EFFECT OF ACETIC ACID AND PROPIONIC ACID ON BACTERIOLOGICAL QUALITY OF BEEF

By

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THESIS

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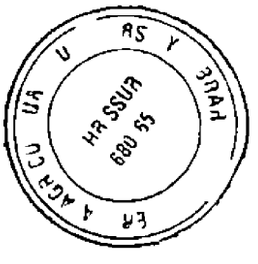

Dedicated to all of
my well wishers

DECLARATION

I hereby declare that this thesis entitled "**EFFECT OF ACETIC ACID AND PROPIONIC ACID ON BACTERIOLOGICAL QUALITY OF BEEF**" is a bonafide record of research work done by me under the valuable guidance of Dr E Nanu, Professor Department of Veterinary Public Health, College of Veterinary and Animal Sciences Mannuthy during the course of research and that the thesis has not previously formed the basis for the award to me of any degree diploma, associateship fellowship or other similar title, of any other university or society

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CERTIFICATE

Certified that this thesis entitled **"EFFECT OF ACETIC ACID AND PROPIONIC ACID ON BACTERIOLOGICAL QUALITY OF BEEF"** is a record of research work done independently by Miss C Latha under my guidence and supervision and that it has not previously formed the basis for the award of any degree fellowship, or associateship to her

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Introduction

INTRODUCTION

Meat well known for its high levels of protein fat vitamins and minerals is an important constituent of human diet Among the meat of various species of animals beef is the most commonly used meat in Kerala as there is no social taboo for this meat

Meat provides all the necessary nutrients desired for growth and development and this makes it an attractive item for human food So also meat is a suitable medium for growth of microorganisms getting access to it during preparation preservation and handling Although muscle by itself in its natural form is free from any contamination, microorganisms invariably get introduced into meat during slaughter and dressing Contamination of animal carcasses during slaughter procedures is an undesirable but inevitable process and the extent of contamination is highly variable on the surfaces of red meat carcasses

Microbial growth in fresh meat is important to the meat industry because it is the main factor associated with reduced quality of meat spoilage resulting in economic loss Tropical environment favours the growth of bacteria on meat which results in rapid spoilage Thus the keeping

quality of meat is highly dependent on the microbial load and it is known that keeping quality is affected by poor hygienic practices in production

The organisms which contaminate meat are either spoilage organisms or potential pathogens Some of the pathogenic organisms elaborate toxins causing food poisoning while others cause infection to the consumer The spoilage organisms influence the keeping quality and shelf life Therefore the bacterial load in meat is important both from commercial as well as public health point of view To get the most reliable indication of hygienic condition it is necessary to know the identity and numbers of all the microorganisms present but it is unfortunately not practicable Routine examination of foods for the multiplicity of pathogenic microorganisms and their toxic products is also impractical in most laboratories Such difficulties have led to the widespread use of indicator organisms whose presence in foods indicates exposure to conditions that might introduce hazardous organisms and or allow proliferation of such organisms Total viable count will indicate the load of Living bacteria in meat Because nearly all the pathogens are adapted to grow at body-temperature (37°C) a simple count of micro organisms growing at that temperature (mesophiles) gives some indication of

the possible occurrence of pathogens Ingram and Roberts (1976) showed that in a slaughter house which looked unhygienic there were unusually high proportions and large absolute numbers of such organisms Presence of coliforms and faecal streptococci are indicative of possible faecal contamination

The importance of extending shelf-life of fresh meat is well recognized in the inadequate chilling facilities for transportation and distribution In a developing country like India the facilities available for slaughter of animals infact is inadequate and far below the accepted level The high humidity and temperature favour the growth of bacteria resulting in early spoilage In addition the maintenance of the quality of meat accidentally contaminated or produced under relatively poor hygienic conditions is of utmost importance Such quality must be maintained before the meat reaches the consumer Thus the problems with the meat which have a poor shelf-life become very acute since it inflicts serious economic loss to the trader if he cannot sell the meat within the limited time This problem is particularly acute for developing nations with inadequate production distribution transportation and storage facilities

Meat preservation methods are aimed at to retard the rate of bacterial multiplication stop their growth

completely or destroy the microbes responsible for spoilage Because of too much handling of meat during operations of slaughter under unsatisfactory environmental conditions it is beneficial to use additional means of sanitizing carcasses at the end of slaughter line Several physical methods have been suggested for this purpose including showering or spraying with water thermal treatment using hot water infra red radiation and Gamma radiation and also chemical methods such as application of chlorinated water, storage in CO_2, and application of organic food grade acids such as acetic acid, lactic acid citric acid, tartaric acid, fumaric acid, ascorbic acid propionic acid malic acid, succinic acid, adipic acid and sorbic acid Though the bactericidal effect of some of the sanitizers was very high, they had certain limitation in acceptibility and safety when indiscriminately used at high concentration

The marketing of meat in Kerala is by keeping the carcass at ambient temperature till the meat is sold out As the tropical conditions favour bacterial multiplication, it is desirable to manipulate the carcass to keep it under limited bacterial load The present study is undertaken to understand the effect of two widely acclaimed organic acids to be used as carcass sanitizers to know their bactericidal/ bacteriostatic effect to maintain the carcass limiting the growth of bacteria and avoiding spoilage in the retail market For this a laboratory model is adopted

Review of Literature

REVIEW OF LITERATURE

Microbial quality of meat

The microbiological quality of varieties of meat and carcasses of different species of animals were investigated by many workers

A study was made on the microbial contamination of fresh beef during the period between the slaughter and retail display by Stringer et al (1969) and observed that the mean $\log_{10}$ number of organisms per square inch was 4 70, 4 78 and 5 94 on beef immediately after slaughter, prior to shipment and on arrival at the retail store respectively

Vanderzant and Nickelson (1969) examined the microbial quality of fresh beef carcasses and beef carcasses stored at 1°C for 3 days and reported that staphylococci were the predominant bacteria present on these carcasses compared to yeast and mold

The microbial count of 213 samples of raw refrigerated ground beef was estimated by Duitschaever et al (1973) and reported that mesophilic and psychrophilic counts on 64% of

the samples were in excess of 10 million/g Enterococcus count ranged between less than 10 to 10 000/g About 95% of the samples had coliform count in excess of 100/g ranging from < 10 to 100 000/g

In a study Nottingham et al (1975) reported that the mean aerobic plate count of beef carcass was 650/cm^2 after ageing In their opinion for aerobic plate counts of mesophilic and psychrophilic organisms spread plates were preferable to pour plates and incubation at 25°C gave a higher and more reproducible count than incubation at 37°C

Emswiler et al (1976) reported that the total aerobic count of unfrozen raw ground beef samples was 10^6 or fewer/g Eighty one per cent samples yielded 100 or fewer coliforms/g and 94% samples had 100 or fewer Escherichia coli/g In the samples stored at $^{-}$1 7 $\pm$ 0 6°C for 18 days there was an increase in total aerobic and psychrophilic counts by one log when examined at different intervals between third and 18th days of storage Counts of E coli decreased and Staphylococcus aureus and Clostridium perfringens did not change significantly

The bacteriological quality of 955 raw ground samples obtained from the super market were examined by Goepfert (1976) and reported that aerobic plate count ranged between

< 10^3/g and > $5x10^7$/g In these samples the range of counts of coliforms was between < 10/g to > 10^4/g and that of *E coli* between < 10/g to $5x10^2$/g

Howe *et al* (1976) studied the calf carcass contamination by *E coli* from the gut contents during slaughter and reported that in one-third of the animals *E.coli* strains found on the surface of the carcass belonged to the same serotype as those found in the rectal sample of the same calf indicating cross contamination of the carcasses

The microbiological quality of retail ground beef prepared in centralized operation and in four local stores were evaluated by Shoup and Oblinger (1976) They reported that aerobic plate count of < 10^5 to 10^6/g in samples from centralized operation and < 10^5 to > 10^8/g in the samples from local stores The corresponding values of coliform counts were < 10^2 to 10^3/g and < 10^2 to 10^6/g

A bacteriological examination of beef carcasses was conducted by Lazarus *et al* (1977) and reported that the mean total bacterial count on fresh carcass was 3 66 $\log_{10}$ number/ 6 45 cm^2 and the mean count on third, seventh and 12th days post slaughter were 2 29 2 43 and 5 35 $\log_{10}$ number/6 45 cm^2 respectively

The level of bacterial contamination on surface of cow carcass was investigated by Niskanen and Pohja (1977) and reported that contact plates gave a mean bacterial count of 9 CFU/cm^2 where as the swab method showed on average count of 1266 CFU/cm^2

Deshpande (1979) studied the bacteriological quality of meat samples collected from slaughter house and meat shops He found the mean aerobic mesophilic count of meat samples taken from the slaughter house was 12 5 x 10^8/g The count in samples collected from one of the shops was 30 x 10^7 and for the other shop it was 40 x 10^7/g Presumptive coliform count ranged from 0 to 24 000/g and faecal streptococci count varied from 31 to 24,000/g

Gill and Newton (1980) studied the development of spoilage flora and the growth of individual psychrotrophs and pathogens on beef steaks They reported that 60% of the spoilage flora consisted of psychrotrophic pseudomonas in samples stored at 20°C under aerobic conditions whereas in samples stored at 30°C it was < 20%

Kleeberger et al (1980) estimated the bacterial count of fresh beef and beef samples stored for 2 days at 15°C or stored for 4 days at 7°C Their study revealed that bacterial count increased from 10^4/g of fresh meat to 10^7 to 10^8/g after storage

Total viable count of beef carcasses obtained from three abattoirs were investigated by Roberts et al (1980) and reported that the mean $\log_{10}$ count/cm^2, for these abattoir carcasses were 2 9 2 96 and 1 93

The microbial contamination of the beef carcasses and air in eight abattoirs was evaluated by Fournaud and Bertaud (1981) and reported that the level of contamination was considerably higher in lean beef than in the fatty parts of the carcasses

Kuttynarayanan (1981) reported that aerobic plate count of beef carcasses varied from $30x10^6$ to $150x10^6$/g and coliform count ranged from $1\ 2x10^5$ to $160x10^5$/100 g of meat Out of 84 samples tested all the samples except one were positive for faecal streptococci and its counts ranged between 1 and 5 log/g of meat

Maxcy (1981) reported the surface contamination in the form of discrete colony forming units was the main source of bacteria associated with meat spoilage In his opinion the fate of these bacteria was determined by the micro-environment at the meat atmosphere interface where the constraints determine the nature of the developing microflora

Nortje and Naude (1981) evaluated the aerobic mesophilic and psychrotrophic counts at specific sites on each of 156 beef carcass surface and reported that the mean initial mesophilic count ranged between 4 5 and 7 $7x10^2/cm^2$ was reduced to 2 $5x10^2/cm^2$ after the chilling process

In a survey on hygienic quality of beef and pork carcasses in Norway Johanson et al (1983) reported that bacterial counts on pork carcasses were consistently higher than those on beef Beef slaughtering left a relatively clean carcass surface after the hide has been removed while on pork a contaminated skin remained on the carcass during and after the slaughter process

In an investigation Iyer (1984) examined the bacteriological quality of buffalo carcasses and reported that the total plate count ranged from 7 0 to 9 1 $\log_{10}/cm^2$ The range of coliform count and faecal streptococci count were 4 5 to 5 8 $\log_{10}/cm^2$ and 4 6 to 5 9 $\log_{10}/cm^2$ respectively

The number and distribution of bacteria on beef carcasses derived from seven member states abattoirs of the European communities were investigated by Roberts et al (1984) and reported that there was high variation in total viable counts on carcasses collected from different abattoirs and those collected from the same abattoir on different occasions

The bacterial contamination and retention on carcass surface along the processing line in the slaughter hall was evaluated by Kriaa *et al* (1985) They found contamination varied along the processing line but the pattern was dependent on the contamination at the dressing station The bacterial count remained unchanged or decreased during the first 12 minutes and then increased even without additional contamination

Zamora and Zaritzky (1985) reported that the total bacterial count of beef samples stored in polyethylene bags at 4°C has rapidly reached to 10^7 CFU/cm^2 with a short lag phase and in samples stored at 0°C showed an increase in lag phase and the growth rate was decreased

Dempster (1986) examined the bacteriological quality of minced beef samples and reported that the total bacterial count ranged between 5 57 $\log_{10}$/g and 8 45 $\log_{10}$/g *E coli* count varied from 1 2 $\log_{10}$/g to 3 71 $\log_{10}$/g

Hudson *et al* (1986) reported that the mean total viable count of minced ground beef collected from supermarket averaged 5 72 $\log_{10}$number/g and the mean count in shop samples was 5 62 $\log_{10}$/g

Among 896 bacterial isolates obtained by Lotfi *et al* (1986) 156 were coliform organisms from emergency slaughtered cattle and buffaloes

The bacterial load of retail samples of beef mince and beef rump was evaluated by Scriven and Singh (1986) They found that total plate count was similar for all samples but the population of coliforms and S aureus were higher in minced meat than in rump The mean total plate count of minced beef was $5\ 0x10^7$ and that of beef rump was $4\ 6x10^6$ Mean coliform counts were 1159 and 11 for beef mince and beef rump respectively

The microbiological profile of beef carcass was determined at the end of the slaughter line and after different stages of chilling by Stolle (1988) and reported that consistently higher contamination was found on the lateral surfaces of the carcasses especially on the forequarters The average total viable count was 0 34 $\log_{10}$ CFU/cm^2 indicated a fairly good standard of slaughter hygiene

Nortje et al (1989) investigated the microbiological quality of beef carcass supplied to the retail outlets of different supermarket and reported that there was no consistency in the level of contamination on various parts of the carcasses but the fore quarters were more contaminated than the hind quarters

In a study Sherikar et al (1989) examined the microbial spoilage and shelf-life of buffalo meat stored at refrigeration temperature (7 ± 1°C) for 168 h and reported that the mean initial total viable count was 5 72 $\log_{10}$ CFU/g and coliform count was 3 37 $\log_{10}$ CFU/g Deteriorative changes in meat started at 72 h of storage and was totally spoiled on 7th day when the mean TVC and faecal coliform count reached 8 22 $\log_{10}$ CFU/g and 5 36 $\log_{10}$ CFU/g, respectively

Nortje et al (1990) investigated the microbial profile of beef carcasses and reported that the mean enterococci count in clean and dirty abattoir carcasses were < 2 9 $\log_{10}$ number/cm^2 and 3 7 $\log_{10}$ number/cm^2 respectively The count in clean retail samples was 2 4 $\log_{10}$ number/cm^2 and in dirty meat samples was 3 6 $\log_{10}$ number/cm^2

Total viable count of buffalo meat stored at refrigeration temperature for 168 h was estimated at different periods by Saoji et al (1990) They reported the mean total viable count at 0 h was 5 8 $\log_{10}$ CFU/g and it was 8 93 $\log_{10}$ CFU/g at 168 h of storage They also recorded the count as considerably high at 24 and 72 h of storage

Charlebois (1991) reported that, out of 54 beef carcasses examined in abattoirs 93 7% of the samples showed

faecal coliform count in between 0 and 100/cm^2 Very few (4 3%) showed 101 500/cm^2 and only 2% showed counts above 500/cm^2 They could not find any significant difference in mean faecal coliform count between fore and hind quarters

A study on the microbiological quality of market beef samples was undertaken by Okodugha and Aligha (1991) Their study revealed that the mean aerobic plate count was 6 34 log_{10} number/g and the mean coliform count was 4 95 log_{10} number/g

Harris and stiles (1992)reported that the fresh ground beef prepared commercially had an aerobic colony count between 3 2x10^4 CFU/g and 3 0x10^6 CFU/g and after 10 days of storage at 4°C the mean count was 5x10^8 CFU/g

The bacteriological quality of sheep and goat carcasses as well as meat of these species of animals has been investigated by a few workers Vanderzant and Nickelson (loc cit) examined the microbiological quality of the lamb carcass shortly after death and after 3 days storage at 1°C and showed that staphylococci were the predominant isolate

In a study Gill et al (1976) evaluated the effect of delayed evisceration of lamb carcasses and reported that the

muscle and lymph nodes of unevisceratedlamb carcasses hung for 24 h at 20°C remained sterile

The surface bacterial load of fresh lamb carcass and ground lamb under chilled condition was investigated by Ali et al (1982) and reported that the average aerobic plate count on lamb carcass surface was 1 1 x 10^6 CFU/cm^2 and for chilled ground lamb it was 3 1 x 10^5 CFU/g

Bhagirathi et al (1983) determined the bacterial contamination of fresh market mutton and reported that the total count ranged from 10^3 to 10^5/g They also observed that on exposure of the mutton in atmospheric condition for 6 to 8 h increased the total aerobic count by 2 2 to 5 0 log number/g

Iyer (loc cit) conducted microbiological examination of sheep and goat carcasses and reported that the mean total plate counts were 6 83 and 5 75 $\log_{10}$ number/cm^2 for sheep and goat carcasses respectively Coliform count of sheep and goat carcasses were averaged to 4 62 and 4 08 $\log_{10}$ number/cm^2 respectively Average faecal streptococci count of sheep carcass was 4 46 $\log_{10}$ number/cm^2 and that of goat carcass was 3 95 $\log_{10}$ number/cm^2

Prieto et al (1991) studied the change in bacterial counts on lamb carcass surface during storage at 3 $\pm$ 1°C on

days 0 5,10 and 15 The mean mesophilic count on lamb carcass immediately after slaughter, was 4 96 $\log_{10}$ CFU/cm^2 The mesophilic and psychrotrophic counts associated with spoilage averaged to 7 4 $\log_{10}$ CFU/cm^2 and 7 95 $\log_{10}$ CFU/cm^2 respectively

A few investigators have reported the bacteriological quality of pork and pig carcasses

Vanderzant and Nickelson (loc cit) isolated various species of bacteria yeast and mold from fresh pig carcasses and after 3 days of storage at 1°C They found staphylococcus as the predominant bacteria present on the carcass surface

The source of surface carcass contamination of pigs at slaughter was studied by determining presumptive coliform count by Linton et al (1977) They found all 16 pig carcasses from the slaughter line of a commercial abattoir were contaminated with presumptive coliform organisms whereas 6 of 8 pig carcasses slaughtered at Meat Research Institute were contaminated with these organisms

Wojton and Kossakowska (1977) evaluated the use of different bacteriological test to assess the sanitary quality

of 200 pig carcasses and reported that aerobic and facultative anaerobic counts correlated well with the counts of *E coli* and enterococci

Murthy and Bachil (1980) reported that total aerobic count of fresh pork was 5 6 $\log_{10}$ CFU/g They found the first sign of offodour of pork started when the count reached to 7 35 $\log_{10}$ CFU/g and clear evidence of spoilage started when the count reached 9 09 $\log_{10}$ CFU/g

Bacteriological examination of pig carcasses was conducted by Iyer (loc cit) and reported that the total plate count coliform count and faecal streptococci count of pig carcasses varied from 5 5 to 7 7 $\log_{10}$ number/cm^2 3 8 to 5 3 $\log_{10}$ number/cm^2 and 3 6 to 5 1 $\log_{10}$ number/cm^2 respectively

