EFFECT OF REFRIGERATION ON THE QUALITY OF BEEF FRANKFURTER AND CHICKEN PEPPERONI

AMBILI V. S.

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Department of Veterinary Public Health
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR - 680 651
KERALA, INDIA

DECLARATION

I hereby declare that this thesis entitled **"EFFECT OF REFRIGERATION ON THE QUALITY OF BEEF FRANKFURTER AND CHICKEN PEPPERONI"** is a bonafide record of research work done by me 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.

Mannuthy,

AMBILI V.S.

CERTIFICATE

Certified that the thesis entitled **"EFFECT OF REFRIGERATION ON THE QUALITY OF BEEF FRANKFURTER AND CHICKEN PEPPERONI"** is a record of research work done independently by **Dr. Ambili V.S.**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

Dr. C. Latha
(Chairperson, Advisory Committee)
Assistant Professor
Department of Veterinary Public Health
College of Veterinary and
Animal Sciences, Mannuthy

Mannuthy
1-10-2004

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Dr. Ambili V.S.,** a candidate for the degree of Master of Veterinary Science in Veterinary Public Health, agree that the thesis entitled **"EFFECT OF REFRIGERATION ON THE QUALITY OF BEEF FRANKFURTER AND CHICKEN PEPPERONI"** may be submitted by Dr. Ambili V.S., in partial fulfillment of the requirement for the degree.

Dr. C. Latha
(Chairperson, Advisory Committee)
Assistant Professor
Department of Veterinary Public Health
College of Veterinary and Animal Sciences, Mannuthy

Dr. E. Nanu
Professor and Head
Department of Veterinary Public Health
College of Veterinary and
Animal Sciences, Mannuthy
(Member)

Dr. P. Kuttinarayanan
Associate Professor
Department of Livestock
Products Technology
College of Veterinary and
Animal Sciences, Mannuthy
(Member)

Dr. G. Krishnan Nair
Associate Professor
Department of Microbiology
College of Veterinary and
Animal Sciences, Mannuthy
(Member)

External Examiner

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

Introduction

1. INTRODUCTION

Food of animal origin is an important source of protein with high biological value. In developing countries, urbanization, rising incomes and the globalization of trade have brought about changes in food habits leading to an increase in per capita consumption of meat. Developing countries account for 56 per cent of the world's total meat production. The world meat production during the year 2002 was 245.9 million tonnes (FAO, 2002).

India has a livestock population of 470 million which includes 219 million cattle, 94 million buffaloes, 58 million sheep, 123 million goats, 17 million pigs and 402 million broilers. According FAO, the total number of animals slaughtered for meat production in India rose from 66.30 million head in 1980 to 106.24 million head in 2000. The per capita availability of meat in India is only 14.24 g/day during 2002 as against the 30 g/day recommended by ICMR. (FAO, 2002)

Ready-to-cook and ready-to-eat meat products are gaining tremendous consumer acceptance, now-a-days. Sausages are emerging as a popular item among the public and in our state there is a steady increase in the demand for processed meat products. The quality control of such products is of great concern and a hygienically processed product should satisfy the following basic requirements.

a) Conditions of sausage production must be such that no toxigenic organism should be present in or gain access to the product prior to, during or after processing.

b) The final sausage product should not contain microorganisms likely to be pathogenic to man.

c) The total bacterial count and the number of spoilage organisms in the product should be reasonably low so that no decomposition or development of undesirable flavor occurs during the period of processing, distribution or storage (FAO, 1985).

In order to achieve these qualities it demands the selection of good quality meat and other raw materials required for the product and also the observance of high standards of hygiene during the various stages of production, processing, packaging, storage and distribution of these products. The application of Hazard analysis critical control point system provides a systematic approach to achieve this end.

Good shelf-life depends on the microbiological quality, conditions maintained at storage, transport and retailing. On set of spoilage and survivability of pathogenic and spoilage microflora in sausages during refrigerated storage is a matter of concern among the health conscious public and food microbiologists.

Findings of this study will help the producers to control or reduce the microbial load at the production level and thus to improve the shelf-life of the product and safeguard consumer health. The study was undertaken with the following objectives.

I. To assess the quality of fresh chicken pepperoni and beef frankfurter and the effect of chilling and freezing on the quality of these products by

1) Estimating the level of

a) Total Viable Count

b) Coliform Count

c) *Escherichia coli* Count

d) Faecal streptococcal Count

e) Psychrotrophic Count

f) Yeast and mold count

and by detecting the presence of

a) *Escherichia coli*

b) *Aeromonas*

c) Salmonellae

d) *Pseudomonas aeruginosa*

e) Lactobacilli

2) Physico chemical quality by detecting the changes in

a) pH

b) TBARS (thiobarbituric acid reacting substances) number

3) Organoleptic quality by observing

a) color

b) odor

c) sliminess

II To identify the critical control points of bacterial contamination.

Review of Literature

2. REVIEW OF LITERATURE

2.1 FRESH AND CHILLED STORAGE

2.1.1 Total Viable Count

Steinke and Foster (1951a) evaluated the effect of different artificial casings on microbial changes in liver sausage stored at 5°C. They observed an increase in total aerobic count from 5.2 x 10^3/g to 12 x 10^3/g after 15 days of storage.

In a study on the outer zone microbial flora of liver sausage during storage at 5°C, Steinke and Foster (1951b) reported that the total aerobic count increased from 9.3 x 10^3/g to 11 x 10^3/g after 15 days. They also evaluated the outer zone microbial flora of bologna stored at 5°C and observed an aerobic count of 4.2 x 10^3/g on zero day, which increased to 4.9 x 10^3/g on 15^{th} day of storage.

According to Ogilvy and Ayres (1952) the total aerobic count of frankfurters stored at 4.4°C in CO_2 free package for 10 days increased from the level of 10^3/g to 10^5/g.

In a study to assess the effect of storage at 2°C for 24 days on quality of chicken frankfurters, Baker *et al.* (1972) observed that total counts were well below 10^{-3}level.

Palumbo *et al.* (1976) evaluated the microbial quality of pepperoni from nine commercial sources and reported that the total viable count of sausages collected from different sources A, B, C, D, E, F, G, H and imported sausages were 1.3 x 10^8, 1 x 10^4, 2.5 x 10^7,2.3 x 10^7, 6.0 x 10^6, 1 x 10^8, 7 x 10^6, 1.2 x 10^7 and 2.5 x 10^6/g respectively.

Blickstad and Molin (1983) studied the microflora of frankfurter sausage after storage in different gas atmospheres at 4°C. The total aerobic count of

frankfurter samples on zero day was 1.7 log/g. In vacuum packaged samples the count was increased to 2 log/g after 20 days of storage. In case of Co_2 gas atmosphere storage the count was increased from 1.7 log/g to 1.8 log/g and in nitrogen gas atmosphere storage it was reduced from 1.7 log/g to 1.5 log/g after 20 days of storage.

Microbiological quality of vacuum packed cooked ring sausage at 4°C was evaluated by Korkeala *et al.* (1985). Aerobic plate count was 2.64 $\log_{10}$ cfu/g on first day and it increased to 5.60 $\log_{10}$ cfu/g on 28^{th} day and 5.84 $\log_{10}$ cfu/g after 35 days of storage.

Microbiological changes during aerobic and vacuum-packed storage of butcher's sausage at 6°C was studied by Adams *et al.* (1987). Upon aerobic storage, total viable count was increased from 5.4 $\log_{10}$ cfu/g on zero day to8.8 $\log_{10}$ cfu/g on 15^{th} day of storage. In vacuum-packed samples the count rose to 7.8 $\log_{10}$ cfu/g on 15^{th} day.

In a study on bacteriological quality of refrigerated (5 ±1°C) dry cooked chicken sausage, Anand *et al.* (1990) observed an increase in total aerobic count from 3.71 log/g to 6.64 log/g on 10^{th} day of storage. In raw sausage samples the initial count was 4.54 log/g, which gradually increased to 7.08 log/g during eight days of refrigerated storage. In moist cooked sausages on day of preparation the count was 3.56 log/g, which after 11 days rose to 6.83 log/g.

von Holy *et al.* (1991) studied the microbial population associated with vacuum packed Vienna sausage stored at 5ºC and reported a total aerobic plate count of 3.58 $\log_{10}$ cfu/g in unspoiled samples aged four to ten days from manufacture and 7.54 $\log_{10}$ cfu/g in spoiled samples aged 30 to 125 days from manufacture.

Krishnan and Sharma (1993) studied the effect of chilled storage on aerobic plate count of beef sausages and reported an increase in count from 3.75 log/g on zero day to 3.89 log/g on seventh day of chilled storage.

In a study Sharma *et al.* (1994) conducted bacteriological analysis of 118 samples of uncooked pork sausage. The total aerobic plate count was in the range of 1.8 x 10^8 to 9.2 x 10^8 cfu/g in the samples tested.

Pipova *et al.* (1997) studied changes in the microbial picture during the production of poultry Salami. In Salami emulsion they reported a total plate count of 4.8 x 10^6/g.

A microbiological study of Greek salami was carried out by Samelis *et al.* (1998). Four batches of sausages were subjected to study and the aerobic mesophilic counts estimated were 6.25, 6.32, 5.30 and 6.39 log cfu/g respectively for batches A, B, C and D. After a period of 28 days of ripening at 15-16°C the count was increased to 8.00, 8.17, 8.08 and 8.04 log cfu/g respectively.

Rao *et al.* (1999) conducted a study on bacteriological quality of smoked chicken sausage stored at 4 ± 1°C. They reported an increase in total viable count from 4.10 log cfu/g on day of manufacture to 5.25 log cfu/g after fourth week of storage.

Samelis *et al.* (2000) studied the bacterial quality of frankfurters stored at 4°C. The total viable count on zero day was 3.90 log cfu/g, which was increased to 6.80 log cfu/g on 15 days of storage at 4°C.

Bacterial population during chilled storage of vacuum packaged frankfurter was analyzed by Yuste *et al.* (2000). The mesophilic count in samples stored at 8°C was 4.63 log cfu/g and 8.41 log cfu/g, on first day and after three weeks of storage respectively. In samples stored at 2°C the count was 7.80 log cfu/g after three weeks.

Effect of antioxidants on the quality of chevon sausage during refrigerated storage (4±1°C) was studied by Verma and Sahoo (2000). In control group without addition of antioxidants the aerobic mesophilic count was 3.75 ± 0.04/log

cfu/g on day of manufacture and it increased to 7.35 ± 0.04 log cfu/g after 12 days of storage at 4 ± 1°C.

Effect of refrigerated (4 ± 1°C) storage on quality characteristics of vacuum-packed bologna was studied by Gines *et al.* (2003). On the day of formulation and packing the aerobic bacterial count was at the level of 2 $\log_{10}$ cfu/g. Over a period of 28 days the counts increased to 6 $\log_{10}$ cfu/g level.

2.1.2 Coliform Count

Cunningham and Bowers (1977) conducted studies on microbial count of chicken patties stored at 3°C. They reported a coliform count of 3 x 10^2/g in fresh chicken patties prepared with 100 per cent chicken and 4 x 10^2/g in patties made with chicken and 10 per cent soy.

Simard *et al.* (1983) compared the effect of vacuum and N_2 packaging on microbial quality of frankfurters stored at 3°C. On zero day the coliform count was 1.7 $\log_{10}$/g and 3.2 $\log_{10}$/g in vacuum packaging and N_2 packaging respectively. After 14 days of storage at 3°C the counts were 1.3 $\log_{10}$/g and 1.5 $\log_{10}$/g respectively.

The bacterial quality of meat and meat products consumed by Egyptian people was evaluated by Zahra *et al.* (1985). The highest coliform counts exceeding the standards were detected in sausage, raw kofta and kabab, whereas no coliforms were present in luncheon meat.

Microbiological quality changes in cooked ring sausage during storage at 4°C was evaluated by Korkeala *et al.* (1985). They opined that coliforms were not detected even after 35 days of chilled storage.

The bacteriological investigation of minced meat and meatballs by Kaloianov *et al.* (1987) in Bulgaria revealed an average coliform count of 31748/g.

Anand *et al.* (1990) conducted a study on raw chicken sausage stored under refrigeration (5±1°C) and reported an increase in coliform count from 3.51 log/g on zero day to 5.41 log/g on eighth day. In case of dry cooked sausage the count increased from 0.87 log/g to 4.24 log/g on eighth day of storage and the count on day 10 was 5.33 log/g. In moist cooked sausages coliforms were not detected on day of preparation. After two days the count was 0.50 log/g. The count gradually increased to 4.62 log/g after 11 days at 5 ± 1°C.

Pipova *et al.* (1997) studied the changes in microbial profile during the production of poultry salami. In raw salami emulsion they reported a very high coliform count of 7 x 10^5/g, which was considerably reduced after the heat treatment.

Microbiological quality of 80 soybean sausage samples (50 per cent frankfurter and 50 per cent sausage mortadela) was evaluated by Monge *et al.* (2000). They reported that 20 per cent of the sausage samples showed coliform levels above 10^4/g.

Soriano *et al.* (2000) examined samples of pork sausage, pork loin, chicken croquettes, chicken breast and meatballs from restaurants. They detected coliforms at a level of <3 to >2,400 MPN/g in meat product samples.

Herida *et al.* (2001) collected 88 samples of ground meat from retail stores in Mexico. Microbial analysis revealed that most of the samples were positive for faecal coliforms. Over 40 per cent of the samples had a coliform count above 10^6 level.

Kakar and Udipi (2002) assessed microbiological quality of ready to eat meat and meat products sold in Mumbai city. The mean faecal coliform count of samples recorded were 6.22, 6.65, 2.62, 5.31, 5.88, 5.28, 4.86, 2.68 and 2.39 $\log_{10}$/g in chicken, chicken pizza, chicken lollypop, chicken patties, chicken roll, mutton chops, mutton burger, mutton kabab and mutton samosa respectively.

Yilmaz *et al.* (2002) evaluated the effects of different cooking processes on microbial flora of meatballs. The study revealed the presence of coliforms at a concentration of 1.1 x 10^5 cfu/g in raw meatballs and the count was reduced by two to three log cycles in grilling and oven cooking. The microwave heating of the meatballs reduced the count by three to four log cycles.

2.1.3 *Escherichia coli* Count

Palumbo *et al.* (1976) colleted pepperoni samples from nine commercial sources and evaluated the microbial quality. *E. coli* count, reported were 1 x 10^5, 3 x 10^2, 3 x 10^5, <1 x 10^2, 3.5 x 10^2, <1 x 10^2, 3 x 10^4, 1.8 x 10^3 and 1 x 10^4/g in samples from sources A, B, C, D, E, F, G, H and imported sausage respectively.

Bacteriological analysis of uncooked pork sausage was carried out by Sharma *et al.* (1994). One hundred and eighteen samples were analyzed and it was reported that the viable count of *E. coli* ranged from 2 x 10^5 to 4.3 x 10^5 cfu/g.

Yilmaz *et al.* (2002) evaluated the effects of different cooking processes on microbial flora of meatballs. The study revealed the presence of *E. coli* at a concentration of 1 x 10^2 cfu/g in raw meatballs. Microwave heating reduced the counts more effectively when compared with grilling and oven cooking.

2.1.4 Faecal Streptococcal Count

In a study on vacuum packed frankfurters stored at 0-3°C, Simard *et al.* (1983) reported presence of streptococci in 2.8 per cent of samples on 21st day of storage.

Korkeala *et al.* (1985) evaluated cooked ring sausage samples from five different sources for microbiological and sensory quality changes during storage at 4°C. Faecal streptococcal count reported on 15th day of storage in samples from source A was 3.65 log cfu/g. In samples from source C the count on 18th day was 2.65 log cfu/g. Samples from source E had a streptococcal count of 2.30

log cfu/g on seventh day of storage. Streptococci were not detected in samples from sources B and D.

Microbial profile of chicken sausages during storage at 5 ± 1°C was studied by Anand *et al.* (1990). In raw sausage samples the initial streptococcal count was 3.19 log/g. They reported an initial count of 2.28 log/g and 2.15 log/g in dry cooked and moist cooked sausage respectively. After eight days of storage the counts were 4.10 in raw sausage samples, 2.97 in dry cooked samples and 2.86 log/g in moist cooked samples. On 10^{th} day of storage the counts in dry cooked and moist cooked sausage were 3.23 and 2.99 log/g respectively.

A study on microbial population of vacuum packed chilled (5°C) Vienna sausage was carried out by von Holy *et al.* (1991). They reported a mean enterococcal count of 2 log cfu/g in unspoiled samples aged 4 to 10 days and the count in spoiled samples aged 30-125 days was 2.13 $\log_{10}$ cfu/g.

Samelis *et al.* (1998) conducted a study on microbial quality of four batches of Greek salami and reported that the initial counts were 2.47, 3.47, 2.30 and 2.84 log cfu/g in samples of batches A, B, C and D respectively. After 28 days of ripening the counts increased to 4.69, 5.17, 5.11 and 5.0log cfu/g respectively.

Kakar and Udipi (2002) assessed microbiological quality of ready to eat meat and meat products sold in Mumbai city. The mean faecal streptococcal counts of samples were 5.02, 4.90, 1.64, 3.64, 5.12, 4.29, 4.14, 1.68 and 1.69 log10 cfu/g in chicken, chicken pizza, chicken lollypop, chicken pattice, chicken roll, mutton chops, mutton burger, mutton kabab and mutton samosa respectively.

2.1.5 Yeast and Mold Count

Steinke and Foster (1951b) studied the microbial quality of liver sausages stored at 5°C. On the day of packaging yeast count was less than 10/g which increased to 5.7 x 10^3 on 15^{th} day of storage.

In a study on effect of carbondioxide on microbial growth of frankfurters stored at 7.2°C, Ogilvy and Ayres (1952) reported an increase in yeast count from 10/g on zero day to 10^8/g on tenth day of storage with zero level CO_2. Carbondioxide at five per cent level increased the count only to 10^5/g on tenth day.

Palumbo *et al.*(1976) collected pepperoni samples from nine commercial sources and evaluated the microbial quality. Yeast and mold counts reported were 3.3 x 10^3/g, <1 x 10^2/g, 9.6 x 10^3/g, 1.0 x 10^5/g, 1.0 x 10^2/g, <1 x 10^2/g, 1.1 x 10^5/g, 1.6 x 10^4/g and <1x 10^2/g in samples collected from sources A, B, C, D, E, F, G, H and imported sausage respectively.

Cunningham and Bowers (1977) studied the microbial quality of chicken patties stored at 3°C. In fresh chicken patties the yeast and mold count was 5700/g. After 10 days of storage count was 4 x 10^3/g.

Simard *et al.* (1983) estimated the yeast and mold count of frankfurters stored at 3°C in vacuum packaging and N_2 packaging and reported an initial count of 2.7 log/g and 3.3 log/g, which decreased to 0.5 log/g and 1 log/g respectively after 14 days of storage.

Korkeala and Lindroth (1987) assessed the differences in microbial growth in the surface layer and at the centre of vacuum packed cooked ring sausage stored at 8°C. They found that after 28 days of storage at 8°C the yeast count was 2.82 log cfu/g in the surface layer and yeast was absent in the samples analyzed from the centre of the sausage.

Microbial changes during aerobic and vacuum-packed storage of British fresh sausage at 6°C was studied by Adams *et al.* (1987). Upon aerobic storage yeast count increased from 10^5 log cfu/g on zero day to 10^7 log cfu/g after 10 days of storage at 6°C. In vacuum packed samples the count was two log cycles lower than the samples in aerobic packaging, after 10 days of storage.

Anand *et al.* (1990) analysed microbial profile during refrigerated storage of chicken sausages at 5 ± 1°C. They reported an yeast and mold count of 1.15 log/g on zero day and on day two, four, six and eight of storage the counts were 1.45, 1.80, 2.25 and 2.49 log/g respectively.

Enumeration and characterization of microbial populations associated with spoiled vacuum packed Vienna sausages kept at 5°C was carried out by vonHoly *et al.*(1991). Yeast and mold count in unspoiled samples aged between four and ten days from manufacture was 1.48 $\log_{10}$ cfu/g. The count was 0.96 $\log_{10}$ cfu/g in spoiled samples aged between 30 and 125 days from manufacture.

Microbiological study on Greek Salami sausage was carried out by Samelis *et al.* (1998). The mean yeast counts detected were 4.30, 3.74, 3.69 and 3.69 log cfu/g on the day of manufacture in samples of batches A, B, C and D respectively. After a period of 28 days of ripening at 15-16°C the counts were 3.69, 3.65, 4.95 and 4.47 log cfu/g respectively.

Spoilage microflora of frankfurters stored at 4°C in vacuum packaging and aerobic packaging was analysed by Samelis *et al.* (2000). In fresh samples the yeast count was below 2 log cfu/g. After 30 days of vacuum packed storage at 4°C the count increased to 2.72 log cfu/g and in aerobic packing count increased to 3.85 log cfu/g.

Pavankumar *et al.* (2003) assessed the shelf-life of vacuum packed Tandoori chicken stored at 4 ± 1°C. In fresh samples the yeast and mold count was 1.25 log cfu/g and after 15 days of storage the count was enhanced to 1.9 log cfu/g.

2.1.6 Psychrotrophic Count

Simard *et al.* (1983) compared the effect of vacuum and nitrogen packaging on microbial quality of refrigerated (3°C) frankfurter. On zero day the psychrotrophic count in vacuum packed and nitrogen packed samples were 3.4

log_{10}/g and 4.0 log_{10}/g respectively. After 14 days of storage at 3°C the count in vacuum packaged samples was 4.5 log_{10}/g and N_2 packed samples it was 4.3 log_{10}/g.

In a study on refrigerated (5±1°C) raw chicken sausages Anand *et al.*(1990) observed an increase in psychrotrophic count from 2.21 log/g on zero day to 4.06 log/g on eighth day. In case of dry cooked chicken sausages initial count was 1.45 log/g, which was increased to 3.97 log/g on 10^{th} day.

Lee *et al.* (1997) estimated the psychrotrophic count of chicken breakfast sausages stored at 4 ± 1°C. The initial count was 4.72 log10 cfu/g. The count was 4.95, 6.44, 7.45, 8.27 and 8.76 log cfu/g on second, fourth, sixth, eighth and tenth day respectively.

Bacterial population of vacuum packaged frankfurter stored at different chilling temperatures was analsyed by Yuste *et al.* (2000) and reported that the psychrotrophic count was 4.37 log cfu/g on day of manufacture. After three weeks of storage at 2°C the count was 7.79 log cfu/g, and at 8°C the count was 8.41 log cfu/g.

Changes in microbiological quality of restructured cured chicken during storage at 4 ± 1°C was evaluated by Mandal *et al.* (2002). Psychrophilic count on day of preparation was 2.53 ± 0.02 log cfu/g, after 10 days of storage it increased to 3.71 ± 0.08 log_{10} cfu/g.

2.1.7 Isolation and Identification of Bacteria

2.1.7.1 Aeromonas

Krovacek *et al.* (1985) studied a case of food poisoning outbreak involving *Aeromonas hydrophila*. The bacteriological examination of leftover samples of smoked sausage revealed the presence of *A. hydrophila.* Investigations on the virulence profile of the isolates detected its ability to

produce beta-hemolysin, cytotoxins, cytotonic toxins, enterotoxins and adhesion to and invasion of human intestinal cells in culture

Hazen *et al.* (1987) concluded that pH did not seem to play a significant role in the distribution of *A. hydrophila* in natural aquatic habitats. The organism was isolated from samples having a pH range of 5.2- 9.8 and its growth was unaffected at pH five to nine.

