group (Table 7). The next better 0.28 log cfu/cm^2 reduction was obtained from comparison of 24 hours sprayed chilled carcass with its control group (Table 8). The least reduction 0.005 log cfu/cm^2 was obtained from the difference of sprayed 30 minute with 24 hours sprayed chilled carcass (Table 9).

Table 7 shows, topical spray of 2% lactic acid on carcass 30 minute after spray had different log mean *E.coli* count than the non-sprayed carcass or control 30 minute group. The *E.coli* count for the control 30 minute group was 1.40±.027 log cfu /cm^2 while the treated or sprayed carcass 30 minutes group was found 0.89± 0.034 log cfu/cm^2. The obtained t-test result showed significant difference ($p<0.05$).

Furthermore Table 7 shows, significant difference ($p<0.05$) for TPC, application of 2% lactic acid on carcass 30 minute after spray was showed different log mean TPC count than

control group 30 minute. The TPC for the non-sprayed or control group was 3.89±.012 log cfu /cm^2 while the treated 30 minutes group was 3.58±.005 log cfu/cm^2 (Table 7).

Table 7. Microorganisms (log cfu/cm^2) examined on the 30 minute control (n=21) and 2 % lactic acid sprayed carcass after 30 minute (n=21).

Microbial quality type	Control (30minute) X± SEM	Sprayed (30 minute) X± SEM	Mean difference ± SEM	95% Confidence Interval of the Difference		t-test	P-value
				Lower	Upper		
E.coli	1.40±.027	.89±.034	.50571±.039	.42451	.58692	12.990	0.000*
TPC	3.89±.012	3.58±.005	.31433±.014	.28539	.34328	22.653	0.000*

* Significant at p<0.05

Table 8 shows the log cfu/cm^2 of *E.coli* count after 2% lactic acid sprayed chilled carcass or treated 24 hours was 0.29 ±.027 log cfu/cm^2 and for non-sprayed carcass or control 24 hours groups was found 1.15±.034 log cfu/cm^2. Comparison of the means of *E.coli* count for Control 24 hours and sprayed chilled carcass or treated 24 hours was confirmed significant difference (p<0.05).

Table 8. Microorganisms (log cfu/cm^2) examined on the control 24 hours group (n=21) and 2 % lactic acid sprayed chilled carcass after 24 hours (n=21).

Microbial quality type	Control (24hours) X± SEM	Sprayed (24hours) X± SEM	Mean difference ± SEM	95% Confidence Interval of the Difference		t-test	P-value
				Lower	Upper		
E.coli	1.15±.034	0.29±.027	0.864 ± .038	0.78340	0.94422	19.391	0.000*
TPC	3.85±.008	3.58±.007	0.2751±.009	0.25441	0.29578	27.744	0.000*

* Significant at p<0.05

Log mean of TPC after spray 2% lactic acid sprayed chilled carcass or treated 24 hours was 3.58±.007 log cfu/cm^2 and for 24 hours control groups was found 3.85 ± .007 log cfu/cm^2. Comparison of the means of TPC count for control 24 hours and treated 24 hours confirmed significant difference $p<0.05$ (Table 8).

Table 9. Microorganisms (log cfu/cm^2) examined on the sprayed 30 minute (n=21) and 2 % lactic acid sprayed chilled carcass after 24 hours (n=21).

Microbial quality type	Sprayed (30minutes) X± SEM	Sprayed (24hours) X± SEM	Mean difference ± SEM	95% Confidence Interval of the Difference		t-test	P-value
				Lower	Upper		
E.coli	0.89±.034	0.29±.027	0.60952± .041	0.52481	0.69424	15.009	0.000*
TPC	3.58±.005	3.57±.007	0.0059±.007	-0.00839	.02020	0.861	0.399

* Significant at $p<0.05$

Table 9 shows, the difference between carcasses sprayed with 2% lactic acid after 30 minutes spray with carcass sprayed chilled carcass or treated 24hours. Log mean of *E.coli* count after 30 minutes spray of 2% lactic acid was .89±.034 log cfu/cm^2 and carcass sprayed chilled carcass or treated 24hours was .29±.027 log cfu/cm^2. The difference between the two groups were statistically significant at $p<0.05$.

Non significant difference ($p>0.05$) was observed for TPC examined on the log means of 2% lactic acid sprayed carcass 30 minutes and carcass sprayed chilled or treated 24hours. Application of 2% lactic acid on carcass 30 minute after spray was 3.58±.005 log cfu/cm^2 while the treated 24hours group was 3.57±.007 log cfu/cm^2(Table 9).

4.1.1.3. Application of 1% lactic Acid on *E.coli* and TPC

Spray of 1 % lactic acid resulted highest reduction 0.729 log cfu /cm^2 of *E.coli* when 24 hours sprayed chilled carcass compared with the control group (Table 11). The next higher 0.58 log cfu/cm^2 reduction was obtained from the difference between sprayed 30 minute and 24 hours sprayed chilled carcass (Table 12). The smallest result 0.35 log cfu/cm^2 was obtained from the difference between sprayed 30 minute carcass and control group (Table 10).