The bacterial load of retail samples of pork mince and pork rump was investigated by Scriven and Singh (loc cit) and reported that the mean total plate count of pork mince and pork rump were 5 1 x 10^7 and 4 1 x 10^6/g respectively Mean coliform count of pork mince was 2091/g and that of pork rump was 119/g

Gupta *et al* (1987) examined the bacteriological

quality of fresh pork samples collected from different abattoirs and meat shops Their study revealed that bacterial load of fresh pork samples collected from the retail shops were significantly higher than those of the samples collected from slaughter houses The standard plate count and coliform count of the samples ranged from 5 77 to 9 14 $\log_{10}$ number/g and 4 3 to 7 81 $\log_{10}$ number/g respectively

Antibacterial Effect of Acetic Acid

Acetic acid is one of the short chain organic acids used as a sanitizer in the meat industry for its efficient antibacterial activity and safety The antibacterial effect of acetic acid alone at different strength and in combination with other acids and chemicals on carcasses of various species of animals have been investigated by many workers

The decontaminating effect of acetic acid on beef carcass has been reported by many workers Anderson *et al* (1977) studied the efficacies and optimum application conditions of acetic acid 4% chlorine 200 250 ppm and quaternary ammonium compound 3 78 g/l on beef and reported a significant reduction and the mean reduction in bacterial count immediately after treatment were 1 47 log/cm^2 0 31 log/cm^2 and 0 79 log/cm^2 respectively whereas after 48 h of application the

reduction in counts were 1 79 log/cm^2 0 53 log/cm^2 and 0 03 log/cm^2 respectively

Bala et al (1977) reported that 7 days aged chilled beef short loins were spray sanitized with 4% acetic acid solution (54 60°C) for one minute significantly reduced the microbial load on its surface without affecting the colour stability and colour score

The sanitizing efficiency of cold water, hot water steam sodium hypochlorite and 3% acetic acid on plate beef during storage at 3 3°C and 90% relative humidity was investigated by Anderson et al (1979) They reported that compared to the untreated control the time taken to reach counts of 10^8 bacteria/cm^2 were one day less with steam or water treated samples, 2-3 days more with hypochlorite treated samples 5 days more with hot water treated samples and 16-17 days more with acetic acid treated samples

Anderson et al (1980) compared the bacteriological quality of half beef carcasses washed with tap water (40° C) under pressure and washed and sanitized with 3% acetic acid solution at 40° C and reported that the mean aerobic plate count was reduced by 0 17 log/cm^2 or 5 3% and 1 4 log/cm^2 or 96 8% on carcasses washed with tap water and washed and sanitized with acetic acid solution respectively

Quartey Papafio et al (1980) tested formic acetic and propionic acids in various combinations and individually for antibacteria effect on beef and reported 2% formic acid and 1% formic acid with 1% acetic acid were most effective destroying 84 and 73% of test cultures respectively The most effective sanitizer was 2% formic acid which reduced the microbial count by 1 56 log/g immediately after treatment

A study was undertaken by Osthold et al (1984) to determine the bactericidal effect of acid mixture containing 2% acetic acid 1% lactic acid, 0 25% citric acid and 0 1% ascorbic acid on beef carcasses which were stored at 7 and 10°C after spraying They found improvement in the bacterial quality of carcass as well as a selective inhibitory effect on enterobacteriaceae and coliforms

Experimentally inoculated beef samples with 5 2 x 10^6 Salmonella typhimurium, Shigella sonnei, Yersinia enterocolitica, Escherichia coli, Pseudomonas aeruginosa or Streptococcus faecalis were dipped in 1 2% acetic acid solution by Bell et al (1986) and reported that the average recoverable numbers of these bacteria reduced by 65% E coli was the most resistant having a reduction of 46% only

Acuff et al (1987) could not find any significant influence in aerobic plate count of beef steaks decontaminated by spraying with 1% lactic acid 1% acetic acid and an acid mixture containing 1% lactic acid 2% acetic acid 0 25% citric acid and 0 1% ascorbic acid followed by vacuum packaging and storing at 4 ± 1°C for 84 days

In a study Anderson et al (1987) compared the antibacterial effect of hand washing and machine washing and machine sanitizing with 1 5% acetic acid solutions at two different temperatures (14 4°C and 52°C) on half beef carcasses They found that machine washing and sanitizing was more effective in reducing the load of E coli, enterobacteriaceae and aerobic bacteria than hand washing

Hamby et al (1987) compared the effect of intermittent spray chilling and single spray treatment with water, 1% acetic acid or 1% lactic acid on the microbiological and sensory properties of beef cuts taken after 48 h postmortem, vacuum packed and stored at 2° C for 28 days They noticed significant reduction in aerobic plate count on the rib and clod areas of carcass treated with acetic acid and reduction of aerobic plate count in all sampling areas sprayed with 1% lactic acid in case of intermittent spray chilling Single

spray of lactic acid resulted in significant reduction in aerobic plate count on strip loins and rib areas whereas treatment with 1% acetic acid did not show significant effect

In a study Anderson and Marshall (1989) compared the sanitizing efficacies of 1 2 or 3% acetic acid solutions at 25 40 55 and 70° C on beef semitendinosus muscle which were contaminated with fresh manure suspension or suspensions of E coli and S typhimurium They found that 3% acetic aicd solution at 70° C was the most effective sanitizer and it showed the greatest effect on total aerobic plate count followed by enterobacteriaceae count and E coli S typhimurium count was affected least by change in temperature

Anderson and Marshall (1990 a) evaluated the sanitizing effect of dipping in varying concentrations of acid mixture (0-3%) containing 2% acetic, 1% lactic, 0 25% citric and 0 1% ascorbic acids at different temperature (20-70° C) on beef core samples artificially inoculated with cultures of S typhimurium E coli and a mixture of bull manure to simulate the aerobic plate count and enterobacteriaceae present on beef surface Examination of the treated samples after 16 h or storage at 1° C revealed that an increase in

either mixed acid concentration or temperature resulted in 1 log reduction in aerobic plate count and S typhimurium less than 1 log in enterobacteriaceae and about half a log reduction in E coli

The bacteriostatic or bactericidal action of 1, 2, 3 and 4% acetic and lactic acid treatment on buffalo meat stored at 7 ± 1° C for 168 h was investigated by Saoji et al (1990) and reported that the bacteriostatic and bactericidal effect of both acids increased with increase in concentration and the acids showed more pronounced antibacterial effect on gram negative bacteria They recommended 3% acetic acid or 2% lactic acid for decontamination and preservation of buffalo meat upto 7 days at refrigeration temperature

Tomancova and Steinhauser (1990) studied the effect of 1% acetic acid, 2% lactic acid and combination of 1% solutions of each of the acids on shelf-life and sensory changes of vacuum packed meat They reported that the shelf life of samples treated with acetic acid lactic acid and the combination of acids increased by 15-17 days 18-20 days and 20-24 days respectively

Dickson (1991) investigated the effectiveness of modified spray chilling with acetic acid solution in reducing

the bacterial load of S typhimurium L monocytogenes and E coli 0157 H7 on lean beef and fat tissue He reported that the reductions upto 3 log cycles (99 9%) were obtained for all the threc bacterial species on fat and it was less on lcan beef tissue with the same tr^atment but the bacterial population was reduced as compared to the control samples

A comparative study was carried out on the preservative effect of 1 2, 3 and 4% acetic lactic acid combinations and acetic propionic acid mixtures on buffalo meat steaks treated and stored at refrigeration temperature for 168 h (Surve et al 1991) They found 3% acetic lactic acid combination reduced bacterial count of the treated samples without affecting the colour and odour and the antibacterial effect of these acid mixtures were pronounced on gram negative organisms than gram positive ones

Anderson et al (1992) evaluated the sanitizing effect of 1 5% and 3% acetic acid and lactic acid solutions of similar strength and two mixtures containing acetic, lactic, citric and ascorbic acids in different concentrations and at temperatures of 20 45 and 70° C on the surface of the beef core samples inoculated with aerobes enterobacteriaceae, S typhimurium and E coli The analysis of bacterial load revealed considerable reduction and the rate of reduction was proportionately higher to the temperature of the solution

The bactericidal effect of 2% acetic acid on beef tissue surface both lean and fat contaminated with S typhimurium was evaluated by Dickson (1992) and reported that the reduction in population of S typhimurium was consistent irrespective of initial cell population on lean and fat tissues Acetic acid treatment reduced the bacterial count by 0 5 to 0 8 log_{10}/g cycles but there was no significant difference between the treated and control samples

Dickson and Anderson (1992) developed different methods including washing and sanitizing with organic acids to reduce the level of contaminating bacteriae on carcasses They showed that the efficiency is dependent on the concentration of the acids and its temperature contact period and sensitivity of the organisms to the compound used Organic acids were reported to have an immediate effect on the microflora of meat primarily when applied during the slaughtering and dressing operations

Siragusa and Dickson (1992) reported that2% acetic acid or 1 7% lactic acid immobilized in calcium alginate gel reduced the L monocytogenes count on lear beef tissue artificially inoculated with L monocytogenes and stored at 5° C for 7 days by 1 5 log_{10} units versus 0 25 log unit and

1 3 $\log_{10}$ units versus 0 03 log unit decrease from the acid treatments alone respectively They showed that the alginate immobilization of acids did not enhance the bactericidal effect on fat tissue but potential for use in sanitizing and preserving lean raw beef

Sanitizing effects of acetic acid on sheep and goat carcasses have been evaluated by a few workers

Ockerman et al (1974) studied the effect of 6 12, 18 and 24% acetic acid sprays on the microbial load of lamb carcasses They reported that all concentrations of acetic acid reduced the bacterial load on the treated lamb carcasses during 12 days of storage at 3 ± 1° C and 18% was the most effective concentration of the acid

Anderson et al (1988) evaluated the effect of dipping and spraying of freshly slaughtered lamb carcasses with1 5% and 3% acetic acid solution at 25° C and 55° C and reported that dipping in 3% acetic acid solution at 55° C was the most effective to reduce bacterial load

Investigations have been made to assess the decontaminating effect of acetic acid on pig carcasses The effect of spraying acetic acid on pig carcasses was evaluated

by Biemuller et al (1973) and reported that acetic acid at pH 1 5 and 2 0 was effective in reducing salmonella contamination and produced 4 log reduction in aerobic plate count They also found that the effect of acetic acid treatment was persistent even after 24 h of treatment

An experimental study was conducted by Reynolds and Carpenter (1974) to determine the suitable concentration of acetic propionic acid in solution used for decontaminating pork carcasses without affecting product quality and appearance and reported that the treatment with 1 5 M acetic propionic acid (60 40 w/w) solution at pH 2 3 reduced the total count by 2 log cycle with no apparent detrimental effect on carcass

Cacciarelli et al (1983) reported that the pork loins spray washed with water followed by sanitizing with 2% acetic acid solution had significantly lower aerobic anaerobic and lactobacilli count than in pork loins spray washed with water alone or spray washing followed by sanitizing with 200 ppm sodium hypochlorite solution immediately after treatment as well as on days 14, 21 and 28 days of storage at 4° C after vacuum packing

In a study Mendonca et al (1989) investigated the antimicrobial effect of dipping fresh pork chops in different

concentrations of organic acids and salt solutions for two minutes and storage at 2 4° C for six weeks after vacuum packing They found pork chops treated with 3% acetic acid showed significantly lower aerobic microbial numbers and effectively inhibited enterobacteriaceae than in other treatments Treatment with 1% acetic acid with or without 1% lactic acid was ineffective

The sanitizing effect of acetic acid on poultry carcass has been investigted by Mountney and O'Malley (1955) They tested the sanitizing effect on cut up poultry parts with water containing various acids at p^H 2 5, sorbic acid at p^H 3 1 and 10 ppm chlortetracycline and reported that acetic, adipic and succinic acids increased the shelf life by six days more than the control and three days more than the chlortetracycline treated samples Adipic and succinic acids gave best overall results and acetic acid was found unacceptable because of its pungent odour and its effect on the skin

The sanitizing effect of acetic acid on rabbit carcass has been investigated by a few workers Bothast et al (1968) reported that dipping of rabbit carcass in 40% acetic acid solution at 23° C for 90 seconds caused marked reduction in viable count but carcass turned completely black in colour

Rao (1991) found that 2% acetic adipic and succinic acids and combination of these acids in the ratio 2 1 1 significantly reduced the microbial load on rabbit carcass surface and prolonged the shelf-life to 19 days from 7 days of the untreated samples He found 2% acetic acid was better than other acids

The antibacterial effect of acetic acid in different concentrations on various bacteria present in the scalding tank water (Oxrend et al 1986 Lillard et al 1987) and its inhibitory effect in media against S typhimurium S enteritidis E coli S aureus, P aeruginosa, M avium and B subtilis (Rubin 1978 Adams and Hall 1988 Zorawski et al 1991) has been reported Yamamoto et al (1990) investigated the effect of various organic acids on thermal resistance of spores of Cl botulinum 62A, Cl sporogenes and Cl perfringens

Antibacterial effect of propionic acid

On perusal of literature, it is observed that antibacterial effect of propionic acid on carcasses of various species of animlas have been scantily investigated and reported

Quartey Papafio et al (loc cit) tested propionic acid

individually and in combination with acetic acid for antimicrobial effect on beef carcasses and reported that the percentage of test culture destroyed was 55% by 2% propionic acid alone and by a combination of 1% acetic and 1% propionic acid

Preservative effect of 1, 2, 3 and 4% solution of acetic propionic acid mixtures on buffalo meat steaks stored at refrigeration temperature was studied by Surve *et al* (loc cit) and reported that these acid mixtures had pronounced antibacterial effect on gram negative organisms than gram positive ones

An investigation was made by Reynolds and Carpenter (loc cit) to detect the suitable proportion of acetic acid and propionic acid in a mixture to reduce the microorganisms and reported that the treatment of pork carcasses with 1 5M acetic propionic acid (60 40 w/w) at p^H 2 3 reduced total count by two log cycles without showing any apparent detrimental effect on carcasses

The antibacterial effect of propionic acid on various bacterial organisms has been reported by many workers Cole *et al* (1968) reported that propionic acid was effective in controlling haemolytic *E coli* and in reducing the count of non haemolytic *E coli* in the duodenum and jejunum

Fklund (1980) studied the effect of benzoate sorbate propionate and alkyl esters of P-hydroxy benzoic acid (parabens) solutions on growth and amino acid uptake process of E coli B subtilis and P aeruginosa and reported that parabens caused growth inhibition of these bacteria by transport inhibition but the uptake inhibition caused by benzoate sorbate and propionate seems to be inadequate to explain the bacterial growth inhibition

Vanstaden et al (1980) reported the effect of 2 3 5 7 and 10% propionic acid on known numbers of S typhimurium E coli and Cl perfringens added to or contained in carcass meal E coli was totally inhibited by 2% and S typhimurium was inhibited by 5% propionic acid Total aerobic bacterial count was reduced by about 74 7% and the number of viable clostridia was reduced by 94 05% with 3% propionic acid over 14 days period The increased strength of propionic acid above 5% did not have appreciable additional effect

The antimicrobial activity of a commercial disinfectant stall saure containing 0 5% propionic acid and other organic acids such as acetic formic and citric acid was tested by Winter balder et al (1984) and reported that above 0 625% stall saure had disinfecting effect on E coli P aeruginosa and S faecium

Hinton and Linton (1988) reported that treatment of Salmonella contaminated feed with BPO 12 a mixture containing formic and propionic acid only slightly reduced the rate of isolation of salmonella but treatment of the feed a week prior to contamination gave protection against subsequent recontamination with salmonella

Cherrington et al (1990) reported that incubating cultures of E coli with propionic acid (5 m mol/l) at $p^H 5$ produced was temporary bacteriostasis lasting 30 mt They found the rate of RNA DNA Protein lipid and cell wall synthesis were reduced DNA synthesis was particularly sensitive to the presence of propionic acid

Yamamoto (1990) investigated the addition of 0 2% propionic acid or several other organic acids like acetic adipic lactic malic citric fumaric gluconic tartaric succinic or sorbic acids decreased thermal resistance of Cl botulinum 62A spores by 35-65%

Propionic acid (5m mol/l) at p^H 5 inhibited the DNA synthesis of E coli K12 without physically damaging DNA molecule or starving the cells for essential thymine (Cherrington et al 1991 a)

Cherrington et al (1991 b) reported that 90% of E coli and salmonella species were killed within 1 h of

incubation in propionic acid solution 0 5 0 7 mol/l at pH 5 whereas formic acid solution in the same concentration took 3 h to produce this effect

Studies were conducted on decontamination of meat with sanitizers other than acetic and propionic acids by various investigators Washing the animal with cold or hot water or sprays (Smith and Graham, 1978 Anderson et al 1981 Davey 1989) and application of chlorinated water (Kotula et al , 1981 , Odlaug 1981) were done as a means of sanitizing carcasses High efficacies of sanitizing meat surfaces with lactic acid have been reported (Snijders et al 1979 1985 Woolthuis et al 1984 Smulders and Woolthuis 1985 Woolthuis and Smulders 1985 Smulders et al , 1986 Anderson and Marshall 1990b Dixon et al , 1991 Prasai et al 1991 1992) Antibacterial effect of sorbic acid was studied by TO and Robach 1980 Eklund 1983 Zamora and Zaritsky, 1987 Sayeed and Sankaran 1991 Cox et al 1974 and Thomson et al 1976 used succinic acid as sanitizing agent on carcasses

Sampling Techniques and Diluents

The result of bacteriological examination of carcasses as well as meat is influenced by the method of collection of samples quantity of sample collected the area of sampling diluent used method of processing of samples and the media

used To assess the bacteriological status of carcasses various sampling techniques have been used by many workers Samples have been collected by surface swab technique (Biemuller et al 1973 Kotula et al 1974 Ockerman et al 1974 Roberts et al 1980 Johanson et al 1983 Whelehan et al 1986 Anderson et al 1987 Mendonca et al 1989 Prieto et al 1991) tissue excision method (Anderson et al 1977 Cacciarelli et al 1983 Osthold et al 1984 Acuff et al 1987 Hamby et al 1987 Saoji et al 1990 Okodugha and Aligba, 1991 Jericho et al 1993) agar sausage method (Nortje and Naude 1981 Nortje et al 1989 1990) and membrane filtration method (Bell et al 1986)

A few workers have evaluated the bacterial quality of carcasses by more than one methods Agar contact method and swab technique was compared by Niskanen and Pohja (1977) and reported that swabbing was more preferable for sampling animal carcasses Stolle (1988) used swabbing rinsing and excision methods to evaluate the bacteriological status of the carcasses Fliss et al (1991) compared direct agar contact tissue excision and swab techniques and reported that tissue excision method was most effective in assessing the microbiological quality of carcasses and agar contact technique was least effective