Okrend *et al.* (1987) studied the incidence and toxigenicity of *Aeromonas* species. Isolates from pork sausage, poultry, ground beef, ground pork, beef liver, and chicken liver and gizzard were studied. The study revealed that Aeromonads were present in all the samples except two pork product samples. Pathogenicity studies showed that majority of the isolates were hemolysin and cytotoxin producers. Three species viz. *Aeromonas hydrophila* (48 per cent), *Aeromonas sobria* (22 per cent) and *Aeromonas caviae* (29 per cent) were isolated from meat and poultry samples. Ninety three per cent of *A. hydrophila,* 69 per cent of *A. sobria* and 18 per cent of *A. caviae* were hemolytic.

Fricker and Tompsett (1989) investigated presence of *Aeromonas* in different food samples. Of these, 79.3 per cent of poultry samples, 38.6 per cent of beef samples, 32.3 per cent of pork samples, 84.2 per cent of offal and 37.5 per cent of cooked meat samples tested were contaminated with *Aeromonas*. *A. hydrophila, A. sobria* and *A. caviae* were the three major species isolated.

Gobat and Jemmi (1993) investigated the distribution of mesophillic *Aeromonas* species in raw and ready to eat, meat and fish products in Switzerland. In smoked cooked sausage 15.6 per cent and in mortadella 12.9 per cent samples were positive for Aeromonads. Among the isolates 61.2 per cent were identified as *Aeromonas hydrophila,* 22.5 per cent as *A. caviae* and 16.3 per cent as *A. sobria*.

A study on *Aeromonas hydrophila* isolates was conducted by Handfield *et al.* (1996). Isolates from raw meat and ready to eat products were tested for

pathogenecity by hemolysis and hemagglutination assays. They reported that 41 per cent of the food isolates caused lysis of two per cent (vol/vol) rabbit erythrocyte suspension and 87 per cent of isolates agglutinated six per cent (vol/vol) rabbit erythrocyte suspension.

A study was conducted to determine the prevalence of *Aeromonas* spp. in beef sausage, ground beef, poultry and pork in Eastern Canada by Singh (1997). The author compared two commercially available media (Ryan's *Aeromonas* medium and GSP agar *Pseudomonas Aeromonas* selective agar) and the starch ampicillin medium for the recovery of *Aeromonas* spp. The recovery was superior using starch ampicillin agar. Seventy nine per cent of ground beef samples and cent per cent samples of other products were positive for *Aeromonas* spp. From beef sausage samples *Aeromonas hydrophila* (89 per cent), *A. sobria* (4 per cent) and *A. caviae* (4 per cent) were isolated.

Devlieghere *et al.* (2000) investigated growth of *Aeromonas hydrophila* in modified atmosphere packed cooked meat products stored at 4°C. The organism was shown to multiply very rapidly at refrigerated temperatures. At high water activity (0.992), a generation time of 13.6 h was noticed at 4°C.

Soriano *et al.* (2000) carried out microbiological analysis of cooked meat samples including long pork sausage, pork loin, chicken croquettes, chicken breast, and meatballs. *Aeromonas hydrophila* was detected from cooked meat samples.

Vaid and Garg (2002) conducted studies to assess the nature of growth of *Aeromonas* in refrigerated meat. Fresh minced mutton samples were inoculated with *A. hydrophila* culture suspension and were kept at 7°C or 25°C for a period till visible spoilage appeared. They found that the organism competed very well with the usual micro flora of meat at 7°C and 25°C. Their findings suggested that Aeromonads were psychrotrophic in nature and that meat acted as a good substrate for their growth.

Yadav and Kumar (2003) tested samples of fish, pasteurized milk, flavored milk and ice cream for the presence of *Aeromonas* spp. They could isolate 19 isolates of *A. hydrophila*, 13 isolates of *A. sobria* and eight isolates of *A. cariae*. It was observed that 63 per cent of *A. hydrophila* cultures, 84.6 per cent of *A. sobria* cultures and 37.5 per cent of *A. caviae* cultures were hemolytic. Cytotoxin assay revealed that 86 per cent of hemolytic isolates were cytotoxic. Results of the study demonstrated a significant correlation among the hemolytic and cytotoxic activities of *Aeromonas* spp.

2.1.7.2 Escherichia coli

Prior and Casaleggio (1978) analyzed 25 bologna samples stored at 5°C. They opined that count of *E. coli* during storage for 12 days was less than 3/g.

A survey of the bacteriological status of fresh and baked pork sausages was conducted by Oyekole and Hassan (1984). In the study *E. coli* was isolated from 48 per cent and 19 per cent of fresh and baked sausage respectively.

Bacterial quality of meat and meat products consumed by Egyptian people was evaluated by Zahra *et al.* (1985). Heavy loads of bacteria were observed in sausage, fresh beef, frozen beef and minced meat. They reported *E. coli* as one of the predominant organisms in the analysed samples, especially fresh beef.

Bacteriological investigation of minced meat and meatballs by Kaloianov *et al.* (1987) in Bulgaria revealed *E. coli* in 10.22 per cent samples.

Yoder (1989) reported that Congo red binding could be directly related to virulence of *Escherichia coli.*

Timm *et al.* (1999) investigated 158 samples of raw sausages. Verotoxin producing *E. coli* were detected in 14 (8.8 per cent) samples within a period of four months. Of the 14 isolates four were VT1, eight were VT2 and two were VT1/VT2 producers.

Monge *et al.* (2000) evaluated microbiological quality of 80 soybean sausage samples (50 per cent frankfurter and 50 per cent sausage mortadela) and *E. coli* was not isolated from any of the samples.

Soriano *et al.* (2000) examined samples of pork sausage, pork loin, chicken croquettes, chicken breast and meatballs collected from restaurants. They detected *E. coli* in 8.8 per cent of the samples tested but *E. coli* O157:H7 could not be detected in any of the samples. They highlighted the importance of implementing a hazard analysis critical control point programme in the processing plant.

Herida *et al.* (2001) collected 88 samples of ground meat from retail stores in Mexico. The microbial analysis detected *E. coli* in 76 per cent of samples, but none was serotype O157:H7. They opined that the microbiological quality of product was unsatisfactory and it could be an important cause of food poisoning.

Soni *et al.* (2002) carried out serogrouping and pathogenicity study on *Escherichia coli* isolates by Congo red binding test. Out of 17 serotypes tested, 15 produced brick red colonies on the Congo red medium. A good correlation was observed between Congo red binding and pathogenic potential.

Rajil *et al.* (2003) conducted invitro pathogenicity studies of *Escherichia coli* isolates obtained from clinical cases of colibacillosis in poultry and dead in shell embryos. In order to identify invasive *Escherichia coli*, Congo red binding assay was carried out. Invasive *E. coli* were identified by their ability to take up Congo red dye. Positive isolates produced red colonies in Congo red medium and negative isolates produced colorless colonies. The results showed that 55 per cent of the isolates from clinical cases were Congo red positive.

2.1.7.3 *Salmonellae*

Weissman and Carpenter (1969) investigated incidence of Salmonellae in meat and meat products. They analyzed fresh pork sausage, smoked pork sausage, frankfurters, smoked link sausage and miscellaneous sausage products. Salmonellae were absent in frankfurters and smoked link sausages whereas it could be isolated from 38.3 per cent of fresh pork sausage samples, nine per cent of smoked pork sausage and 6.2 per cent of miscellaneous sausage products.

Roberts *et al.* (1975) analysed samples of pork sausages collected from two large scale, two medium scale manufacturers, and several local butchers. Out of 3309 samples 786 samples were found to contain salmonellae.

Banks and Board (1983) studied the incidence of contamination with *Salmonella* in British fresh sausages and the ingredients used in their manufacture. The organism was present in 65 per cent of pork sausage and 55 per cent of pork and beef sausage. The number of organisms isolated from pork sausage was at the range of 7-40/g, 6-24/g in beef and pork sausages and 0.8-378/g in ingredients. Salmonella serotypes isolated were *S. derby, S. dublin, S. newport, S. stanley, S. typhimurium, S. heidelberg, S. infantis and S. agona*.

Oyekole and Hassan (1984) conducted a survey on the bacteriological status of fresh and baked pork sausages produced in Nigeria. Salmonellae were present in 42 per cent of the fresh pork sausage samples and in 56 per cent of the baked samples.

. Al-Rajab *et al.* (1986) analysed a total of 223 retail samples consisting of chicken pieces, Kebab, Kufta, ground meat, beef burger, steak beef and liver samples for the presence of *Salmonella* spp. Salmonellae were present in 26 per cent of chicken samples, 11 per cent of Kebab and Kufta, 18 per cent ground meat, 10 per cent of beef burger, and 10 per cent of liver samples analysed. *Salmonella* was absent in steak beef samples. The species isolated were

S. infantis, S. zanzibar, S. anatum, S. dublin, S. amsterdam, S. cerco, S. duesseldorf, S. copenhagen and S. montevideo.

An outbreak of salmonellosis was investigated by van Netten *et al.* (1986). Seventeen persons contracted the disease, as a result of consumption of fermented pork sausage prepared by a butcher. *Salmonella typhimurium* was identified as the major cause of outbreak. In addition to that *Staphylococcus aureus* and *Clostridium perfringens* were also isolated from the product.

Ihnot *et al.* (1998) studied the behaviour of *Salmonella typhimurium* DT 104 during the manufacture and storage of pepperoni. The batter was inoculated with the culture at a concentration of 4.4 x 107 organism/g of batter. After fermentation at 36°C and 92 per cent relative humidity counts of pathogen decreased by about 1.3 $\log_{10}$ units. An additional 1.6 $\log_{10}$ unit decrease was observed following drying at 13°C and 65per cent relative humidity. After storage for 56 days under vacuum at 4 or 21°C, counts of the pathogen were reduced by 4.6 and 6.6 log10 units respectively when compared with the initial levels in the batter.

Samelis *et al.* (1998) investigated the nature of microflora of Greek dry salami during the ripening period. Salmonellae were absent in all the samples tested.

Herida *et al.* (2001) analyzed 88 samples of ground meat from retail stores in Mexico. They detected *Salmonella* in 11.40 per cent of the samples tested.

Mattick *et al.* (2002) investigated the prevalence and number of *Salmonella* in sausages. They tested 109 packs of uncooked economy or catering sausages. Salmonellae were present in 9.1 per cent of chilled sausage samples.

2.1.7.4 Pseudomonas aeruginosa

Sherikar *et al.* (1979) recorded that *Pseudomonas aeruginosa* was absent in samples of cocktail sausage and Oxford sausage analyzed. However the organism was isolated from 1.2 per cent samples of pork products tested.

In a study on frankfurter sausages stored in different gas atmospheres at 4°C, Blickstad and Molin (1983) reported the presence of *Pseudomonas* in sausage samples taken directly from the processing plant. Mesophillic *Pseudomonas* was present in 17 per cent of frankfurter samples.

Simard *et al.* (1983) analysed microflora of vacuum or nitrogen packed chilled (0-3°C) frankfurters. In vacuum packed storage they could isolate *Pseudomonas* in 35.7 per cent samples on zero day, 21.1 per cent samples on seventh day and 14.3 per cent samples on 14^{th} day. The incidence in samples on 21^{st}, 28^{th} and 49^{th} day was 11.3, 3.8 and 1 per cent respectively. In N2 packed samples on zero day 49.4, seventh day 42.6 and 14^{th} day 25 per cent had *Pseudomonas*. On 21^{st}, 28^{th} and 49^{th} day the distribution was 13.6, 6.5 and 4 per cent respectively.

Samelis *et al.* (1998) studied the microflora of traditional Greek dry salami produced by one of the five largest meat industries in Greece. Four batches of samples were analyzed during the ripening period of 28 days. Initial *Pseudomonas* counts were 5.34, 4.17, 3.12 and 5.17 log cfu/g in samples belonging to batches A, B, C and D. After 28 days of ripening at 15-16°C the count decreased to <2.00 logcfu/g in all the samples tested.

2.1.7.5 Lactobacilli

Ogilvy and Ayres (1952) could isolate 120 isolates of Lactobacilli from spoiled samples of frankfurters when stored under refrigeration in glass containers with various carbondioxide concentrations. They opined that number

of lactobacilli reached very high levels just before the development of spoilage changes in appearance, flavor or pH.

Drake *et al.* (1957) isolated Lactobacilli from the surface slime on refrigerated frankfurters. They examined 36 packages of frankfurters stored at 2°C and 10°C. Twenty five isolates were obtained from samples stored at 2°C and 41 isolates from samples stored at 10°C.

A rapid and simple identification method for Lactobacilli from meat and meat products was prescribed by Schillinger and Karllucke (1987). From fermented sausages and vacuum-packed meat products they identified different strains of Lactobacilli viz., *L.brevis, L.carnis, L.coryniformis, L.farciminis, L.plantarum, L. halotolerans, L. hillgardii, L. sake* and *L. viridescens*.

Allen and Foster (1958) conducted studies on spoilage of bologna, pressed ham, smoke patties and P&P loaf. They could isolate both heterofermentative and homofermentative lactobacilli from the samples. The major species identified was *Lactobacillus viridescens*. They opined that lactic acid bacteria multiplied to very high numbers even at lower temperature.

Blickstad and Molin (1983) analyzed microflora of frankfurter sausage and smoked pork loin stored at 4°C in different gas atmospheres. They could isolate *Lactobacillus* spp. from samples stored in vacuum, CO_2 packaging and N_2 packaging on 48^{th} and 140^{th} day of storage at 4°C. Different species of Lactobacilli isolated were *L. delbrueckii, L. plantarum* and *L. viridescens*.

Simard *et al.* (1983) analyzed microflora of vacuum or nitrogen packed frankfurters. Samples were stored at 0 –3°C. Lactobacilli were detected in both types of packaging. In vacuum packed samples Lactobacilli were detected in 2 per cent samples on zero day, 9.2 per cent samples on seventh day, 36.5 per cent samples on 14^{th} day, 63.4 per cent samples, on 21^{st} day, 82.5 per cent samples on 28th day and 96.3 per cent samples on 49^{th} day. In $N_{2,}$ packed samples the distribution of Lactobacilli was 2.6per cent, 7.7per cent, 33.3 per cent 54.0 per

cent 84.12 per cent and 85.22 per cent on days 0, 7, 14, 21, 28 and 49 respectively.

Korkeala *et al.* (1985) evaluated the microbiological and sensory quality changes in cooked ring sausage during storage at 4°C. They collected samples from five different sources. Lactobacilli were detected from samples collected from four sources.

Korkeala and Lindroth (1987) detected lactobacilli in vacuum packed cooked ring sausages stored at 8°C on 28th day of storage. On the surface layer the count was 8.48 log cfu/g and in the centre of the sausage the mean count was 2.28/log cfu/g.

Korkeala *et al.* (1988) investigated the cause of ropiness in meat products and the chemical characteristics of the ropy slime. They opined that the ropy slime observed on the surface of vacuum packed cooked meat product was mainly produced by homofermentative Lactobacilli.

Presence of lactic acid bacteria in raw materials of cooked ring sausages was reported by Makela *et al.* (1990). Highest lactic acid bacterial count was found in the pork skin emulsion (4.4 log cfu/g) and the count in pork and beef trimmings was 3.6 log cfu/g. Skim milk powder and potato flour also were found to contain lactic acid bacteria. Out of 208 isolates studied, 168 were found to grow at 8°C.

Borch *et al.* (1996) reviewed mechanism of bacterial spoilage of meat and cured meat products. They reported that lactic acid bacteria caused spoilage of refrigerated meat products by producing defects such as sourness, off flavor, discoloration, gas production, slime production and lowering of pH. Surface greening was caused by homofermentative *Lactobacillus* spp., heterofermentative *Lactobacillus* spp, and *Leuconostoc* spp. Ropy slime producing *L. sake* and *L. amelibiosum* were recovered from the processing rooms at meat plants where the meat products were handled after heat processing.

Characterization of 254 strains of lactobacilli isolated from dry fermented sausages collected from 15 different sources was carried out by Hugas *et al.* (1993). They opined that the main criteria for the identification of isolates to species level were, production of lactic acid isomers, presence of mDpm acid in cell walls, deamination of argininine and fermentation of mannitol and melibiose. The species identified were *L. sake* (55 per cent), *L. curvatus* (26 per cent), *L. bavaricus* (11 per cent) and *L. plantarum* (8 per cent).

Korkeala and Bjorkroth (1997) studied the microbial spoilage and contamination of vacuum-packaged cooked sausages. They observed that lactic acid bacteria formed the major component of the microbial population on various types of vacuum packaged cooked sausages. Predominantly *L. curvatus* and *L. sake* were isolated. Spoilage changes like white liquid slime formation; ropiness and sourness were attributed to the growth of lactobacilli in sausages.

A microbiological study on Greek salami was carried out by Samelis *et al.* (1998). Four batches of samples were studied. These sausages were prepared without addition of starter cultures and fermentation was produced by an active natural lactic flora consisting of strains of *L. sake.*

2.1.8 pH

Steinke and Foster (1951b) assessed the effect of chilled (5°C) storage on microbial changes in liver sausage and bologna. In liver sausage sample the pH on zero day was 6.51, which remained same on 15^{th} day of storage at 5°C. Meanwhile in case of bologna pH on zero day was 6.35, which was reduced to 6.27 on 14^{th} day of storage at 5°C.

Smith and Palumbo (1973) recorded that bologna sausage prepared from fresh meat had a pH of 5.6 and when prepared from aged meat (14 days at 5°c) the pH was 4.5. The pH of bologna prepared from thawed meat was 4.6.

Palumbo *et al.* (1976) carried out chemical analysis of pepperoni samples collected from nine sources. The pH reported were 5.5, 5.2, 6.1, 4.8, 5.8, 4.9, 5.1, 4.7 and 5.3 in samples collected from sources of A, B, C, D, E, F, G, H and imported sausages respectively.

Blickstad and Molin (1983) analyzed frankfurter sausage stored at 4°C in different gas atmospheres. Initial pH recorded was 5.9 and on 20th days of storage in carbondioxide and nitrogen packing the pH was reduced to 5.6 and 5.8 respectively.

Krishnan and Sharma (1993) conducted studies on chilled and frozen stored ready to eat buffalo beef sausages. On the day of preparation pH was 6.3 ± 0.1. After seven days of storage at 4-5°C pH was slightly increased to 6.4 ± 0.1.

Quality evaluation of chicken meat sausages was carried out by Reddy and Vijayalakshmi (1998). Four different formulations were studied and the initial pH of the different preparations were 5.64 ± 0.01, 5.69 ± 0.01, 5.80 ± 0.01 and5.78 ± 0.01.

Borch *et al.* (1996**)** investigated various aspects of bacterial spoilage of meat and cured meat products. They reported a reduction in pH from 6.00-6.5 to 5.00-5.3 during refrigerated storage, due to lactic acid production. They opined that the microbial growth in cooked meat products was not affected by initial pH value.

The microbiological and physicochemical changes occurred during the fermentation and ripening at 15-16°C, of four batches of Greek dry salami manufactured without starter cultures were analyzed by Samelis *et al.* (1998). On the day of preparation pH of the sausage samples were 6.3, 6.3, 6.2 and 6.1 respectively in batches A, B, C and D. After 28 days of ripening pH was lowered to 5.1 in samples of batches A and B. Samples of batches C and D had pH 5.2 and 5.0 respectively.

Rao *et al.* (1999) assessed the stability of smoked chicken sausages, containing milk proteins, stored at 4 ± 1°C. Initial pH of sausage prepared without milk protein was 6.36 which was lowered to 6.30 after 4 weeks storage .The pH of fresh samples recorded were 6.38, 6.42 and 6.42 respectively in sausages prepared by addition of milk co-precipitate, skim milk powder and sodium caseinate to sausage emulsion. After four weeks of storage at 4 ± 1°C, p^H was lowered to 6.36, 6.38 and 6.41 respectively.

Effect of antioxidant vitamins on the quality of chevon sausage during storage at 4 ± 1°C was assessed by Verma and Sahoo (2000). The pH of the sausage emulsion prepared without addition of antioxidants was 6.13 ± 0.01. When ascorbic acid was added to the emulsion pH was 6.08 ± 0.01 and addition of tocopherol acetate lowered the pH to 6.09 ± 0.01. When both the antioxidants were added pH recorded was 6.03 ± 0.01. The pH of freshly prepared sausages without addition of antioxidants was 6.22 ± 0.01. After 12 days of storage at 4 ± 1°C it was reduced to 5.53 ± 0.02. In sausage samples containing both the antioxidants pH on zero day was 6.23 ± 0.02, which was lowered to 5.65 ± 0.03 on 12^{th} day of storage at 4 ± 1°C.

Gines *et al.* (2003) studied the effect of storage at 4±1°C on quality characteristics of Bologna sausages made with citrus fibre. On day of preparation pH of sausage was 5.88. Small differences were evident for the pH levels during the time of storage, reaching a value of 6.22 ± 0.10 after 28 days of storage. No significant difference in pH was noticed with addition of different concentrations of citrus fibre.

2.1.9 TBARS Number

Witte *et al.* (1970) described a new extraction method for estimation of thiobarbituric acid value. An extracting solution containing 20 per cent trichloro-acetic acid in 2 *M* phosphoric acid was used for determining thiobarbituric acid values of pork and beef stored at 4°C and –20°C. In beef samples stored at 4°C

mean TBA values gradually increased from 0.102 mg malonaldehyde/kg on second day of storage to 0.153 on seventh day of storage. In case of pork samples the value increased from 0.103 on second day of storage to 0.208 after seven days.

Baker *et al.* (1972) studied the effect of refrigerator storage (2°C for 24 days) on TBA value of chicken frankfurter. A slight increase in values was reported over the 24 days of storage and the maximum TBA value reported was 0.88 mg malonaldehyde/kg.

Krishnan and Sharma (1993) carried out distillation method for the determination of TBA value of buffalo beef sausages. The initial value reported was 0.49 mg malonaldehyde/kg.

Storage stability of chicken meat sausages was assessed by Reddy and Vijayalakshmi (1998). For sausages prepared with two different formulations using cooked meat the TBA values obtained were 0.64 ± 0.02 and 0.63 ± 0.03 mg malonaldehyde/kg.

Effect of preblending with antioxidant vitamins on lipid oxidation of chilled (4 ± 1°C) chevon sausage was assessed by Verma and Sahoo (2000). In fresh sausage samples without addition of antioxidants, TBARS number reported was 0.67 ± 0.02 mg malonaldehyde/kg. After 12 days of storage at 4 ± 1°C the value increased to 1.84 ± 0.03 mg/kg. When ascorbic acid was added as antioxidant, on zero day the TBARS number was 0.51 ± 0.02 mg malonaldehyde/kg. After 12 days at 4±1°C it increased to 1.51 ± 0.03 mg/kg. When tocopherol acetate was used as antioxidant in the sausage, on day of preparation the value was 0.44 ± 0.01 mg/kg and after 12 days it was 1.59 ± 0.02 mg/kg. When both the antioxidants were added, on zero day TBARS number was 0.30 ± 0.02, which increased to 1.37 ± 0.05 mg/kg after 12 days of chilled storage.

Effect of storage conditions viz., chilled (4 ± 1°C) storage under lighting and darkness on quality of Bologna sausage made with citrus fiber was evaluated by Gines *et al.* (2003). In order to assess the rate of lipid oxidation in samples TBA values were recorded. They reported that TBA values of samples stored under lighting conditions were higher and increased more rapidly than under darkness, throughout the period of storage. In Bologna samples without citrus fiber the initial TBA value was 4.26, which increased to 7.99 mg malonaldehyde/kg on 28th day of chilled storage, whereas for samples stored in darkness the value on 28th day was 6.71 mg malonaldehyde/kg.