The TPC highest reduction obtained from the difference between sprayed 30 minute and control group was 0.22 log cfu/cm^2 (Table 10) then 0.188 log cfu/cm^2 reductions was obtained from comparison of 24 hours sprayed chilled carcass with its control group (Table 11). The least reduction -0.01 log cfu/cm^2 was obtained from the difference of sprayed 30 minute with 24 hours sprayed chilled carcass (Table 12).

Table 10 shows, 1% lactic acid sprayed carcass 30 minute after spray had different log mean *E.coli* count than the non-sprayed carcass or control 30 minute group. The *E.coli* count for the control 30 minute group was 1.32±.049log cfu /cm^2 while the treated or sprayed carcass 30 minutes group was found 0.97±.025 log cfu/cm^2. The obtained result showed significant difference ($p<0.05$).

Table 10. Microorganisms (log cfu/cm^2) examined on the 30 minute control (n=21) and 1 % lactic acid sprayed carcass after 30 minute (n=21).

Microbial quality type	Control (30minute) X± SEM	Sprayed (30 minute) X ± SEM	Mean difference ± SEM	95% Confidence Interval of the Difference		t-test	P-value
				Lower	Upper		
E.coli	1.32±.049	0.97±.025	0.35000±.049	0.24586	0.45414	7.011	0.000*
TPC	3.96±.007	3.74±.009	0.2241± .009	0.20382	0.24428	23.100	0.000*

* Significant at $p<0.05$

Additionally, Table 10 shows, significant difference ($p<0.05$) for TPC, application of 1% lactic acid on carcass 30 minute after spray was showed different log mean TPC count than control group 30 minute. The TPC for the non-sprayed or control group was 3.96 ±.007 log cfu /cm^2 while the treated 30 minutes group was found 3.74 ±.009 log cfu/cm^2.

Table 11. Microorganisms (log cfu/cm^2) examined on the control 24 hours group (n=21) and 1% lactic acid sprayed chilled carcass after 24 hours (n=21).

Microbial quality type	Control (24hours) X± SEM	Sprayed (24hours) X± SEM	Mean difference ± SEM	95% Confidence Interval of the Difference		t-test	P-value
				Lower	Upper		
E.coli	1.12±.040	0.39±.034	0.72952± 05	0.62419	0.83485	14.447	0.000*
TPC	3.93±.005	3.75±.006	0.1880±.007	0.17253	0.20347	25.357	0.000*

* Significant at $p<0.05$

Table 11 reveals, the log mean of *E.coli* count after 1% lactic acid sprayed-chilled carcass or treated 24 hours was .39±.034 log cfu/cm^2 and for non-sprayed carcass or control 24 hours groups was found 1.12±.040 log cfu/cm^2. Comparison of the means of *E.coli* count for Control 24 hours and sprayed chilled carcass or treated 24 hours was confirmed significant difference $p<0.05$(Table 11). Comparison of the means of TPC count for control 24 hours and treated 24 hours confirmed significant difference ($p<0.05$). Log mean of TPC after spray 1% lactic acid sprayed chilled carcass or treated 24 hours was 3.75 ±.006 log cfu/cm^2 and for 24 hours control groups was found 3.93 ± .005 log cfu/cm^2 (Table 11).

For *E.coli* count Table 12 shows, difference between carcasses sprayed with 1% lactic acid after 30 minutes spray with carcass sprayed chilled carcass or treated 24hours. The difference between the two groups were statistically significant at $p<0.05$. Log mean of *E.coli* count

after 30 minutes spray of 1% lactic acid was .97±.025 log cfu/cm^2 and carcass sprayed chilled carcass or treated 24 hours was .39±.034 log cfu/cm^2 (Table 12).

Table 12. Microorganisms (log cfu/cm^2) examined on the sprayed 30 minute (n=21) and 1 % lactic acid sprayed chilled carcass after 24 hours (n=21).

Microbial quality type	Sprayed (30minutes) X± SEM	Sprayed (24hours) X± SEM	Mean difference ± SEM	95% Confidence Interval of the Difference		t-test	P-value
				Lower	Upper		
E.coli	0.97±.025	0.39±.034	0.58048±.037	0.50297	0.65799	15.622	0.000*
TPC	3.74±.009	3.75±.006	-0.0102± .009	-0.02950	0.00912	-1.101	0.284

* Significant at p<0.05

Furthermore TPC result indicated non significant difference (p>0.05) on the log means of 1 % lactic acid sprayed carcass 30 minutes and carcass sprayed chilled or treated 24hours. Application of 1% lactic acid on carcass 30 minute after spray was 3.74 ±.009 log cfu/cm^2 while the sprayed 24 hours group was 3.75±.006 log cfu/cm^2 (Table 12).

4.1.1.4. Treatments Effect on *E.coli* Count and TPC

Table 13 shows *E.coli* count after 30 minute spray of treatments mean log cfu/cm^2 goat carcass decontamination resulted of 2.5% acetic acid (0.955±0.261 log cfu/cm^2), 2% lactic acid (0.895 ±0.154 log cfu/cm^2) and 1% lactic acid (0.966 ±0.113 log cfu/cm^2) non significant different to each other. However, they all were statistically different ($P < 0.05$) from the control group (1.370 ±0.159 log cfu/cm^2).