Many investigators have used swab technique for assessing the bacterial quality of carcass and meat but the extent of area covered was not uniform The area swabbed by different workers was 2 5 sq inch (Stringer et al 1969) 8 10 cm^2 (Biemuller et al 1973 Mendonca et al 1989) 12 3 cm^2 (Kotula et al 1974 Ockerman et al 1974) 13 cm^2 (Reynolds and Carpenter 1974) 15 cm^2 (Prieto et al 1991) 40 cm^2 (Stolle et al 1988) 50 cm^2 johanson et al 1983) and 100 cm^2 (Roberts et al 1980 Whelehan et al 1986)

In tissue excision technique the quantity of meat used for evaluating the bacterial quality of carcass and meat by various workers was not uniform The quantity of raw meat used for sampling was 3 5 g (Kondaiah et al 1985) 10 0 g (Gupta et al 1987 Sherikar et al 1989 Okodugha and Aligba, 1991 Saoji et al 1990) and minced meat taken was 25 0 g (Hudson et al 1986)

For bacterial analysis of meat samples various diluents have been used by many investigators A comparative study was made to evaluate the suitability of Butterfield s buffered phosphate solution 0 1% peptone in distilled water 0 5% peptone in distilled water 0 85% NaCl in distilled water and pure distilled water as diluent for total bacterial count estimation by Oblinger and Kennedy (1976) and reported that

Butterfield s phosphate buffer was comparatively better than the others

Most of the investigators used 0 1% peptone water as diluent (Bala et al , 1977 Cacciarelli et al 1983 Acuff et al , 1987 Hamby et al 1987 Anderson and Marshall 1989 1990 Mendonca et al 1989 Okodugha and Aligba 1991 Prieto et al , 1991 Anderson et al 1992 Harris and Stiles 1992 The other diluents used by investigators include normal saline (Biemuller et al 1973 Reynolds and Carpenter 1974 and Saoji et al 1990) and Butterfield s phosphate buffer was used by Dickson, 1991 1992 and Siragusa and Dickson 1992

Materials and Methods

MATERIALS AND METHODS

Meat samples were collected from cattle slaughtered in the university slaughter house following standard procedure for slaughter The dressing of carcass was done off the floor on rails Ten samples each were used for the study on the effect of acetic acid and propionic acid

Collection of meat sample

The samples were collected from the external aspect of the carcasses using sterile precautions The size of the sample was about 300 cm^2 area having a thickness of about 1 5 cm It was further divided and was transported to the laboratory immediately in a sterile galvanised tray covered with its lid for further processing and examination

Processing of sample

Each meat sample was divided nto three equal parts of about 100 cm^2 area using sterile instruments A strip was about 40 0 cm long Two S shaped sterile hooks were attached to the two ends of the meat strips They were labelled as T_1 T_2 and C_A (Treatment 1 Treatment 2 and control)

Acid treatment of samples

a Acetic acid

Of the three strips of a sample one (T_1) was completely dipped in freshly prepared 1% acetic acid solution in distilled water for 15 seconds Similarly strips T_2 was dipped in 2% acetic acid in distilled water and the third strip was kept as control (C_A) without any treatment At the end of this period the strips were taken by holding the hooks and allowed to drain in standing position at room temperature under similar conditions

b Propionic acid

Meat samples were collected in similar manner as above and the strips labelled as T_3, T_4 and C_P (Treatment-3, Treatment-4 and control) T_3 was dipped in 1% and T_4 in 2% propionic acid solution in distilled water respectively for 15 seconds allowed to drain in standing position at room temperature as in the case of acetic acid treatment C_P was kept as control without any treatment

Bacteriological examination

The bacteriological quality of each sample was assessed by determining the total viable count coliform count and

faecal streptococci count of the external surface at 1 5 9 and 24 h after treatment In the case of control the bacterial load was assessed immediately after taking the sample also

Collection of samples for bacteriological examination

An area of 25 cm^2 was exposed with a sterile aluminium template having 5 cm internal measurement This area was swabbed with moist sterile absorbent cotton swab The area was swabbed first in one direction with one side of a swab then at right angles of the original direction with the other side of the swab and finally from one corner to the opposite corner with the tip of the same swab The swab was then transferred into a flask containing 25 0 ml sterile 0 1% peptone water (diluent)

Preparation of sample

The flask with its contents was thoroughly shaken to disperse the bacteria from the swab into the diluent This suspension forms the stock solution From this 10 0 ml was transferred to a flask containing 90 0 ml diluent with a sterile pipette so as to form one in 10 dilution Further ten fold serial dilutions were made in 0 1% peptone water by transferring 1 ml inoculum to 9 ml diluent The inoculations

into the media were made observing sterile precautions and using suitable dilutions depending upon the period of storage types of bacteria and treatment The colony forming units (CFU) were enumerated after incubation For each transfer separate sterile pipette was used

Bacterial count

1 Total viable count·

Total viable count of aerobic organisms was determined according to procedure recommended by American Public Health Association (1976) From the diluted inoculum 1 ml was transferred into duplicate sterile petriplates About 15-20 ml sterile molten standard plate count agar (Composition and method of preparation appended) (Hi-media) maintained at 45°C was poured and mixed with the inoculum by gentle rotatory movement (clockwise anti clock wise, forward and backward) The plates were then left at room temperature for the medium to solidify These plates were incubated at 37°C for 24 h At the end of the incubation period the inoculated plates showing 30 to 300 CFU were selected and these colonies were counted with the help of the colony counter Number of CFU per cm^2 was estimated from the mean colony forming units present in duplicate plates applying the dilution factor and was expressed as $\log_{10}CFU/cm^2$ of the sample

2 Coliform count

The coliform count was carried out according to the method described by Nordic Committee on Food Analysis (1966 No 62 UDC 576 851 48) From the selected ten fold dilution 0 1 ml each of the inoculum was transferred on duplicate Violet Red Bile Agar (VRBA)(Composition and method of preparation appended)(Hi-media) plates and spread evenly by a sterile L shaped glass rod The inoculated plates from each dilution in duplicate were incubated at 37°C for 24 h At the end of the period of incubation the purplish red colonies with a diameter of 0 5 mm or more surrounded by a red precipitation zone were counted The number of CFU/cm^2 of the sample surface was estimated from the mean colony count after applying dilution factor and was expressed as $\log_{10}$ number/cm^2 of the sample surface

3 Faecal streptococcal count·

Faecal streptococcal count was determined following the spread plate technique described by Nordic Committee on Food Analysis (1968 No 68 UDC 576 851 21) KF-Streptococcal Agar (Composition and method of preparation appended) (Hi media) plates were inoculated in duplicate with 0 1 ml of selected dilution from each sample The inoculum was

spread uniformly on the surface of the plates with a sterile L shaped glass rod These inoculated plates were incubated at 37°C for 48 h After the incubation period colonies with pink to dark red colour surrounded by narrow white zone were counted The number of CFU/cm^2 of the sample surface was estimated from the mean colony count after applying dilution factor and was expressed as log_{10}CFU/cm^2 of the sample surface

Statistical Analysis

Data has been analysed statistically using the T test and analysis of variance as per the methods of Snedecor and Cochran (1967)

Results

RESULTS

In the present study separate experiments were conducted to assess the effect of acetic and propionic acid treatments on the bacterial quality of beef stored at ambient temperature The effect of acid treatments at 1% and 2% concentrations on the total viable count (TVC) coliform count and faecal streptococcal count(FS) were analysed at specified periods

Acetic Acid Treatment

The surface bacterial load on beef samples collected immediately after slaughter from ten carcasses were estimated The bacteriological count per centimetre square (cm^2) of each untreated (control) sample was estimated immediately after collection and at 1 h 5 h, 9 h and 24 h of storage at ambient tempeiature Similarly the samples treated with acetic acid 1% (treatment-1) and 2% (treatment-2) stored at ambient temperature were also tested at 1 5 9 and 24 h of storage

Total Viable Count

The mean $\log_{10}$ TVC/cm^2 of control and treatment at different time intervals is shown in Table 1 The difference in counts in controls between zero hour (immediately after slaughter) and 24 h of storage indicated that there was a mean increase of 1 60 $\log_{10}$ CFU/cm^2 In the case of treatment-1 the mean increase in TVC during the period of storage between 1 h and 24 h post treatment was 1 19 $\log_{10}$CFU where as the corresponding increase in TVC in treatment-2 was 1 08

From the above table it can be seen that the mean TVC has increased by 0 17 $\log_{10}$ CFU/cm^2 in the control samples between 0 and 1 h and 0 14 between 1 and 5 h and 0 21 between 5 and 9 h and 1 08 between 9 and 24 h In the case of treatment-1 the increase in mean values were 0 18 between 1 and 5 h 0 16 between 5 and 9 h and 0 85 $\log_{10}$ CFU between 9 and 24 h The corresponding rate of increase in treatment-2 were 0 18 0 15 and 0 75 respectively for intervals between 1 and 5 h 5 and 9 h and 9 and 24 h of treatment

Statistical analysis of the data showed a highly significant ($P < 0\ 01$) difference in TVC between control and

Table 1 Total viable count on acetic acid treated beef stored at ambient temperature

Treatment	Mean $\log_{10}$ CFU/cm^2 ± S E Period of storage in hours				
	0	1	5	9	24
Control	4 85 ± 0 034	5 02^{a} ± 0 016	5 16^{a} ± 0 027	5 37^{a} ± 0 014	6 45^{a} ± 0 006
Acetic Acid 1%		4 69^{b} ± 0 030	4 87^{b} ± 0 026	5 03^{b} ± 0 012	5 88^{b} ± 0 032
Acetic acid 2%		4 55^{c} ± 0 022	4 73^{c} ± 0 032	4 88^{c} ± 0 028	5 63^{c} ± 0 032

Figures bearing the same superscripts do not differ significantly within the columns

Table 1a ANOVA of the effect of 1% and 2% acetic acid treatment and the period of storage on total viable count on beef

Source of variance	d f	Period of storage in hours							
		M S S				F			
		1	5	9	24	1	5	9	24
Treatment	2	0 6013	0 4708	0 6485	1 7734	108 308**	58 961**	173 767**	253 56**
Error	27	0 0056	0 0080	0 0037	0 0070				

* P < 0 05

** P < 0 01

treatments 1 and 2 at all intervals of observation (Table 1a) Similarly there was significant difference in the mean TVC between treatment 1 and 2 the count being significantly lower in treatment 2 than treatment 1 at all time intervals (Table 1)

Figure 1 reveals the trend in bacterial multiplication on the surface of control and treatments over a period of 24 h of storage at ambient temperature It can be seen that the mean increase in TVC in controls depends on the initial bacterial load on the surface at zero hour The progressive increase in TVC was observed at intervals of 1, 5 9 and 24 h of storage due to bacterial multiplication

In treatment 1 and 2 a decline in TVC was observed at 1 h of post treatment At 5, 9 and 24 h of storage TVC was found to increase from that at 1 h However an increasing trend in bacterial multiplication was observed in all samples over the period of storage

The effect of treatment 1 and 2 stored at ambient temperature on TVC is shown in figure 2 The antibacterial effect of acetic acid increased as the strength of the acid increased ie the log per cent decrease of TVC in treatment-2

Figure 1 EFFECT OF ACETIC ACID TREATMENT ON TOTAL VIABLE COUNT ON BEEF STORED AT AMBIENT TEMPERATURE

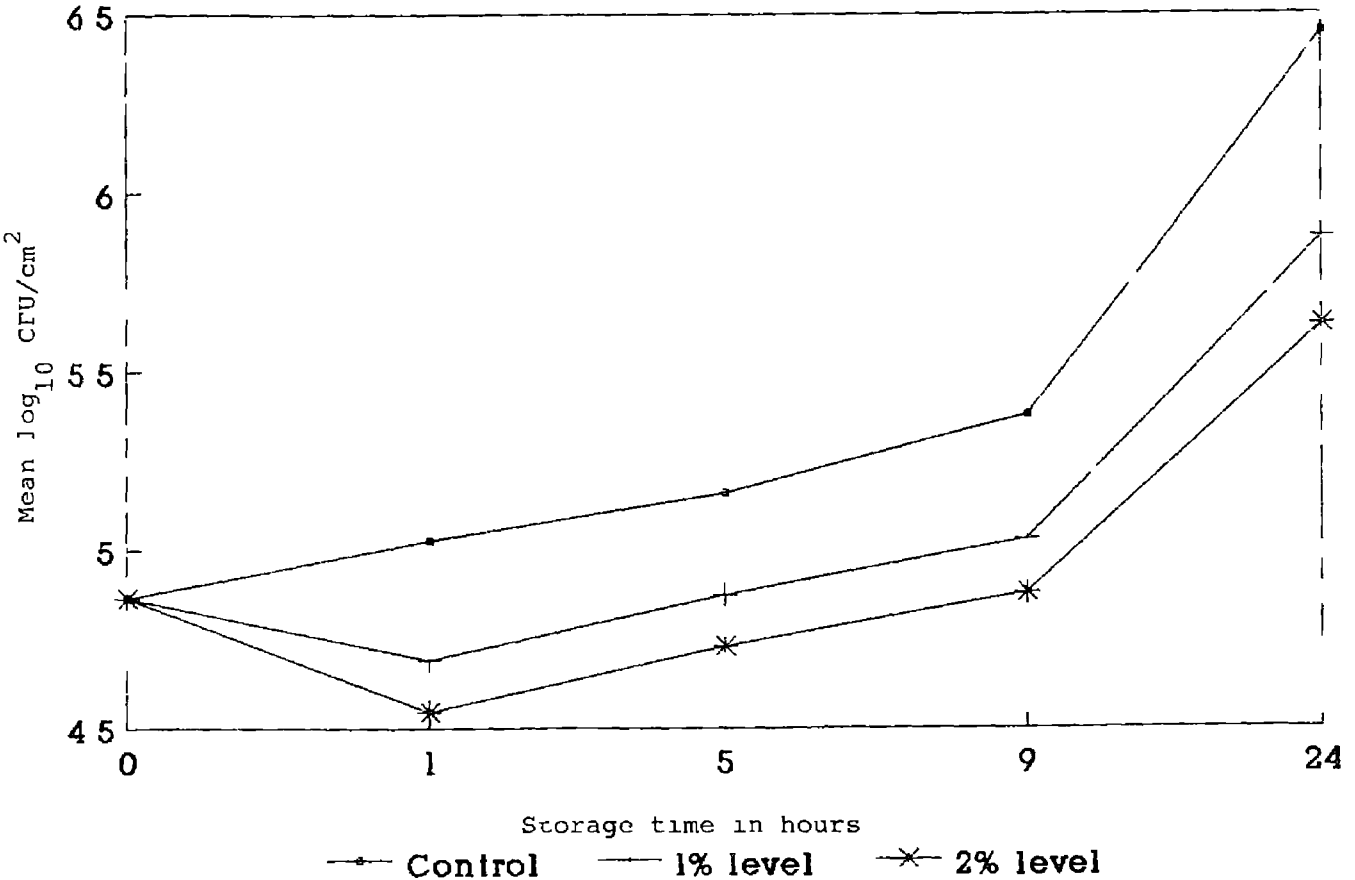
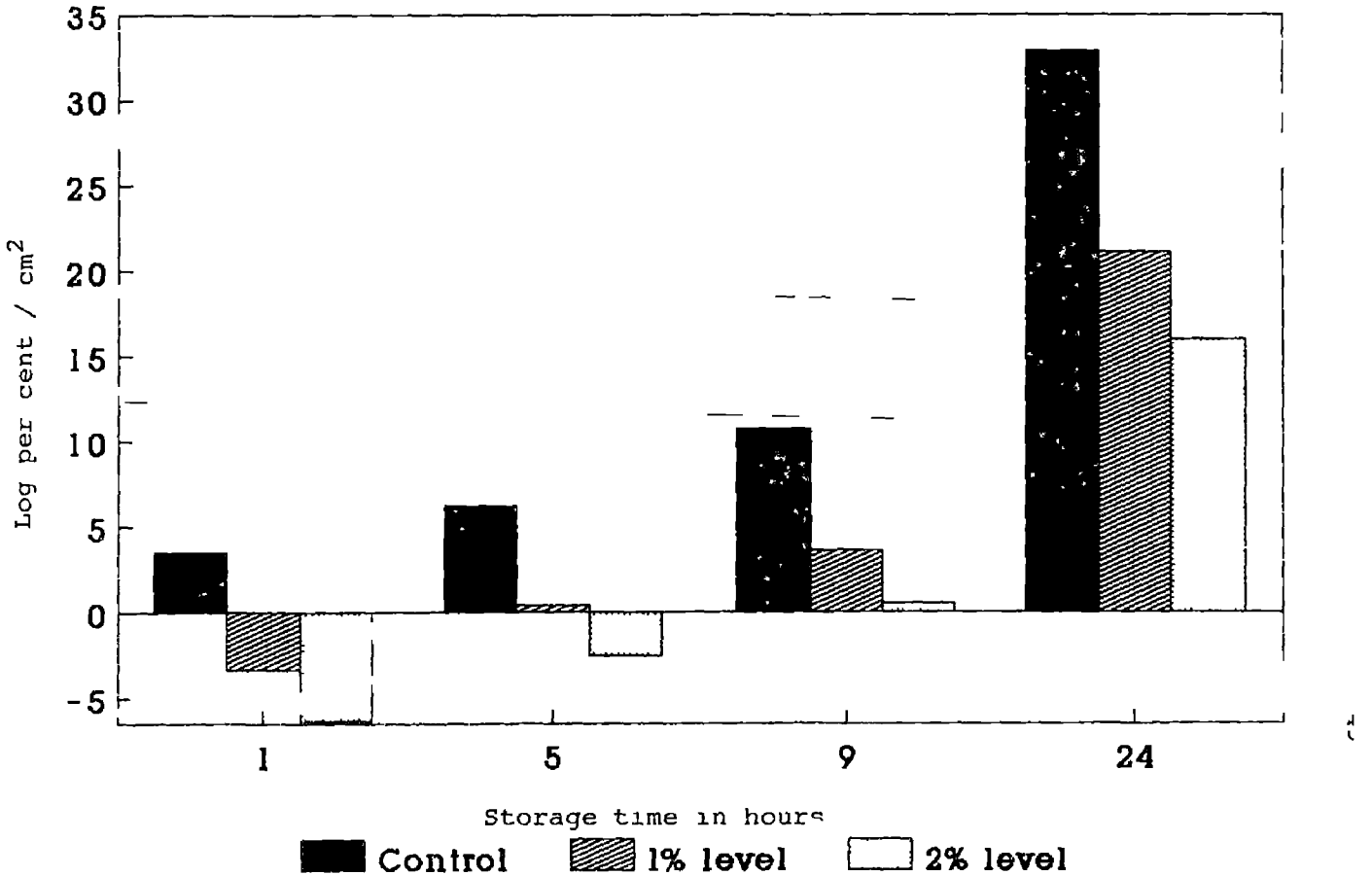


Figure 2 LOG PER CENT CHANGE IN TOTAL VIABLE COUNT ON ACETIC ACID TREATED BEEF STORED AT AMBIENT TEMPERATURE



ın comparıson to treatment-1 was hıgher The fıgure 2 also revealed that the effect of acetıc acıd treatment remaın for longer duratıon as the concentratıon ıncreases