2.1.10 Organoleptic Evaluation

Ogilvy and Ayres (1952) studied the microbial growth and organoleptic qualities of frankfurters stored at 4.4°C in glass containers with various concentrations of carbondioxide. Presence of yeast and mold colonies, sliminess and greening were observed in spoiled samples stored at different carbondioxide concentration. Watery slime development by Lactobacilli, yeast and mold colonies and greening were present in samples stored at 50 per cent carbondioxide concentration.

Allen and Foster (1958) conducted a study involving storage of vacuum-packed processed meats viz., bologna and smoke patties under refrigeration storage (1.1°C and 7.2°C). Bologna samples developed an atypical flavour on 40th day of storage at 7.2°C, whereas smoke patties developed slime and atypical flavour on 20th day of storage.

Korkeala *et al.* (1985) evaluated the microbiological and sensory quality changes in cooked ring sausages during storage at 4°C. They reported that one week after the sell- by date the samples developed sliminess, fermented or musty odor and sour taste.

A study on shelf-life of British fresh sausage in vacuum packs stored at 6 ± 1°C was conducted by Adams *et al.* (1987). They reported that after nine days

of storage the sausage had a stale, yeasty odor and there were areas of grey surface discoloration with a visible slime.

Anand *et al.* (1990) observed that raw chicken sausages had a shelf-life of seven days at 5 ±1ºC and dry and moist cooked sausages had comparatively a longer shelf-life of nine and ten days respectively.

Shelf-life of ready to eat buffalo beef sausages stored in chilled (4-5ºC) condition was evaluated by Krishnan and Sharma (1993). No significant changes in color, odor and surface appearance were observed in sausage during storage for seven days.

Korkeala and Bjorkroth (1997) reviewed microbiological spoilage of vacuum packaged cooked sausages. Organoleptic quality changes observed in spoiled sausages were acidity (sour taste), white liquid slime formation, gas formation and ropiness. When lactic acid bacteria population reached 6 x 10^7 cfu/g, pH decreased sharply and sourness was developed. Gas formation inside the packages could be attributed to metabolic by-products of heterofermentative lactobacilli.

2.2 FROZEN STORAGE

2.2.1 Total Viable Count

In frozen chicken patties stored at –18ºC Anand *et. a.l* (1991) reported aerobic plate count of 3.78±0.04, 3.51± 0..4, 3.53± 0.00, 3.47± 0.02 and 3.42± 0.02 on $15^{th,}$ 30^{th} ,$45^{th,}$ 60^{th} and 90^{th} day of storage respectively.

Krishnan and Sharma (1993) studied the effect of frozen (–10°C) storage on aerobic plate count of beef sausages and reported that the count decreased from 3.75 log/g in fresh samples to 3.55 log/g in samples stored frozen for two weeks. The count was 3.56 after six weeks and 3.57 log/g after eight weeks of storage at –10°C.

Reddy and Vijayalakshmi (1998) studied the quality of frozen chicken meat sausage. They reported a mesophilic count of 4.62 ± 0.05 log/cm^2 in fresh chicken meat sausage, which was reduced to 4.04 ± 0.01 log/cm^2 on 60th day of frozen storage.

2.2.2 Psychrotrophic Count

Emswiler *et al.* (1976) reported an increase in psychrotrophic count in ground beef stored at –1.7 ± 0.6°C from 4.86 log count/g on day of preparation to 5.52 log count/g over a period of 18 days of storage.

Chicken patties stored at –18°C were analyzed for microbial qualities by Anand *et al.*(1991). On day of manufacture the psychrotrophic count was 3.26 ± 0.02 $\log_{10}$ cfu/g, which showed a slight increase to 3.39 ± 0.02 $\log_{10}$ cfu/g on 90th day of storage.

Frozen British beef sausages were found to contain psychrotrophs at the level of 2 x 10^5 cfu/g (Khalafalla and Sherif, 1993).

In a study on effect of different methods of freezing on the microbial flora of beef patties, Kraft *et al.* (1979) reported that the cryogenic freezing produced significantly greater reduction in microbial counts than the mechanical freezing. The psychrotrophic count reported on zero day was 7 log/g, which was lowered to 4.35, 4.42 and 4.82 on 150th day of storage using liquid nitrogen, liquid carbon dioxide and by mechanical freezing respectively.

Bhoyar *et al.* (1998) analysed chicken steaks stored at –18 ± 1°C for microbial quality changes. Psychrotrophic count of steaks decreased from 1.99 log cfu/g in fresh steaks to 1.89 log cfu/ in steaks after 60 days of storage.

2.2.3 Isolation and Identification of Bacteria

2.2.3.1 *Aeromonas*

Psychrotrophic microflora of frozen beef sausage was analysed by Khalafalla and Sherif (1993). In the study Aeromonads could be detected from all the 50 samples analyzed. Mean *Aeromonas* count was 4 x 10^2 log cfu/g.

2.2.3.2 Escherichia coli

In a study Emswiler *et al.* (1976) reported an initial *E. coli* count of 1.24 $\log_{10}$/g in ground beef samples stored at –1.7 ± 0.6°C. The counts reported on third, sixth and ninth day of storage were 1.13, 1.07 and 0.90 $\log_{10}$/g respectively. The count was 0.82, 10.0 and 1.04 $\log_{10}$/g after 12, 15 and 18 days of storage respectively.

Fifty samples of fresh beef sausage stored in freezer were examined by Khalafalla and Sherif (1993) and they reported that *E. coli* was present in 20 per cent of the samples. They opined that the maintenance of food temperature close to freezing retarded spoilage and prevented multiplication of pathogens.

2.2.3.3 *Salmonellae*

Mattick *et al.* (2002) investigated the prevalence and number of Salmonellae in uncooked economy or catering sausages. They tested 53 packs of frozen sausage. Salmonellae were present in 7.5 per cent of the samples

2.2.3.4 Pseudomonas aeruginosa

A total of 50 samples of frozen beef sausage were analysed for enumeration, isolation and identification of psychrotrophic bacteria by Khalafalla and Sherif (1993). They reported the presence of *Pseudomonas* in all the samples tested.

2.2.3.5 *Lactobacilli*

Recovery of lactobacilli from frozen food is minimum because of the lethal effect of freezing on lactobacilli (Hall *et al.*, 2001).

2.2.4 pH

Krishnan and Sharma (1993) conducted studies on frozen (-10°C) stored ready to eat buffalo beef sausages. On the day of preparation pH was 6.3 ± 0.1. In frozen samples there was no change in pH when compared with fresh sausage.

Ho *et al.* (1995) carried out a study on storage stability of vacuum packaged frozen pork sausage. Three different formulations were prepared by different combinations of fat, water and antioxidant. The pH of uncooked fresh sausages belonging to three different formulations were 5.94, 5.98 and 6.00 respectively. The pH analysis of sausages showed that antioxidants and packaging system did not affect the pH.

Reddy and Vijayalakshmi (1998) carried out quality evaluation of frozen chicken meat sausages. Four different formulations were studied and the pH of the different preparations were 5.64 ± 0.01, 5.69 ± 0.01, 5.80 ± 0.01 and 5.78 ± 0.01. After 60 days of storage at –18 ± 1°C the mean pH recorded was 5.76 ± 0.01.

2.2.5 TBARS Number

Chang *et al.* (1961) mentioned that the formation of carbonyl addition products would possibly account for the apparent loss in malonaldehyde during freezing and so lowering of TBA values.

In a study conducted by Witte *et al.* (1970), TBA values of frozen pork samples were lowered from 0.140 on first day of storage to 0.129 on sixth day of storage. Changes in TBA values of beef during storage at –20°C were insignificant.

Krishnan and Sharma (1993) carried out distillation method for the determination of TBA value of buffalo beef sausages stored at –10°C. The initial value of 0.49 mg malonaldehyde/kg was reduced to 0.40 mg/kg on second week and the value after eight weeks of frozen storage was 0.14 mg malonaldehyde/kg.

Storage stability of vacuum packaged frozen pork sausages was assessed by Ho *et al.* (1995). TBARS (thiobarbituric acid reacting substances) values of three different formulations were recorded. First formulation contained no antioxidant. Second formulation contained combination of butylated hydroxytoluene, propylgallate and citric acid and third formulation contained extracts of rosemary. Initial TBARS values indicated no differences among formulations. During storage at –20°C for 16 weeks TBARS value of all treatments increased gradually. Values for fresh pork sausage without antioxidants increased rapidly. Products containing rosemary extracts and chemical antioxidants had a low TBARS value (<1 mg. malonaldehyde/kg) throughout storage. Results of the study indicated that addition of antioxidants was effective in preventing lipid oxidation in high fat sausage products.

Storage stability of chicken meat sausages was assessed by Reddy and Vijayalakshmi (1998). For sausages prepared with two different formulations using cooked meat the TBA values obtained were 0.64 ± 0.02 and 0.63 ± 0.03 mg malonaldehyde/kg, and for sausages prepared with raw meat after four and six weeks of frozen storage were 0.14 and 0.19 mg/kg. After eight weeks of frozen storage the value recorded was 0.14 mg malonaldehyde/kg.

2.2.6 Organoleptic Evaluation

Krishnan and Sharma (1993) evaluated spoilage changes in frozen ready to eat buffalo beef sausages. They reported that there was no significant difference in color, odor and surface appearance after eight weeks of storage at -10ºC.

Sensory evaluation of chicken meat sausages was carried out by Reddy and Vijayalakshmi (1998). Sausages prepared with raw meat scored significantly superior for appearance flavor, juiciness, firmness and overall acceptability. Loss of volatile compounds, retention of moisture and fat resulted in lowering the flavor, juiciness and overall acceptability scores of cooked meat sausages on 30 day of storage at –18 ± 1°C. The scores for flavor, juiciness and firmness were significantly lower at 45 days of storage.

2.3 CRITICAL CONTROL POINTS

de-Wit and Kampelmacher (1982) analyzed handwashing samples collected from workers in slaughterhouses. They found that all workers examined carried *E. coli* on their hands during work. Salmonellae were also detected in hand washing samples.

Tebutt (1986) evaluated various working practices in shops selling raw and cooked meats. Butcher's shops, supermarkets and premises of general dealer shops were compared. *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus faecalis* were widely distributed in all three groups of working premises. Contamination of hands, towels and nailbrushes was attributed to poor working practices. The study revealed that there was poor correlation between microbiological results and visual inspection.

Kaloianov *et al.* (1987) investigated the hygienic condition of three meat-dressing plants. The study was made on a total of 1,042 samples of washings taken from equipment, working tools, machines and hands and aprons of workers prior to the beginning of work and during work. They opined that during work there was considerable increase in the number of indicative organisms. No pathogenic Staphylococci and salmonellae were isolated. Results of the study showed that proper cleaning, washing and disinfection practices could reduce the residual microflora to a minimum 11.

de-Wit and Kampelmacher (1988) investigated the extent of bacterial contamination of hands of workers in food service establishments. No salmonellae could be detected but high number (>10^5 /hand) of *Enterobacteriaeceae* and *Staphylococcus aureus* occurred in about eight per cent of the hand washing samples.

In a study on the importance of implementing HACCP in the production of meat and poultry products, Tompkin (1990) opined that raw meat, spices and other ingredients were the major sources of contamination in the preparation of dry fermented sausage. According to the study, there were certain product characteristics like microflora of ingredients, seasonings added, brine content, carbohydrate content, acid content, smoke, phosphate content, metal ion content, nitrite content and addition of sodium lactate and potassium sorbate, which influenced the micorbial content of ready to eat meat and poultry products.

Makela *et al.* (1990) analysed the raw materials of cooked ring sausages for the presence of spoilage causing lactic acid bacteria of sausages in a meat processing plant and reported that the these materials formed a source for its spoilage. Highest counts were found in pork skin emulsion and meat trimmings. Lactic acid bacteria were also found in skim milk powder and potato flour.

Anand *et al.* (1990) evaluated the microbial quality of chicken sausages during preparation and storage. They analyzed minced meat samples and sausage casings. Total aerobic counts reported were 4.43 log/g and 3.13 log/g in minced meat and casings respectively. Coliform counts were 1.93 log/g and 2.16 log/g respectively. Faecal streptococcal count of minced meat samples was 2.62 log/g whereas streptococci were absent in samples of casing. Samples of spices and condiments were also analyzed. All the samples had a total aerobic count higher than 10^3 cfu/g level. Coliforms were also detected in those samples.

Rao and Ramesh (1992) conducted a study in order to identify the critical points of microbial contamination in the slaughter line. They analyzed water;

knives, floors, wall processing equipment and hand wash of workers for microbial quality. The study revealed a total viable count at level of 10^2 cfu / cm^2 or per ml in floor washings, wall and equipments, before slaughtering operation. The mean count of water and knives was at the level of 10 cfu/cm^2 or per ml. They observed a four fold increase of the count in the samples collected after slaughter.

Tarwate *et al.* (1993) evaluated the hygienic condition of slaughter line and surroundings. They examined knife, axe, saw blade, hooks, floor, wall, platform, hand swab and water and reported that the total viable count of these samples varied from 6.70 $\log_{10}$ cfu/cm^2 to 2.9 $\log_{10}$ cfu/cm^2. The coliform count ranged between 6.9 $\log_{10}$ cfu/ cm^2 and 4.2 $\log_{10}$ cfu/ cm^2. Water samples had the mean count of 2.07 $\log_{10}$ cfu/ml. They could detect the presence of potential pathogens such as *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium* spp., *Escherichia coli* and *Shigella* spp. and spoilage organism.

Korkeala and Bjorkroth (1997) studied contamination and spoilage of vacuum packaged cooked sausages. Cold storage rooms, slicing and packaging rooms were identified as critical points of bacterial recontamination. During the chilling process, contamination occurred mainly through the air and during packaging and slicing major sources were workers and equipment.

Eisel *et al.* (1997) estimated the microbial load of air floor and processing equipment in a processing plant. The mean total viable count of air samples was 0.6 cfu/m^3. Processing equipment had a count of 1 cfu/cm^2. Coliforms and *Escherichia coli* were not recovered from these samples.

Materials and Methods

3. MATERIALS AND METHODS

The present study was undertaken to assess the effect of refrigeration (chilling and freezing) on the microbial, physico-chemical and organoleptic qualities of beef frankfurter and chicken pepperoni. The sausage samples were collected from a meat processing plant located at Kochi in Kerala.

The microbial quality of fresh and chilled samples of beef frankfurter and chicken pepperoni were evaluated by estimating the Total viable count (TVC), Coliform count (CC), *Escherichia coli* count (ECC), Faecal streptococcal count (FSC), Psychrotrophic count (PC) and yeast and Mold count (YMC). The quality of the frozen products was detected by estimating the Total viable count and Psychrotrophic count.

Fresh, chilled and frozen samples of the products were tested for the isolation of *Aeromonas*, *Escherichia coli*, Salmonellae, *Pseudomonas aeruginosa* and Lactobacilli.

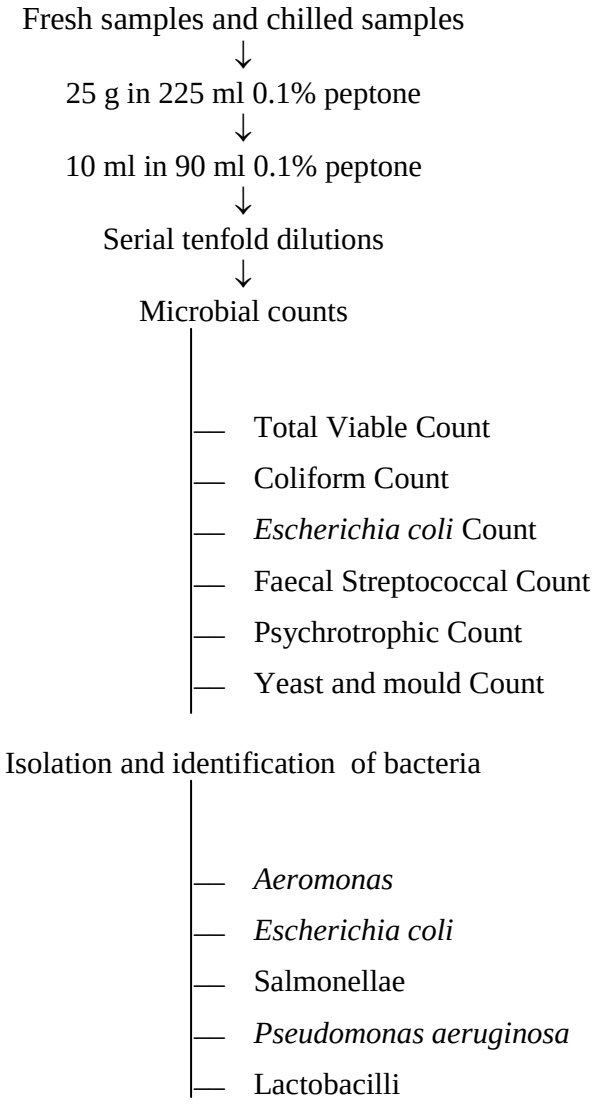
The organoleptic qualities of the products such as color, odor and presence of sliminess and the changes in pH and TBARS number were also assessed.

3.1 BEEF FRANKFURTER

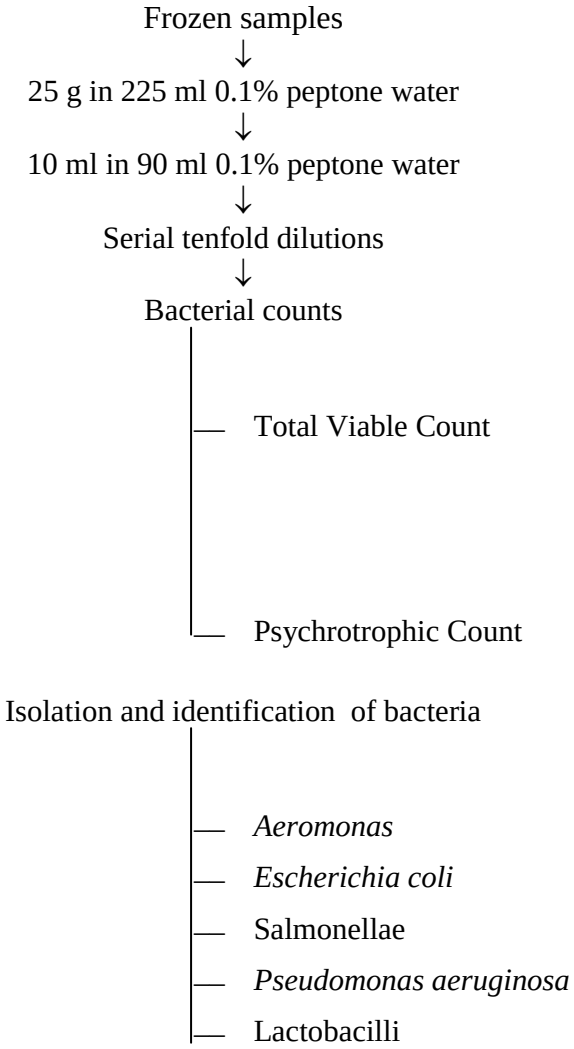
3.1.1 Collection of Samples

In the present study, beef frankfurter samples were collected from six batches. From each batch 24 samples, each consisted of 250 g, was collected randomly on the day of production and brought to the laboratory in a thermocool container. The distribution of the samples, storage temperature, period of storage, day of examination and number of samples examined on each day are shown in table 1.

Flowchart 1. Microbial analysis



Flow Chart 2. Microbial analysis



Flow Chart 3. Physico-chemical analysis and organoleptic evaluation

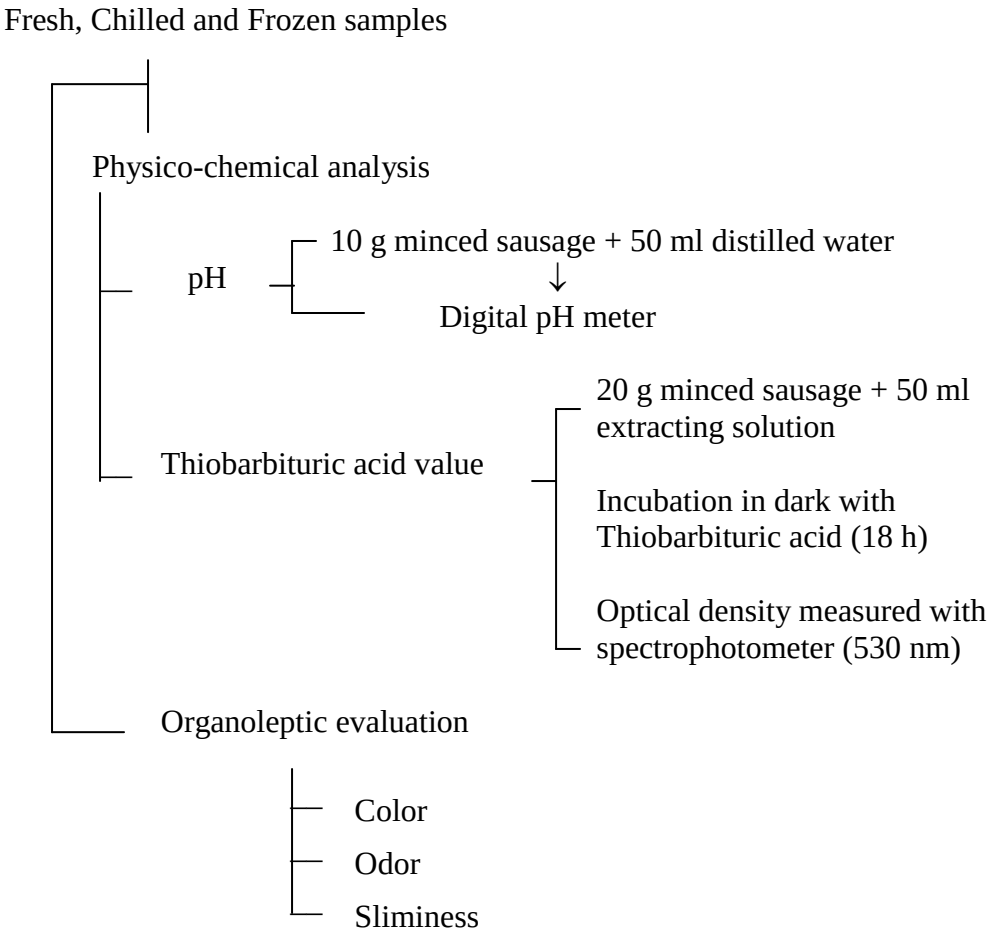


Table 1. Distribution of samples of beef frankfurter collected for analysis

Fresh		Chilled (4-7°C)		Frozen (-20 °C)	
Samples examined		Samples examined		Samples examined	
Day	Number	Day	Number	Day	Number
0 day	12	2	12	15	12
		4	12	30	12
		6	12	45	12
		8	12	60	12
		10	12	75	12
				90	12
Total	12		60		72

3.1.2 Fresh and Chilled Storage

Fresh and chilled samples of beef frankfurter were subjected to evaluation of microbial, physico- chemical and organoleptic quality.

3.1.2.1 Evaluation of Microbial Quality

Microbial quality of the samples was evaluated by estimation of microbial counts, isolation and identification of pathogenic and spoilage causing bacteria.