Table 13. Treatments Effect on *E.coli* Count (30 minute) ANOVA

30 minute ANOVA (n=21 per each treatment)	
Treatments	***E.coli* load log cfu/cm2** $\bar{x}$ **± SD**
2% lactic acid	0.895 ± 0.154^{a}
2.5% acetic acid	0.955 ± 0.261^{a}
1% lactic acid	0.966 ± 0.113^{a}
Control	1.370 ± 0.159^{b}

* Means not sharing a common superscript letter in a column are significantly different at ($P < 0.05$) as assessed by LSD test.

Table 14. Treatments Effect on *E.coli* Count (24hours) ANOVA

24hr ANOVA (n=21 per each treatment)	
Treatments	***E.coli* load log cfu/cm2** $\bar{x}$ **± SD**
2% lactic acid	0.286 ± 0.123^{a}
2.5% acetic acid	0.350 ± 0.112^{ab}
1% lactic acid	0.387 ± 0.154^{b}
Control	1.125 ± 0.180^{c}

* Means not sharing a common superscript letter in a column are significantly different at ($P < 0.05$) LSD test.

Table 14 shows, *E.coli* count after 24hours sprayed chilled carcasses of 2.5% acetic acid, 2% lactic acid and 1% lactic acid showed significant different ($P < 0.05$) as compared to the control group. However, 2% lactic acid and 1% lactic acid showed significant different ($P < 0.05$).

Table 15. Treatments Effect on TPC (30 minute) ANOVA

30 minutes ANOVA (n=21 per each treatment)	
Treatments	**TPC load log cfu/cm2** $\bar{x}$ **± SD**
2% lactic acid	3.584 ± 0.0216^{a}
2.5% acetic acid	3.611 ± 0.033^{b}
1% lactic acid	3.736 ± 0.0395^{c}
Control	3.909 ± 0.0537^{d}

* Means not sharing a common superscript letter in a column are significantly different at ($P < 0.05$) as assessed by LSD test.

Table 15 shows, TPC after 30 minute spray of treatments 2.5% acetic acid , 2% lactic acid (, 1% lactic acid and the control group showed significant different ($P < 0.05$).

Table 16. Treatments Effect on TPC (24hours) ANOVA

ANOVA (n=21 per each treatment)	
Treatments	**TPC load log cfu/cm2** $\bar{x}$ **± SD**
2% lactic acid	3.578 ± 0.0298^{a}
2.5% acetic acid	3.601 ± 0.050^{a}
1% lactic acid	3.746 ± 0.0288^{b}
Control	3.839 ± 0.048^{c}

* Means not sharing a common superscript letter in a column are significantly different at ($P < 0.05$) as assessed by LSD test.

Table 16 shows, TPC after 24hours sprayed chilled carcasses of 2.5% acetic acid and 2% lactic acid showed non significant different. Both treatments 2.5% acetic acid and 2% lactic acid showed significant different ($P < 0.05$) when compared with 1% lactic acid. All

treatments 2.5% acetic acid , 2% lactic acid and 1% lactic acid were statistically different ($P < 0.05$) from the control group(Table 16).

4.1.2. pH

Table 17 shows, pH value after 30 minute spray of 2% lactic acid and 2.5% acetic acid non significantly different. However, pH value of control and 1% lactic acid were significantly different at ($P < 0.05$). Furthermore, 2% lactic acid and 2.5% acetic acid showed significantly different at ($P < 0.05$) when compared with pH value of 1% lactic acid for each location.

Table 17. pH value of Treatments (30 minute)

Treatments	carcass location			
	Midloin	Flank	Brisket	Lateral Thorax
2% lactic acid	5.65 ± 0.175^{a}	5.61 ± 0.092^{a}	5.68 ± 0.131^{a}	5.70 ± 0.173^{a}
2.5% acetic acid	5.90 ± 0.100^{a}	5.78 ± 0.230^{a}	5.85 ± 0.255^{a}	5.75 ± 0.132^{a}
1% lactic acid	6.10 ± 0.173^{b}	5.98 ± 0.288^{b}	6.00 ± 0.200^{b}	6.20 ± 0.086^{b}
Control	6.74 ± 0.265^{c}	6.46 ± 0.330^{c}	6.50 ± 0.173^{c}	6.26 ± 0.818^{c}

*Values are means of triplicates (± SD). Means not sharing a common superscript letter in a column are significantly different at ($P < 0.05$) as assessed by LSD tests.

Table 18 shows, pH value after 24hours for sprayed chilled carcasses with 2% lactic acid showed significant difference at ($P < 0.05$). The 1% lactic acid were statistically different ($P < 0.05$) from the control group. All treatments were statistically different to each other at ($P < 0.05$) after 24 hours.

Table 18. pH value of Treatments (24hours)

Treatments	carcass location			
	Midloin	Flank	Brisket	Lateral Thorax
2% lactic acid	5.57 ± 0.026^{a}	5.63 ± 0.043^{a}	5.59 ± 0.085^{a}	5.67 ± 0.070^{a}
2.5% acetic acid	5.79 ± 0.190^{b}	5.75 ± 0.132^{b}	5.80 ± 0.059^{b}	5.69 ± 0.200^{b}
1% lactic acid	6.00 ± 0.150^{c}	5.98 ± 0.192^{c}	5.82 ± 0.130^{c}	6.11 ± 0.257^{c}
Control	6.30 ± 0.360^{d}	6.28 ± 0.158^{d}	6.21 ± 0.276^{d}	6.32 ± 0.368^{d}

*Values are means of triplicates (± SD). Means not sharing a common superscript letter in a column are significantly different at ($P < 0.05$) as assessed by LSD tests.