At 1 h of storage TVC ın control sample ıncreased by 3 54 per cent of the $\log_{10}$ CFU count at 0 h In the above perıod the TVC ın treatments 1 and 2 reduced by 3 32 and 6 33 per cent respectıvely In the control sample at 5 h of storge TVC ıncreased by about 6% $\log_{10}$ CFU from that at 0 h whıle ıt was lower by 0 41% ın treatment-1 and 2 53% ın treament-2 The percentage ıncrease of TVC was 10 76, 3 59 and 0 54 ın the control, treatment-1 and 2 respectıvely at 9 h of storage The correspondıng ıncrease ın TVC at 24 h of storge was about 33 ?1 and 16 ın control treatment 1 and 2

The treatment-1 took 5 h to reach equal to or a lıttle above the ınıtıal TVC level of the control, whereas ın treatment-2 ıt was 9 h

Colıform Count

The mean colıform count on the surface of meat samples ın control, treatment-1 and 2 at specıfıed tıme ıntervals are shown ın Table 2 The ınıtıal count of colıforms per square centımetre averaged 2 67 $\log_{10}$ CFU and ıt reached 4 04 $\log_{10}$ CFU at storage at ambıent temperature for 24 h Thus showıng

Table 2 Coliform count on acetic acid treated beef stored at ambient temperature

Treatment	Mean $\log_{10}$ CFU/cm^2 ± S E Period of storage in hours				
	0	1	5	9	24
Control	2 67 ± 0 044	2 71^{a} ± 0 027	2 93^{a} ± 0 020	3 11^{a} ± 0 032	4 04^{a} ± 0 012
Acetic acid 1%		2 44^{b} ± 0 030	2 64^{b} ± 0 035	2 83^{b} ± 0 023	3 78^{b} ± 0 037
Acetic acid 2%		1 93^{c} ± 0 058	2 34^{c} ± 0 043	2 55^{c} ± 0 044	3 43^{c} ± 0 039

Figures showing the same superscripts do not differ significantly within the columns

Table 2a ANOVA of the effect of 1% and 2% acetic acid treatment and the period of storage on colirorm count on beer

Source of Variance	d f	Period of storage in hours							
		M S S				F			
		1	5	9	24	1	5	9	24
Treatment	2	1 7982	0 8674	0 7699	0 9314	108 149**	74 993**	66 313**	91 659**
Error	27	0 0166	0 0116	0 0116	0 0102				

* P < 0 05
** P < 0 01

a mean increase of 1 37 $\log_{10}$ CFU Between 1 h and 24 h the increase in coliform in control was 1 27 $\log_{10}$ In treatment-1 the average increase in coliform count during the period between 1 and 24 h was 1 54 and corresponding value in treatment-2 was 1 50

The mean $\log_{10}$ increase in coliform count per square centimetre in controls were 0 16 0 18 and 0 93 during the period of storage between 1 and 5 h, 5 and 9 h and 9 and 24 h respectively In treatment 1 corresponding values were 0 20, 0 19 and 0 95 In treatment-2 the increase in count were 0 41, 0 21, 0 88 $\log_{10}$ between 1 and 5 h, 5 and 9 h and 9 and 24 h respectively

The results of statistical analysis of the data are shown in Table 2a There was highly significant difference in coliform count ($P < 0\ 01$) of control and treatment-1 and 2 samples at all intervals of storge from 1 to 24 h The significant difference in counts of control and treatments were indicated with the help of superscripts in the column of Table 2 It can be seen that the treated samples have low coliform count as compared to the controls Similarly there was significant difference between treatment-1 and 2 that means the coliform count was significantly low in treatment 2 than that of treatment 1

The trend of multiplication of coliform bacteria in both treatment 1 and 2 and control at specified period of intervals is shown in figure 3 It was observed that a progressive multiplication of coliform has taken place on the surface of beef samples collected immediately after slaughter and stored at ambient temperature over the period of 24 h In the case of treatment 1 an initial reduction of coliform was observed at 1 h Thereafter the $\log_{10}$ cycle increase was slow till 9 h of storage and the increase was steady thereafter (between 9 and 24 h) In the case of treatment-2 the reduction in coliform count was higher than that of treatment-1 at 1 h of storage The $\log_{10}$ cycle increase in count was rapid between 1 and 5 h and thereafter rate of growth appears to be slow upto 9 h Between 9 and 24 h the $\log_{10}$ cycle increased rapdily on the sample surface

The effect of acetic acid treatment of beef samples with respect to coliform count during the period of storage at ambient temperature is shown in figure 4 It is seen that there was a progressive increase in $\log_{10}$ per cent in controls (untreted) at all stages of storage The lethal and inhibitory effect of acetic acid varies depending on its concentration and the period of storage In control sample at 1 h of storage there was 4 log per cent increase in coliform count per cm^2 in comparison to the initial counts at

Figure 3 EFFECT OF ACETIC ACID TREATMENT ON COLIFORM COUNT ON BEEF STORED AT AMBIENT TEMPERATURE

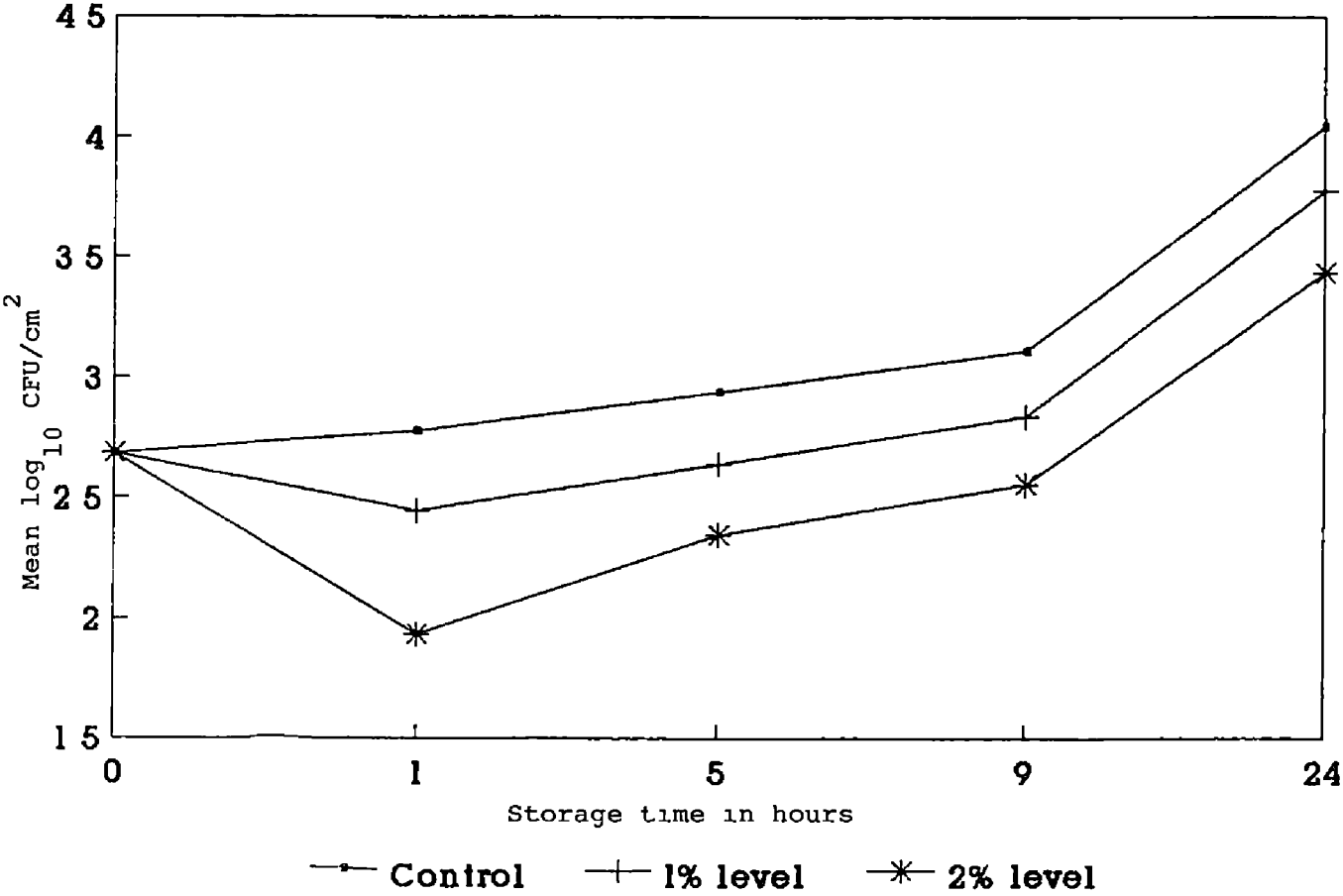
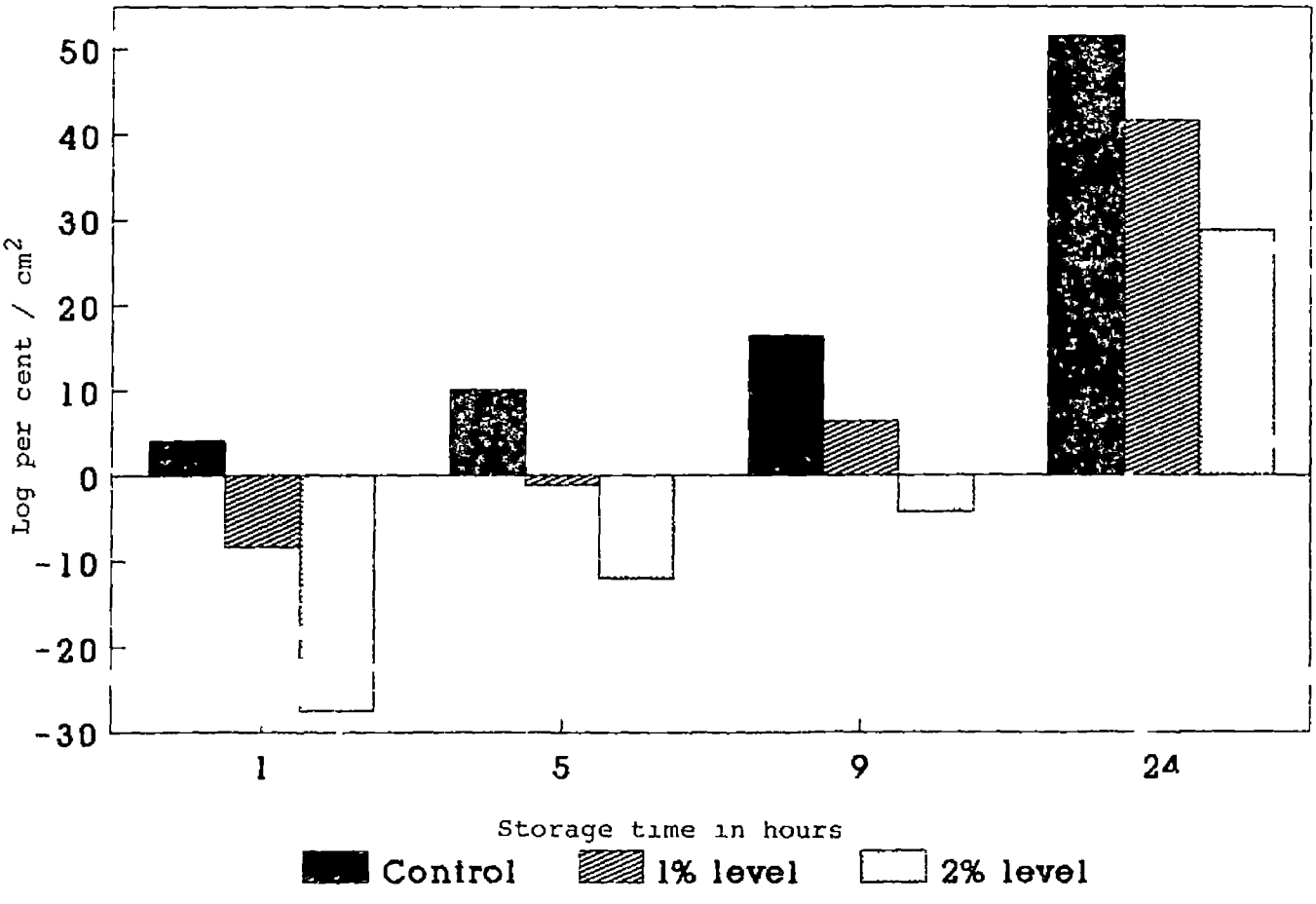


Figure 4 LOG PER CENT CHANGE IN COLIFORM COUNT ON ACETIC ACID TREATED BEEF STORED AT AMBIENT TEMPERATURE



0 h In the case of treated samples there was decrease in log per cent in coliform counts and the rates of decrease were 8 4 log per cent in treatment-1 and 27 53% in treatment-2 At 5 h of storage the increase in coliform count in control was about 10 log per cent whereas in treatments reduction in log per cent was 1 13 for treatment-1 and 12 04 in treatment-2 in comparison to the counts at 0 h At 9 h in control the log per cent increase in coliform was 16 54 whereas it was 6 30 in treatment-1 The count of coliform in treatment 2 at 9 h of storage was 4 28% log less than the coliform count at 0 h At 24 h of storge all samples haveshown higher values of coliform and the rate of increase when compared to the count at 0 h were 51 61, 41 64 and 28 77 per cent log respectively for control, treatment-1 and treatment 2

Faecal Streptococcal Count

The mean $\log_{10}$ value of faecal streptococci counts (FS) per square centimetre, On the surface of control, treatment-1 and treatment-2 samples, stored at ambient temperature for 24 h are shown in Table 3 A gradual increase in FS count over the period of 24 h of storage was observed The mean increase in count in control samples from

Table 3 Faecal streptococcal count on acetic acid treated beef stored at ambient temperature

Treatment	Mean log$_{10}$ CFU/cm^2 ± S E Period of storage in hours				
	0	1	5	9	24
Control	2 42 ± 0 02a	2 53^{a} ± 0 030	2 69^{a} ± 0 035	2 90^{a} ± 0 032	3 84^{a} ± 0 047
Acetic acid 1%		2 16^{b} ± 0 043	2 41^{b} ± 0 033	2 64^{b} ± 0 044	3 45^{b} + 0 038
Acetic acid 2%		1 81^{c} ± 0 051	2 14^{c} ± 0 047	2 38^{c} ± 0 036	2 92^{c} ± 0 016

Figures showing the same superscripts do not differ significantly within the columns

Table 3a ANOVA of the effect or 1% and 2% acetic acid treatment and the period of storage on faecal streptococci count on beef

Source of Variance	d f	Period of storage in hours							
		M S S				F			
		1	5	9	24	1	5	9	24
Treatment	2	1 2879	0 7632	0 6732	2 1581	71 395**	50 599**	47 470**	166 351**
Error	27	0 0180	0 0151	0 0142	0 0130				

* P < 0 05

** P < 0 01

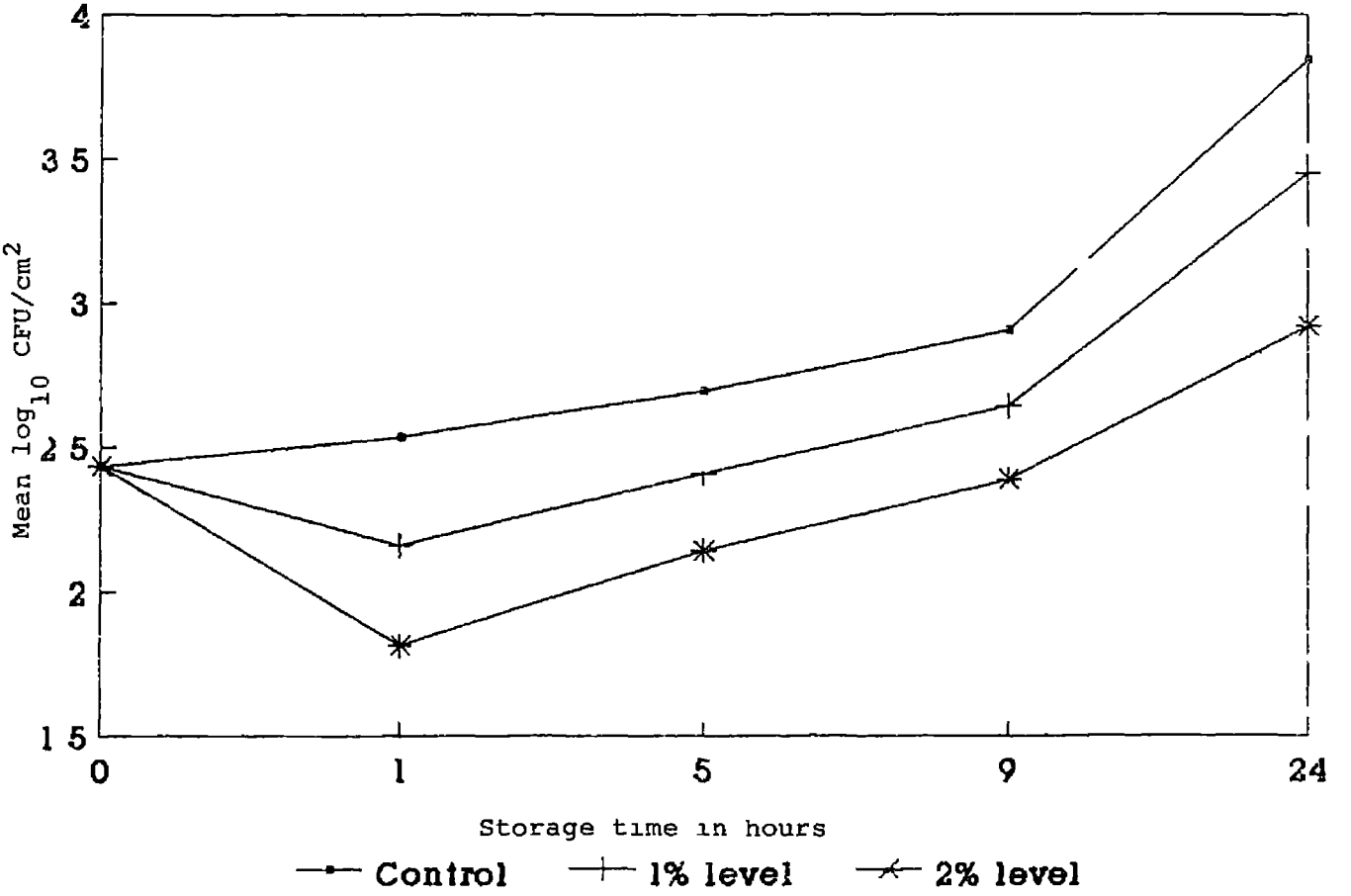
0 h to 24 h was 1 42 $\log_{10}$ CFU/cm^2 The mean $\log_{10}$ increase during the period between 1 and 5 h 5 and 9 h, and 9 and 24 h were 0 16, 0 21 and 0 94 $\log_{10}/cm^2$ respectively In treatment 1 mean increase in FS count between 1 and 24 h of storage was 1 29 CFU/cm^2 During storage the mean increase in count was 0 25 between 1 and 5 h Between 5 and 9 h, 9 and 24 h of storage the mean increase were 0 23 and 0 81 respectively