Processing of samples

Fresh sample

Organoleptic qualities of the samples such as color, odor and sliminess were evaluated before processing. Each sample was opened aseptically and sausage links were cut into small pieces and minced in a sterile stainless steel waring blender for three minutes at 8000 rpm. From the minced sample, 25 g was weighed and transferred to a sterile conical flask containing 225 ml of 0.1per cent peptone water (diluent) and blended using a cyclomixer so as to extricate the

microorganisms from the sample into the diluent. This formed the initial test sample. From the processed sample 10 ml was transferred into a sterile conical flask containing 90 ml of the diluent and mixed properly. Further 10 fold serial dilutions were prepared by transferring one milliliter of the inoculum into nine milliliter of the diluent. From each sample dilutions were made up to 10^{-6} and selected dilutions were used for assessing various microbial counts.

Chilled sample

Chilled sample was thawed to room temperature and then processed as in the case of fresh sample.

3.1.2.1.1 Microbial counts

Total viable count

Total viable count of each sample was estimated by pour plate technique, as described by Mortan (2001). From the selected dilution of each sample, one milliliter of the inoculum was transferred on to duplicate petri dishes of uniform size. To each of the inoculated plates, about 15-20ml sterile molten standard plate count agar (Hi-media) maintained at 45°C, was poured and mixed with the inoculum by gentle rotatory movement in clock-wise, anti-clock wise, forward and backward directions. The inoculated plates were allowed to solidify at room temperature and incubated at 37°C for 24 h. At the end of incubation period Petri dishes with a bacterial count between 30 and 300 colonies were selected and the colony counts were taken with the help of a colony counter. The number of colony forming units (cfu) per gram of sausage was calculated by multiplying the mean colony count of the duplicate plates with the dilution factor and expressed as $\log_{10}$ cfu/g.

Coliform count

Coliform count per gram of sample was estimated as per the procedure described by Kornaki and Johnson (2001). Surface spread method was carried out

in duplicate plates of Violet Red Bile Agar (VRBA) (Hi-media), using 0.1 ml of selected dilution per Petridish. The plates were incubated at 37°C for 24 h. At the end of incubation period, purplish red colonies with a diameter of at least 0.5 mm, surrounded by a reddish zone of precipitate were counted as coliforms. Number of organisms per gram of the sample was calculated by multiplying the mean count of the duplicate plates with the dilution factor and the count was expressed as $\log_{10}$ cfu/g.

Escherichia coli count

The number of *Escherichia coli* per gram of sausage sample was estimated as per the method prescribed by Indian Standards (1980).Surface spread method was carried out in duplicate plates of Eosin Methylene Blue (EMB) Agar (Hi-media), using 0.1 ml of selected dilution per petridish. The plates were incubated at 37°C for 24 h. After the incubation period colonies with a dark center and a distinct indelible ink greenish black metallic sheen on deflected light were counted as *Escherichia coli*. The number of organisms per gram of sample was calculated as in the case of total viable count and expressed as $\log_{10}$ cfu/g.

Faecal streptococcal count

Faecal streptococcal count was estimated as per the standard procedure given by the Nordic Committee on Food Analysis (1968). Surface spread method was carried out in duplicate plates of Karl Friedrich (KF) streptococcal agar (Hi-media), using 0.1 ml of selected dilution per Petri dish. The plates were incubated at 37°C for 48 h. After the period of incubation pink to dark red colonies with a diameter between 0.5 and 3 mm and surrounded by a narrow whitish zone were counted as faecal streptococci. The number of organisms per gram of sausage was calculated and expressed as described in total viable count.

Psychrotrophic count

Psychrotrophic count of each sample was assessed by spread plate technique as suggested by Cousin *et al*. (2001). Surface spread method was carried out in duplicate plates containing plate count agar (Hi-media), using 0.1 ml of selected dilution per Petridish. The plates were incubated at 7 ± 1°C for 10 days. At the end of incubation, the colonies were counted with the help of a colony counter. The number of colony forming units per gram of the sample was calculated as described for total viable count and the count was expressed as $\log_{10}$ cfu/g.

Yeast and mold count

Method described by Beuchat and Consin (2001) was followed for estimation of Yeast and Mold Count. Surface spread method was carried out in duplicate plates of Potato dextrose agar (H-media), using 0.1 ml of selected dilution per Petridish. Plates were incubated at 25°C for 5 days. At the end of incubation the colonies were counted with the help of a colony counter. The number of colony forming units per gram of the sample was calculated as described for total viable count and the count was expressed as $\log_{10}$ cfu/g.

3.1.2.1.2 Isolation and identification of bacteria

Each sausage sample was tested for the isolation and identification of *Aeromonas, Escherichia coli*, Salmonellae, *Pseudomonas aeruginosa* and lactobacilli.

Aeromonas

For the isolation of *Aeromonas* from fresh beef frankfurter samples a loopful of the initial test sample was streaked on to duplicate plates of *Aeromonas* starch DNA agar (Hi-media) supplemented with ampicillin and incubated at 30°C for 24 h (Palumbo *et al.*, 2001). After the incubation period agar plates were flooded with Lugol's iodine and honey yellow colored colonies were selected.

Psychrotrophic count

Psychrotrophic count of each sample was assessed by spread plate technique as suggested by Cousin *et al.* (2001). Surface spread method was carried out in duplicate plates containing plate count agar (Hi-media), using 0.1 ml of selected dilution per Petridish. The plates were incubated at 7 ± 1°C for 10 days. At the end of incubation, the colonies were counted with the help of a colony counter. The number of colony forming units per gram of the sample was calculated as described for total viable count and the count was expressed as $\log_{10}$ cfu/g.

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Three or four such characteristic colonies were transferred on to nutrient agar slants and incubated at 30°C for overnight. The isolates were subjected to further characterization and identification by cultural, morphological and biochemical reactions described by Barrow and Feltham (1993.) and are shown in Flow Chart 4.

In order to isolate the organism from chilled and frozen samples of beef frankfurter, 10 ml of the initial test sample was transferred to a sterile conical flask containing 90 ml of alkaline peptone water (pH 8.7 ± 0.1) and incubated at 30°C for overnight for enrichment (Lopez *et al*., 1998). At the end of incubation, a loopful of the inoculum from the enriched sample was streaked on to duplicate plates of *Aeromonas* starch DNA Agar and incubated at 30°C for 24 h. Further characterization and identification of the isolates were carried out as described previously in case of fresh beef frankfurter samples.

Escherichia coli

For the isolation of *Escherichia coli* from fresh samples of beef frankfurter, a loopful of inoculum from the initial test sample was inoculated on to duplicate plates of Eosin Methylene Blue Agar and incubated at 37°C for 24 h (Indian Standards, 1980). At the end of incubation period three or four colonies with a dark center and a distinct indelible-ink greenish black metallic sheen on deflected light were transferred on to nutrient agar slants and incubated at 37°C overnight. Those isolates were subjected to further characterization and identification by cultural, morphological and biochemical reactions as described by Barrow and Feltham (1993) and are shown in Flow Chart 5. Isolates were serotyped at National *Salmonella* and *Escherichia* Centre, Central Research Institute, Kasauli, Himachal Pradesh.

In order to isolate *Escherichia coli* from chilled and frozen samples of beef frankfurter 10 ml of the initial test sample was added to 90 ml of Trypticase soy broth and incubated at 37°C for 6 h for enrichment (Hara-Kudo *et al*., 1999).

From the enriched sample a loopful of inoculum was streaked on to duplicate plates of Eosin Methylene Blue Agar. Further isolation and identification of the isolates was carried out as described in case of fresh beef frankfurter samples.

Salmonellae

In order to isolate salmonellae, 10 ml each of the initial test sample was transferred into a sterile conical flask containing 90 ml of tetrathionate broth (Hi-media) and to an equal quantity of selenite cystine broth (Hi-media) (Cox *et al*., 1981). The contents of the flask were mixed thoroughly and the flask containing tetrathionate broth was incubated at 37°C for 48 h and the flask containing selenite cystine broth was incubated in a water bath at 43°C for 48 h. At the end of 24 and 48 h of incubation, a loopful of the culture from each of tetrathionate broth and selenite cystine broth was inoculated on to of duplicate plates of Mac Conkey Agar and incubated at 37°C for 24 h. At the end of incubation, translucent, colorless colonies with opaque center (Andrews *et al*., 2001) were selected from Mac Conkey Agar plates and were transferred to nutrient agar slants and incubated at 37°C for overnight and stored at refrigeration temperature for further characterization. The cultural, morphological and biochemical characteristics of the isolates were identified according to the procedure described by Edwards and Ewing (1972) and Barrow and Feltham (1993) and are shown in Flow Chart 6.

Pseudomonas aeruginosa

For the isolation of *Pseudomonas aeruginosa*, a loopful of initial test sample was streaked on to duplicate plates of Pseudomonas Agar base (Hi-media) supplemented with Cetrinix (FD 029, Hi-media) and plates were incubated at 30°C for 24 h.

At the end of incubation, pigmented/non pigmented smooth circular colonies were transferred on to nutrient agar slants and incubated at 30°C for overnight. The isolates were stored at refrigeration temperature. The cultural,

morphological and biochemical characteristics of the isolates were tested according to the procedure described by Barrow and Feltham (1993) and are shown in Flow Chart 7.

Lactobacilli

In order to isolate lactobacilli, a loopful of inoculum from the initial test sample was inoculated on to duplicate plates of *Lactobacillus* MRS agar (Mann Rogosa Sharpe) (Hi-media). Plates were incubated at 30°C for 48 h in an anaerobic jar (Murano and Hudnall, 2001). At the end of incubation, cream colored, circular, smooth colonies were transferred on to nutrient agar slants and incubated at 30°C for overnight. The isolates were tested for further characterization and identification by cultural, morphological and biochemical reactions described by Barrow and Feltham (1993) and are shown in Flow Chart 8.

In-vitro Pathogenicity Studies

1. ***Escherichia coli***

   a. Congo red Binding Assay

Congo red binding assay of the *Escherichia coli* isolates were carried out by the method given by Rajil *et al.* (2003). The media used was Tryptone Soya Agar supplemented with 0.03 per cent congo red dye (Nesslers) and 0.15 per cent bile salts (Loba Chemie). *E. coli* isolates were cultured on duplicate plates of the congo red medium and incubated at 37°C for 24 h. After incubation, the cultures were left at room temperature for 48 h to facilitate annotation of results. Invasive *Escherichia coli* were identified by their ability to take up congo red dye and production of characteristic brick red colonies.

2. ***Aeromonas***

Hemolysis and hemagglutination assays of the *Aeromonas* isolates were carried out according to the procedure described by Handfield *et al.* (1996). In the present study instead of Trypticase soy broth, brain heart infusion broth was used for culturing the *Aeromonas* isolates.

Hemolysis assay

Rabbit erythrocytes suspended in sterile 20 *mM* phosphate-buffered saline (pH 7.2) (PBS) were centrifuged (3400 xg for 10 min. at 4°C), washed three times and resuspended in PBS to obtain two percent (vol/vol) suspension. *Aeromonas* isolates were cultured in five milliliter of Brain heart infusion broth and incubated at 30°C for 18 h. Supernatant fluid was collected after centrifugation at 10,000 xg for 30 min at 4°C and 50 µl of the supernatant was mixed with an equal volume of two per cent (vol/vol) suspension of washed erythrocytes in a 96-well microtitre plate. The mixture was incubated at 37°C for one hour and then at 4°C for one hour. An erythrocyte suspension in PBS was included in each assay as a negative control. Hemolysis was produced by pathogenic organisms and was recorded by visual examination.

Haemagglutination assay

Aeromonas isolates were streaked on Trypticase soya agar plates (Hi-media) and incubated at 30°C for 18 h. After the incubation, two loopfuls of culture was emulsified in 0.5 ml of sterile PBS. An equal volume (50 µl) of this suspension was mixed with 50 µl of six per cent (vol/vol) suspension of washed erythrocytes placed on a microscopic slide. A negative control consisting of erythrocyte suspension in PBS was included in each assay. Hemagglutination was recorded positive if the reaction occurred within 10 min. The occurrence of haemagglutination within the period was indicative of pathogenicity of the isolates and the result was recorded by visual examination.

3.1.2.2 *Evaluation of Physico-chemical Quality*

Measurement of pH

Ten gram of minced sausage sample, in duplicate, was homogenized with 50 ml of distilled water for 10-15 sec. (Krishnan and Sharma, 1993) in a waring blender. The pH was recorded using a digital pH meter (Cyberscan 2500).

TBARS number estimation

Sausage samples were subjected to estimation of TBARS number as per the method described by Witte *et al.* (1970). After thorough mincing of each sample, 20 g of comminuted sausage was taken in a stainless steel waring blender with 50 ml of chilled extracting solution (20 per cent Trichloroacetic acid in 2 *M* phosphoric acid) kept at 4°C and blended at 8000 rpm for 1.5 min. The slurry obtained was transferred into 100 ml volumetric flask and the volume was made upto 100 ml with distilled water and homogenized by shaking. From the homogenate 50 ml was filtered through Whatman No.1 filter paper. From the filtrate, five milliliter each was transferred into duplicate test tubes. To each of these tubes five milliliter of 2-Thiobarbituric acid solution (0.005 *M* in distilled water) was added. The tubes were stoppered and the solution was mixed by inverting the tubes several times and kept in dark for 15 h at room temperature. Another test tube containing five milliliter each of 20 per cent Trichloroacetic acid in 2 *M* phosphoric acid and 0.005 *M* Thiobarbituric acid was also incubated as blank. After the period of incubation the optical density was measured at 530 nm with a Genesys 10 uv thermospectronic spectrophotometer. The optical density was multiplied by a factor 5.2 to obtain the TBARS number.

3.1.2.3 *Evaluation of Organoleptic Quality*

A semi-trained four member sensory panel evaluated sensory characters viz., color and odor of sausage samples. The color was scored according to a six point standardized scale as suggested by Woolthuis and Smulders (1985).

Flow Chart 4. Isolation and identification of *Aeromonas*

Initial test sample solution ↓ 10 ml + 90 ml alkaline peptone water at pH 8.7 for enrichment for (chilled and frozen samples) overnight a loopful to *Aeromonas* starch DNA agar plate (ampicillin)* (30°C – 24 h) ↓	**Characteristics/Reactions**
Honey yellow colored colonies when flooded with Lugol's iodine ↓	
Nutrient agar slants ↓	
Gram's reaction and cell morphology morphology ↓	Gram negative short rods
Motility ↓	+
Aerobic growth ↓	+
Anaerobic growth ↓	+
Catalase ↓	+
Oxidase ↓	+
Glucose (acid) ↓	+
OF ↓	**F**
Arginine hydrolysis ↓	+
Lysine decarboxylase ↓	+/-
Ornithine decarboxylase ↓	-
Indole ↓	+
VP ↓	+/-
Aesculin hydrolysis ↓	+/-
Carbohydrate utilization	
Arabinose	+/-
Salicin	+
Sucrose	+
Mannitol	+

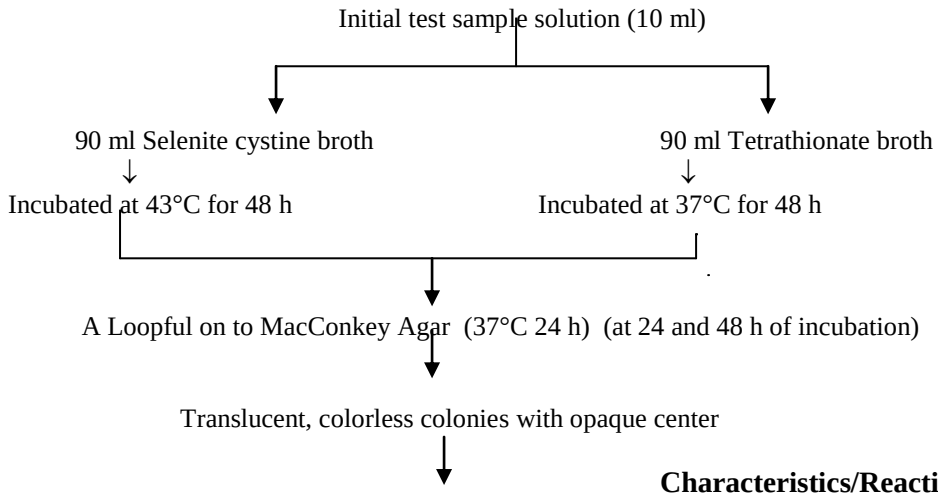
F = fermentation**,** + = positive**,** **-** = negative * supplement

Flow Chart 5. Isolation and identification of *Escherichia coli*

	Characteristics/Reactions
Initial test sample solution	
↓	
Enrichment: 10 ml sample + 90 ml Brain heart infusion broth (for chilled and frozen samples) (37°C 24 h)	
↓	
A loopful streaked on to Eosin Methylene Blue Agar	
↓	
Colonies with dark center with distinct indelible ink, greenish black metallic sheen on deflected light)	
↓	
Nutrient agar	
↓	
Grams' staining reaction and cell morphology	Gram negative small rods
↓	
Motility test	+
↓	
Growth aerobically	+
↓	
Catalase	+
↓	
Oxidase	-
↓	
Glucose (acid)	+
↓	
OF test	**F**
↓	
Urease	-
↓	
ONPG	+
↓	
Indole	+
↓	
MR	+
↓	
VP	-
↓	
Citrate Utilization test	-
↓	
Carbohydrate utilization	
↓	
Lactose	+
Glucose	+
Mannitol	+
Inositol	-
Maltose	+
Eijkman test	+

F = fermentation**,** + = positive**,** - = negative

Flow Chart 6. Isolation and Identification of *Salmonella*



		Characteristics/Reactions
↓		
Gram's reaction and cell morphology		Gram negative small rods
↓		
Motility		+
↓		
Growth aerobically		+
↓		
Catalase		+
↓		
Oxidase		-
↓		
OF test		**F**
↓		
Urease		-
↓		
TSI		Alkaline slant, acid butt, with or without H_2S
↓		
Lysine decarboxylase test		+
↓		
Arginine hydrolysis test		+
↓		
Phenylalanine deaminase test		-
↓		
Indole		-
↓		
MR		+
↓		
VP		-
↓		
Citrate utilization		-
↓		
Carbohydrate utilization	- mannitol	+
	lactose	-
	salicin	-

F=fermentation + = positive, - = negative

Flow Chart 7. Isolation and identification of *Pseudomonas aeruginosa*

A loopful of initial test sample	
↓	
A loopful to duplicate pseudomonas agar base (cetrinix)* (30°C 24h)	
↓	
Pigmented/non pigmented smooth round colonies	
↓	
Nutrient agar slants	
↓	
Grams' reaction	Gram negative rods
↓	
Motility test	+
↓	
Oxidase	+
↓	
Catalase	+
↓	
OF test	0
↓	
Indole	-
↓	
Arginine hydrolysis	+
↓	
Lysine	-
↓	-
Ornithine	
↓	
Citrate utilization	+
↓	
Urease	+
↓	
Nitrate reduction	+
↓	
Carbohydrate utilization	
↓	
Gluose	+
Mannitol	+

F =fermentation , + = positive, - = negative
***** supplement

Flow Chart 8. Isolation and identification of *Lactobacillus*

Initial test sample
↓
A loopful to Lactobacillus MRS Agar (30°C 48 h) anaerobically
↓
Cream circular smooth colonies
↓
Nutrient agar slants
↓
Grams' reaction and cell morphology — Gram positive short rods
↓
Motility test — -
↓
Catalase — -
↓
Oxidase — -
↓
Glucose (acid) — +
↓
OF test — F
↓
Growth at 15°C
↓
Growth at 45°C
↓
growth in 7% sodium chloride
Acid from
↓
Arabinose
Galactose
Lactose
Maltose
Mannitol
Mellibiose
Raffinose
Salicin
Sorbitol
Trehalose
↓
Aesculin hydrolysis
↓
Nitrate reduction
↓
Arginine hydrolysis

3.1.2.2 *Evaluation of Physico-chemical Quality*

Measurement of pH

Ten gram of minced sausage sample, in duplicate, was homogenized with 50 ml of distilled water for 10-15 sec. (Krishnan and Sharma, 1993) in a waring blender. The pH was recorded using a digital pH meter (Cyberscan 2500).

TBARS number estimation

Sausage samples were subjected to estimation of TBARS number as per the method described by Witte *et al.* (1970). After thorough mincing of each sample, 20 g of comminuted sausage was taken in a stainless steel waring blender with 50 ml of chilled extracting solution (20 per cent Trichloroacetic acid in 2 *M* phosphoric acid) kept at 4°C and blended at 8000 rpm for 1.5 min. The slurry obtained was transferred into 100 ml volumetric flask and the volume was made upto 100 ml with distilled water and homogenized by shaking. From the homogenate 50 ml was filtered through Whatman No.1 filter paper. From the filtrate, five milliliter each was transferred into duplicate test tubes. To each of these tubes five milliliter of 2-Thiobarbituric acid solution (0.005 *M* in distilled water) was added. The tubes were stoppered and the solution was mixed by inverting the tubes several times and kept in dark for 15 h at room temperature. Another test tube containing five milliliter each of 20 per cent Trichloroacetic acid in 2 *M* phosphoric acid and 0.005 *M* Thiobarbituric acid was also incubated as blank. After the period of incubation the optical density was measured at 530 nm with a Genesys 10 uv thermospectronic spectrophotometer. The optical density was multiplied by a factor 5.2 to obtain the TBARS number.

3.1.2.3 *Evaluation of Organoleptic Quality*

A semi-trained four member sensory panel evaluated sensory characters viz., color and odor of sausage samples. The color was scored according to a six point standardized scale as suggested by Woolthuis and Smulders (1985).

1 = extremely acceptable color

2 = moderately acceptable

3 = slightly acceptable

4 = slightly discolored

5 = moderately discolored

6 = extremely discolored

The odor was scored as per a four point standardized scale prescribed by Acuff *et al.* (1987)

1 = no off odor

2 = slight off odor

3 = moderate off odor

4 = extreme off odor.

Sliminess on sausage was recorded by visual and tactile examination.

The experiment was repeated six times.

3.1.3 Frozen Storage

Frozen samples of beef frankfurter were subjected to evaluation of microbial, physico-chemical and organoleptic qualities.

3.1.3.1 *Evaluation of Microbial Quality*

Microbial quality was evaluated by estimation of microbial counts, isolation and identification of spoilage and pathogenic bacteria.

Processing of sample

Frozen sample was thawed for 18 h at 4-5°C, prior to testing (Krishnan and Sharma, 1993) and processed as in the case of fresh sample.

3.1.3.1.1 Microbial counts

Total viable count

Total viable count of frozen samples of beef frankfurter was estimated as in the case of fresh and chilled samples.

Psychrotrophic count

Psychrotrophic count of frozen samples of beef frankfurter was estimated as described in the case of fresh and chilled samples.

3.1.3.1.2 Isolation and identification of bacteria

Frozen samples of beef frankfurter were tested for isolation and identification of pathogenic and spoilage bacteria as performed in case of fresh and chilled sample.

3.1.3.2 *Evaluation of Physico-chemical Quality*

The TBARS number and pH were estimated as described in case of fresh and chilled samples.

3.1.3.3 *Evaluation of Organoleptic Quality*

Sensory characters such as color and odor of the frozen sausage samples were evaluated as described in case of fresh and chilled beef frankfurter. Sliminess on the sausage was assessed by visual and tactile examination.

3.2 CHICKEN PEPPERONI

3.2.1 Collection of Samples

In the present study, chicken pepperoni samples were collected from six batches. From each batch 24 samples, each consisting of 250 g, were collected randomly on the day of production and brought to the laboratory in a thermocool

container. The distribution of the samples, storage temperature, period of storage, day of examination and number of samples examined on each day were similar to that given in table 1.

3.2.2 Fresh and Chilled Storage

Fresh and chilled samples of chicken pepperoni were subjected to evaluation of microbial, physico-chemical and organoleptic qualities.