4.2. Discussion

The highest log cfu/cm^2 reduction obtained for *E.coli* count was from 2% lactic acid, followed by 2.5% acetic acid and least performer 1% lactic acid. According to Gunter and peter, (2007) critical microbial condition happens when the E.coli load is within the range of 2 log cfu/cm^2 to 3 log cfu/cm^2. *E.coli* counts exceeding 3log cfu/cm^2 are not acceptable and alarm signals and meat hygiene along the slaughter and handling chain must be urgently improved. The *E.coli* results obtained in this study was within the recommended range for goat carcass which is below 2 log cfu/cm^2. Amsalu *et al,* (2013) has done *E.coli* count on goat carcass under Ethiopian export abattoirs, it was reported as 2.2 log cfu/cm^2 to 2.9 log cfu/cm^2. While Assegide, (2008) has showed *E.coli* count on goat carcass with mean value ranging from 1.7 log cfu/ cm^2 to 2.8 log cfu/cm^2.

Devrin and Mustafa, (2010) on sheep carcass have showed that mean log *E.coli* count for 1% lactic acid was 0.31 cfu/ cm^2 and for 2% lactic acid was 0.09 cfu/ cm^2 and during the conclusion higher reduction was observed for the 2% lactic acid treatment with anticipated increase shelf life. In a similar way the present study on *E.coli* count after 24 hours sprayed-chilled carcass revealed presence of significant difference ($P<0.05$) on application of 2% lactic acid and 1% lactic acid on goat carcass. Smulders *et al.*,(1986) also reported that 2% lactic acid was found to be more effective when compared to 1% and 1.5% because increased concentration of lactic acid has effective antimicrobial action and able to penetrate bacterial cell by means of diffusion and interfere with intra cellular enzymes. Other study on beef carcass has revealed that the application of 2% acetic acid on *E. coli* O157:H7 was reduced 0.65 log cfu/cm^2 as compared with the control (Arthur *et al.,* 2008).

In the present study TPC highest reduction was obtained at 2% followed by 2.5% acetic acid. The least reduction observed was at 1% lactic acid. This finding relates with the expectation that effectiveness of organic acids as antimicrobials depends on their concentration (Beth *et al.*,2005). Present study results were comparable with Gunter and Peter, (2007) recommended range of TPC for goat carcass which is less than 4 log cfu/ cm^2. Critical TPC condition happens when count is within the range of 4 log cfu/cm^2 to 5 log cfu/cm^2. According to Pipek *et.al.*, (2004) decontamination of beef carcasses by 2% solution of lactic acid on the surface of beef carcass reduced the total count of psychrophilic and mesophilic microorganisms. The effect was higher on more contaminated parts of the carcasses. Such treatment can be used to prolong shelf life and to increase food safety of beef carcasses. While for 2% lactic acid applied on sheep carcass the reduction rate for TPC was 1.77 log cfu/cm^2 (Devrin and Mustafa, 2010).

Good quality meat usually has a pH value of 5.4 to 5.8 (Gunter and Peter, 2007). From the obtained result 2% lactic acid was highly comparable. Next to 2% lactic acid, 2.5% acetic acid performed better. The result obtained for 2.5% was compatible with Amsalu *et al*, (2013) research when 2.5% acetic acid was sprayed relatively lower pH was measured in sprayed chilled carcasses with mean pH=5.77 than non sprayed chilled carcasses mean pH=5.98.

According to Bibek, (2004) the better performance of 2% lactic acid than 2.5% acetic acid was due to its pKa is 1 unit less, meaning lactic acid deprotonates ten times as easily as acetic acid does (Bibek, 2004). In the present study 1% lactic acid performed least. pH values above 6 are generally considered as unsuitable for storage because of the favourable development of microorganisms (Ameha, 2008).

5. Conclusion and Recommendations

5.1. Conclusion

This study generated an empirical data to answer which level of lactic acid performed better under Ethiopian goat carcass export business. This study not only tried to test two different concentrations of lactic acid effects on goat carcass but also compared it with the common 2.5% acetic acid application under standard export abattoir working condition.

This study also has demonstrated that application of organic acid (2 % lactic acid, 2.5% acetic acid and 1% lactic acid) resulted in a significant reduction in *E.coli* count and TPC of goat carcass for export as compared to untreated goat carcass. After 24 hours relatively lower pH was measured in 2% lactic acid sprayed chilled carcasses than other treatments. Therefore, pH improvement of 2% lactic acid is higher than 2.5% acetic acid, 1% lactic acid treatments and untreated carcass.

By considering the overall performance of 2% lactic acid, it was concluded that application of 2% lactic acid would improve microbial and pH quality of goat carcass.

5.2. Recommendations

- Despite application of 2.5% acetic acid by Ethiopian export abattoirs as goat carcass decontamination, still microbial quality related complaints are raised by some customers. Hence, 2% lactic acid can be used as alternative to 2.5% acetic acid for goat carcass decontamination.