In treatment-2 the increase in FS count over 24 h of storage was 1 11 $\log_{10}$ CFU/cm^2 At intervals of 1 and 5 h 5 and 9 h and 9 and 24 h the increase in FS count were 0 33 0 24 and 0 54 $\log_{10}$ CFU/cm^2 respectively

The results were analysed statistically and shown in Table 3a It can be seen that there was highly significant difference ($P < 0\ 01$) in FS count at all periods of storage between samples of control and treatments and between treatment-1 and treatment-2 The significant difference is indicated with the help of superscripts in the columns of the Table 3

The trend of growth of FS on the beef samples under control and treatments stored at ambient temperature is shown in figure 5 The multiplication of FS dependent on the

Figure 5 **EFFECT OF ACETIC ACID TREATMENT ON FAECAL STREPTOCOCCAL COUNT ON BEEF STORED AT AMBIENT TEMPERATURE**

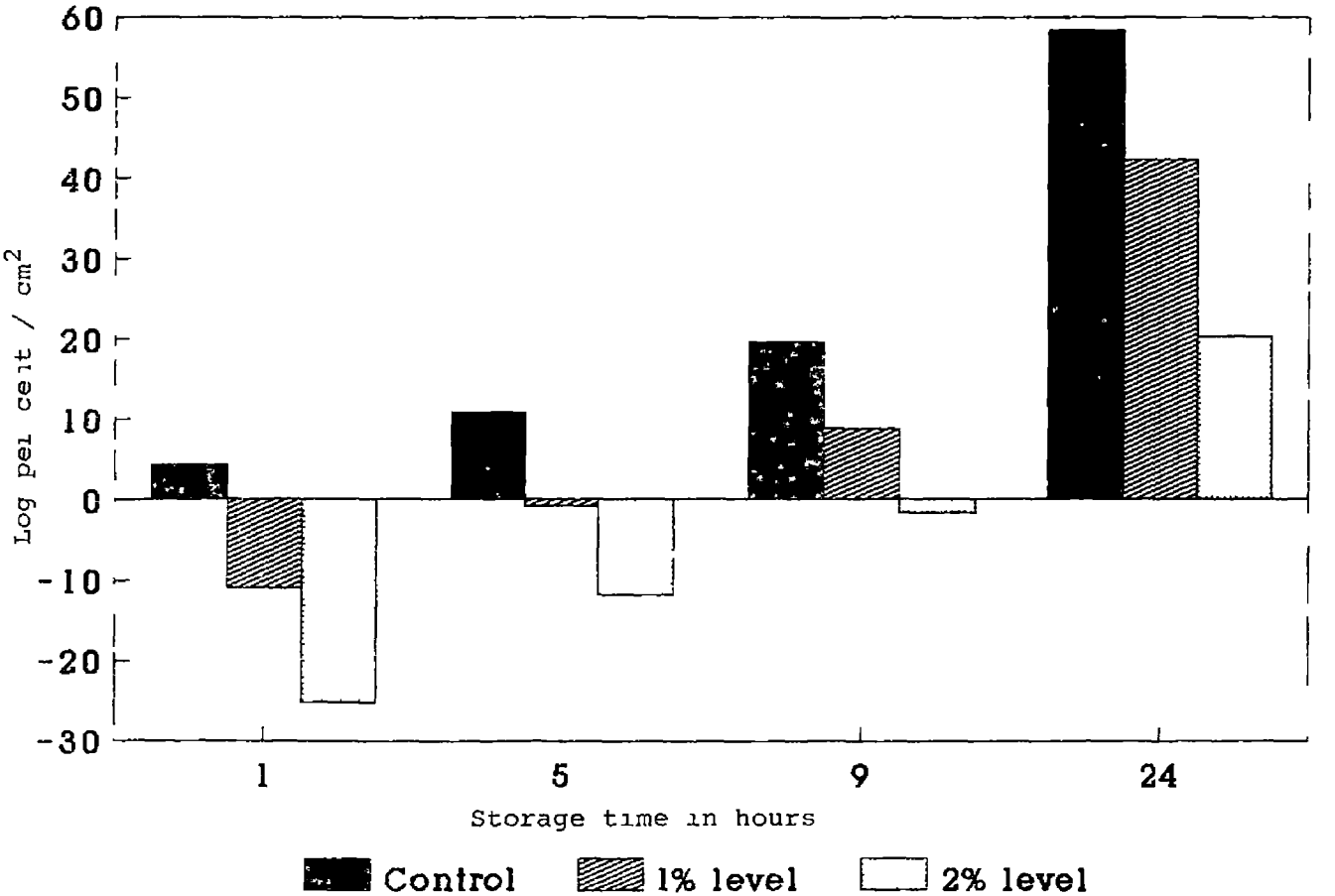


initial (0 h) load of the organism In the case of treatments the trend of multiplication was dependent on the number of viable organisms that remain in the samples after treatment

The effect of treatments on FS over a period of 24 h of storage at ambient temperature is shown in figure 6 Bactericidial/bacteriostatic effect of acetic acid on FS dependent on its concentration and duration of storage It was directly proportional to the concentration and inversely proportional to the duration of storage After 1 h of storage the increae in log per cent of FS in control sample was found to be 4 41 mean $\log_{10}$ CFU/cm^2 At 5 h further increase to 10 97 and at 9 h 19 68 and at 24 h it has reached 58 50% of the initial count

In the case of treatment-1 there was a reduction in FS count of 10 97 log per cent at 1 h and 0 78 log % at 5 h The count increased by 8 83 at 9 h and 42 29% at 24 h In treatment-2 the FS count was found to be reduced till 9 h of storage The per cent log reduction values were 25 21% at 1 h, 11 84% at 5 h and 1 73% at 9 h in comparison to the FS count at 0 h At 24 h the FS count in treatment 2 has increased by 20 34 log per cent than the count at 0 h

Figure 6 LOG PER CENT CHANGE IN FAECAL STREPTOCOCCAL COUNT ON ACETIC ACID TREATED BEEF STORED AT AMBIENT TEMPERATURE



Propionic Acid Treatment

The surface bacterial load on beef samples collected immediately after slaughter from ten carcasses were estimated The bacteriological count per square centimetre of each untreated (control) sample was estimated immediately after collection and at 1 h, 5 h 9 h and 24 h of storage at ambient temperature Similarly the samples treated with propionic acid 1% (treatment-3) and 2% (treatment-4) stored at ambient temperature were also tested at 1, 5 9 and 24 h of storage

Total Viable Count

The mean $\log_{10}$ TVC/cm^2 of control and treatments at different time interval is shown in Table 4 The difference in counts in controls between zero hour (immediately after slaughter) and 24 h of storage indicated that there was a mean increase of 1 67 $\log_{10}$ CFU/cm^2 In the case of treatment-3 the mean increase in TVC during the period of storage between 1 h and 24 h post treatment was 1 45 $\log_{10}$ CFU whereas the corresponding increase in TVC in treatment-4 was 1 12

From the above table it can be seen that the mean TVC has increased by 0 18 $\log_{10}$ CFU/cm^2 in the control sample

Table 4 Total viable count on propionic acid treated beef stored at ambient temperature

Treatment	Mean $\log_{10}$ CFU/cm^2 ± S E				
	Period of storage in hours				
	0	1	5	9	24
Control	4 77 ± 0 025	4 95^{a} ± 0 014	5 28^{a} ± 0 022	5 43^{a} ± 0 008	6 44^{a} ± 0 005
Propionic acid- 1%		4 64^{b} ± 0 018	4 96^{b} ± 0 008	5 10^{b} ± 0 008	6 09^{b} ± 0 020
Propionic acid- 2%		4 49^{c} ± 0 014	4 69^{c} ± 0 025	4 92^{c} ± 0 007	5 60^{c} ± 0 020

Figures showing the same superscripts do not differ significantly within the columns

Table 4a ANOVA of the erfect of 1% and 2% propionic acid treatment and the period of storage on total viable count on beef

Source of Variance	d f	Period of storage in hours							
		M S S				F			
		1	5	9	24	1	5	9	24
Treatment	2	0 5758	0 8661	0 6591	1 8088	233 901**	220 697**	1108 54**	661 277**
Error	27	0 0025	0 0039	0 0006	0 0027				

* P < 0 05

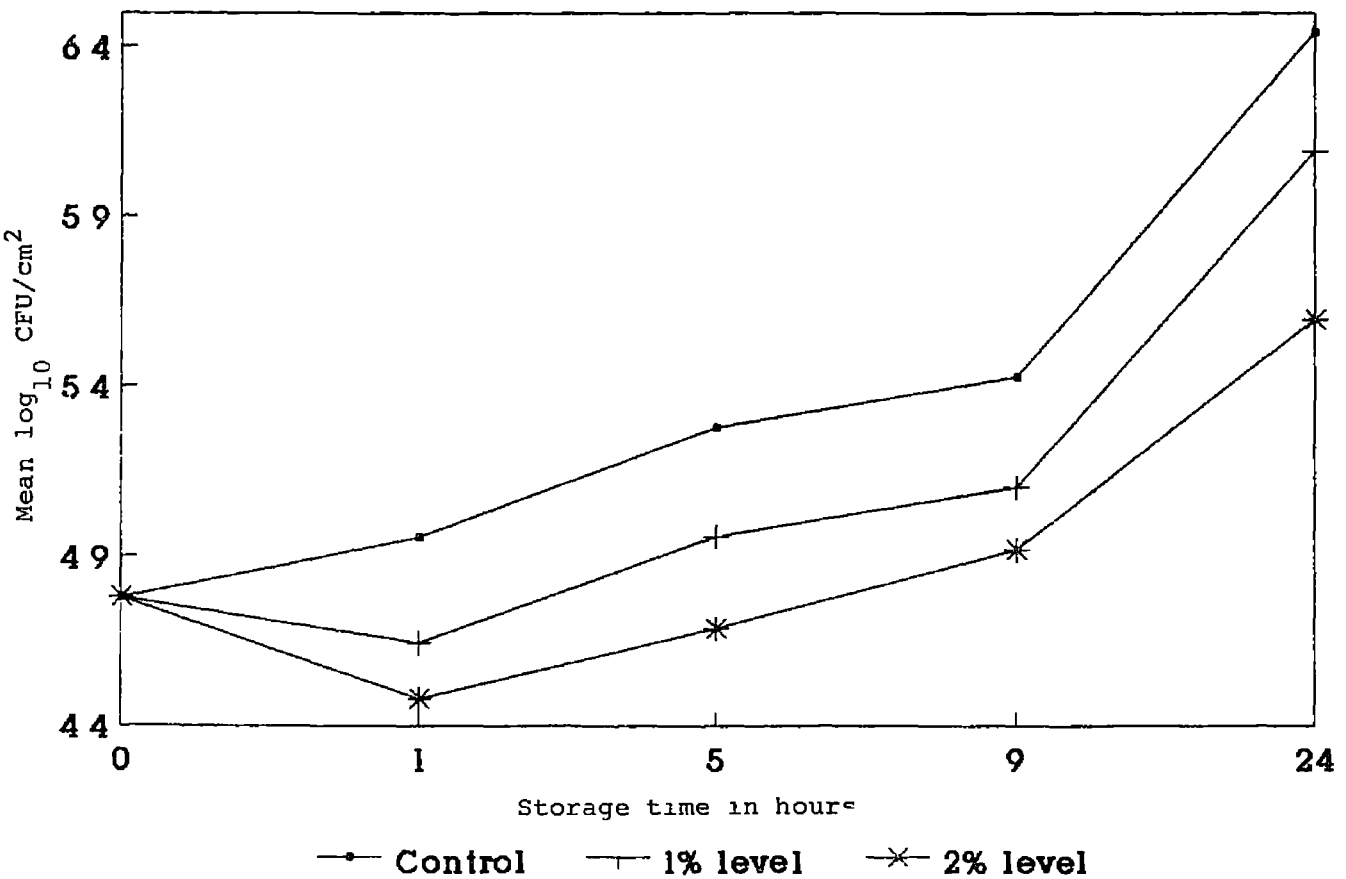
** P < 0 01

between 0 and 1 h and 0 33 between 1 and 5 h and 0 15 between 5 and 9 h and 1 01 between 9 and 24 h In the case of treatment-3 the increase in mean values were 0 32 between 1 and 5 h, 0 14 between 5 and 9 h and 0 99 $\log_{10}$ CFU between 9 and 24 h The corresponding rate of increase in treatment 4 were 0 21, 0 23 and 0 68 $\log_{10}$ CFU/cm^2 respectively for intervals between 1 and 5 h, 5 and 9 h and 9 and 24 h of treatment

Statistical analysis of the data showed a highly significant ($P < 0\ 01$) difference in TVC between control and treatments 3 and 4 at all intervals of observation (Table 4a) Similarly there was significant difference in the mean TVC between treatments 3 and 4 being significantly lower in treatment-4 than treatment-3 at all time intervals (Table 4)

Figure 7 reveals the trend in bacterial multiplication on the surface of control and treatments over a period of 24 h of storage at ambient temperature It can be seen that a progressive increase in TVC in control samples during the period of storage due to bacterial multiplication In treatments 3 and 4 a decline in TVC was observed at 1 h post treatment At 5, 9 and 24 h of storage TVC was found to increase from that at 1 h However an increasing trend in bacterial multiplication was observed in all samples over the period of storage

Figure 7 EFFECT OF PROPIONIC ACID TREATMENT ON TOTAL VIABLE COUNT ON BEEF STORED AT AMBIENT TEMPERATURE

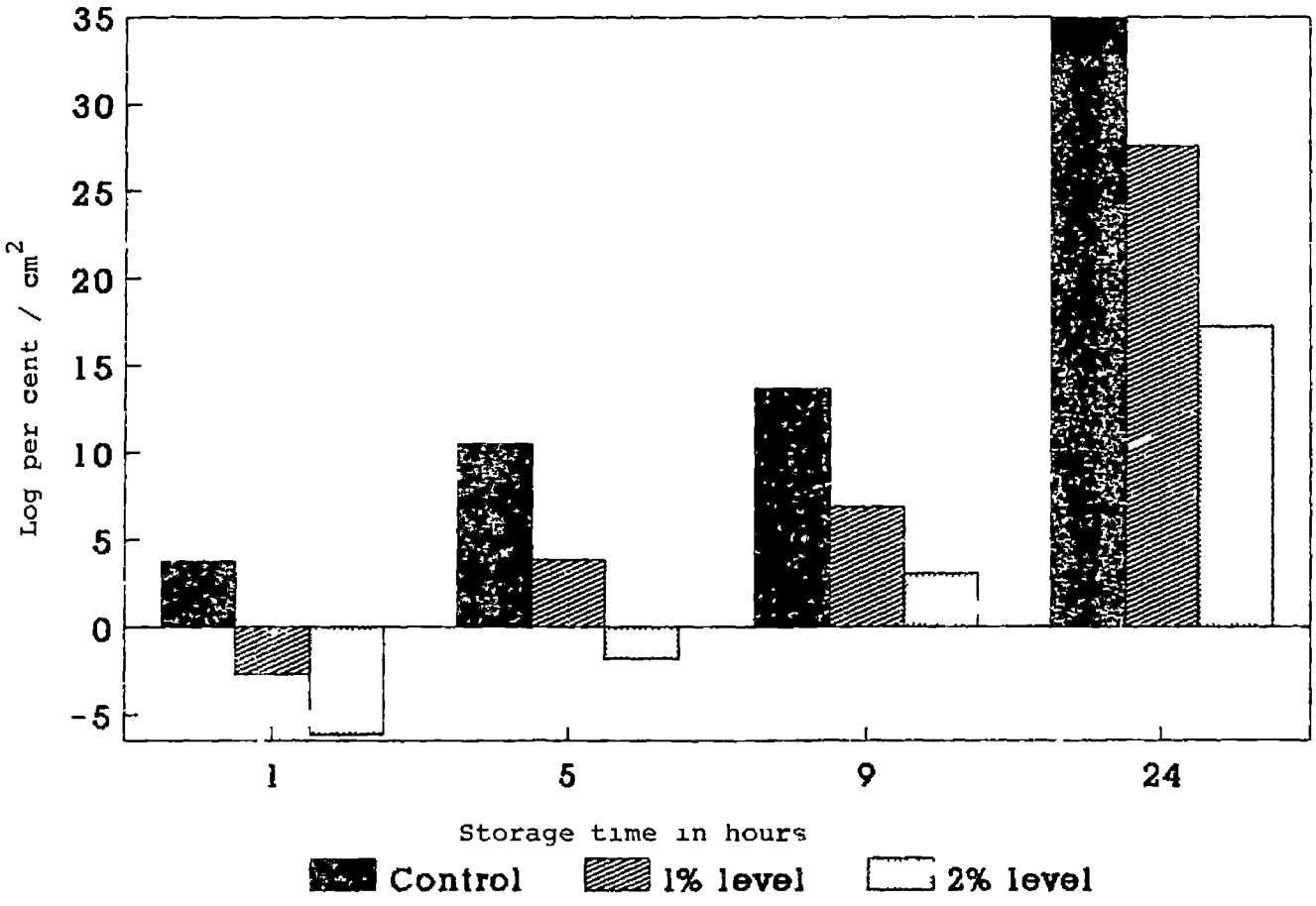


The effect of treatment 3 and 4 on samples stored at ambient temperature on TVC is shown in figure 8 The antibacterial effect of propionic acid increased as the strength of the acid increased ie the log per cent decrease of TVC in treatment-4 in comparison to treatment-3 was higher The figure 8 also revealed that the effect of propionic acid remains for longer duration as the concentration increases At 1 h of storage TVC in control sample increased by 3 77 log per cent in comparison to the initial count at 0 h In the case of treatments there was decrease in log per cent in TVC which were 2 70 log per cent in treatment-3 and 6 14 in treatment-4 At 5 h of storage the increase in TVC in control was about 10 log per cent and in treatment-3 was 3 83 whereas in treatment-4 reduction in log per cent was 1 82 in comparison to the counts at 0 h At 9 h in control the log per cent increase in TVC was 13 66 whereas it was 6 85 in treatment-1 and 3 04 in treatment-2 At 24 h of storage all samples have shown higher values of TVC and the rate of increase when compared to the count at 0 h were 34 94, 27 59 and 17 22 per cent log respectively for control treatment-3 and treatment 4

Coliform Count

The mean coliform counts on surface of meat samples in control treatments-3 and 4 at specified time intervals are

Figure 8 LOG PER CENT CHANGE IN TOTAL VIABLE COUNT ON PROPIONIC ACID TREATED BEEF STORED AT AMBIENT TEMPERATURE



shown in Table 5 The initial count of coliform per cm^2 average 2 47 log_{10} CFU and it reached 4 12 log_{10} CFU at storage at ambient temperature for 24 h thus showing a mean increase of 1 65 log_{10} CFU Between 1 h and 24 h the increase in coliform in control was 1 45 In treatment-3 the average increase in coliform count during the period between 1 and 24 h was 1 57 and corresponding value in treatment-4 was 1 48