3.2.2.1 *Evaluation of Microbial Quality*

Microbial quality was evaluated by estimation of microbial counts, isolation and identification of pathogenic and spoilage bacteria.

Processing of samples

Fresh sample

Organoleptic qualities of the samples such as color, odor and sliminess were evaluated before processing. Each sample was opened aseptically and sausage links were cut into small pieces and minced in a sterile stainless steel waring blender for three minutes at 8000 rpm. From the minced sample, 25 g was weighed and transferred to a sterile conical flask containing 225 ml of 0.1 per cent peptone water (diluent) and blended using a cyclomixer so as to extricate the microorganisms from the sample into the diluent. This formed the initial test sample. From the processed sample 10 ml was transferred into a sterile conical flask containing 90 ml of the diluent and mixed properly. Further ten-fold serial dilutions were prepared by transferring one millilitre of the inoculum into nine millilitre of the diluent. From each sample, dilutions were made up to 10^{-6} and selected dilutions were used for assessing various microbial counts.

Chilled sample

Chilled sample was thawed to room temperature and then processed as in the case of fresh sample.

3.2.2.1.1 Microbial counts

Estimation of various microbial counts was done as described in case of beef frankfurter.

3.2.2.1.2 Isolation and identification of bacteria

Each chicken pepperoni sample was tested for isolation and identification of pathogenic and spoilage bacteria as performed in case of beef frankfurter samples.

3.2.2.2 *Evaluation of Physico-chemical Quality*

TBARS number and pH were estimated as described in case of beef frankfurter.

3.2.2.3 *Evaluation of Organoleptic Quality*

Sensory characters such as color and odor of the sausage samples were evaluated as described in case of beef frankfurter. Sliminess on the sausage was assessed by visual and tactile examination.

3.2.3 Frozen Storage

Frozen samples of chicken pepperoni were subjected to evaluation of microbial, physico-chemical and organoleptic qualities.

3.2.3.1 *Evaluation of Microbial Quality*

Microbial quality was evaluated by estimation of microbial counts, isolation and identification of pathogenic and spoilage bacteria.

Processing of sample

Frozen sample was thawed for 18 h at 4-5°C, prior to testing (Krishnan and Sharma, 1993) and processed as in the case of fresh sample.

3.2.3.1.1 Microbial counts

Total viable count

Total viable count of frozen samples of chicken pepperoni was estimated as in the case of fresh and chilled samples.

Psychrotrophic count

Psychrotrophic count of frozen samples of chicken pepperoni was estimated as in the case of fresh and chilled samples

3.2.3.1.2 Isolation and identification of bacteria

Each frozen chicken pepperoni sample was tested for isolation and identification of pathogenic and spoilage bacteria as performed in case of fresh and chilled samples.

3.2.3.2 *Evaluation of Physico-chemical Quality*

TBARS number and pH were estimated as described in case of fresh and chilled sample.

3.2.3.3 *Evaluation of Organoleptic Quality*

Sensory characters such as color and odor of the frozen sausage samples were evaluated as described in case of fresh and chilled sausage. Sliminess on the sausage was assessed by visual and tactile examination.

3.3 ASSESSMENT OF CRITICAL CONTROL POINTS

3.3.1 Assessment of Critical Control Points in the Preparation of Beef frankfurter

Critical control points (CCP) in the manufacture of beef frankfurter were determined after analyzing the bacterial quality of samples collected at points

viz., mincing of meat, mixing the minced meat with spices, filling into casings, linking, cooking, chilling and packing.

3.3.1.1 Air

Total viable count

Direct exposure method described by Evancho *et al.* (2001) was employed for the estimation of total viable count in the air samples of processing, cooking and chilling room before and after processing. In order to estimate the count, duplicate Petridishes (90mm diameter) containing sterile nutrient agar medium were exposed in the rooms for 15 min. The plates were brought to the laboratory in thermocool container and incubated at 37°C for 24 h. The number of colonies developed in the duplicate plates was counted and the mean count was expressed as cfu/ft/min.

Yeast and mold count

In order to estimate the yeast and mold count, procedure described by Evancho *et al*. (2001) was followed. Duplicate plates of Potato Dextrose Agar (Hi-media) medium were exposed in the rooms and the plates were brought to the laboratory in a thermocool container and incubated at 25°C for five days, and the mean count was expressed as cfu/ft/min.

3.3.1.2 Water

Collection of samples

(1) ***Water used for washing the casings and for chilling the cooked sausage***

Water samples were collected and analyzed following the procedure described by Indian Standards (1978). A clean sterile bottle of 250 ml capacity was held by its bottom and plunged its neck downwards below the surface of the water. The bottle was then turned until the neck pointed slightly upwards. After collecting water, it was raised above the surface of water and the stopper was

replaced. The water samples were transported to the laboratory in a thermocool container.

(2) ***Ice used in the preparation of sausage batter***

Ice cubes were collected in a sterile wide mouthed conical flask of 250 ml capacity up to half of its volume. The flask was sealed and brought to the laboratory in a thermocool container.

(3) ***Hand washing***

On each visit the hand washing of a randomly selected individual involved in processing operations was collected. The individual's hand was washed in 100 ml of 0.1 percent sterile peptone water and washing was collected in sterile conical flask and brought to the laboratory in a thermocool container.

Processing of samples

(1) ***Water used for washing the casings and chilling the cooked sausage***

Samples brought to the laboratory were agitated vigorously. In order to estimate the bacterial load per milliliter of water sample, 10 ml was transferred to 90 ml of normal saline solution so as to form one in 10 dilution of the sample. Further serial 10 fold dilutions were prepared by transferring one milliliter of inoculum to nine milliliter of the diluent. Dilutions were made up to $10^{-4.}$

The selected serial dilutions of each sample were used to estimate the Total viable count (TVC), Coliform count (CC), *Escherichia coli* count (ECC) and Faecal streptococcal count (FSC) as per the method described by Mortan (2001), Kornaki and Johnson (2001), Indian Standards (1980)and the Nordic Committee on Food Analysis (1968) respectively. The counts obtained were expressed as $\log_{10}$ cfu/ml.

(2***) Ice used in preparation of sausage batter***

Processing of samples and enumeration of various bacterial counts were done as described in case of water samples.

(3). ***Hand washing***

Processing of samples and enumeration of various bacterial counts were done as described in case of water samples.

3.3.1.3 Packaging Materials

In order to assess the microbiological quality of polyethylene bags (250 g capacity), 20 ml of sterile peptone water was poured into the bag and was agitated vigorously by holding in both horizontal and vertical position (Evancho *et al.*, 2001). The polyethylene bag with its content was brought to the laboratory in a thermocool container by keeping it in an upright position. Serial ten fold dilutions up to 10^{-4} were prepared and selected dilutions were used for estimation of bacterial counts as in the case of water samples.

3.3.1.4 Processing Equipment

(1). **Linking table**

Swab contact method (Evancho *et al.*, 2001) was followed for the collection of samples from linking table. For sampling linking table, a sterile cotton swab was moistened with 0.1 per cent peptone water and excess diluent was removed by pressing the swab against the interior wall of the vial. The swab head was rubbed slowly and thoroughly over 100 cm^2 surface, which was marked with a sterile aluminium template (10 x 10 cm^2 internal area). The swab was rubbed three times, reversing the direction between strokes. After swabbing the cotton swab was cut with sterile scissors, transferred into sterile flask containing 100 ml of 0.1 per cent peptone water and brought to the laboratory in a thermocool container.

The flask was thoroughly agitated with the help of a cyclomixer at 8000 rpm for three min, so as to extricate the bacteria attached to the cotton swab into the diluent. Serial 10 fold dilutions were prepared and bacterial counts were estimated as in the case of water samples. The mean count was expressed in $\log_{10}$ cfu/cm^2.

(2). **Sausage filler, mincer and mixer**

Rinse method (Evancho *et al.*, 2001) was followed for the collection of samples from sausage filler, mincer and mixer. One hundred milliliter of sterile 0.1 per cent peptone water was poured into the processing equipment and then allowed to flow by gravity through the equipment. Samples of rinse water were collected from the discharge end of the equipment into a sterile conical flask and brought to the laboratory in a thermocool container for the estimation of the bacterial load. Serial 10 fold dilutions were prepared and estimation of bacterial counts were done as in the case of water samples.

3.3.1.4 Raw Materials Used in the Preparation of Beef Frankfurter

Collection of samples

For assessing the bacterial load, about 250g of frozen beef, or frozen chicken and pork in case of chicken pepperoni, minced meat, mixed meat, casings and spices were collected aseptically and brought to the laboratory in sterile polyethylene bags in a thermocool container.

Processing of samples

(1). ***Frozen meat***

From each meat sample an initial test sample of 100 g was prepared by cutting a thin layer of approximately five millimeter thickness from the exterior of the cut surface of the sample. These pieces were further cut into smaller pieces and 10 g of sample was weighed and transferred into a sterile stainless steel

waring blender containing 90 ml of 0.1 per cent peptone water. The sample was then blended for three minutes at 8000 rpm to extricate the bacteria into the diluent. Further consecutive 10 fold serial dilutions were prepared and selected dilutions were used for estimation of bacterial counts as in the case of water samples. The mean count was expressed in $\log_{10}$ cfu/g.

(2) ***Minced meat, mixed meat, casings and spices***

From the initial sample 10 g was weighed and transferred into a sterile stainless steel waring blender containing 90 ml of 0.1 per cent peptone water to form one in ten dilution. The sample was blended for three minutes at 8000 rpm to extricate the bacteria from the sample into the diluent. Further consecutive 10 fold serial dilutions were prepared and selected dilutions were used for estimation of bacterial counts as in the case of water samples. The count obtained was expressed in $\log_{10}$ cfu/g.

The experiment was repeated six times.

3.3.2 Assessment of Critical Control Points in the Preparation of Chicken pepperoni

The critical control points in the manufacture of chicken pepperoni were assessed as in the case of beef frankfurter. The experiment was repeated six times.

Results

4. RESULTS

In the present investigation the effect of chilling and freezing on beef frankfurter and chicken pepperoni with respect to microbial, physico-chemical and organoleptic qualities was assessed and compared with fresh sample. All the samples were tested for the isolation and identification of certain bacterial pathogens of public health significance. The raw ingredients, processing equipment, hand washing of personnel engaged in processing line, packaging materials and environmental samples were also tested to identify the source of contamination.

4.1 BEEF FRANKFURTER

4.1.1 Fresh and Chilled Storage

Fresh and chilled samples of beef frankfurter were subjected to estimation of various microbial counts and isolation and identification of certain bacteria of public health significance on day zero, two, four, six, eight and ten of storage.

4.1.1.1 Total Viable Count

Mean total viable count of fresh and chilled beef frankfurter samples are given in table 2 (Fig.1).

Table 2. Mean total viable count of fresh and chilled beef frankfurter

Days of storage	Count (log_{10} cfu/g) Mean ± SE
0	5.86 ± 0.15^{a}
2	6.15 ± 0.06^{b}
4	6.44 ± 0.16^{c}
6	6.69 ± 0.12^{d}
8	7.03 ± 0.04^{e}
10	7.25 ± 0.02^{f}

Figures bearing the same superscript do not differ significantly.

Statistical analysis of the mean counts was carried out by student's 't' test, which revealed a gradual increase in the mean count of samples examined during chilled storage. Mean count of fresh and chilled samples on all days differ significantly ($P \leq 0.05$) from each other.

Distribution of fresh and chilled samples of beef frankfurter based on level of total viable count is given in table 3.

Table 3. Distribution of fresh and chilled beef frankfurter samples based on total viable count

Days of storage	Total viable count (cfu/g)		
	10^5	10^6	10^7
0	6 (50)	6 (50)	
2	6 (50)	6 (50)	
4	5 (41.67)	5 (41.67)	2 (16.67)
6		7 (58.33)	5 (41.67)
8		6 (50)	6 (50)
10			12 (100)

Figures in parenthesis indicate per cent

The count in 50 per cent of fresh and chilled samples on day two of storage was at the level of 10^6 cfu/g. On day four of storage the count in 16.67 per cent of chilled samples reached up to the level of 10^7 cfu/g and on day 10 the count in cent per cent of samples was at the above level.

4.1.1.2 Coliform Count

Mean coliform count of fresh and chilled beef frankfurter samples are given in table 4 (Fig.1).

Table 4. Mean coliform count of fresh and chilled beef frankfurter

Days of storage	Count ($\log_{10}$ cfu/g) Mean ± SE
0	ND
2	1.41 ± 0.15^a
4	2.51 ± 0.23^b
6	2.58 ± 0.22^b
8	2.54 ± 0.22^b
10	2.82 ± 0.24^c

Figures bearing the same superscript do not differ significantly
ND: Not detected

Coliforms were not detected in fresh beef frankfurter samples. Student's 't' test of the data revealed that coliform count of chilled samples stored for two days was significantly (P≤0.05) different from that of the samples stored for four, six, eight and ten days. The count of the samples on day 10 of storage was significantly (P≤0.05) higher than that of the samples on second, fourth, sixth and eighth day of storage.

Distribution of fresh and chilled beef frankfurter based on level of coliform count is given in table5.

Table 5. Distribution of fresh and chilled beef frankfurter samples based on coliform count

Days of storage	Coliform count (cfu/g)		
	10^1	10^2	10^3
0	ND		
2	5 (41.67)		
4		12 (100)	
6		12 (100)	
8		12 (100)	
10		9 (75)	3 (25)

Figures in parenthesis indicate per cent
ND – Not detected

Coliforms could not be detected in cent per cent of fresh samples. However the count was at 10^2 cfu/g level in cent per cent of the samples on day four, six and eight of storage. The organism was detected in all samples, stored on day 10 and the count in 25 per cent samples was at the level of 10^3 cfu/g.

4.1.1.3 Escherichia coli Count

Mean *E. coli* count of fresh and chilled beef frankfurter are given in table 6. (Fig.1)

Table 6. Mean *Escherichia coli* count of fresh and chilled beef frankfurter

Days of storage	Count (log_{10} cfu/g) Mean ± SE
0	ND
2	ND
4	1.00 ± 0.66^{a}
6	0.39 ± 0.39^{a}
8	0.64 ± 0.46^{a}
10	0.62 ± 0.46^{a}

Figures bearing the same superscript do not differ significantly.
ND: Not detected

Escherichia coli were not detected in fresh samples and samples kept chilled for two days. Mean count of the samples on four, six, eight and ten days of storage were subjected to student's't' test. Analysis revealed that the mean count of samples on day four, six, eight and ten did not differ significantly.

Distribution of fresh and chilled beef frankfurter samples based on level of *E. coli* count is given in table 7.

Table 7. Distribution of fresh and chilled beef frankfurter samples based on *E. coli* count

Days of storage	*E. coli* Count (cfu/g)
	10^{1}
0	ND
2	ND
4	3 (25)
6	1 (8.33)
8	2 (16.67)
10	3 (25)

Figures in parenthesis indicate per cent
ND – Not detected

E. coli was not detected in cent per cent samples on day zero and two. The organism was detected in 25 per cent samples on fourth and tenth day of storage at a level of 10^1 cfu/g. On sixth and eighth day 8.33and 16.67 per cent samples respectively had the organism at 10^1 cfu/g level.

4.1.1.4 Faecal Streptococcal Count

Mean faecal streptococcal counts of fresh and chilled samples of beef frankfurter were subjected to student's 't' test to detect the significant difference between the mean counts (Table 8 and Fig.1).

Table 8. Mean faecal streptococcal count of fresh and chilled beef frankfurter

Days of storage	Count (log_{10} cfu/g) Mean ± SE
0	2.02 ± 0.05^a
2	2.28 ± 0.08^b
4	2.57 ± 0.04^c
6	2.66 ± 0.08^c
8	3.25 ± 0.12^d
10	3.30 ± 0.15^d

Figures bearing the same superscript do not differ significantly

Analysis of the data revealed that mean faecal streptococcal count increased gradually during storage. Significant ($P \leq 0.05$) difference in counts was observed on all the days of storage except between fourth and sixth day and eighth and tenth day.

Distribution of fresh and chilled samples of beef frankfurter based on level of faecal streptococcal count is given in table 9.

Table 9. Distribution of fresh and chilled beef frankfurter samples based on level of faecal streptococcal count

Days of storage	Faecal streptococcal count (cfu/g)			
	10^1	10^2	10^3	10^4
0	4 (33.33)	8 (66.67)		
2	2 (16.67)	10 (83.33)		
4		12 (100)		
6		6 (50)	6 (50)	
8		6 (50)	6 (50)	
10		6 (50)	4 (33.33)	2 (16.67)

Figures in parenthesis indicate per cent

On day of production majority (66.67 per cent) of samples had a count at 10^2 cfu/g level. Cent per cent samples on fourth day had the count at 10^2 cfu/g level. On sixth and eighth day 50 per cent samples had the count at 10^3 cfu/g level. On tenth day of storage 16.67 per cent had a higher count at 10^4 cfu/g level.

4.1.1.5 Psychrotrophic Count

Mean psychrotrophic count of fresh and chilled beef frankfurter samples are given in table 10 (Fig.1).

Table 10. Mean psychrotrophic count of fresh and chilled beef frankfurter

Days of storage	Count (log_{10} cfu/g) Mean ± SE
0	5.62 ± 0.02^a
2	5.64 ± 0.02^a
4	5.69 ± 0.02^a
6	5.87 ± 0.03^b
8	5.98 ± 0.03^c
10	6.13 ± 0.15^d

Figures bearing the same superscript do not differ significantly

Mean psychrotrophic counts were subjected to statistical analysis by student's't' test. Analysis revealed a significant ($P \leq 0.05$) increase in counts from sixth day onwards during the chilled storage. Counts on eighth day and tenth day

were significantly (P≤0.05) higher than the count on sixth day. A significantly (P≤0.05) higher mean count was obtained on tenth day of storage when compared to the count on eighth day.

Distribution of fresh and chilled beef frankfurter samples based on psychrotrophic count is given in table 11.

Table 11. Distribution of fresh and chilled beef frankfurter samples based on psychrotrophic count

Days of storage	Psychrotrophic count (cfu/g)	
	10^5	10^6
0	12 (100)	
2	12 (100)	
4	11 (91.67)	1 (8.33)
6	11 (91.67)	1 (8.33)
8	9 (75)	3 (25)
10	1 (8.33)	11 (91.67)

Figures in parenthesis indicate per cent

Cent per cent samples on day of production and second day of chilled storage had the count at 10^5 cfu/g level. On fourth and sixth day 8.33 per cent each had count at 10^6 cfu/g level. On tenth day of storage 91.67 per cent had the count at 10^6 cfu/g level.

4.1.1.6 Yeast and Mold Count

Mean yeast and mold count of fresh and chilled beef frankfurter samples were subjected to statistical analysis by student's 't' test. Analysis revealed that the increase in count was significant (P≤0.05) throughout the storage period (Table 12 and Fig.1).

Table 12. Mean yeast and mold count of fresh and chilled beef frankfurter

Days of storage	Count (log_{10} cfu/g) Mean ± SE
0	2.64 ± 0.06^{a}
2	2.70 ± 0.05^{b}
4	2.92 ± 0.04^{c}
6	3.35 ± 0.08^{d}
8	3.63 ± 0.12^{e}
10	3.73 ± 0.15^{f}

Figures bearing the same superscript do not differ significantly.

Distribution of fresh and chilled beef frankfurter based on level of yeast and mold count is given in table 13.

Table 13. Distribution of fresh and chilled beef frankfurter samples based on yeast and mold count

Days of storage	Yeast and mold count (cfu/g)		
	10^2	10^3	10^4
0	12 (100)		
2	12 (100)		
4	8 (66.67)	4 (33.33)	
6		12 (100)	
8		8 (66.67)	4 (33)
10		6 (50)	6 (50)

Figures in parenthesis indicate per cent

On day of production and second day of chilled storage cent per cent samples had yeast and mold count at 10^2 cfu/g level. An equal per cent had the count at 10^3 cfu/g level on sixth day of storage. On tenth day of storage 50 per cent of samples had a higher count at the level of 10^4 cfu/g.

4.1.1.7 Correlation Between Various Microbial Counts of Fresh and Chilled Beef Frankfurter

Total viable count

Correlation between mean total viable count and all other counts of beef frankfurter during the storage at 4-7°C is given in table 14.

Table 14. Correlation coefficient between mean total viable count and other microbial counts of fresh and chilled beef frankfurter

Microbial counts	Total viable count					
	Days of storage					
	0	2	4	6	8	10
CC	ND	0.40	0.50	-0.86**	-0.88**	0.53
ECC	ND	ND	0.43	-0.28	-0.42	0.00
FSC	-0.47	0.82**	-0.34	0.93**	0.94**	-0.42
PC	0.12	0.87**	0.81**	0.59*	0.61*	-0.08
YMC	0.73 **	0.88**	0.89**	0.61*	0.93**	-0.31

CC: Coliform count, ECC: *Escherichia coli* count, FSC: Faecal streptococcal count, PC: Psychrotrophic count, YMC: Yeast and mold count, * = P $\leq$0.05, ** = P $\leq$0.01. ND: not detected

Total viable count and coliform count were negatively correlated with a high significance (P≤0.01) on sixth and eighth day of storage.

Total viable count and faecal streptococcal count were associated positively at a high level of significance (P≤0.01) on second, sixth and eighth day of chilled storage.

Relationship between total viable count and psychrotrophic counts was positive and highly significant (P≤0.01) on second and fourth day and there was significant (P≤0.05) positive correlation between the two counts on sixth and eighth day of storage.

Highly significant positive correlation (P≤0.01) was observed between total viable count and yeast and mold count on zero, second, fourth and eighth

day of storage. The association was positive and significant (P≤0.05) on sixth day.

Coliform count

Correlation between mean coliform count and other microbial counts of beef frankfurter stored at refrigeration temperature (4-7°C) is given in table 15.

Table 15. Correlation coefficient between mean coliform count and other microbial counts of fresh and chilled beef frankfurter

Microbial counts	Coliform count					
	Days of storage					
	0	2	4	6	8	10
TVC	ND	0.40	0.50	-0.86**	-0.88**	0.53
ECC	ND	ND	0.94**	0.43	0.60*	0.58*
FSC	ND	-0.26	-0.22	-0.82**	-0.80**	-0.68*
PC	ND	0.48	0.26	-0.57	-0.57	0.43
YMC	ND	0.24	0.19	-0.36	-0.88**	-0.75**

TVC: Total viable count, ECC: *Escherichia coli* count,FSC: Faecal streptococcal count,PC Psychrotrophic count,YMC: Yeast and mold count,* = P ≤0.05, ** = P ≤0.01. ND:not detected

Coliforms were not detected on the day of production from any of the samples tested.

Association between coliform count and *E. coli* count was positive and highly significant (P≤0.01) on fourth day of storage. The relationship was positive and significant (P≤0.05) on eighth and tenth day of storage. *E. coli* were noted detected in fresh and chilled samples on second day of storage.

Coliform count and faecal streptococcal count were correlated negatively with a high significance (P≤0.01) on sixth and eighth day of storage. On tenth day the association was negative and significant (P≤0.05).

A highly significant (P≤0.01) negative correlation was observed between coliform count and yeast and mold count on eighth and tenth day of storage.

***Escherichia coli* count**

Correlation between mean *Escherichia coli* count and other microbial counts of fresh and chilled beef frankfurter is given in table 16.