- Since application of 2 % lactic acid resulted higher performance in *E.coli* count, Total plate count and in pH improvement for goat carcass. It can be used in the Hazard Analysis Critical Control Point (HACCP) system in the export abattoirs. Therefore, can

contribute goat carcass quality improvement programmes, food safety and extends shelf life of the export carcass.

5.3. Scope of Future Activities

- The study was conducted by considering lactic acid (2% and 1% concentrations) and acetic acid (2.5% concentration). In spite of this, further researches should be conducted by taking in to account:
    - Other potentials organic acids,
    - Different concentration levels,
    - More microbiological parameters,
    - Organic acids application temperatures,
    - Different slaughtering conditions and chilling rates.
- Further studies must be conducted to see the sensory quality of the exported carcass by targeting destination market consumers need.

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Annex

A. T test E *coli* Results for 1% lactic acid, 2.5% acetic acid and 2% lactic acid

A_1. 2.5% acetic acid

Table 1 the number of microorganisms (log cfu/cm2) examined on the control (n=21) and treated 2.5% acetuc acid groups(n=21) 30 minute after application

Microbial quality type	Control (30minute) X± SD	Treated (30 minute) X ± SD	95% Confidence Interval of the Difference		t-test	P-value
			Lower	Upper		
E.coli	1.3624±.22724	.9548±.26189	.23004	.58520	4.788	.000*

* Significant at p<0.05

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	control30min	1.3624	21	.22724	.04959
	treated30min	.9548	21	.26189	.05715

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	control30min - treated30min	.40762	.39013	.08513	.23004	.58520	4.788	20	.000

Table 2 the number of microorganisms (log cfu/cm2) examined on the control 24 hours (n=21) and treated at 2.5% acetuc acid (n=21) 24 hours after spray

Microbial quality type	Control (24hours) X± SD	Treated (24hours) X ± SD	95% Confidence Interval of the Difference		t-test	P-value
			Lower	Upper		
E.coli	1.1290±.15827	.3490±.11072	.69609	.86391	19.391	.000*

* Significant at p<0.05

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	control24hr	1.1290	21	.15827	.03454
	treated24hr	.3490	21	.11072	.02416

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	control24hr - treated24hr	.78000	.18434	.04023	.69609	.86391	19.391	20	.000

Table 3 the number of microorganisms (log cfu/cm2) examined on the treated 30 minute (n=21) and treated with 2.5% acetuc acid groups(n=21) 24 hours after spray

Microbial quality type	Treated (30minutes) X± SD	Treated (24hours) X ± SD	95% Confidence Interval of the Difference		t-test	P-value
			Lower	Upper		
E.coli	.9548±.26189	.3490±.26189	.49253	.71890	11.163	.000*

* Significant at p<0.05

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	treated30min	.9548	21	.26189	.05715
	treated24hr	.3490	21	.11072	.02416

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	treated30min - treated24hr	.60571	.24865	.05426	.49253	.71890	11.163	20	.000

A_2. 2% lactic acid

Table 4 the number of microorganisms (log cfu/cm2) examined on the control (n=21) and treated 2 % lactic acid groups(n=21) 30 minute after application

Microbial quality type	Control (30minute) X± SD	Treated (30 minute) X ± SD	95% Confidence Interval of the Difference		t-test	P-value
			Lower	Upper		
E.coli	1.4010±.12337	.8952±.15555	.42451	.58692	12.990	000*

* Significant at p<0.05

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	control30min	1.4010	21	.12337	.02692
	treated30min	.8952	21	.15555	.03394

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	control30min - treated30min	.50571	.17840	.03893	.42451	.58692	12.990	20	.000

Table 5 the number of microorganisms (log cfu/cm2) examined on the control 24 hours (n=21) and treated at 2 % lactic acid (n=21) 24 hours after spray

Microbial quality type	Control (24hours) X± SD	Treated (24hours) X ± SD	95% Confidence Interval of the Difference		t-test	P-value
			Lower	Upper		
E.coli	1.1495±.15377	.2857±.12143	.78340	.94422	19.391	.000*

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	control24hr	1.1495	21	.15377	.03356
	treated24hr	.2857	21	.12143	.02650

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	control24hr - treated24hr	.86381	.17665	.03855	.78340	.94422	22.409	20	.000

Table 6 the number of microorganisms (log cfu/cm2) examined on the treated 30 minute (n=21) and treated with 2 % lactic acid groups(n=21) 24 hours after spray

Microbial quality type	Treated (30minutes) X± SD	Treated (24hours) X ± SD	95% Confidence Interval of the Difference		t-test	P-value
			Lower	Upper		
E.coli	.8952±.15555	.2857±.12143	.52481	.69424	15.009	.000*

* Significant at p<0.05

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	treated30min	.8952	21	.15555	.03394
	treated24hr	.2857	21	.12143	.02650

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	treated30min - treated24hr	.60952	.18610	.04061	.52481	.69424	15.009	20	.000

A_3. 1% lactic acid

Table 7 the number of microorganisms (log cfu/cm2) examined on the control (n=21) and treated 1 % lactic acid groups(n=21) 30 minute after application