The mean log_{10} increase in coliform count per square centimetre in controls were 0 20, 0 20 and 1 05 during the period of storage between 1 and 5 h, 5 and 9 h and 9 and 24 h respectively In treatment-3 the corresponding values were 0,22 0 24 and 1 11 In treatment-4 the difference were 0 39 0 25 and 0 84 log_{10} CFU between 1 and 5 h, 5 and 9 h and 9 and 24 h respectively

The results of statistical analysis of the data are shown in Table 5a There was highly significant difference ($P < 0\ 01$) in coliform count of control and treatment-3 and 4 samples at all intervals of storage from 1 to 24 h The significant differnce in counts of control and treatments were indicated with the help of superscripts in the columns of Table 5 It can be seen that the treated samples have low coliform count as compared to the controls Similarly there was significant difference betwen treatment-3 and 4 that

Table 5 Coliform count on propionic acid treated beef stored at ambient temperature

Treatment	Mean $\log_{10}$ CFU/cm^2 ± S E Period of storage in hours				
	0	1	5	9	24
Control	2 47 ± 0 020	2 67^{a} ± 0 015	2 87^{a} ± 0 008	3 07^{a} ± 0 014	4 12^{a} ± 0 017
Propionic acid - 1%		2 44^{b} ± 0 018	2 66^{b} ± 0 012	2 90^{b} ± 0 008	4 01^{b} ± 0 003
Propionic acid - 2%		2 06^{c} ± 0 022	2 45^{c} ± 0 025	2 70^{c} ± 0 027	3 54^{c} ± 0 048

Figures showing the same superscripts do not differ significantly within the columns

Table 5a ANOVA of the effect of 1% and 2% propionic acid treatment and the period of storage on coliform count on beef

Source of Variance	d f	Period of storage in hours							
		M S S				F			
		1	5	9	24	1	5	9	24
Treatment	2	0 9612	0 4436	0 3494	0 9382	279 450 **	156 704 **	105 659 **	109 494 **
Error	27	0 0034	0 0028	0 0033	0 0086				

* P < 0 05
** P < 0 01

means the coliform count was significantly low in treatment 4 than that of treatment 3

The trend of multiplication of coliform bacteria in both treatment-3 and 4 and control at specified periods of intervals in shown in figure 9 It was observed that a progressive multiplication of coliform has taken place on the surface of beef samples collected immediately after slaughter and stored at ambient temperature over the period of 24 h In the case of treatment-3 an initial reduction of coliform was observed at 1 h Thereafter the $\log_{10}$ cycle increase was slow till 9 h of storage and the increase was steady In the case of treatment-4 the reduction in coliform count was higher than that of treatment-3 at 1 h of storage A very rapid multiplication of coliform was noticed in treatment-4 during the period between 1 and 5 h of storage and the trend of multiplication of the organism from 5 to 24 h of storage was similar to that of control

The effect of propionic acid treatment of beef samples with respect to coliform count during the period of storage at ambient temperature is shown in figure 10 It was seen that there was a progressive increase in $\log_{10}$ per cent in controls at all stages of storage The lethal and inhibitory effect of propionic acid varies depending on its

Figure 9 EFFECT OF PROPIONIC ACID TREATMENT ON COLIFORM COUNT ON BEEF STORED AT AMBIENT TEMPERATURE

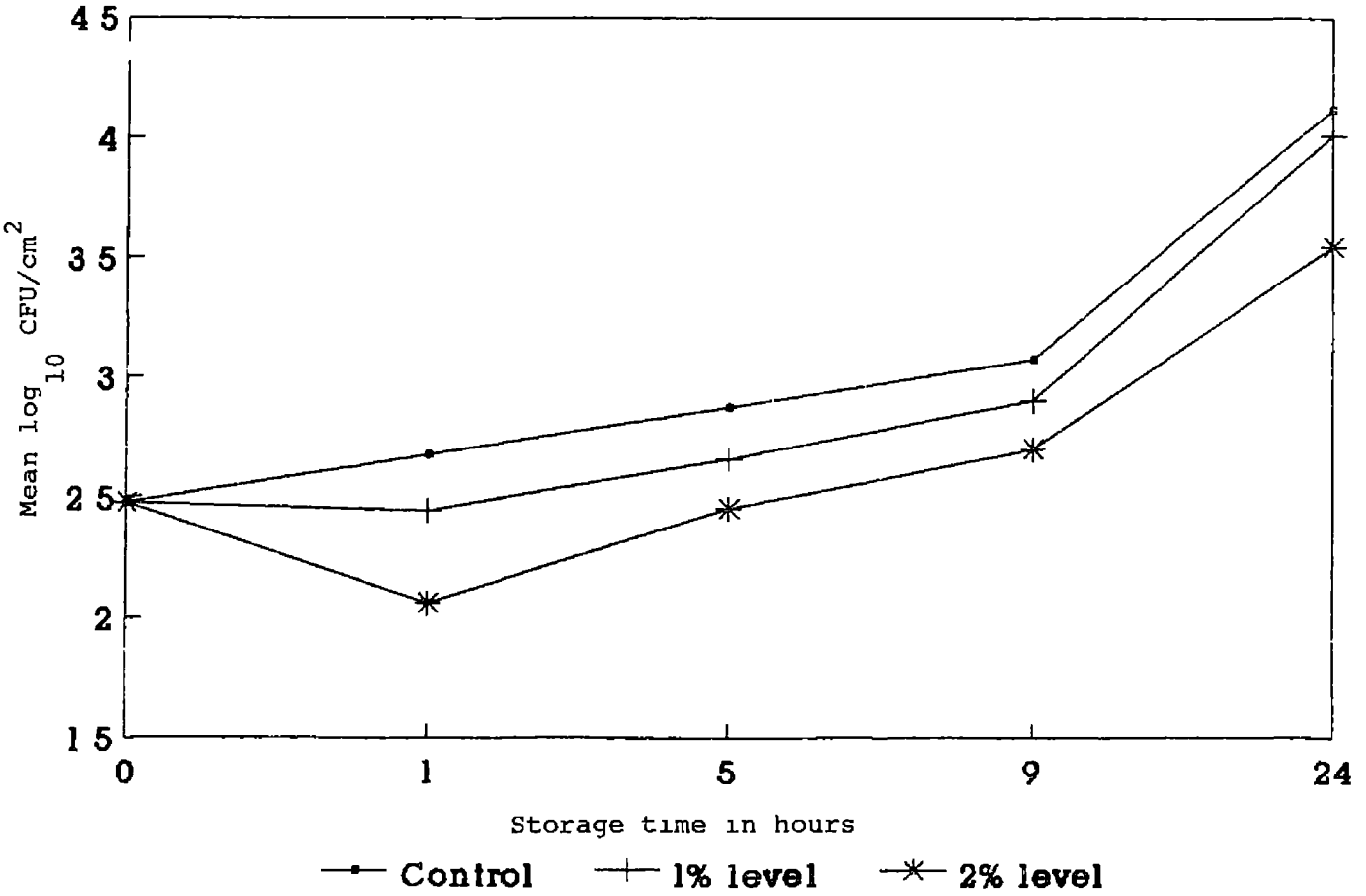
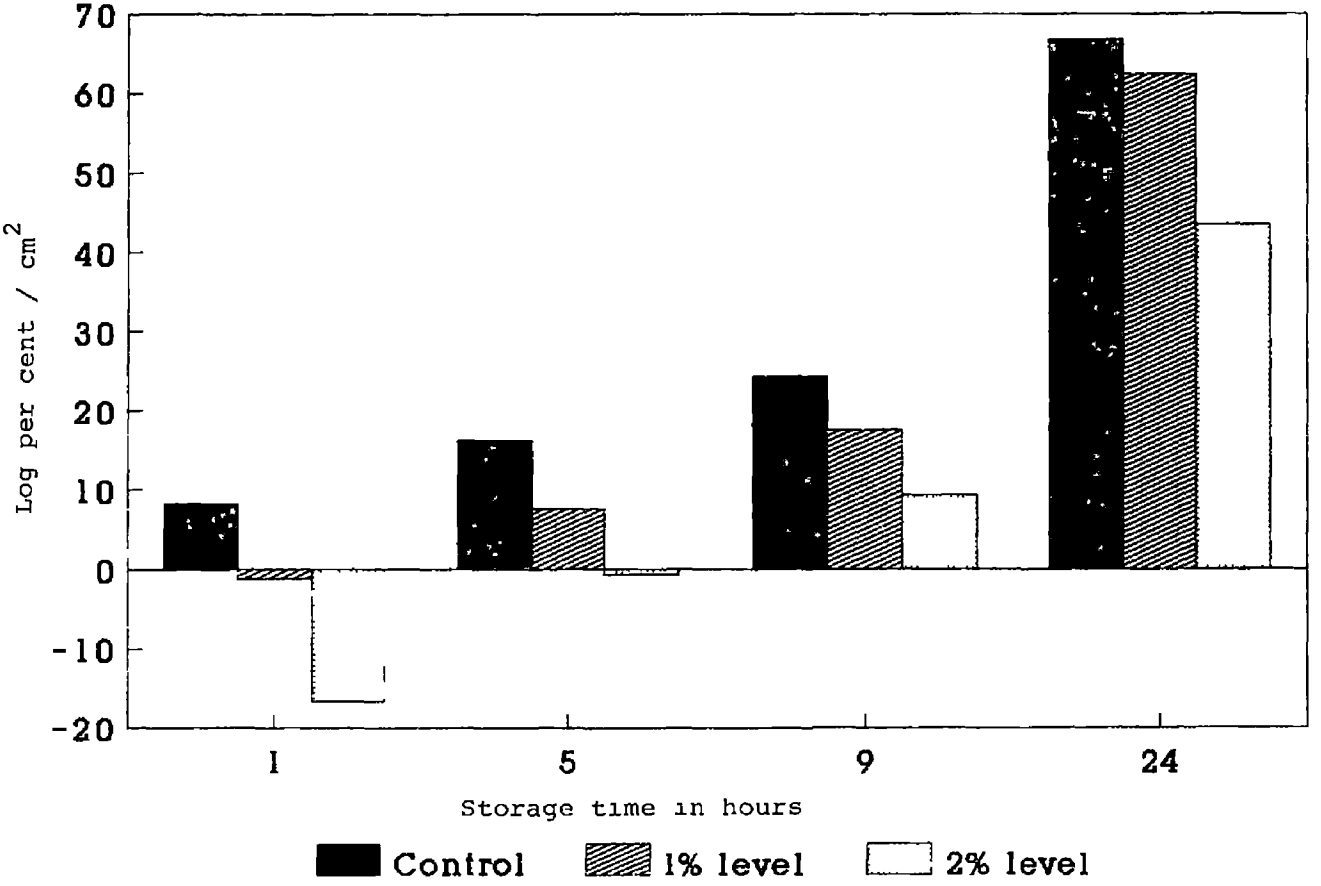


Figure 10 LOG PER CENT CHANGE IN COLIFORM COUNT ON PROPIONIC ACID TREATED BEEF STORED AT AMBIENT TEMPFRATURE



concentration and the period of storage In control sample at 1 h of storage there was 8 26 log per cent increase in coliform count per square centimetre in comparison to the initial count at 0 h In the case of treatments there was decrease in log per cent in coliform count and the rates of decrease were 1 21 log per cent in treatment-3 and 16 64 log per cent in treatment-4 At 5 h of storage the increase in coliform count in control was 16 28 log per cent and in treatment-3 was 7 53 whereas in treatment-4 the reduction in log per cent was 0 77 in comparison to the counts at 0 h At 9 h in control the log per cent increase in coliform count was 24 33 whereas it was 17 41 in treatment-3 and 0 23 in treatment-4 At 24 h of storage all samples has shown higher values of coliform and the rate of increase when compared to the count at 0 h were 66 84, 62 43 and 43 48 log per cent respectively for control, treatment-3 and treatment-4

Faecal Streptococcal Count:

The mean $\log_{10}$ value of faecal streptococcal counts (FS) per cm^2 on the surface of control treatment 3 and treatment 4 samples stored at ambient temperature for 24 h are shown in Table 6 Samples collected immediately after slaughter showed a mean faecal streptococcal count of 2 39

Table 6 Faecal streptococcal count on propionic acid treated beef stored at ambient temperature

Treatment	Mean $\log_{10}$ CFU/cm^2 ± S E				
	Period of storage in hours				
	0	1	5	9	24
Control	2 39 ± 0 020	2 53^{a} ± 0 023	2 91^{a} ± 0 016	2 98^{a} ± 0 017	3 73^{a} ± 0 033
Propionic acid 1%		2 18^{b} ± 0 042	2 62^{b} ± 0 026	2 89^{b} ± 0 017	3 44^{b} ± 0 033
Propionic acid 2%		1 95^{c} ± 0 025	2 35^{c} ± 0 028	2 66^{c} ± 0 041	2 89^{c} ± 0 018

Figures showing the same superscripts do not differ significantly within the columns

Table 6a ANOVA of the effect of 1% and 2% propionic acid treatment and the period of storage on faecal streptococci count in beef

Source of Variance	d f	Period of storage in hours			
		M S S			F
Treatment	2	0 8452	0 7617	0 2779	1 7928
					**
					86 831
					**
					133 964
					**
					36 706
					**
					211 602
Error	27	0 0097	0 0057	0 0076	0 0085

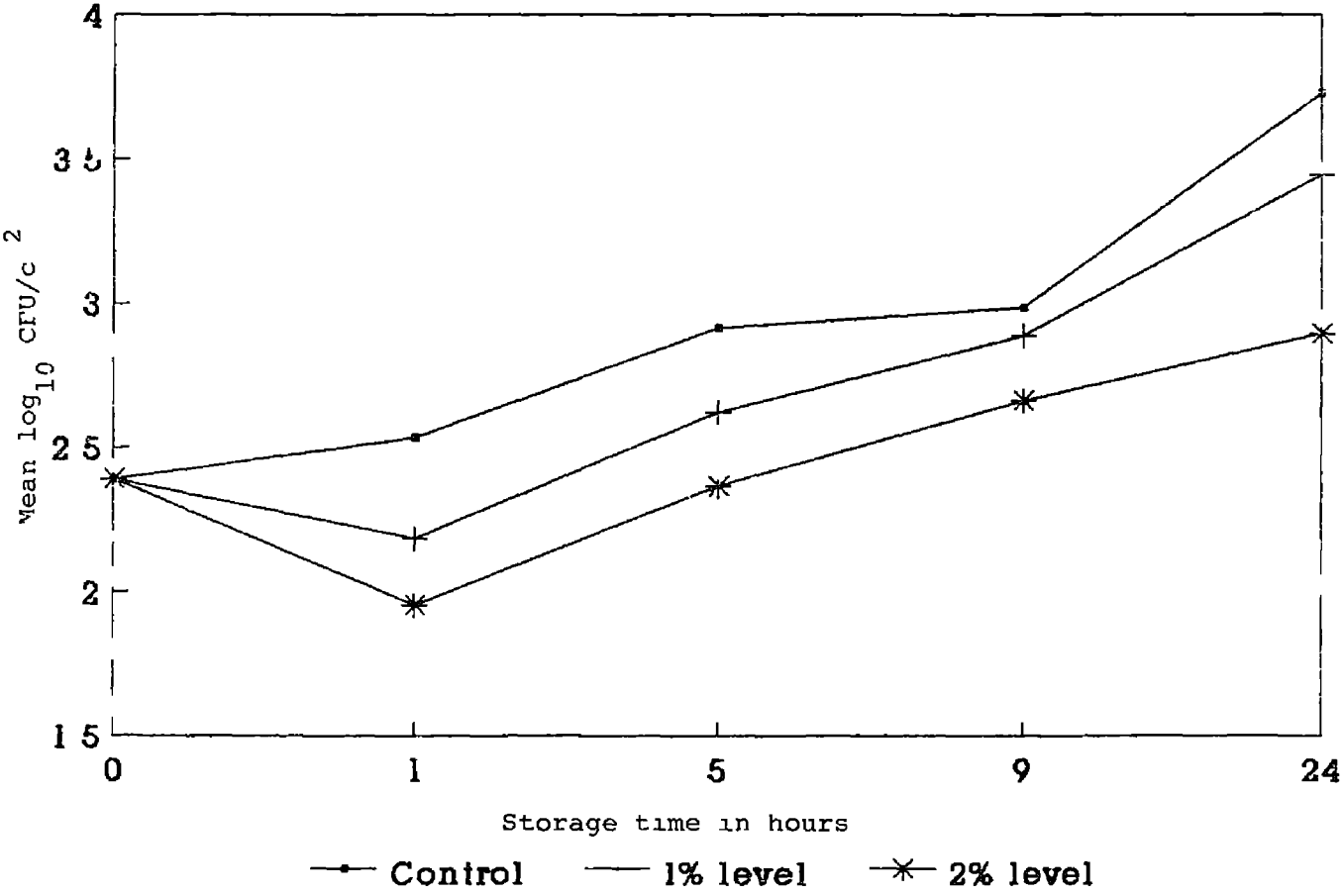
* P < 0 05
** P < 0 01

$\log_{10}$ CFU/cm^2 Increase in count indicated bacterial multiplication during the period of storage The mean increase in count in control samples from 0 h and 24 h was 1 34 $\log_{10}$ CFU/cm^2 The mean $\log_{10}$ increase during the period between 1 and 5 h 5 and 9 h and 9 and 24 h were 0 38 0 07 and 0 75 $\log_{10}$ CFU/cm^2 respectively In treatment-3 mean increase in FS count between 1 and 24 h of storage was 1 26 CFU/cm^2 During storge the mean increase in count was 0 44 between 1 and 5 h Between 5 and 9 h 9 and 24 h of storage the mean increase were 0 27 and 0 55 respectively In treatment-4 the increase in FS count over 24 h of storage was 0 94 $\log_{10}$ CFU/cm^2 At intervals of 1 and 5 h 5 and 9 h and 9 and 24 h the increase in FS count were 0 41 0 30 and 0 23 $\log_{10}$ CFU/cm^2 respectively

The results were analysed statistically and shown in Table 6a It can be seen that there was highly significant difference ($P < 0\ 01$) in FS count at all period of storage between samples of control and treatments and between treatment-3 and 4 The significant difference is indicated with the help of superscripts in the columns of Table 6

The trend of growth of FS on the beef samples under control and treatments stored at ambient temperature is shown in figure 11 The multiplication of FS dependent on