Table16. Correlation coefficient between mean *E. coli* count and other microbial counts of fresh and chilled beef frankfurter

Microbial counts	*Escherichia coli* count					
	Days of storage					
	0	2	4	6	8	10
TVC	ND	ND	0.43	-0.28	-0.42	0.00
CC	ND	ND	0.94**	0.43	0.60*	0.58*
FSC	ND	ND	0.15	-0.24	-0.45	-0.40
PC	ND	ND	0.17	0.55	0.66*	0.69*
YMC	ND	ND	0.42	-0.18	0.34	0.14

TVC: Total viable count, CC: Coliform count,FSC: Faecal streptococcal count,PC Psychrotrophic count,YMC: Yeast and mold count,* = P $\leq$0.05, ** = P $\leq$0.01. ND:not detected

E. coli count and coliform count were correlated with a high significance (P $\leq$0.01) on fourth day of storage and the relationship was significant on eighth and tenth day also.

E. coli count and psychrotrophic count were significantly (P$\leq$0.05) associated on eighth and tenth day of storage.

Faecal streptococcal count

Correlation between mean faecal streptococcal count and other microbial counts of fresh and chilled beef frankfurter is given in table 17.

Table 17. Correlation coefficient between mean faecal streptococcal count and other microbial counts of fresh and chilled beef frankfurter

Microbial counts	Faecal streptococcal count					
	Days of storage					
	0	2	4	6	8	10
TVC	-0.47	0.82**	-0.34	0.93**	0.94**	-0.42
CC	ND	-0.26	-0.22	-0.82**	-0.80**	-0.68*
ECC	ND	ND	0.15	-0.24	-0.45	-0.40
PC	-0.29	-0.76**	-0.22	-0.46	0.70*	-0.48
YMC	-0.01	-0.96**	-0.47	-0.60*	0.91**	0.76**

TVC: Total viable count, CC: Coliform count,ECC: *Escherichia coli* count,PC Psychrotrophic count,YMC: Yeast and mold count,* = P $\leq$0.05, ** = P $\leq$0.01. ND:not detected

Faecal streptococcal count and psychrotrophic counts were positively and significantly (P≤0.05) correlated on eighth day of storage and on second day the correlation was negative and highly significant (P≤0.01).

Relationship between faecal streptococcal count and yeast and mold count was highly significant (P≤0.01) and negative on second day of storage and was negative and significant (P≤0.05) on sixth day. Analysis of data revealed that the correlation was positive and highly significant (P≤0.01) on eighth and tenth day of storage.

Psychrotrophic count

Correlation between Psychrotrophic count and other microbial counts of fresh and chilled beef frankfurter is given in table 18.

Table 18. Correlation coefficient between mean psychrotrophic count and other microbial counts of fresh and chilled beef frankfurter

Microbial counts	Psychrotrophic count					
	Days of storage					
	0	2	4	6	8	10
TVC	0.12	0.87**	0.81**	0.59*	0.61*	-0.08
CC	ND	0.48	0.26	-0.57	-0.57	0.43
ECC	ND	ND	0.17	0.55	0.66*	0.69*
FSC	-0.29	-0.76**	0.22	-0.46	0.70*	-0.48
YMC	0.25	0.76**	0.62*	0.72**	0.40	-0.64*

TVC: Total viable count, CC: Coliform count, ECC:*Escherichia coli* count, FSC: Faecal streptococcal count, YMC: Yeast and mold count,* = P $\leq$0.05, ** = P $\leq$0.01. ND:not detected

Psychrotrophic count and yeast and mold count were associated with a high significance on second and sixth day of chilled storage. Relationship between the two organisms was significant (P $\leq$0.05) on fourth day and significant (P $\leq$0.05) and negative on tenth day of storage.

Yeast and mold count

Correlation between mean yeast and mold count and other microbial counts of fresh and chilled beef frankfurter is given in table 19.

Table 19. Correlation coefficient between mean yeast and mold count and other microbial counts of fresh and chilled beef frankfurter

Microbial counts	Yeast and mold count					
	Days of storage					
	0	2	4	6	8	10
TVC	0.73**	0.88**	0.89**	0.61*	0.93**	-0.31
CC	ND	0.24	0.19	-0.36	-0.88**	-0.75**
ECC	ND	ND	0.42	-0.18	0.34	0.14
FSC	-0.01	-0.96**	-0.47	-0.60*	0.91**	0.76**
PC	0.25	0.76**	0.62*	0.72*	0.40	-0.64*

TVC: Total viable count, CC: Coliform count, ECC:*Escherichia coli* count, FSC: Faecal streptococcal count,PC Psychrotrophic count,,* = P $\leq$0.05, ** = P $\leq$0.01. ND:not detected

Yeast and mold count and total viable count were associated with a high ($P \leq 0.01$)significance on days zero, two. four and eight of storage. A significant ($P \leq 0.05$) relationship was noticed on sixth day also.

4.1.1.8 Isolation and Identification of Bacteria

The pathogenic and spoilage organisms isolated from fresh and chilled samples of beef frankfurter are given in table 20.

Table 20. Pathogenic and spoilage bacteria isolated from fresh and chilled beef frankfurter

Bacteria	Samples tested on each day	Number of positive samples					
		Days of storage					
		0	2	4	6	8	10
Aeromonas	12	3	1	4	3	3	3
Escherichia coli	12	ND	ND	2	3	4	4
Salmonellae	12	ND	ND	ND	ND	ND	ND
Pseudomonas aeruginosa	12	ND	ND	2	ND	1	1
Lactobacilli	12	ND	ND	ND	1	3	ND

ND: Not detected

Aeromonas

Fresh and chilled samples of beef frankfurter were tested to detect the presence of *Aeromonas* spp. Twenty five per cent of fresh samples revealed the presence of organism. An equal per cent of samples tested on sixth, eighth and tenth day were also positive for the presence of *Aeromonas*. On fourth day among the 12 samples tested four samples (33.33 per cent) had the organism (Table 20).

Different species of *Aeromonas* isolated from fresh and chilled beef frankfurter samples are given below (Table 21).

Table 21. *Aeromonas* species isolated from fresh and chilled beef frankfurter

Species	Total number of isolates	Number of positive samples					
		Days of storage					
		0	2	4	6	8	10
A. hydrophila	1	ND	ND	1	ND	ND	ND
A. sobria	8	1	ND	2	1	2	2
A. caviae	8	2	1	1	2	1	1
Total	17	3	1	4	3	3	3

ND: not detected

From fresh beef frankfurter samples one isolate of *A. sobria* and two isolates of *A. caviae* could be detected. From chilled samples of beef frankfurter, one isolate of *A. hydrophila*, seven isolates of *A. sobria*. and six isolates of *A. caviae* could be isolated.

A total of 17 isolates of *Aeromonas* were obtained from fresh and chilled beef frankfurter samples. Among that 5.88 per cent of isolates were *A. hydrophila* and 47.05 per cent each belonged to *A. sobria* and *A. caviae* species.

Hemolysis and hemagglutination assays

In order to identify enteropathogenic *Aeromonas*, hemolysis and hemagglutination assays of the isolates were carried out. Results are given in table 22 and illustrated in Plate 1.

Table 22. Hemolysis and hemagglutination assays of *Aeromonas* isolates from fresh and chilled beef frankfurter

Species	Total number of isolates	Number of positive isolates	
		Hemolysis	Hemagglutination
A. hydrophila	1	1 (100)	1 (100)
A. sobria	8	6 (75)	2 (25)
A. caviae	8	6 (75)	2 (25)

Figures in parenthesis indicate per cent

A. hydrophila isolated from chilled beef frankfurter was hemolytic and hemagglutinating. Seventy five per cent each of *A. sobria* and *A. caviae* were hemolytic whereas 25 per cent each were hemagglutinating.

Escherichia coli

Fresh and chilled samples of beef frankfurter were tested for the isolation and identification of *Escherichia coli.*

E. coli was not detected in fresh samples and chilled samples on day two of storage. The organism could be isolated from 16.67 and 25 per cent of samples tested on fourth and sixth day of storage respectively. On eighth and tenth day of storage 33.33 per cent of samples were positive for the presence of the organism (Table 20).

From positive samples one isolate each was selected and subjected to further characterization by biochemical tests, serotyping and Congo red binding test. Results of serotyping are shown in Table 23.

Table 23. Distribution of *E. coli* isolates from fresh and chilled beef frankfurter based on serotypes

Isolate	Serotype
1	O2
2	O2
3	O2
4	O2
5	O2
6	O2
7	O2
8	O2
9	O2
10	O2
11	O2
12	rough (UT)
13	rough (UT)

UT - Untypable

A total of 13 isolates were obtained from chilled beef frankfurter samples. Among that 84.62 per cent of the isolates belonged to serotype O2 (Enterohemorrhagic *Escherichia coli*) and 15.38 per cent of isolates were untypable.

Isolates of *E. coli* were subjected to Congo red binding test. Results are given in table 24 and illustrated in Plate 2.

Table 24. Congo red binding test of *E. coli* isolates from fresh and chilled beef frankfurter

Isolate	Congored binding test
1	+
2	+
3	+
4	-
5	+
6	-
7	+
8	-
9	+
10	+
11	+
12	-
13	-

Among the 13 isolates of *E. coli* subjected to congo red binding test 61.53 per cent of isolates were congo red binding and produced brick red colonies on congo red agar, indicating their property of invasiveness. Five of the isolates (38.46 per cent) were not congo red binding.

Salmonellae

Salmonellae could not be isolated from any of the fresh and chilled samples of beef frakfurter (Table 20).

Pseudomonas aeruginosa

Fresh and chilled samples of beef frankfurter were subjected to isolation and identification of *Pseudomonas aeruginosa*. The organism could not be isolated from fresh samples. The samples on second and sixth day of chilled storage also did not reveal its presence, whereas it was detected in 16.67 per cent of samples on fourth day and 8.33 per cent samples each on eighth and tenth day of storage (Table 20).

Lactobacilli

Fresh and chilled samples of beef frankfurter were subjected to isolation and identification of *Lactobacillus* spp. Lactobacilli were not detected in fresh samples and samples chilled for two and four days. On sixth day of storage 8.33 per cent samples were positive for the presence of the organism. On eighth day out of 12 samples tested 25 per cent samples revealed the presence of the organism (Table 20).

Table 25. Different species of Lactobacillus isolated from fresh and chilled samples of beef frankfurter

Species	Total number of isolates	Number of positive samples					
		Days of storage					
		0	2	4	6	8	10
L. brevis	2	ND	ND	ND	1	1	ND
L. curvatus	1	ND	ND	ND	ND	1	ND
L. sake	1	ND	ND	ND	ND	1	ND
Total	4	ND	ND	ND	1	3	0

ND – Not detected

Lactobacilli belonging to three different species could be isolated from the chilled samples tested. Two isolates of *L. brevis* and one isolate each of *L. curvatus* and *L. sake* were obtained.

4.1.1.9 pH

Fresh and chilled beef frankfurter samples, stored at 4-7°C for 10 days were subjected to estimation of pH at two day interval. The results are given in Table 26 (Fig.2).

Table 26. Mean pH of fresh and chilled beef frankfurter

Characteristic	Days of storage					
	0	2	4	6	8	10
pH (Mean ± SE)	6.89 ± 0.02[a]	6.68 ± 0.06[b]	6.39 ± 0.05[c]	6.27 ± 0.07[c]	5.87 ± 0.03[d]	5.82 ± 0.02[d]

Figures bearing same superscript do not differ significantly.

Student's 't' test revealed that mean pH of fresh samples was significantly ($P \leq 0.05$) higher than that of chilled samples. There was significant reduction in pH of samples analysed on fourth, sixth, eighth and tenth day of storage when compared to that of samples on day two. A significant ($P \leq 0.05$) reduction in pH was observed throughout the storage except between fourth and sixth day and eighth and tenth day.

4.1.1.10 TBARS Number

TBARS number of beef frankfurter stored at 4-7°C was estimated at two days interval up to 10 days.

The values are given in the table 27 (Fig.2).

Table 27. TBARS number of fresh and chilled beef frankfurter

characteristic	Days of storage					
	0	2	4	6	8	10
TBARS number (mg malonaldehyde/ kg) Mean ± SE	1.31 ± 0.04[a]	1.59 ± 0.03[b]	1.37 ± 0.03[ab]	1.79 ± 0.08[c]	1.98 ± 0.07[d]	2.14 ± 0.11[e]

Figures bearing same superscript do not differ significantly.

Student's 't' test revealed that there was significant (P≤0.05) increase in the values during storage, except between zero and fourth day and second and fourth day. After 10 days of storage the value was increased by 0.83 units.

4.1.1.11 Organoleptic Evaluation

Fresh and chilled samples of beef frankfurter were subjected to sensory evaluation. Semi-trained four member panel of the department evaluated color, odor and presence of sliminess of the chilled samples of the sausage at two days interval up to 10 days.

Table 28. Color and odor scores of fresh and chilled beef frankfurter

Days of storage	(Mean scores ± SE)	
	Color	Odor
0	1 ± 0.00[a]	1 ± 0.00[a]
2	1 ± 0.00[a]	1 ± 0.00[a]
4	1.33 ± 0.14[b]	1.42 ± 0.15[b]
6	2.25 ± 0.13[c]	1.58 ± 0.15[b]
8	3.08 ± 0.19[d]	2.27 ± 0.14[c]
10	4.16 ± 0.21[e]	2.67 ± 0.15[c]

Fresh samples and chilled samples after two days of storage had similar color and odor scores. From fourth day onwards color scores increased gradually.

Student's 't' test revealed that the color scores increased significantly ($P \leq 0.01$) through out storage. Grayish discoloration was noticed mainly on the less smoked inner surface of sausage links. Odor scores showed a highly significant ($P \leq 0.05$) increase on all subsequent days when compared to second day. On 10^{th} day of storage slight change in color was noticed with a score of 4.16 ± 0.21 and a slight off odor was noticed with a score of 2.67 ± 0.15.

Fresh and refrigerated samples of beef frankfurter were tested for presence of sliminess during the storage. Distribution of samples based on development of sliminess is given in table 29

Table 29. Distribution of fresh and chilled beef frankfurter samples based on presence of sliminess

Days of storage	Distribution based on development of sliminess
0	ND
2	ND
4	3 (25)
6	6 (50)
8	10 (83.33)
10	12 (100)

ND: not detected

Development of sliminess started from fourth day onwards and 25 per cent of samples showed slime formation on the inner less smoked surface. On 10^{th} day sliminess was noticed on all the samples tested.

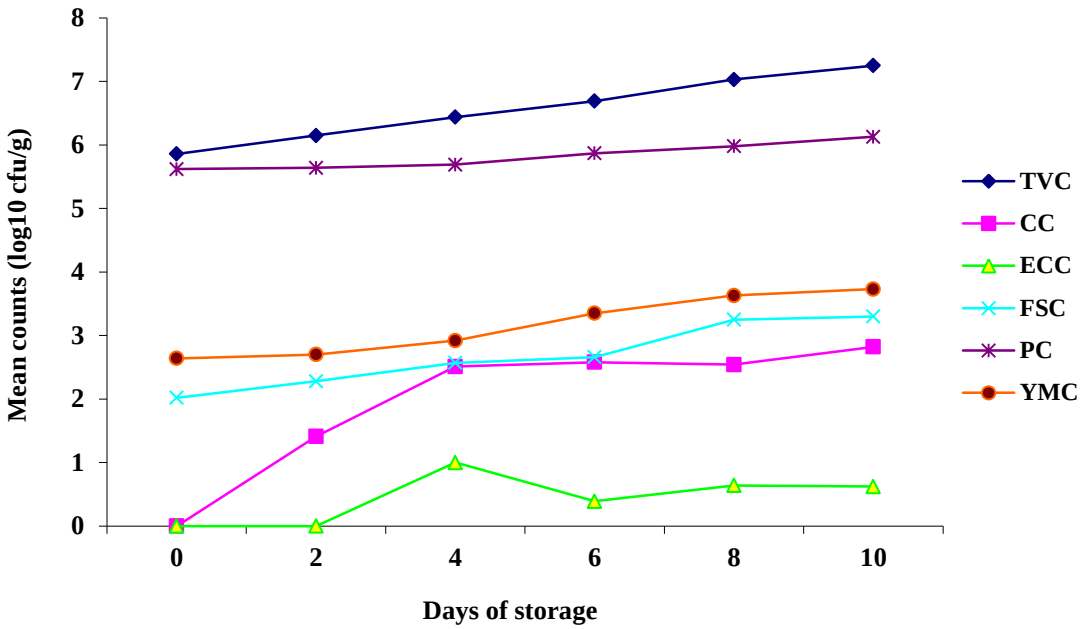


Fig. 1: Mean microbial counts of fresh and chilled beef frankfurter

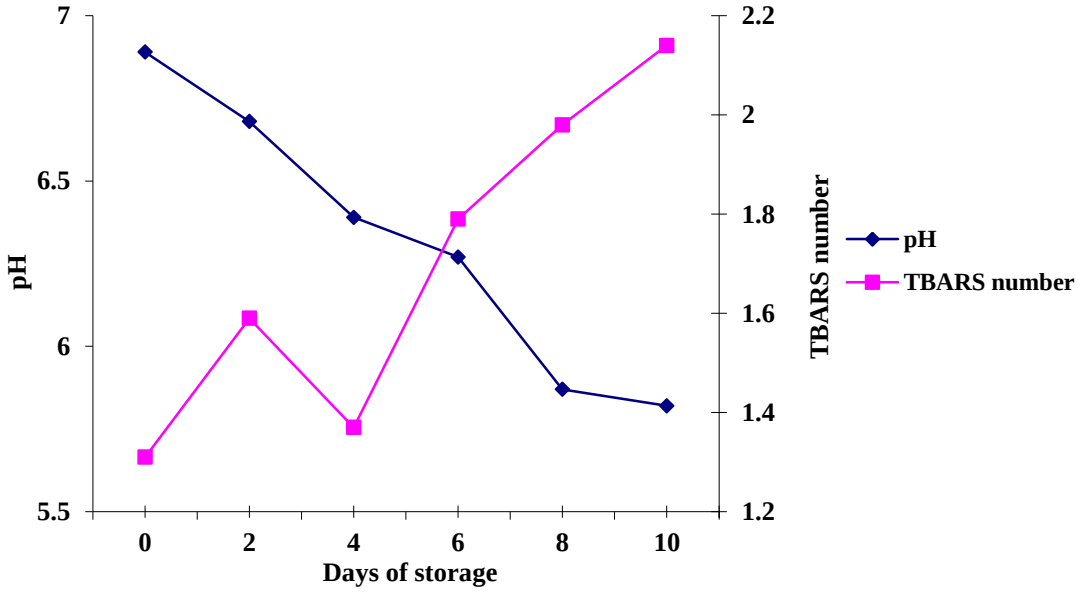
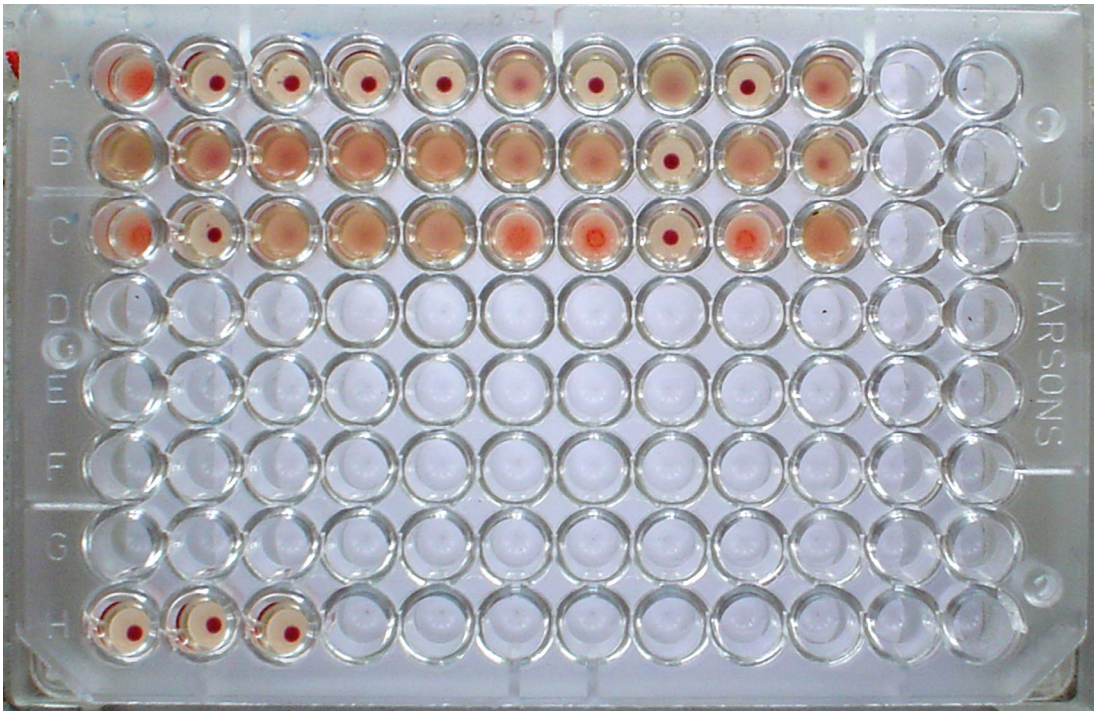
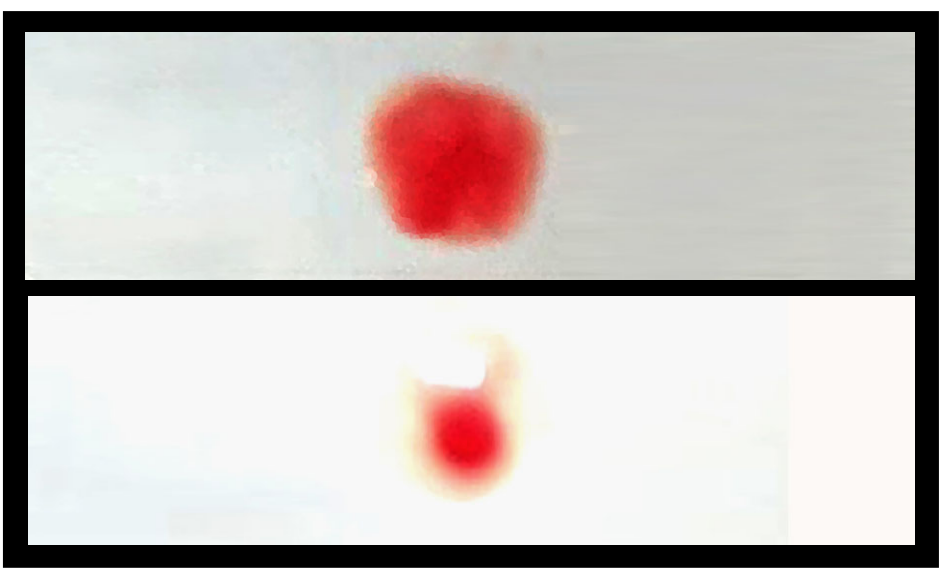


Fig. 2: Mean pH and TBARS number of fresh and chilled beef frankfurter



*

A



**

B

Plate 1. A) Test for Hemolytic activity of Aeromonas isolates.
* Negative control with button formation.
B) Test for Hemagglutinating activity of Aeromonas isolates.
** Negative control without agglutination.