Microbial quality type	Control (30minute) X± SD	Treated (30 minute) X ± SD	95% Confidence Interval of the Difference		t-test	P-value
			Lower	Upper		
E.coli	1.3162±.22489	.9662±.11408	.24586	.45414	7.011	.000*

* Significant at p<0.05

paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	control30min	1.3162	21	.22489	.04907
	treated30min	.9662	21	.11408	.02489

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	control30min - treated30min	.35000	.22878	.04992	.24586	.45414	7.011	20	.000

Table 8 the number of microorganisms (log cfu/cm2) examined on the control 24 hours (n=21) and treated at 1 % lactic acid (n=21) 24 hours after spray

Microbial quality type	Control (24hours) X± SD	Treated (24hours) X ± SD	95% Confidence Interval of the Difference		t-test	P-value
			Lower	Upper		
E.coli	1.1152±.18476	.3857±.15354	.62419	.83485	14.447	.000*

* Significant at p<0.05

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	control24hr	1.1152	21	.18476	.04032
	treated24hr	.3857	21	.15354	.03351

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	control24hr - treated24hr	.72952	.23140	.05050	.62419	.83485	14.447	20	.000

Table 9 the number of microorganisms (log cfu/cm2) examined on the treated 30 minute (n=21) and treated with 1 % lactic acid groups(n=21) 24 hours after spray

Microbial quality type	Treated (30minutes) X± SD	Treated (24hours) X ± SD	95% Confidence Interval of the Difference		t-test	P-value
			Lower	Upper		
E.coli	.9662±.11408	.3857±.15354	.50297	.65799	15.622	.000*

* Significant at p<0.05

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	treated30min	.9662	21	.11408	.02489
	treated24hr	.3857	21	.15354	.03351

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	treated30min - treated24hr	.58048	.17028	.03716	.50297	.65799	15.622	20	.000

B. T test TPC results for 1% lactic acid, 2.5% acetic acid and 2% lactic acid

B_1. 2.5% acetic acid

Table 1 the number of microorganisms (log cfu/cm2) examined on the control (n=21) and treated 2.5% acetuc acid groups(n=21) 30 minute after application

Microbial quality type	Control (30minute) X± SD	Treated (30 minute) X ± SD	95% Confidence Interval of the Difference		t-test	P-value
			Lower	Upper		
TPC	3.9062±.03519	3.6110±..03341	.27252	.31796	27.103	.000*

* Significant at p<0.05

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	control30	3.9062	21	.03519	.00768
	treated30	3.6110	21	.03341	.00729

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	control30 - treated30	.29524	.04992	.01089	.27252	.31796	27.103	20	.000

Table 2 the number of microorganisms (log cfu/cm2) examined on the control 24 hours (n=21) and treated at 2.5% acetuc acid (n=21) 24 hours after spray

Microbial quality type	Control (24hours) X± SD	Treated (24hours) X ± SD	95% Confidence Interval of the Difference		t-test	P-value
			Lower	Upper		
TPC	3.8480±.05550	3.6010±.05007	.21400	.28009	15.596	.000*

* Significant at p<0.05

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	control24hr	3.8480	21	.05550	.01211
	treated24hr	3.6010	21	.05007	.01093

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	control24hr - treated24hr	.24705	.07259	.01584	.21400	.28009	15.596	20	.000

Table 3 the number of microorganisms (log cfu/cm2) examined on the treated 30 minute (n=21) and treated with 2.5% acetuc acid groups(n=21) 24 hours after spray

Microbial quality type	Treated (30minutes) X± SD	Treated (24hours) X ± SD	95% Confidence Interval of the Difference		t-test	P-value
			Lower	Upper		
TPC	3.6110±.03341	3.6010±.05007	-.01225	.03225	.937	.360*

* Not Significant at p<0.05

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	treated30	3.6110	21	.03341	.00729
	treated24hr	3.6010	21	.05007	.01093

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	treated30 - treated24hr	.01000	.04889	.01067	-.01225	.03225	.937	20	.360

B_2. 2% lactic acid

Table 4 the number of microorganisms (log cfu/cm2) examined on the control (n=21) and treated 2 % lactic acid groups(n=21) 30 minute after application

Microbial quality type	Control (30minute) X± SD	Treated (30 minute) X ± SD	95% Confidence Interval of the Difference		t-test	P-value
			Lower	Upper		
TPC	3.8982±.05388	3.5839±.02171	.28539	.34328	22.653	.000*

* Significant at p<0.05

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	control30min	3.8982	21	.05388	.01176
	treated30min	3.5839	21	.02171	.00474

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	control30min - treated30min	.31433	.06359	.01388	.28539	.34328	22.653	20	.000

Table 5 the number of microorganisms (log cfu/cm2) examined on the control 24 hours (n=21) and treated at 2 % lactic acid (n=21) 24 hours after spray

Microbial quality type	Control (24hours) X± SD	Treated (24hours) X ± SD	95% Confidence Interval of the Difference		t-test	P-value
			Lower	Upper		
TPC	3.8530±.03491	3.5780±.02957	.25441	.29578	27.744	.000*

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	control24hr	3.8530	21	.03491	.00762
	treated24hr	3.5780	21	.02957	.00645