Figure 11 **EFFECT OF PROPIONIC ACID TREATMENT ON FAECAL STREPTOCOCCAL COUNT ON BEEF STORED AT AMBIENT TEMPERATURE**

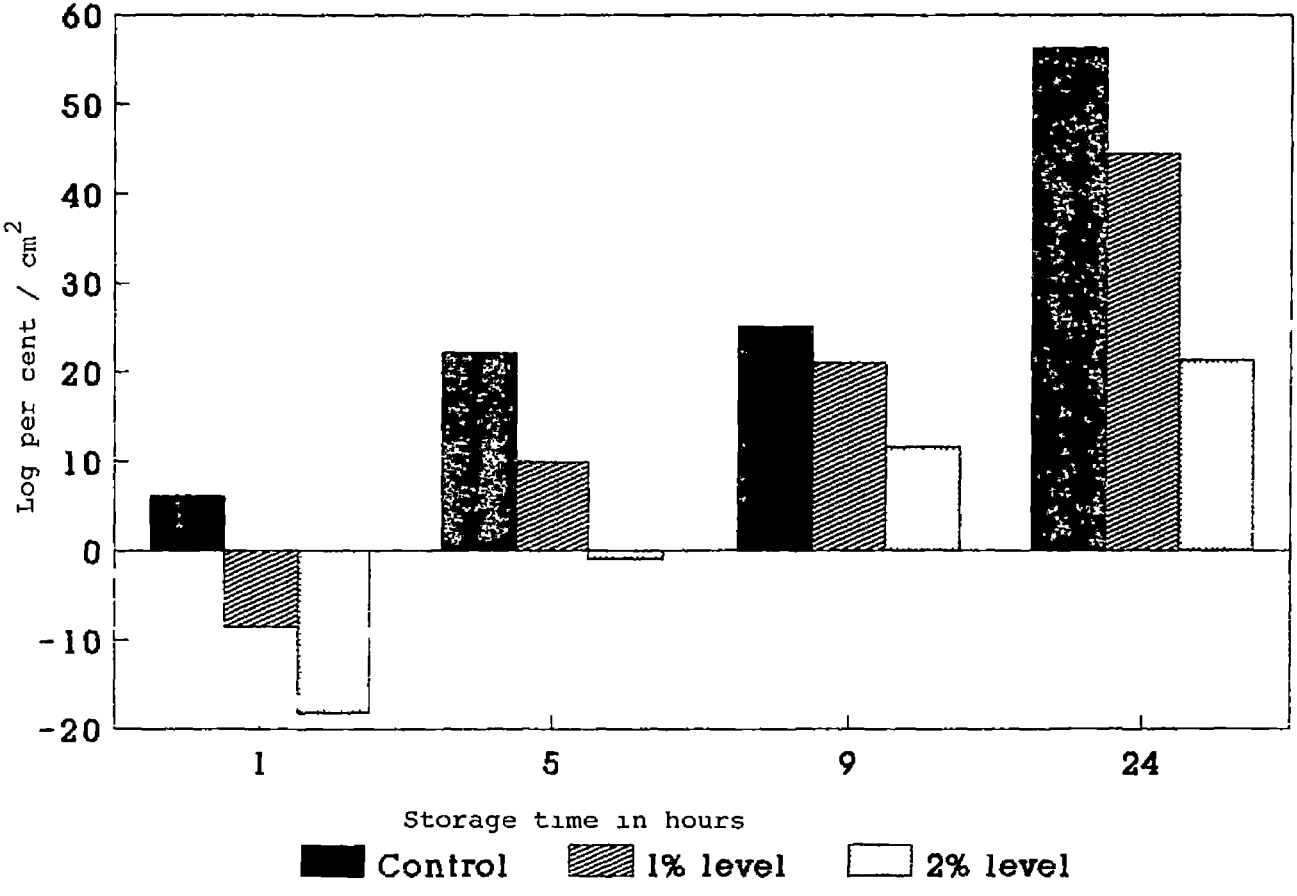


the initial load at 0 h of organism In the case of treatments the trend of multiplication was dependent on the number of viable organisms that remained in the sample after treatment

The percentage changes in FS count per square centimetre of control, and treatment samples during 1 5 9 and 24 h of storage is presented in figure 12 Antibacterial effect of propionic acid on FS depends on the concentration and storage time It was directly proportional to the concentration and inversely proportional to the duration of storage After 1 h of storage the increase in log per cent of FS in control sample was found to be 6 08 mean $\log_{10}$ CFU/cm^2 At 5 h further increase to 22 18 and at 9 h 25 12 and at 24 h it has reached 56 27 log per cent of the initial count

In the case of treatment 3 there was a reduction in FS count by 8 55 log per cent at 1 h The count increased by 9 81% at 5 h and 21 01% at 9 h and 44 44% at 24 h In treatment-4 the FS count was found to be reduced by 18 11% at 1 h and 0 92% at 5 h The count increased by 11 49% at 9 h and 21 34% at 24 h of storage in comparison to the FS count at 0h

Figure 12 LOG PER CENT CHANGE IN FAECAL STREPTOCOCCAL COUNT ON PROPIONIC ACID TREATED BEEF STORED AT AMBIFNT TEMPERATURE



Discussion

DISCUSSION

Carcasses derived from healthy and physiologically normal animals may be regarded as sterile In the conversion or live animal to meat for consumption, microbiological contamination inevitably occurs during processing Although the extent of contamination is highly variable most of the initial contamination occurs on the surface of carcasses and internal muscles remain essentially sterile Much contamination is contributed by the hide during the dehiding process, since exposed surface of the hide and hair accumulate dust dirt and faecal material (Ayres 1955) Contamination can also occur from the dirt on processing equipments, from the hands of butchers and meat handlers during evisceration and from the vehicles used for the transport of carcasses

The majority of the microflora transferred to the tissue surfaces from the above sources though non pathogenic are aesthetically undesirable and deleterious to shelf-life However, pathogens such as Salmonella Campylobacter Listeria and other pathogenic bacteriae may be infrequently transferred to carcass during slaughter operation Growth of

microorganisms results in discolouration putrefaction and slime formation of meat Such growth is the primary reason for quality deterioration and subsequent spoilage of meat resulting in public health hazard and economic loss Thus production and processing of meat is a critical operation It should be handled with all care and precautions with thorough knowledge of its inherent characteristics and means and measures to prevent contamination and for preservation

The initial bacterial load of meat has a direct bearing on its shelf-life Meat possessing a high initial bacterial load will have extremely short shelf-life in comparison with meat having low initial bacterial load

Growth of spoilage bacteria is most rapid at ambient temperature They continue to grow at refrigeration temperature also Mere act of refrigeration is not sufficient in preventing growth of spoilage bacterial flora on meat surface So different techniques have been used to reduce surface bacterial load of carcasses Organic acids are used for decontamination of carcass surface and meat cuts Sanitizing carcasses and retail cuts with organic acid reduces bacterial population and the storage life of the product is extended All food grade acids have some beneficial effect in reducing the bacterial contamination on

meat surfaces (Anderson et al 19?? Saoji et al 1990 Dickson and Anderson 1992) Antimicrobial activity exerted by organic acid depends upon p^H reduction minimizing dissociation of the acid and/or maximizing toxicity of the acid molecule (Ingram et al 1956) Weak organic acids tend to be more effective than strong acids because they acidify the interior of the cell (Anderson et al 1992)

A p^H below 5 5 is needed to slow microbial growth The effectiveness of an acid in lowering p^H depends on its strength, that is the extend to which it is ionized and its concentration For a given hydrogen ion concentration weak organic acids are more toxic than strong inorganic ones This suggests that the undissociated organic acid molecules exert a toxic effect and contribute towards its preservative action (Osthold et al , 1984) Antimicrobial activity vary with the type of acids and the microbial species

In the present investigation beef steaks were dipped in 1% and 2% solutions of acetic acid and propionic acid for 15 seconds and hung at ambient temperature for 24 h to assess the effect of these acids on the surface bacterial load during storage

Acetic acid treatment

Total viable count

In 10 beef carcasses used for this study mean TVC of the samples collected from these carcasses immediately after slaughter (0h), was 4 85 $\log_{10}$ CFU/cm^2 There is wide variation in reported TVC by different workers 4 7 $\log_{10}$/ $inch^2$ by Stringer et al (1969), and 3 06 $\log_{10}$/6 45 cm^2 by Lazarus et al (1977) on fresh beef and between 7 and 9 1 $\log_{10}/cm^2$ on buffalo carcass (Iyer 1984) The observation in the present study indicated the level of hygienic quality of production Mean bacterial count observed in this study could not be compared with the findings of others since the conditions under production vary The increase in count as seen at intervals of storage indicate bacterial multiplication The overall mean increase in TVC over the 24 h of storage was 1 60 $\log_{10}$ CFU/cm^2

The effect of acetic acid on TVC is evident from table 1a Highly significant difference ($P< 0 01$) in TVC was observed among control and treatments at all intervals of storage Table 1 shows that the effect of acetic acid increases as the concentration increases It is also seen that the bacteriostatic effect of acetic acid on TVC of

samples stored at ambient temperature decreases as the storage period increases irrespective of the concentration of acetic acid

The difference in surface bacterial load at 1 h of storage between control and treatment-1 was 0 33 $\log_{10}$ CFU/cm^2 whereas, the corresponding difference between control and treatment-2 was 0 47 $\log_{10}$ CFU/cm^2 From this it can be inferred that the initial lethal effect of acetic acid is high as the concentration of acetic acid increases Anderson et al (1979) observed that the initial count of beef surface reduced by 1 47 $\log_{10}$ /cm^2 immediately after treatment with 4% acetic acid while, Quartey-papafio et al (1980) reported that the surface bacterial count of beef samples reduced by 0 89 $\log_{10}$/g immediately after treatment with 3% acetic acid Their observation also strengthen the observation of the present study The difference in $\log_{10}$ TVC/cm^2 was observed at all intervals of storage Mean difference in count between control and treatment-1 was 0 57 $\log_{10}$ CFU/cm^2 at 24 h storage In treatment-2 the reduction in TVC was by 0 82 $\log_{10}$ CFU/cm^2 than in the control sample at the end of 24 h of storage This difference may be attributed to initial bactericidal action and / or continued sustained effect of 1% and 2% acetic acid treatment during the period of 24 h of storage This may be due to the exertion of

of inhibition on growth of bacteria by acetic acid with undissociated molecules that can penetrate the bacterial cell by means of diffusion and interferance with intracellular enzymes as reported by Smulders et al (1986)

The trend of surface bacterial multiplication on control and the two levels of treatment at ambient storage can be appreciated from figure 1 From figure 2 it can be seen that treatment of beef with 1% acetic acid is sufficient to keep the meat for 5 h and with 2% acetic acid for 9 h at ambient temperature without exceeding the initial bacterial load immediately after slaughter

Coliforms.

All samples collected from 10 beef carcasses yielded coliforms Mean count was 2 67 $\log_{10}$ CFU/cm^2 This count on carcass surface immediately after slaughter indicate faecal contamination of the carcass The count observed in this study was less than the range of 4 5 to 5 8 $\log_{10}/cm^2$ as reported by Iyer (loc cit) The quality guidelines recommended by Massachusettes agency is 100/g Presence of high coliform count on carcass surface indicate the chances for the presence of enteropathogens as coliforms are considered as indicator organisms Tompkin(1983) During 24 h storage the mean increase in count on control samples was

1 37 $\log_{10}$ CFU/cm^2 (Table 2) Multiplication of coliforms on beef surface at ambient temperature of storage was indicated by an increase in count at all intervals of storage

The effect of acetic acid treatment on coliforms on beef surface at ambient temperature of storage was indicated by highly significant difference in coliform count between the control and treated samples at all intervals of storage (Table 2a) Mean difference in count between control and treatment-1 was 0 33 $\log_{10}$ CFU/cm^2 at 1 h of storage whereas, the corresponding difference in count between control and treatment-2 was 0 84 $\log_{10}$ CFU/cm^2 This difference in $\log_{10}$ CFU/cm^2 can also be observed among control and treatments at 5 9 and 24 h of storage The difference in coliform count between control and treatment 1 was 0 26 $\log_{10}$ CFU/cm^2 at 24 h of storage and the difference in count between control and treatment-2 was 0 61 $\log_{10}$ CFU/cm^2 From the above observations it was evident that acetic acid exerted an initial bactericidal action and also checked the multiplication of coliforms over 24 h of storage depending on the concentration of acetic acid The reduction in coliform count at all intervals of storage was found dependent on the concentration of acetic acid solution and it may be attributed to the initial bactericidal action and/or

residual bacteriostatic effect of acetic acid

The trend in the multiplication of coliforms on control and treated samples on beef surface can be appreciated from figure 3 The treatment of beef samples with 1% acetic acid solution has helped to keep the meat for 5 h at ambient temperature limiting its coliform count within its initial load immediately after slaughter Beef samples treated with 2% acetic acid solution can be kept for 9 h with a coliform count not more than the initial load observed immediately after slaughter Osthold et al (loc cit) has reported selective inhibitory effect on enterobacteriaceae and coliforms by using a combination of acids including acetic acid Similarly acetic acid has been reported to have beneficial effect in reducing the microb al count as reported by Bell et al (1986) and Anderson et al (1987)

Faecal Streptococal Count (FS)

Faecal streptococcal count is used as an index of food sanitary quality during processing and storage of food products (Jay, (loc cit) In the present study all samples yielded faecal streptococci Mean FS count observed in these samples was 2 42 $\log_{10}$ CFU/cm^2 The presence of faecal streptococci on carcass surface immediately after slaughter

can be due to contamination of carcass during the process of dressing or post slaughter contamination Deshpande (1979) Kuttynarayanan (1981) and Iyer (loc cit) has reported the presence or faecal streptococci on meat and their reports were found to vary widely

On storage at ambient temperature there was multiplication of the organisms as evidenced by the increase in count at all intervals of storage During 24 h of storage the mean increase in count was 1 42 $\log_{10}$ CFU/cm^2

Examination of beef treated with 1% and 2% acetic acid and stored at ambient temperature shows a highly significant difference ($P < 0.01$) in counts of control and treated samples at all intervals of storage (Table 3a) The bactericidal effect of acetic acid on faecal streptococci present on beef surface increases as the concentration of acid increases (Table 3) It could be seen that at 1 h of storage the mean difference in count between treatment-1 and control sample was 0 37 $\log_{10}$ CFU/cm^2 The difference in count between control and treatment-2 at the above period of storage was 0 72 $\log_{10}$ CFU/cm^2 Similar difference in count was observed at 5, 9 and 24 h of storage Mean difference in count at 24 h of storage between control and treatment-1 was 0 39 $\log_{10}$ CFU/cm^2 whereas the corresponding mean difference

in count of control and treatment-2 was 0 92 $\log_{10}$ CFU/cm^2 This difference in FS count at all intervals of storage clearly shows that the effect of acetic acid on faecal streptococci increases with increasing concentration This effect of acetic acid may be attributed to the initial bactericidal action of acetic acid solution and/or the continued bacteriostatic action during storage

The trend in the multiplication of faecal streptococci on control and treated samples of beef during 24 h storage at ambient temperature is given in figure 5 From figure 6, it can be seen that treatment of beef with 1% acetic acid effectively checked the faecal streptococci multiplication for 5 h at ambient temperature and the count was below the initial count observed in samples collected immediately after slaughter Treatment of beef with 2% acetic acid has helped to keep the beef for 9 h with FS count not more than that observed in samples collected immediately after slaughter No reports are found on the effect of storage on FS count

Propionic acid treatment:

In this experiment samples were collected from 10 beef carcasses immediately after dressing Bacterial load on all samples surface were estimated immediately after collection

(Oh) Thereafter control and samples treated with 1% propionic acid (Treatment-3) and 2% propionic acid (Treatment-4) were examined at 1 5 9 and 24 h of storage at ambient temperature The bacterial count, viz total viable count coliform count and faecal streptococci count were used to evaluate the decontaminating efficiency of propionic acid

Though propionic acid is highly specific against molds (Jay (loc cit) its antibacterial effect has been reported by many workers (Quartey papafio et al ,(loc cit) Surve et al 1991)

Total Viable Count

Mean TVC of beef samples collected immediately after slaughter and used for studies on the effect of propionic acid was 4 77 $\log_{10}$ CFU/cm^2 TVC of beef samples collected immediately after slaughter is an indication of initial contamination and not due to bacterial multiplication Bacterial multiplication on beef surface during storage at ambient temperature was indicated by increase in TVC per cm^2 of samples at all intervals of storage Mean increase in TVC over 24 h storage was 1 67 $\log_{10}$ CFU/cm^2

Treatment of beef with 1% and 2% propionic acid showed highly significant ($P < 0.01$) difference in TVC at 1 5,9 and

24 h of storage (Table 4a) Highly significant difference in surface bacterial count between control and treatments and between the two levels of treatment was evident A closer examination of the above table reveals that the bactericidal and bacteriostatic effect of propionic acid at ambient temperature increases as its concentration increases Two per cent propionic acid solution was more effective than 1% in reducing bacterial count

Mean difference in TVC between control and treatment-3 at 1h of storage was 0 31 $\log_{10}$ CFU/cm^2 and the corresponding difference between control and treatment 4 was 0 47 $\log_{10}$ CFU/cm^2 The initial reduction in bacterial count can be attributed to bactericidal effect of propionic acid at ambient temperature Reduction in TVC was observed among control and treatments at 24 h of storage Mean difference in count between control and treatment-3 was 0 35 $\log_{10}$ CFU/cm^2 whereas the difference in TVC between control and treatment-4 was 0 84 $\log_{10}$ CFU/cm^2 at 24 h of storage The mean difference in TVC between treatment-3 and treatment-4 was 0 49 $\log_{10}$ CFU/cm^2 The above difference in TVC indicate that treatment with 1% and 2% propionic acid can maintain the reduction of bacterial count during 24 h of storage This effect can be attributed to initial bactericidal and/or sustained bacteriostatic effect of propionic acid over 24 h of storage

The trend of bacterial multiplication of beef surface during 24 h of storage in control and treated samples is shown in fig 7 The effect of treatment with 1% and 2% propionic acid on surface bacterial count on beef and their multiplication during storage is shown in figure 8 Van staden et al (1980) has reported a reduction in TVC when carcass meal was treated with propionic acid It can be seen from the figure that treatment of beef with 1% propionic acid solution was able to maintain its initial TVC at 0 h for less than 5 h of post treatment and 2% propionic acid treatment maintained that level for less than 9 h of storage

Coliform count·

All beef samples collected immediately after slaughter were examined for coliforms Mean coliform count in these samples was 2 47 $\log_{10}$ CFU/cm^2 Multiplication of coliforms on beef surface during storage was revealed by increase in count at all intervals of storage Over 24 h of storage coliform count increased by 1 65 $\log_{10}$ CFU/cm^2

Highly significant ($P < 0.01$) difference in the mean coliform count of control and propionic acid treated samples was observed at all specified periods of storage (Table 5a) Highly significant difference ($P<0.01$) in coliform count between control

and treatment 3 control and treatment-4 and between treatment 3 and 4 was observed at 1, 5 9 and 24 h of storage (Table 5)