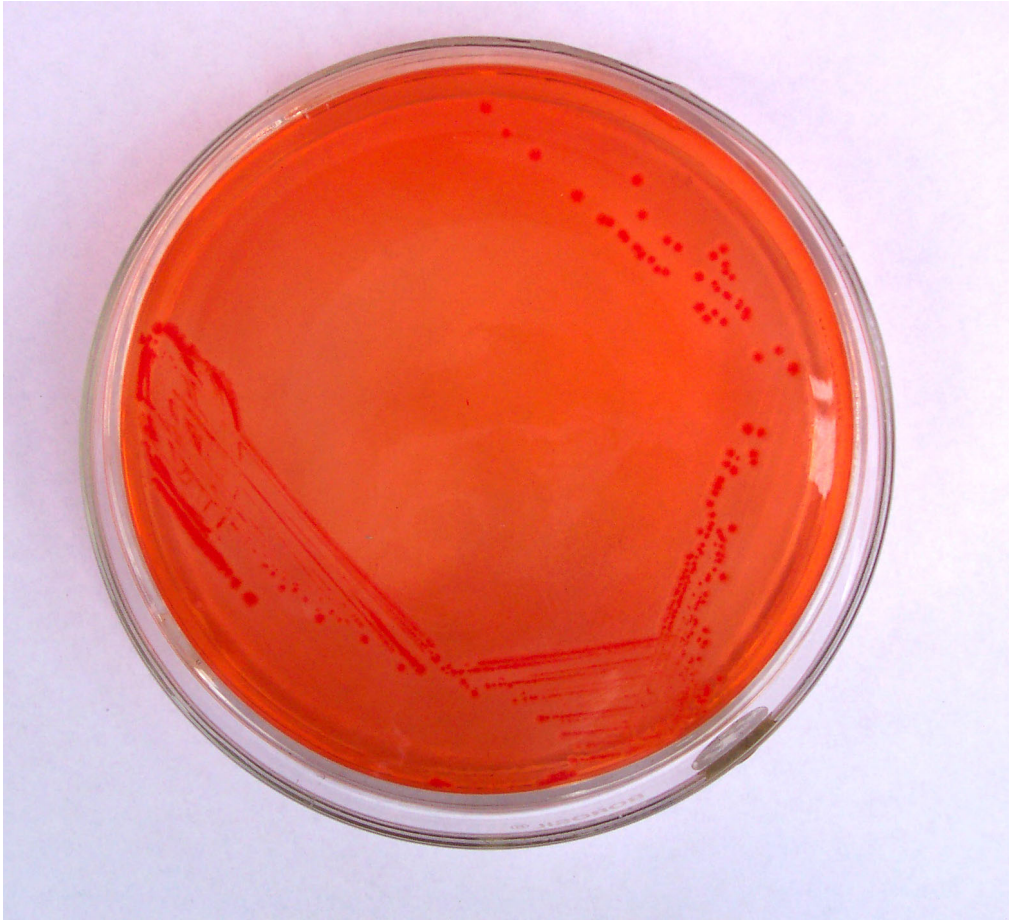


Plate 2. Congored binding test of *Escherichia coli* isolates.

4.1.2 Frozen Storage

Frozen beef frankfurter samples were subjected to estimation of various bacterial counts at an interval of 15 days.

4.1.2.1 Total Viable Count

The mean total viable counts were statistically analyzed by student's 't' test and the results are shown in Table 30 (Fig.3).

Table 30. Mean total viable count of frozen beef frankfurter samples

Days of storage	Count (log_{10} cfu/g) Mean ± SE
0	5.86 ± 0.15^{abc}
15	5.91 ± 0.11^{ac}
30	6.07 ± 0.8^{bd}
45	6.08 ± 0.02^{ab}
60	6.11 ± 0.02^{ab}
75	5.96 ± 0.03^{cd}
90	6.05 ± 0.03^{ab}

Figures bearing the same superscript do not differ significantly.

Mean total viable counts of fresh samples and frozen samples were not different significantly ($P \leq 0.05$). Mean count on 15^{th} day was significantly lower ($P \leq 0.05$) than the count on 30^{th} day. A significant difference was observed ($P \leq 0.05$) between mean counts on 45^{th} and 75^{th} day and 75^{th} and 90^{th} day of frozen storage.

Distribution of frozen beef frankfurter samples based on level of total viable count is given in Table 31.

Table 31. Distribution of frozen beef frankfurter samples based total viable count

Days of storage	Total viable count (cfu/g)	
	10^5	10^6
0	6 (50)	6 (50)
15	6 (50)	6 (50)
30	6 (50)	6 (50)
45	1 (8.33)	11 (91.67)
60		12 (100)
75	6 (50)	6 (50)
90	2 (16.67)	10 (83.33)

Figures in parenthesis indicates percentage

Fresh samples and frozen samples on 15^{th}, 30^{th} and 75^{th} day of storage showed the total viable count at the level of 10^5 (50 per cent) and 10^6 (50 per cent) cfu/g. On 60^{th} day cent per cent samples had the count at 10^6 cfu/g level.

4.1.2.2 Psychrotrophic Count

Psychrotrophic count of frozen beef frankfurter samples is given in Table 32 (Fig.3).

Table 32. Mean psychrotrophic count of frozen beef frankfurter samples

Days of storage	Count (log_{10} cfu/g)
0	5.62 ± 0.02^a
15	5.12 ± 0.02^b
30	5.08 ± 0.05^b
45	4.85 ± 0.04^c
60	4.82 ± 0.03^c
75	4.77 ± 0.05^c
90	4.74 ± 0.04^c

Figures bearing same superscript do not differ significantly.

Mean psychrotrophic counts were subjected to statistical analysis by student's 't' test. The analysis revealed a significant ($P \leq 0.05$) reduction in mean

psychrotrophic count through out the period of frozen storage when compared to fresh samples.

Psychrotrophic count on 30th day of frozen storage was significantly (P≤0.05) higher than the count obtained on 45th, 60th, 75th and 90th day of storage. From 60th day onwards there was no significant difference in counts.

Distribution of frozen samples of beef frankfurter based on level of psychrotrophic count is given in the table 33.

Table 33. Distribution of frozen beef frankfurter samples based on psychrotrophic count

Days of storage	Psychrotrophic count cfu/g	
	10^4	10^5
0		12 (100)
15	1 (8.33)	11 (91.67)
30	4 (33.33)	8 (66.67)
45	10 (83.33)	2 (16.67)
60	11 (91.67)	1 (8.33)
75	11 (91.67)	1 (8.33)
90	12 (100)	

Figures in parenthesis indicate per cent

In fresh samples the psychrotrophic count was at the level of 10^5 cfu/g. During storage there was a gradual reduction in the level of count. On 60th and 75th day of storage 91.67 per cent samples had count at 10^4 cfu/g level and on 90th day of storage all the samples had the count 10^4 cfu/g level.

4.1.2.3 Correlation Between Total Viable Count And Psychrotrophic Count of Frozen Beef Frankfurter

Correlation between mean total viable count and psychrotrophic count of frozen beef frankfurter samples is given in the table 34.

Table 34. Correlation coefficient between mean total viable count and psychrotrophic count of frozen beef frankfurter

	Days of storage						
	0	15	30	45	50	75	90
Correlation between TVC and PC	0.12	0.47	0.93**	0.00	-0.16	0.37	0.73**

TVC: total viable count, PC: psychrotrophic count, ** P <0.01, * P <0.05

The association between total viable count and psychrotrophic count was positive and highly significant (P≤0.01) on 30th and 90th day of storage.

4.1.2.4 Isolation and Identification of Bacteria

The pathogenic and spoilage organisms isolated from frozen samples of beef frankfurter during different days of frozen storage are shown in table 35.

Table 35. Pathogenic and spoilage organisms isolated from frozen beef frankfurter

Bacteria	Samples tested on each day	Number of positive samples						
		Days of storage						
		0	15	30	45	60	75	90
Aeromonas	12	3	2	2	ND	ND	1	2
Escherichia coli	12	ND	ND	ND	ND	ND	ND	ND
Salmonella	12	ND	ND	ND	ND	ND	ND	ND
Pseudomonas aeruginosa	12	ND	1	1	1	ND	ND	ND
Lactobacillus	12	ND	ND	ND	ND	ND	ND	ND

ND : Not detected

Aeromonas

Frozen samples of beef frankfurter were subjected to the isolation and identification of *Aeromonas* spp. On 15th, 30th and 90th day of storage, 16.67 per cent of samples revealed the presence of organism.

Different species of *Aeromonas* isolated from frozen samples of beef frankfurter are given in table 36.

Table 36. *Aeromonas* species isolated from frozen beef frankfurter

Species	Total number of isolates	Number of positive samples					
		Days of storage					
		15	30	45	60	75	90
A. hydrophila	2	ND	1	ND	ND	1	ND
A. sobria	3	1	ND	ND	ND	ND	2
A. caviae	2	1	1	ND	ND	ND	ND
Total	7	2	2	ND	ND	1	2

ND: not detected

A total of seven isolates of *Aeromonas* were obtained from frozen beef frankfurter samples. Among that 28.57 per cent each belonged to *A. hydrophila* and *A. caviae* and 42.85 per cent of isolates were of *A. sobria* species.

Hemolysis and hemagglutination assays

Hemolysis and hemagglutination assays were carried out in order to identify the enteropathogenic *Aeromonas*. Results are shown in table 37.

Table 37. Hemolysis and Hemagglutination assays of *Aeromonas* isolates from frozen beef frankfurter

Isolate	Total number of isolates	Number of positive isolates	
		Hemolysis	Hemagglutination
A. hydrophila	2	ND	ND
A. sobria	3	1 (33.33)	2 (66.67)
A. caviae	2	1 (50)	1 (50)

Figures in parenthesis indicate per cent ND: not detected

Two isolates of *A. hydrophila* obtained from frozen beef frankfurter were non-hemolytic and non-hemagglutinating. Fifty per cent isolates of *Aeromonas caviae* and 33.33 per cent of *A. sobria* respectively showed hemolysis. Fifty per

cent isolates of *A. caviae* and 66.67 percent of *A. sobria* were positive for hemagglutination of six per cent v/v suspension of rabbit erythrocytes.

Escherichia coli

Escherichia coli was not detected in any of the frozen samples tested (Table 35).

Salmonellae

Salmonellae were not detected in any of the frozen samples tested (Table 35).

Pseudomonas aeruginosa

Frozen samples of beef frankfurter were tested to isolate and identify *Pseudomonas aeruginosa* on days 15, 30, 45, 60, 75 and 90 of frozen storage. Among the 12 samples tested one sample each (8.33 per cent) revealed the presence of organism on 15^{th}, 30^{th} and 45^{th} day of storage (Table 35). The organism could not be detected on all other days.

Lactobacilli

Lactobacilli were not detected from any of the frozen samples of beef frankfurter (table 35).

4.1.2.5 pH

Frozen samples were also subjected to estimation of pH on day 15, 30, 45, 60 and 90 of storage. The values are given in the table 38 (Fig.4).

Table 38. Mean pH of frozen beef frankfurter

Characteristic	Days of storage						
	0	15	30	45	60	75	90
pH (Mean ± SE)	6.89 ± 0.02[a]	6.16 ± 0.04[bd]	6.33 ± 0.01[b]	6.23 ± 0.04[c]	6.12 ± 0.02[d]	6.19 ± 0.01[c]	6.23 ± 0.03[bc]

Figures bearing the same superscript do not differ significantly.

pH of fresh samples was significantly (P≤0.05) higher than that of frozen samples. The pH on 15th, 30th and 90th day were not different significantly. pH on 60th day of storage was significantly (P≤0.05) lower than that reported on other days of frozen storage except 15th day.

4.1.2.6 TBARS Number

During frozen storage samples of beef frankfurter were subjected to estimation TBARS number. The values are given in the table 39 (Fig.4).

Table 39. Mean TBARS number of frozen beef frankfurter

Characteristic	Days of storage						
	0	15	30	45	60	75	90
TBARSnumber (mg malonaldehyde/ kg) Mean ± SE	1.31 ± 0.04[a]	1.29 ± 0.02[a]	1.17 ± 0.01[b]	1.15 ± 0.01[b]	1.11 ± 0.01[c]	1.15 ± 0.04[b]	1.04 ± 0.02[d]

A significant (P≤0.05) reduction in mean TBARS number was noticed for the frozen samples when compared to the fresh samples. There was significant (P≤0.05) difference in values on 30th and 60th day and 45th and 60th day. The mean TBARS number was reduced by 0.27 units on 90 days of frozen storage and the value differed significantly from the mean value on all other days.

4.1.2.7 Organoleptic Evaluation

During the frozen storage samples were analysed for organoleptic qualities such as color, odor and sliminess at 15 days intervals by a semi-trained four member panel. The mean color and odor scores are given in table 40.

Table 40. Color and odor scores of frozen beef frankfurter

Days of storage	(Mean scores± SE)	
	Color	Odor
0	1 ± 0.00	1 ± 0.00
15	1 ± 0.00	1 ± 0.00
30	1 ± 0.00	1 ± 0.00
45	1 ± 0.00	1 ± 0.00
60	1 ± 0.00	1 ± 0.00
75	1 ± 0.00	1 ± 0.00
90	1 ± 0.00	1 ± 0.00

Fresh samples and frozen samples throughout the frozen storage were having similar color and odor scores.

Surface slime formation was not observed in frozen samples.

4.2 CHICKEN PEPPERONI

4.2.1 Fresh and Chilled Storage

Samples of chicken pepperoni were examined for various microbial counts on day zero, two, four, six, eight and ten of storage.

4.2.1.1 Total Viable Count

Mean total viable count of fresh and chilled chicken pepperoni are given in table 41 (Fig.5).

Table 41. Mean total viable count of fresh and chilled chicken pepperoni

Days of storage	Count(log_{10} cfu/g) Mean ± SE
0	6.36 ± 0.13^{a}
2	6.31 ± 0.08^{a}
4	6.77 ± 0.05^{b}
6	6.97 ± 0.03^{c}
8	7.21 ± 0.06^{d}
10	7.17 ± 0.01^{f}

Figures bearing same superscript do not differ significantly

The data were subjected to student's 't' test to evaluate the significant difference between the mean count of the samples stored on day zero, two, four, six eight and ten. Analysis of the data revealed that the mean total viable count increased during the period of storage. The mean count of the samples on fourth, sixth, eighth and tenth day of storage were significantly ($P<0.05$) higher than that of fresh chicken pepperoni. Highest mean count was observed on eighth day of storage at refrigeration temperature.

On the day of production all the samples had count at or below 10^6 cfu/g. In 83.33 per cent of samples stored on day two and four the count was at the level of 10^6 cfu/g. On tenth day of storage cent percent samples had the count at 10^7 cfu/g level (Table 42).

Table 42. Distribution of fresh and chilled chicken pepperoni samples based on total viable count

Days of storage	Total viable count (cfu/g)		
	10^5	10^6	10^7
0	4 (33.33)	8 (66.67)	
2	2 (16.67)	10 (83.33)	
4		10 (83.33)	2 (16.67)
6		9 (75)	3 (25)
8		2 (16.67)	10 (83.33)
10			12 (100)

Figures in parenthesis indicate per cent

4.2.1.2 Coliform Count

The mean coliform count of fresh and chilled chicken pepperoni are given in the table 43 (Fig.5).

Table 43. Mean coliform count of fresh and chilled chicken pepperoni

Days of storage	Count(log_{10} cfu/g) Mean ± SE
0	ND
2	1.18 ± 0.33^{a}
4	2.26 ± 0.41^{b}
6	3.32 ± 0.23^{c}
8	3.76 ± 0.09^{d}
10	3.84 ± 0.05^{d}

Figures bearing the same superscript do not differ significantly
ND: not detected

In fresh sausage samples coliforms were not detected. From second day onwards the count increased significantly ($P<0.05$) throughout the storage period. There was 2.66 log increase in mean count on tenth day of storage when compared with the count on second day of refrigerated storage.

Table 44. Distribution of fresh and chilled chicken pepperoni samples based on coliform count

Days of storage	Coliform count (cfu/g)			
	10^1	10^2	10^3	10^4
0				
2	2 (16.67)	4 (33.33)	1 (8.33)	
4	2 (16.67)	3 (25)	4 (33.33)	
6		4 (33.33)	6 (50)	2 (16.67)
8			11 (91.67)	1 (8.33)
10			9 (75)	3 (25)

Figures in parenthesis indicate per cent
ND : Not detected

Up to fourth day of storage none of the samples had a count at 10^4 cfu/g level. On sixth, eighth and tenth day 16.67, 8.33 and 25 per cent of samples respectively had a count at 10^4 cfu/g level (Table 44).

4.2.1.3 Escherichia coli Count

Escherichia coli was not detected in chicken pepperoni samples during refrigerated storage for 10 days.

4.2.1.4 Faecal Streptococcal Count

Faecal streptococcal count of chicken pepperoni samples stored at 4-7°C for 10 days is given in the table 45 (Fig.5).

Table 45. Mean faecal streptococcal count of fresh and chilled chicken pepperoni

Days of storage	Count($\log_{10}$ cfu/g) Mean ± SE
0	2.35 ± 0.18^{a}
2	3.07 ± 0.13^{b}
4	3.57 ± 0.13^{c}
6	4.16 ± 0.15^{d}
8	3.97 ± 0.18^{d}
10	4.88 ± 0.03^{e}

Figures being the same superscript do not differ significantly

Faecal streptococcal count increased significantly up to sixth day of storage on comparison with the count on zero day. On eighth day the count was reduced and on tenth day it increased significantly ($P \leq 0.05$). A significant ($P \leq 0.05$) difference in mean count was noted between all the days of storage except sixth and eighth day.

Distribution of chicken pepperoni samples based on level of faecal streptococcal count is shown in table 46.

Table 46. Distribution of fresh and chilled chicken pepperoni samples based on faecal streptococcal count

Days of storage	Faecal streptococcal count (cfu/g)				
	10^1	10^2	10^3	10^4	10^5
0	3 (25)	9 (75)			
2		4 (33.33)	8 (66.67)		
4		2 (16.67)	8 (66.67)	2 (16.67)	
6			6 (50)	5 (41.67)	1 (8.33)
8			4 (33.33)	8 (66.67)	
10				11 (91.67)	1 (8.33)

Figures in parenthesis indicate percent

Initially 75 per cent of samples had count at 10^2 cfu/g level. On second and fourth day 66.67 per cent had count at 10^3 cfu/g level. After 10 days of storage highest percentage (91.67 per cent) had a count at 10^4 cfu/g level.

4.2.1.5 Psychrotrophic Count

Psychrotrophic count of chilled chicken pepperoni during 10 days of storage is given in table 47 (Fig.5).

Table 47. Mean psychrotrophic count of fresh and chilled chicken pepperoni

Days of storage	Count($\log_{10}$ cfu/g) Mean ± SE
0	5.66 ± 0.03^{a}
2	5.77 ± 0.04^{b}
4	6.00 ± 0.06^{c}
6	6.80 ± 0.03^{de}
8	6.78 ± 0.02^{d}
10	6.84 ± 0.05^{e}

Figures being the same superscript do not differ significantly

Paired students' 't' test revealed a significant ($P \leq 0.05$) increase in mean psychrotrophic count throughout the storage period except between sixth and eighth day. On tenth day of count was increased by 1.18 log when compared with the initial count.

Distribution of samples based on level of psychrotrophic count is shown in table 48.

Table 48. Distribution of fresh and chilled chicken pepperoni samples based on psychrotrophic count

Days of storage	Psychrotrophic count (cfu/g)	
	10^5	10^6
0	12 (100)	
2	12 (100)	
4	8 (66.67)	4 (33.33)
6		12 (100)
8		12 (100)
10		12 (100

Figures in parenthesis indicate percent

Cent per cent samples had a psychrotrophic count at 10^5 cfu/g level on zero and second day and 10^6 cfu/g level on sixth, eighth and tenth day.

4.2.1.6 Yeast and Mold Count

Mean yeast and mold count of fresh and refrigerated chicken pepperoni is given in table 49 (Fig.5).

Table 49. Mean yeast and mold count of fresh and chilled chicken pepperoni samples

Days of storage	Count($\log_{10}$ cfu/g) Mean ± SE
0	3.66 ± 0.08 [a]
2	3.61 ± 0.06 [a]
4	3.92 ± 0.08 [b]
6	4.22 ± 0.02 [d]
8	4.28 ± 0.03 [d]
10	4.42 ± 0.05 [e]

Figures bearing same superscript do not differ significantly

Analysis of the data revealed that there was significant (P≤0.05) increase in count on all the days tested, except between zero and second day and between sixth and eighth day.

Distribution of samples based on level of yeast and mold count is given in the table 50.

Table 50. Distribution of fresh and chilled chicken pepperoni samples based on yeast and mold count

Days of storage	Yeast and mold count (cfu/g)	
	10^3	10^4
0	11 (91.67)	1 (8.33)
2	12 (100)	
4	9 (75)	3 (25)
6		12 (100)
8		12 (100)
10		12 (100)

Figures in parenthesis indicate per cent

Initially 91.67 per cent of samples had a count at 10^3 cfu/g level. Cent per cent samples on second day had a count at 10^3 cfu/g. On sixth, eighth and tenth day all the samples had a count at 10^4 cfu/g level.

4.2.1.7 Correlation Between Various Microbial Counts of Fresh and Chilled Chicken Pepperoni

Total viable count

Correlation between mean total viable count and other microbial counts of chicken pepperoni during storage at 4-7°C are shown in table 51.

Table 51. Correlation coefficient between mean total viable count and other microbial counts of fresh and chilled chicken pepperoni

Microbial counts	Total viable count					
	Days of storage					
	0	2	4	6	8	10
CC	-0.26	0.42	-0.12	0.59*	-0.75**	0.81**
FSC	-0.67*	0.78**	0.53	0.85**	-0.69*	0.81**
PC	-0.43	0.45	0.18	0.12	-0.65*	0.16
YMC	0.84**	0.28	0.93**	0.49	-0.35	0.75**

TVC: Total viable count, CC: Coliform count,FSC: Faecal streptococcal count,PC Psychrotrophic count,YMC: Yeast and mold count,* = P $\leq$0.05, ** = P $\leq$0.01.

A highly significant (P$\leq$0.01) and positive correlation was observed between total viable count and coliform count of the samples on tenth day of storage. However, the relationship between the counts was significant (P$\leq$0.05) on day six of storage. The association between these organisms was highly significant (P$\leq$0.01) on day eight of storage but it was negative.

Total viable count showed a highly significant (P$\leq$0.01) correlation with faecal streptococcal count on second, sixth and tenth day of storage. The association was negative and significant (P$\leq$0.05) on zero day and eighth day.

E. coli was not detected from any of the samples tested.

A negative significant (P$\leq$0.05) association was observed between total viable count and psychrotrophic count on eighth day of storage.

There was a highly significant (P$\leq$0.01) relationship between total viable count and yeast and mold count on zero, fourth and tenth day of storage.

Coliform count

Correlation between mean coliform count and other microbial counts of fresh and chilled chicken pepperoni are given in table 52.

Table 52. Correlation coefficient between mean coliform count and other microbial counts of fresh and chilled chicken pepperoni

Microbial counts	Coliform count					
	Days of storage					
	0	2	4	6	8	10
TVC	-0.26	0.42	-0.12	0.59*	-0.75**	0.80**
FSC	0.47	0.03	-0.23	0.62*	0.55	0.93**
PC	0.54	0.35	-0.22	0.24	0.74**	0.45
YMC	-0.00	0.39	-0.14	0.45	0.49	0.64*

TVC: Total viable count, CC: Coliform count,FSC: Faecal streptococcal count,PC Psychrotrophic count,YMC: Yeast and mold count,* = P $\leq$0.05, ** = P $\leq$0.01.

Coliform count and faecal streptococcal count were significantly (P$\leq$0.05) and positively correlated on sixth day of storage and the positive association was highly significant (P$\leq$0.01) on tenth day of storage. The relationship was non-significant on all other days of storage.

The correlation between coliform count and psychrotrophic count was non-significant except on eighth day of storage where a highly significant (P$\leq$0.01) positive association was observed.