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	control24hr - treated24hr	.27510	.04544	.00992	.25441	.29578	27.744	20	.000

Table 6 the number of microorganisms (log cfu/cm2) examined on the treated 30 minute (n=21) and treated with 2 % lactic acid groups(n=21) 24 hours after spray

Microbial quality type	Treated (30minutes) X± SD	Treated (24hours) X ± SD	95% Confidence Interval of the Difference		t-test	P-value
			Lower	Upper		
TPC	3.5839±.02171	3.5780±.02957	-.00839	.02020	.861	.399*

* Not Significant at p<0.05

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	treated30min	3.5839	21	.02171	.00474
	treated24hr	3.5780	21	.02957	.00645

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	treated30min - treated24hr	.00590	.03141	.00685	-.00839	.02020	.861	20	.399

B3. 1% lactic acid TPC

Table 7 the number of microorganisms (log cfu/cm2) examined on the control (n=21) and treated 1 % lactic acid groups(n=21) 30 minute after application

Microbial quality type	Control (30minute) X± SD	Treated (30 minute) X ± SD	95% Confidence Interval of the Difference		t-test	P-value
			Lower	Upper		
TPC	3.9596±.03269	3.7356±.03960	.20382	.24428	23.100	.000*

* Significant at p<0.05

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	control30min	3.9596	21	.03269	.00713
	treated30min	3.7356	21	.03960	.00864

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	control30min - treated30min	.22405	.04445	.00970	.20382	.24428	23.100	20	.000

Table 8 the number of microorganisms (log cfu/cm2) examined on the control 24 hours (n=21) and treated at 1 % lactic acid (n=21) 24 hours after spray

Microbial quality type	Control (24hours) X± SD	Treated (24hours) X ± SD	95% Confidence Interval of the Difference		t-test	P-value
			Lower	Upper		
TPC	3.9338±.02364	3.7458±.02882	.17253	.20347	25.357	.000*

* Significant at p<0.05

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	control24hr	3.9338	21	.02364	.00516
	treated24hr	3.7458	21	.02882	.00629

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	control24hr - treated24hr	.18800	.03398	.00741	.17253	.20347	25.357	20	.000

Table 9 the number of microorganisms (log cfu/cm2) examined on the treated 30 minute (n=21) and treated with 1 % lactic acid groups(n=21) 24 hours after spray

Microbial quality type	Treated (30minutes) X± SD	Treated (24hours) X ± SD	95% Confidence Interval of the Difference		t-test	P-value
			Lower	Upper		
TPC	3.7356±.03960	3.7458±.02882	-.02950	.00912	-1.161	.284*

*Not Significant at p<0.05

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	treated30min	3.7356	21	.03960	.00864
	treated24hr	3.7458	21	.02882	.00629

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	treated30min - treated24hr	-.01019	.04241	.00926	-.02950	.00912	-1.101	20	.284

C. *E.coli* ANOVA Results

C_1. 30 minute

ANOVA

E.coli load cfu/cm2

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.971	3	.990	30.318	.000
Within Groups	2.613	80	.033		
Total	5.584	83			

Post Hoc LSD Tests

(I) treatments	(J) treatments	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
2.5% acetic acid	2% lactic acid	.05952	.05578	.289	-.0515	.1705
	1%lactic acid	-.01143	.05578	.838	-.1224	.0996
	control	-.41381*	.05578	.000	-.5248	-.3028
2% lactic acid	2.5% acetic acid	-.05952	.05578	.289	-.1705	.0515
	1%lactic acid	-.07095	.05578	.207	-.1819	.0400
	control	-.47333*	.05578	.000	-.5843	-.3623
1%lactic acid	2.5% acetic acid	.01143	.05578	.838	-.0996	.1224
	2% lactic acid	.07095	.05578	.207	-.0400	.1819
	control	-.40238*	.05578	.000	-.5134	-.2914
Control	2.5% acetic acid	.41381*	.05578	.000	.3028	.5248
	2% lactic acid	.47333*	.05578	.000	.3623	.5843
	1%lactic acid	.40238*	.05578	.000	.2914	.5134

*. The mean difference is significant at the 0.05 level.

C_2. 24 hr *E.coli* anova results

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9.933	3	3.311	160.827	.000
Within Groups	1.647	80	.021		
Total	11.580	83			

Post Hoc LSD Tests

Multiple Comparisons

(I) treatments	(J) treatments	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
2.5% acetic acid	2% lactic acid	.06333	.04428	.157	-.0248	.1515
	1%lactic acid	-.03667	.04428	.410	-.1248	.0515
	control	-.78095*	.04428	.000	-.8691	-.6928
2% lactic acid	2.5% acetic acid	-.06333	.04428	.157	-.1515	.0248
	1%lactic acid	-.10000*	.04428	.027	-.1881	-.0119
	control	-.84429*	.04428	.000	-.9324	-.7562
1%lactic acid	2.5% acetic acid	.03667	.04428	.410	-.0515	.1248
	2% lactic acid	.10000*	.04428	.027	.0119	.1881
	control	-.74429*	.04428	.000	-.8324	-.6562
Control	2.5% acetic acid	.78095*	.04428	.000	.6928	.8691
	2% lactic acid	.84429*	.04428	.000	.7562	.9324
	1%lactic acid	.74429*	.04428	.000	.6562	.8324

*. The mean difference is significant at the 0.05 level.