Effect of propionic acid treatment on coliforms is given in Table 5 At 1 h of storage mean coliform count in treatment-3 was 0 23 $\log_{10}$ CFU/cm^2 less than that of the control At the above period of storage the difference in count between control and treatment-4 was 0 61 $\log_{10}$ CFU/cm^2 From the above results it can be inferred that at 1% and 2% levels propionic acid have bactericidal action on coliform organisms and effect of propionic acid on coliform increases as its concentration increases Difference in the count was maintained at all invervals over the period of 24 h of storage At 24 h storage the count in treatment-3 was 0 11 $\log_{10}$ CFU/cm^2 less than that of control In the case of treatment-4 mean difference in coliform count between control and treated sample was 0 58 The abov observations indicate that the effect of propionic acid solution on coliforms on beef surface was maintained over 24 h of storage and this also depends on the concentration of propionic acid used This effect of propionic acid can be due to its initial bactericidal action and/or sustained bacteriostatic action The antibacterial effect of propionic acid on E coli has been reported by Cole et al (1968) Van Staden et al

(loc cit) and Winterbalder et al (1984) Cherrington et al (1990) has found that propionic acid at p^H 5 can induce a temporary bacteriostatic effect for 30 mt Propionic acid has been reported to inhibit the DNA synthesis of E coli without physically damaging the molecule (Cherrington et al , 1991)

The multiplication trend of coliforms on beef surface both in control and treatments can be seen from figure 9 Figure 10 reveals the effect of treatment-3 and treatment 4 on coliforms during 24 h or storage At the end of 1 h of storage the mean count on samples in treatment-3 was 1 21% less than the mean $\log_{10}$ count observed at pretreatment levels During the corresponding period the number of organisms in treatment-4 was about 17% less than that observed immediately after slaughter Observations at 1 h post treatment shows that reduction in number of coliform bacteria was dependent on the concentration of propionic acid used Between the two treatments, 2% propionic acid was found to be more effective

It may be inferred from the figure that the treatment with 1% propionic acid has helped to keep the beef upto 5 h within the limit of initial count of coliforms at 0 h Beef treated with 2% propionic acid can be stored at ambient temperature for less than 9 h with the count equal to the initial count (at 0 h) immediately after slaughter

Faecal Streptococcal Count

Faecal streptococcal count is one of the bacterial indices used for assessing the sanitary processing or proper storage of food products In the present investigation the mean FS count of samples collected from 10 beef carcasses immediately after slaughter was 2 39 $\log_{10}$ CFU/cm^2 Increase in number of organism on sample surface was observed 1 5 9 and 24 h of storage The findings are similar to the earlier studies with acetic acid

High significant reduction ($P < 0.01$) in FS count between control and treatments is shown in Table 6a Highly significant reduction in the mean number of organism between control and treatment-3 control and treatment-4 and between treatment-3 and 4 was also observed at 1 5,9 and 24 h of storage (Table 6a) Effect of propionic acid on faecal streptococci was revealed by reduction in the bacterial population on beef surface Mean reduction in FS count in treatment 3 at 1 h of storage was 0 35 $\log_{10}$ CFU/cm^2 In treatment-4 it was 0 58 $\log_{10}$ CFU/cm^2 Reduction in bacterial count of samples in treatment 3 and 4 was observed upto 24 h of storage The mean difference in $\log_{10}$ count between control and treatment-3 was 0 29 and it was 0 84 $\log_{10}$ CFU/cm^2 between control and treatment 4 Thus it could be inferred that the reduction in FS count was dependent on

the concentration of propionic acid in the dipping menstra The mean bacterial count in treatment 4 was significantly ($P < 0.01$) lower than the mean count found in treatment-3 This observation reveals that the treatment of beef with 2% propionic acid has a high residual effect over 1% propionic acid at 24 h of storage

Multiplication trend of faecal streptococci on beef samples subjected to treatment-3 and 4 and on control over the 24 h of storage at ambient temperature is shown Fig 11 A comparison of the sanitizing effect of each treatment with the initial count obtained immediately after slaughter is shown in Figure 12 At 1 h of storage the mean reduction in FS count in treatment-3 was about 9% and in treament-4 it was about 18% of the pretreatment levels The bacterial count increases both in control and treated samples during storage which indicate that there is a bacterial multiplication as evidenced at 5, 9 and 24 h of storage but the rate of multiplication of the organism was influenced by the concentration of propionic acid Propionic acid has been found to have a disinfecting effect on Streptococcus faecium by Winterbalder et al (loc cit)

From the above findings it can be inferred that on treatment with 1% propionic acid the meat can be stored for nearly 5 h but with 2% propionic acid it can be stored

nearly upto 9 h limiting its FS count within its initial count immediately after slaughter

Both acetic acid and propionic acid used as sanitizers on beef carcass at two different strength of 1% and 2% were found to have significant effect in reducing bacterial load in comparison to untreated controls kept at ambient temperature upto 24 h Acetic acid 2% was more effective than 1% The samples treated with 1% acetic acid could be maintained for about 5 h without any increase in TVC from its initial load before treatment, whereas, those treated with 2% acetic acid maintained its TVC upto 9 h within the limit of the initial load Similar bacteriostatic effect of acetic acid was noticed in the treated samples in respect of coliforms and faecal streptococci

The bactericidal and bacteriostatic effect of propionic acid on surface of beef carcasses shows that like acetic acid both 1% and 2% brought significant reduction in bacterial load in comparison to untreated upto 24 h of storage at ambient temperature Treatment with 1% propionic acid initially brought in a reduction in TVC at 1 h and thereafter there was gradual increase in growth but was effective in maintaining the bacterial load almost upto 5 h within the limit of the initial load before treatment This effect was lower when compared to 1% acetic acid within the

corresponding time interval Treatment with 2% propionic acid was more effective than 1% and the reduction of total bacteria was highly significant at all intervals of observation and the load of bacteria could be sustained almost upto 9 h within the limit of initial load before treatment Similar phenomena was observed on the effect of propionic acid on coliforms and faecal streptococci on beef stored for 24 h

Both the acids were found to have sanitizing effect on beef and were capable of maintaining the bacterial load on beef surface by treatment with 1% for 5 h and by 2% for 9 h at ambient temperature

It may be concluded that acetic acid and propionic acid can be used as sanitizers on beef in order to enhance its storage period at ambient temperature The period of storage at ambient temperature can be extended without any appreciable bacterial multiplication upto 9 h by treatment with 2% acetic acid

Summary

SUMMARY

Meat is one of the important items of food all over the world The safety and wholesomeness of meat has a strong bearing on public health Presence of bacteria has economic bearing as the spoilage of meat is principally by multiplication of bacteria under favourable circumstances In order to safeguard the interest of public health and meat trade it is necessary to keep the bacterial load on meat in check at all levels of production and storage Over and above the strict hygienic measures adopted/practiced at production point certain measures are suggested to reduce/control bacterial load and multiplication The present study envisages to evaluate the effect of treating beef with acetic acid and propionic acid at 1% and 2% strength and their suitability as a sanitizer on meat under ambient temperature

The study was conducted on 20 adult beef carcasses obtained from the Kerala Agricultural University slaughter house Immediately after slaughter meat samples were collected and subjected to bacteriological evaluation All samples were maintained at ambient temperature during the

period of study An area of 25cm^2 of exposed surface of beef was swabbed for bacteriological evaluation Treatments with acetic acid 1% (Treatment-1) acetic acid 2% (treatment-2) propionic acid 1% (treatment-3) and propionic acid 2% (treatment 4) were compared with the control during all intervals of observation During the study total viable count (TVC) coliform count and count of faecal streptococci were made initially before treatment and at intervals of 1 5 9 and 24 h post treatment The pre treatment counts were expressed as counts at 0h All counts were expressed as $\log_{10}$ CFU/cm^2

The following results were obtained on acetic acid treatment In control the TVC increased by 1 60 $\log_{10}$ CFU/cm^2 from 0 to 24h In treatment-1, there was an increase of 1 19 $\log_{10}$ CFU/cm^2 between 1h and 24 h and in treatment-2 it was 1 08 On storage the rate of increase of TVC in control was 0 17 $\log_{10}$ CFU/cm^2 between 0 and 1 h 0 14 between 1 and 5 h 0 21 between 5 and 9 h and 1 08 between 9 and 24 h In treatment-1 the increase in TVC was 0 18 between 1 and 5h, 0 16 between 5 and 9h 0 85 between 9 and 24 h In treatment-2, between 1 and 5h the increase was 0 18 $\log_{10}$ CFU/cm^2 between 5 and 9 h the increase was 0 15 and between 9 and 24 h it was 0 75 $\log_{10}$ CFU/cm^2 Highly

significant difference in TVC was noticed between control and treatment 1 and treatment 2 and also between treatment-1 and 2 In treatment 1 and 2 there was decline in TVC at 1 h post treatment An increasing trend of bacterial multiplication was observed in all samples over storage The effect of treatment and its persistence was found to be proportional to the strength of the acid The log per cent increase of TVC in control noticed was 3 54 at 1h 6 at 5 h 10 76 at 9 h and 33 at 24 h In treatment-1 the count was reduced by 3 32% at 1 h and 0 41% at 5h At 9 h and 24 h it was found to be increased by 3 59% and 21% respectively The corresponding figures at 1, 5 9 and 24 h in treatment-2, was 6 33, 2 53, 0 54 and about 16%

The coliform count was found increase by 1 37 logs between 0 and 24 h storage in the control Betwen 1 and 24 h it was 1 27 The rate of increase between 1 and 5 h was 0 16 betwen 5 and 9 h, it was 0 18 and between 9 and 24 h 0 93 In treatment-1 the average increase between 1 and 24 h was 1 34 $\log_{10}$ CFU/cm^2 The rate of increase in treatment-1, was 0 20 0 19 and 0 95 at intervals between 1 and 5 5 and 9 and 9 and 24 h respectively In treatment-2 it was 0 41 0 21 and 0 88 for intervals between the above time intervals Highly significant difference in coliform

count between control treatmnet-1 and treatment-2 and also between treatment 1 and 2 was observed The log per cent increase in control was 4 at 1 h 10 at 5 h 16 54 at 9 h and 51 61 at 24 h In treatment-1, there was an initial reduction of 8 4% at 1 h and 1 13% at 5 h At 9 and 24 h the increase was at the rate of 6 30% at 9 h and 41 64% at 24 h of storage In treatment-2 there was reduction upto 9h 27 53% at 1 h 12 04% at 5 h and 4 28% at 9h At 24 h the increase was 28 77% in comparison to account at 0 h

The faecal streptococci count in control was found to increase by 1 42 $\log_{10}$ CFU/cm^2 from 0 to 24 h Between 1 h and 24 h it was 1 31 $\log_{10}$ CFU/cm^2 in control 1 29 in treatment-1 and 1 11 in treatment-2 The rate of increase was 0 16 0 21 and 0 94 in control between 1 and 5 h 5 and 9 h and 9 and 24 h of storage In treatment-1, the rates were 0 25 between 1 and 5 h, 0 23 between 5 and 9 h and 0 81 between 9 and 24 h In treatment-2 the rate of increase was 0 33 0 24 and 0 54 respectively between the above time intervals of storage Highly significant difference between control and treatment-1 and treatment-2 was noticed Between treatment-1 and 2 also highly significant difference in count was noticed The log per cent increase in count on storage were 4 41% at 1 h 10 97% at 5 h, 19 68% at 9 h and 58 50% at 24 h for control For treatment-1 there was

reduction of 10 97% at 1 h and 0 78% at 5h of storage The increase noticed at 9 h was 8 83% and at 24 h it was 42 29% In treatment-2 the reduction was noticed upto 9h 25 21% at 1h 11 84% at 5 h and 1 73% at 9 h At 24 h an increase of 20 34 log per cent was noticed

Propionic acid was also used for the treatment of meat as sanitizer Samples treated with 1% propionic acid (treatment 3) and 2% (treatment-4) along with control were kept at ambient temperature and TVC, coliform count and faecal streptococci counts were monitored at 1,5 9 and 24 h intervals At 24 h a log increase of 1 67 was observed in controls 1 45 in treatment-3 and 1 12 in treatment-4 The rate of increase between 0 and 1 h and 1 and 5 h, 5 and 9 h and 9 and 24 h in controls were 0 18 0 33, 0 15 and 1 01 repectively In treatment-3, rate of increase in TVC was 0 32 between 1 and 5 h, 0 14 between 5 and 9 h, and 0 99 between 9 and 24 h In treatment-4 the corresponding increase were 0 21 0 23, and 0 68 Highly significant difference in TVC between control and treatment 3 and treatment-4 was observed Difference between treatment-3 and 4 were significant at all levels of observation The antibacterial effect and its persistence of propionic acid was found to be dependent on its strength During the period of storage the log per cent change in TVC indicated that

there was an increase at all intervals in the control The increase was at the rate of 3 77 log per cent at 1h 10 03 at 5 h, 13 66 at 9 h and 34 94 at 24 h In treatment-3 there was a reduction of 2 70% at 1 h but increase of 3 83% at 5 h and 6 85% and 27 59 log % at 9 and 24 h respectively In treatment-4 TVC was found to decrease by 6 14 log at 1 h and 1 82 log% at 5 h At 9 and 24 h there was an increase of 3 04 and 17 22 log% respectively

In control there was an increase of coliforms by 1 65 log during 24 h of storage In treatment-3 the increase was 1 57 log and in treatment 4 it was 1 48 The rate of increase of coliforms were 0 20, between 1 and 5 h 0 20 between 5 and 9 h and 1 05 between 9 and 24 h in control In treatment-3, the rate of increae were found to be 0 22 0 24 and 1 11 respectively at intervals between 1 and 5 5 and 9 and 9 and 24 h In treatment-4, the rate of increase was 0 39 between 1 and 5 h 0 25 between 5 and 9 h and 0 84 between 9 and 24 h Highly significant difference in coliform count was observed between control and treatment-3 and treatment-4, and significant difference between treatment 3 and 4 The percentage change in coliform count during the period of storage indicate that the coliform count in control increased always by 8 26 log% at 1 h 16 28% at 5 h 24 33% at 9 h and 66 84% at 24 h In treatment-3 an initial reduction of coliform count by 1 21 log % at 1 h was noticed At 5 h the count increased by 7 53 and at 9 h

17 41 and at 24 h 62 43 log percents were noticed In treatment-4, at 1 and 5 h the coliform count was showing log per cent decreaseof 16 64 and 0 77 respectively At 9 h there was an increase of 9 23 and at 24 h by 43 38 log% in comparison to the count at 0 h

The faecal streptococci count during storage for 24 h has shown an overall increase of 1 34 log in control group between 0 and 24 h In treatment-3 the increase between 1 and 24 h was 1 26 and in treatment 4 it was 0 94 The rate of increase at different intervals for control between 1 and 5 h was 0 38 between 5 and 9 h 0 07 and between 9 and 24 h 0 75 log In treatment-3 there was increase of 0 44 0 27 and 0 55 for intervals between 1 and 5, 5 and 9 and 9 and 24 h respectively The correspond ng rates for treatment 4 were 0 41 0 30 and 0 23 Highly significant difference in faecal streptococcal count were observed between control treatment-3 and treatment 4 The difference in faecal streptococcal count was significant between treatment 3 and 4 The log per cent change in count of faecal streptococci revealed an increase at all intervals of storage of control The count increased by 6 08 at 1 h 22 18 at 5 h 25 12 and 9 h and 56 27 log% at 24 h In treatment 3 there was a reduction of 8 55 log% at 1 h but increase by 9 81 21 0 and 44 44 log% at 5 9 and 24 h of storage respectively In treatment-4 the reduction in faecal streptococci was noticed

by 18 11 and 0 92 log% at 1 and 5 h respectively but increase by 11 49 and 21 34 log% was noticed at 9 and 24 h interval respectively

The results indicate that treatment of beef immediately after slaughter with acetic acid and propionic acid at concentrations of 1 and 2% have significant sanitizing effect by its bactericidal and bacteriostatic effect and for storage of meat for about 9 hours at ambient temperature This effect was found directly proportional to the strength of the acid and inversely proportional to the duration of storage The bacterial load could generally be maintained upto 5 h with 1% acetic acid and upto 9 h with 2% acetic acid within the initial total viable count Propionic acid could also be used but acetic acid was found comparatively better than propionic acid for sanitization of carcasses

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Appendices

Appendix - I

Plate Count Agar

Tryptone	-	5 0 g
Yeast extract		2 5 g
Dextrose		1 0 g
Agar		15 0 g
Aq Dist	-	1000 ml

Dissolved the ingredients in distilled water and adjusted the p^H to 7 0 $\pm$ 0 2 with 0 1N sodium hydroxide solution Sterilized by autoclaving at 121°C for 15 minutes

Appendix II

Violet red bile agar

Peptone	- 7 0g
Yeast extract	- 3 0g
Bile salt mixture	- 1 5g
Lactose	10 0g
Sodium chloride	5 0g
Agar	- 15 0g
Neutral red	0 03g
Crystal violet	- 0 002 g
Aq Dist	- 1000 ml

Dissolved the peptone yeast extract bile salt mixture agar and sodium chloride in distilled water by steaming Then cooled to 50°C and adjusted the p^H to 7 4 $\pm$ 0 2 with 0 1N sodium hydroxide solution Lactose neutral red and crystal violet were added and autoclaved at 121°C for 15 minutes Hot medium was poured into sterile petridishes and allowed to solidify

Appendix III

K F. Streptococcal agar

Proteose peptone	- 10 0g
Yeast extract	- 10 0g
Sodium chloride	- 5 0g
Sodium glycero Phosphate	- 10 0g
Maltose	- 20 0g
Lactose	- 1 0g
Sodium azide	- 0 4g
Agar	- 20 0g
Aq dist	- 1000 ml

Boiled to dissolve the ingredients completely and adjusted the p^H to 7 2 $\pm$ 0 2 with 0 1N sodium hydroxide solution Autoclaved at 121°C for 10 minutes Then cooled to 60°C and 1 ml of 1% TTC (Triphenyl tetrazolium chloride) was added aseptically into each 100 ml of the sterile medium Mixed the medium thoroughly to obtain uniform distribution of TTC in the medium and poured it into sterile petridishes

EFFECT OF ACETIC ACID AND PROPIONIC ACID ON BACTERIOLOGICAL QUALITY OF BEEF

By

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ABSTRACT OF A THESIS

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ABSTRACT

The bacterial contamination of meat surface is posing a threat to public health and meat trade. It is necessary to minimise the bacterial load at all levels of production, storage and marketing. Use of sanitizer is one of the methods suggested for reducing the bacterial load on carcass surface. The study was undertaken to assess the efficiency of acetic and propionic acids at one and two per cent strength as sanitizer on beef. Carcasses obtained from Kerala Agricultural University Slaughter House, were subjected for the study. The samples were maintained at ambient temperature for 24 h. The acid treatment of samples was done immediately after slaughter. The total viable count,coliform count and faecal streptococcal count were estimated by standard methods at zero, one, five, nine, and twenty-four hours of storage. An upward trend of bacterial load was observed during storage. At all intervals, the bacterial load was significantly lower in treated samples compared to that of control. The bacterial load was found to be significantly lower in samples subjected to acid treatments at two per cent level than one per cent. The persistence of the effect was found to be inversely proportional to the duration of storage. The bacterial load could generally be confined with one per cent acetic acid upto five hours and

nine hours with two per cent acetic acid within the initial count Though propionic acid at one and two per cent levels had beneficial effect acetic acid was found to be better