D. TPC ANOVA Results

D_1. 30 minute

ANOVA

TPC load cfu/cm2

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.385	3	.462	305.971	.000
Within Groups	.121	80	.002		
Total	1.505	83			

Post Hoc LSD Tests

Multiple Comparisons

(I) treatments	(J) treatments	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
2.5% acetic acid	2% lactic acid	.02714*	.01199	.026	.0033	.0510
	1%lactic acid	-.12457*	.01199	.000	-.1484	-.1007
	control	-.29790*	.01199	.000	-.3218	-.2741
2% lactic acid	2.5% acetic acid	-.02714*	.01199	.026	-.0510	-.0033
	1%lactic acid	-.15171*	.01199	.000	-.1756	-.1279
	control	-.32505*	.01199	.000	-.3489	-.3012
1%lactic acid	2.5% acetic acid	.12457*	.01199	.000	.1007	.1484
	2% lactic acid	.15171*	.01199	.000	.1279	.1756
	control	-.17333*	.01199	.000	-.1972	-.1495
Control	2.5% acetic acid	.29790*	.01199	.000	.2741	.3218
	2% lactic acid	.32505*	.01199	.000	.3012	.3489
	1%lactic acid	.17333*	.01199	.000	.1495	.1972

D_2. 24hr TPC

ANOVA

TPC load cfu/cm2

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.963	3	.321	196.568	.000
Within Groups	.131	80	.002		
Total	1.093	83			

Post Hoc LSD Tests

Multiple Comparisons

(I) treatments	(J) treatments	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
	2% lactic acid	.02305	.01247	.068	-.0018	.0479
2.5% acetic acid	1%lactic acid	-.14476*	.01247	.000	-.1696	-.1199
	control	-.23819*	.01247	.000	-.2630	-.2134
	2.5% acetic acid	-.02305	.01247	.068	-.0479	.0018
2% lactic acid	1%lactic acid	-.16781*	.01247	.000	-.1926	-.1430
	control	-.26124*	.01247	.000	-.2861	-.2364
	2.5% acetic acid	.14476*	.01247	.000	.1199	.1696
1%lactic acid	2% lactic acid	.16781*	.01247	.000	.1430	.1926
	control	-.09343*	.01247	.000	-.1182	-.0686
	2.5% acetic acid	.23819*	.01247	.000	.2134	.2630
control	2% lactic acid	.26124*	.01247	.000	.2364	.2861
	1%lactic acid	.09343*	.01247	.000	.0686	.1182

*. The mean difference is significant at the 0.05 level.

E. pH Results

E_1. pH 30 minute Result

pH

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.513	3	.504	30.903	.000
Within Groups	.196	12	.016		
Total	1.709	15			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: pH LSD

(I) treatments	(J) treatments	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
	2.5% acetic acid	.65500*	.09035	.000	.4582	.8518
control	2% lactic acid	.81500*	.09035	.000	.6182	1.0118
	1% lactic acid	.40500*	.09035	.001	.2082	.6018
	control	-.65500*	.09035	.000	-.8518	-.4582
2.5% acetic acid	2% lactic acid	.16000	.09035	.102	-.0368	.3568
	1% lactic acid	-.25000*	.09035	.017	-.4468	-.0532
	control	-.81500*	.09035	.000	-1.0118	-.6182
2% lactic acid	2.5% acetic acid	-.16000	.09035	.102	-.3568	.0368
	1% lactic acid	-.41000*	.09035	.001	-.6068	-.2132
	control	-.40500*	.09035	.001	-.6018	-.2082
1% lactic acid	2.5% acetic acid	.25000*	.09035	.017	.0532	.4468
	2% lactic acid	.41000*	.09035	.001	.2132	.6068

*. The mean difference is significant at the 0.05 level.

E_2. pH 24hr Result

ANOVA

pH

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.999	3	.333	63.330	.000
Within Groups	.063	12	.005		
Total	1.063	15			

post Hoc Tests

Multiple Comparisons Dependent Variable: pH LSD

(I) Treatments	(J) Treatments	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
control	2.5% acetic acid	.52000*	.05129	.000	.4083	.6317
	2% lactic acid	.66250*	.05129	.000	.5508	.7742
	1% lactic acid	.30000*	.05129	.000	.1883	.4117
2.5% acetic acid	control	-.52000*	.05129	.000	-.6317	-.4083
	2% lactic acid	.14250*	.05129	.017	.0308	.2542
	1% lactic acid	-.22000*	.05129	.001	-.3317	-.1083
2% lactic acid	control	-.66250*	.05129	.000	-.7742	-.5508
	2.5% acetic acid	-.14250*	.05129	.017	-.2542	-.0308
	1% lactic acid	-.36250*	.05129	.000	-.4742	-.2508
1% lactic acid	control	-.30000*	.05129	.000	-.4117	-.1883
	2.5% acetic acid	.22000*	.05129	.001	.1083	.3317
	2% lactic acid	.36250*	.05129	.000	.2508	.4742

*. The mean difference is significant at the 0.05 level.