Association between coliform count and yeast and mold count was positive and significant (P$\leq$0.05) on tenth day of storage.

Faecal streptococcal count

Correlation between mean faecal streptococcal count and other microbial counts of fresh and chilled chicken pepperoni are given in table 53.

Table 53. Correlation coefficient between mean faecal streptococcal count and other microbial counts of fresh and chilled chicken epperoni

Microbial counts	Faecal streptococcal count					
	Days of storage					
	0	2	4	6	8	10
TVC	-0.67	0.78**	0.53	0.85**	-0.69*	0.81**
CC	0.47	0.03	-0.23	0.62*	0.55	0.93**
PC	0.73**	0.38	0.64*	0.25	0.17	0.53
YMC	-0.36	0.27	0.58*	0.47	0.19	0.62*

TVC: Total viable count, CC: Coliform count, FSC: Faecal streptococcal count,PC Psychrotrophic count,YMC: Yeast and mold count,* = P $\leq$0.05, ** = P $\leq$0.01.

Faecal streptococcal count and psychrotrophic counts were positively correlated with a high significance (P$\leq$0.01) on the day of preparation and there was a significant positive association on fourth day. The correlation was non-significant on all other days of storage.

Faecal streptococcal count and yeast and mold counts were correlated significantly (P$\leq$0.05) and positively on fourth and tenth day of storage.

Psychrotrophic count

Correlation between mean psychrotrophic count and other microbial counts of fresh and chilled chicken pepperoni are given in Table 54.

Table 54. Correlation coefficient between mean psychrotrophic count and Other microbial counts of fresh and chilled chicken pepperoni

Microbial counts	Psychrotrophic count					
	Days of storage					
	0	2	4	6	8	10
TVC	-0.43	0.45	0.18	0.12	-0.65*	0.16
CC	0.54	0.35	-0.22	0.24	0.74**	0.45
FSC	0.73**	0.38	0.64*	0.25	0.17	0.53
YMC	-0.10	0.25	0.28	0.23	0.66*	-0.07

TVC: Total viable count, CC: Coliform count,FSC: Faecal streptococcal count,PC Psychrotrophic count,YMC: Yeast and mold count,* = P $\leq$0.05, ** = P $\leq$0.01.

Psychrotrophic count and yeast and mold counts showed a significant positive ($P \leq 0.05$) association on eighth day of storage. Analysis of data revealed a non-significant correlation on all the other days of storage and a negative correlation was observed on the day of preparation and tenth day.

Yeast and mold count

Correlation between mean yeast and mold count and other microbial counts of fresh and chilled chicken pepperoni is given in Table 55.

Table 55. Correlation coefficient between mean yeast and mold count and other microbial counts of fresh and chilled chicken pepperoni

Microbial counts	Yeast and mold count					
	Days of storage					
	0	2	4	6	8	10
TVC	0.84**	0.28	0.93**	-0.49	-0.35	0.75**
CC	-0.00	0.39	-0.14	0.45	0.49	0.64*
FSC	-0.36	0.27	0.58*	0.47	0.19	0.62*
PC	-0.10	0.25	0.28	0.23	0.66*	-0.07

TVC: Total viable count, CC: Coliform count,FSC: Faecal streptococcal count,PC: Psychrotrophic count,YMC: Yeast and mold count,* = $P \leq 0.05$, ** = $P \leq 0.01$.

There was highly significant ($P \leq 0.01$) association between yeast and mold count and total viable count on zero, fourth and tenth day of storage.

4.2.1.8 Isolation and Identification of Bacteria

Fresh and chilled samples of chicken pepperoni were subjected to isolation of pathogenic and spoilage bacteria. All isolates were identified by cultural, morphological and biochemical characteristics. The pathogenic and spoilage organisms isolated from fresh and chilled samples of chicken pepperoni on different days of storage are shown in table 56.

Table 56. Pathogenic and spoilage bacteria isolated from fresh and chilled chicken pepperoni

Bacteria	Samples tested on each day	Number of positive samples					
		Days of storage					
		0	2	4	6	8	10
Aeromonas	12	2	3	2	3	2	1
Escherichia coli	12	ND	ND	ND	ND	ND	ND
Salmonellae	12	ND	ND	ND	ND	ND	ND
Pseudomonas aeruginosa	12	ND	ND	2	1	1	1
Lactobacilli	12	ND	ND	ND	2	3	2

ND : Not detected

Aeromonas

Aeromonas spp. was isolated from 16.67 percent of fresh chicken pepperoni. The organism was isolated from 25 per cent of the samples stored on day two and six of storage and 16.67 per cent of the samples stored on day four and eight (Table 56).

Different species of *Aeromonas* spp. isolated from fresh and chilled samples of chicken pepperoni are given in table 57.

Table 57. *Aeromonas* species isolated from fresh and chilled chicken pepperoni

Species	Number of isolates	Number of positive samples					
		Days of storage					
		0	2	4	6	8	10
A. hydrophila	7	2	2	ND	2	1	ND
A. sobria	2	ND	ND	ND	1	1	ND
A. caviae	4	ND	1	2	ND	ND	1
Total	13	2	3	2	3	2	1

ND : Not detected

A total of 13 *Aeromonas* isolates consisting of *A. hydrophila* (7), *A. sobria* (2) and *A. caviae* (4), were isolated from fresh and chilled samples of chicken pepperoni.

Hemolysis and hemagglutination assays

In order to identify enteropathogenic *Aeromonas*, hemolysis and haemagglutination assays of the isolates were carried out. Results are given in table 58.

Table 58. Hemolysis and hemagglutination assays of *Aeromonas* isolates from fresh and chilled chicken pepperoni

Species	Total number of isolates	Number of positive isolates	
		Hemolysis	Hemagglutination
A. hydrophila	7	4 (57.14)	4 (57.14)
A. sobria	2	2 (100)	1 (50)
A. caviae	4	2 (50)	ND

Figures in parenthesis indicate per cent. ND: not detected.

Out of seven isolates of *A. hydrophila* obtained from fresh and chilled chicken pepperoni samples 57.14 per cent were hemolytic and hemagglutinating. All the isolates of *A. sobria* were hemolytic and one isolate was hemagglutinating. None of the *A. caviae* isolates were hemagglutinating whereas 50 percent of the isolates were hemolytic.

Escherichia coli

Escherichia coli were not detected from any of the fresh and chilled samples of chicken pepperoni (Table 56).

Salmonellae

Salmonellae could not be isolated from any of the fresh or chilled samples of chicken pepperoni (Table 56).

Pseudomonas aeruginosa

Fresh and chilled samples on day two of storage did not reveal the presence of the organism. The organism was isolated from 16.67 per cent samples on day four of storage and from 8.33 per cent of the samples on day six, eight and ten of storage (Table 56).

Lactobacilli

Lactobacilli could not be isolated from fresh samples and chilled samples on day two and four of storage. On eighth day of storage, the organism was isolated from 25 per cent samples. The organism was also isolated from 16.67 per cent of samples stored on day six and ten (Table 56).

Different species of Lactobacilli isolated from fresh and chilled chicken pepperoni samples are given in table 59.

Table 59. *Lactobacillus* species isolated from fresh and chilled chicken pepperoni

Species	Total number of isolates	Number of positive samples					
		Days of storage					
		0	2	4	6	8	10
L. brevis	3	ND	ND	ND	1	2	ND
L. curvatus	1	ND	ND	ND	ND	ND	1
L. fermentum	2	ND	ND	ND	1	1	ND
L. sake	1	ND	ND	ND	ND	ND	1
Total	7	ND	ND	ND	2	3	2

ND: not detected

From chilled samples four species of Lactobacilli viz., *L. brevis, L. curvatus, L. fermentum* and *L. sake* could be isolated. Out of the seven isolates obtained three were *L. brevis* and two were *L. fermentum*. One isolate each of *L. curvatus* and *L. sake* were detected from samples on tenth day of storage.

4.2.1.9 pH

Fresh and chilled samples of chicken pepperoni were subjected to the estimation of pH and the mean values are given in table 60 (Fig.6).

Table 60. Mean pH of fresh and chilled chicken pepperoni

Characteristic	Days of storage					
	0	2	4	6	8	10
pH (Mean ± SE)	6.28± 0.00[a]	6.21 ± 0.00[b]	6.16 ± 0.01[c]	6.08 ± 0.02[d]	5.89 ± 0.01[e]	5.41 ± 0.43[e]

Figures bearing same superscript do not differ significantly

Analysis of the data revealed that a significant reduction in pH was observed till eighth day of storage. There was significant difference in pH of samples tested on all the days of storage except on eighth and tenth day. The value reduced by 0.87 on tenth day of storage, when compared to the initial value.

4.2.1.10 TBARS number

Fresh and chilled samples of chicken pepperoni were subjected to the estimation of TBARS number. The mean values obtained are given in table 61 (Fig.6).

Table 61. TBARS number of fresh and chilled chicken pepperoni

Characteristic	Days of storage					
	0	2	4	6	8	10
TBARS number (mg malonaldehyde/kg) Mean ± SE	0.981 ± 0.01[a]	1.00 ± 0.05[b]	1.31 ± 0.04[c]	1.41 ± 0.17[d]	1.60 ± 0.04[e]	1.93 ± 0.05[f]

Figures bearing same superscript do not differ significantly

Student's 't' test revealed that mean TBARS number of the fresh samples was significantly ($P \leq 0.05$) lower than that of chilled samples tested on day two, four, six, eight and ten of storage. There was a significant ($P \leq 0.05$) increase in the TBARS number of samples throughout the chilled storage.

4.2.1.11 Organoleptic Evaluation

A semi-trained four-member panel of the department studied organoleptic qualities of fresh and chilled samples of chicken pepperoni. Color, odor and presence of sliminess were evaluated in fresh samples and at two days interval during the chilled storage. The mean color and odor scores are given in the table 62.

Table 62. Color and odor scores of fresh and chilled chicken pepperoni

	(Mean scores ± SE)	
Days of storage	Color	Odor
0	1 ± 0.00^{a}	1 ± 0.00^{a}
2	1 ± 0.00^{a}	1 ± 0.00^{a}
4	1.67 ± 0.14^{b}	1.64 ± 0.14^{b}
6	2.83 ± 0.21^{c}	2.00 ± 0.00^{c}
8	3.83 ± 0.21^{d}	2.00 ± 0.00^{c}
10	4.5 ± 0.11^{e}	2.16 ± 0.11^{c}

Figures bearing same superscript do not differ significantly.

Color and odor scores remained same in fresh samples and on second day of storage. Fourth day onwards the color scores increased significantly ($P<0.05$) throughout the period of storage. The odor scores on sixth, eighth and tenth day of storage were significantly ($P<0.05$) higher than that on fourth day of storage. A slight discoloration and off odor was noticed on 10^{th} day.

Samples of chilled chicken pepperoni were observed for the presence of sliminess during the storage period at two days interval. Distribution of samples based on presence of sliminess is given in the table 63.

Table 63. Distribution of fresh and chilled chicken pepperoni samples based on presence of sliminess

Days of storage	Samples showing sliminess
0	ND
2	ND
4	ND
6	8 (66.67)
8	9 (75)
10	12 (100)

ND: not detected. Figures in parenthesis indicate percent

Sliminess on the surface of chilled sausages was observed from sixth day onwards. Of the 12 samples tested eight developed sliminess on sixth day and sliminess was noticed on cent per cent of the samples on tenth day of storage at 4-7°C.

4.2.2 Frozen Storage

Chicken pepperoni samples stored at –20°C were evaluated for microbial quality as well as for physico-chemical and organoleptic qualities at an interval of 15 days up to 90 days.

4.2.2.1 Total Viable Count

Mean total viable count of frozen chicken pepperoni samples is given in table 64 (Fig.7).

Table 64. Mean total viable count of frozen chicken pepperoni

Days of storage	Count (log_{10} cfu/g)
0	6.36 ± 0.13^{a}
15	5.59 ± 0.07^{bc}
30	5.76 ± 0.05^{bd}
45	5.97 ± 0.10^{de}
60	5.76 ± 0.02^{bd}
75	5.54 ± 0.03^{ce}
90	5.53 ± 0.01^{ce}

Figures bearing same superscript do not differ significantly

The mean total viable count of fresh samples was significantly ($P<0.05$) higher than the count on all other days during frozen storage. Count on 15^{th} day was ($P<0.05$) significantly lower than the count on 45^{th} day.

Count on 30^{th} day was significantly ($P<0.05$) higher than the count on 75^{th} and 90^{th} day of frozen storage. Student's't' analysis showed that total viable count of frozen chicken pepperoni increased significantly ($P<0.05$) upto 45 days of storage and later significantly ($P<0.05$) reduced.

Distribution of samples based on level of total viable count is given in table 65.

Table 65. Distribution of frozen chicken pepperoni samples based on total viable count

Days of storage	Total viable count (cfu/g)	
	10^5	10^6
0	4 (33.33)	8 (66.67)
15	12 (100)	
30	12 (100)	
45	7 (58.33)	5 (41.67)
60	12 (100)	
75	12 (100)	
90	12 (100)	

Figures in parenthesis indicate percent

On day of preparation and 45^{th} day of storage 66.67 and 41.67 per cent of samples respectively, had a higher count at 10^6 cfu/g level. On all the other days of storage the count was noticed at 10^5 cfu/g level only.

4.2.2.2 Psychrotrophic Count

Mean psychrotrophic count of frozen chicken pepperoni samples is given in the table 66 (Fig.7).

Table 66. Mean psychrotrophic count of frozen chicken pepperoni

Days of storage	Count (log_{10} cfu/g)
0	5.86 ± 0.15^{a}
15	4.55 ± 0.04^{bde}
30	4.97 ± 0.04^{c}
45	4.90 ± 0.06^{c}
60	4.46 ± 0.06^{b}
75	4.70 ± 0.03^{d}
90	4.45 ± 0.09^{e}

Figures bearing same superscript do not differ significantly

Mean psychrotrophic counts of frozen samples of chicken pepperoni were subjected to Student's 't' test to find out the significant difference between them. Analysis revealed a significant ($P<0.05$) reduction in count throughout the storage period when compared to the initial count.

A significant ($P<0.05$) increase in count was noted after 30 days of storage when compared to day 15 of storage. On 60^{th} day there was a significant reduction in count compared to count on 30^{th} and 45^{th} day of storage. A significant ($P<0.05$) lowering of count was noted on 90^{th} day when compared to count on 75^{th} day.

Distribution of frozen chicken pepperoni samples based on level of psychrotrophic count is given in table 67.

Table 67. Distribution of frozen chicken pepperoni samples based on psychrotrophic count

Days of storage	Psychrotrophic count(cfu/g)		
	10^4	10^5	10^6
0		6 (50)	6 (50)
15	9 (75)	3 (25)	
30	7 (50)	5 (41.67)	
45	8 (66.67)	4 (33.33)	
60	12 (100)		
75	12 (100)		
90	12 (100)		

Figures in parenthesis indicate percent

In fresh samples 50 per cent had the count at 10^5 cfu/g level and 50 per cent at 10^6 cfu/g level. Cent per cent samples had the count at 10^4 cfu/g level on 60^{th}, 75^{th} and 90^{th} day of storage. On 15^{th}, 30^{th} and 45^{th} day of storage 25, 41.67 and 33.33 per cent samples respectively had the count at 10^5 cfu/g level.

4.2.2.3 Correlation Between Total Viable Count and Psychrotrophic Count of Frozen Chicken Pepperoni

Correlation between mean total viable count and psychrotrophic count of chicken pepperoni stored at –20°C for 90 days is given in table 68.

Table 68. Correlation between mean total viable count and psychrotrophic count of frozen chicken pepperoni

Correlation between	Days of storage						
	0	15	30	45	60	75	90
TVC and PC	-0.43	0.79**	0.51	0.49	0.38	0.51	0.20

TVC:totalviable count, PC:psychrotrophic count ,** P <0.01,* P <0.05

Statistical analysis revealed that the association between total viable count and psychrotrophic count was positive and highly significant (P<0.01) on 15^{th} day of frozen storage. On all other days of frozen storage the association was positive but non significant.

4.2.2.4 Isolation and Identification of Bacteria

Frozen samples of chicken pepperoni were subjected to isolation of pathogenic and spoilage bacteria. All isolates were identified by cultural, morphological and biochemical characteristics. Pathogenic and spoilage organisms isolated from frozen samples of chicken pepperoni on different days of frozen storage are shown in table 69.

Table 69. Pathogenic and spoilage organisms isolated from frozen chicken pepperoni

Bacteria	Samples tested on each day	Number of positive samples						
		Days of storage						
		0	15	30	45	60	75	90
Aeromonas	12	2	1	2	2	3	2	1
Escherichia coli	12	ND	ND	ND	ND	ND	ND	ND
Salmonellae	12	ND	ND	ND	ND	ND	ND	ND
Pseudomonas aeruginosa	12	ND	2	1	ND	1	1	2
Lactobacilli	12	ND	ND	ND	ND	ND	ND	ND

ND:not detected

Aeromonas

Seventy two frozen samples of chicken pepperoni were tested for isolation and identification of *Aeromonas* spp. On 15th and 90th day, 8.33 cent samples each were positive for the presence of the organism. The organism was isolated from 16.67 per cent samples on 30th, 45th and 75th day of storage. Twenty five per cent samples revealed the presence of organism on 60th day of storage (Table 69).

Different species of *Aeromonas* isolated from frozen samples of chicken pepperoni are given in table 70.

Table 70. *Aeromonas* species isolated from frozen chicken pepperoni

Species	Number of isolates	Number of positive samples					
		Days of storage					
		15	30	45	60	75	90
A. hydrophila	4	ND	ND	1	1	1	1
A. sobria	2	ND	ND	1	1	ND	ND
A. caviae	5	1	2	ND	1	1	ND
Total	11	1	2	2	3	2	1

ND: not detected

Out of 11 isolates of *Aeromonas* obtained from frozen samples of chicken pepperoni, 36.36 per cent belonged to *A. hydrophila*, 18.18 per cent was *A. sobria* and 45.45 per cent was identified as *A. caviae*.

Hemolysis and hemagglutination assays

In order to identify enteropathogenic Aeromonas, hemolysis and hemagglutination assays of the isolates were carried out. Results are given in table 71.

Table 71. Hemolysis and hemagglutination assays of Aeromonas isolates from frozen chicken pepperoni

Isolate	Total number of isolates	Number of positive isolates	
		Hemolysis	Hemagglutination
A. hydrophila	4	4 (100)	2 (50)
A. sobria	2	2 (100)	1 (50)
A. caviae	5	3 (60)	5 (100)

Cent per cent isolates of *A. hydrophila* and *A. sobria* obtained from frozen chicken pepperoni were hemolytic, whereas only 60 percent of *A. caviae* were haemolytic. Fifty per cent each of A. hydrophila and *A. sobria* and cent per cent of *A. caviae* agglutinated six per cent v/v suspension of rabbit RBC.

Escherichia coli

E. coli was not isolated from frozen chicken pepperoni samples (Table 69).

Salmonellae

None of the samples of frozen chicken pepperoni were positive for presence of Salmonellae (Table 69).

Pseudomonas aeruginosa

Frozen chicken pepperoni samples were subjected to isolation of *Pseudomonas aeruginosa.*The organism was isolated from 9.7 percent of the samples. On 15th and 90th day 16.67 per cent samples each were positive for the presence of the organism. Out of the 12 samples tested one sample each revealed the presence of organism on 30th, 60th and 75th day of storage. None of the samples on 45th day of storage revealed the presence of organism (Table 69).

Lactobacilli

Lactobacilli were not detected from any of the frozen samples of chicken pepperoni (Table 69).

4.2.2.5 pH

Frozen chicken pepperoni samples were subjected to estimation of pH. The mean values are given in table 72 (Fig.8).

Table 72. Mean pH of frozen chicken pepperoni

Characteristic	Days of storage						
	0	15	30	45	60	75	90
pH (Mean ± SE)	6.28± 0.00^{a}	6.18 ± 0.01^{b}	6.14 ± 0.01^{b}	6.18 ± 0.02^{bc}	6.22 ± 0.02^{c}	6.17 ± 0.02^{bc}	6.14 ± 0.01^{b}

Figures bearing same superscript do not differ significantly

Student's 't' test revealed that pH of fresh samples was significantly ($P<0.05$) higher than that of all the frozen samples. There was no significant difference in pH of chicken pepperoni samples stored at -20°C on 15th, 30th, 45th, 75th and 90th day. A significant ($P<0.05$) increase was noted on 60th day compared to pH on 15th and 30th day of storage.

4.2.2.6 TBARS Number

The mean TBARS numbers of frozen chicken pepperoni samples are given in table 73 (Fig.8).

Table 73. Mean TBARS number of frozen chicken pepperoni

Characteristic	Days of storage						
	0	15	30	45	60	75	90
TBARS number (mg malonaldehyde/ kg) Mean± SE	0.98 ± 0.00[a]	0.96 ± 0.01[b]	0.93 ± 0.01 [c]	0.92 ± 0.02[bc]	0.92 ± 0.01[bc]	0.84 ± 0.01[d]	0.91 ± 0.01[e]

Figures bearing same superscript do not differ significantly

Fresh samples had the highest mean TBARS number. Student's 't' test revealed that there was a significant ($P<0.05$) reduction in the TBARS numbers upto 30th day of frozen storage. On 75th and 90th day a significant ($P<0.05$) lowering of the value was observed. The lowest mean value reported was on 75th day of frozen storage.

4.2.2.7 Organoleptic Evaluation

Samples of chicken pepperoni were stored at –20°C for 90 days and sensory analysis was done at 15 days interval. Mean color and odor scores are given in the table 74.

Table 74. Color and odor scores of frozen chicken pepperoni

Days of storage	(Mean scores ± SE)	
	Color	Odor
0	1 ± 0.00	1 ± 0.00
15	1 ± 0.00	1 ± 0.00
30	1 ± 0.00	1 ± 0.00
45	1 ± 0.00	1 ± 0.00
60	1 ± 0.00	1 ± 0.00
75	1 ± 0.00	1 ± 0.00
90	1 ± 0.00	1 ± 0.00

No change in color and odor was noticed during the frozen storage of chicken pepperoni samples. Scores obtained for fresh and frozen samples were the same throughout the period of storage.

Surface slime formation was not observed in frozen samples.

4.3 ASSESSMENT OF CRITICAL CONTROL POINTS

4.3.1 Beef Frankfurter

4.3.1.1 Air

The mean microbial count of air samples obtained from the processing room before and after the production and packaging of beef frankfurter are given in table 75.

Table75. Mean microbial counts of air samples in beef frankfurter processing room

Processing room	TVC (cfu/ft/min) Mean ± SE	YMC (cfu/ft/min) Mean ± SE
Before processing	1.20 ± 0.68	1.62 ± 0.56
After processing	1.63 ± 0.78	1.81 ± 0.58

TVC: total viable count, YMC: yeast and mold count

Mean total viable count of air samples collected from the processing room was higher after processing of the product than that before processing. Mean fungal count also was found to be considerably increased after processing.

4.3.1.2 Water

The mean total viable count (TVC), coliform count (CC), *Escherichia coli* count (ECC) and faecal streptococcal count (FSC) of water samples collected

from the chilling tank, water used for washing the casings, hand washing samples of personnel engaged in processing line and samples of ice used in mixing sausage batter are given in table 76.

Among the samples, hand washing samples had the highest mean total viable count whereas chilling water samples had the lowest count. Hand washing samples had the highest coliform count. *E. coli* and faecal streptococci were not detected in any of the water samples tested.

The mean bacterial counts of water samples are given in table 76.

Table 76. Mean bacterial count of water samples

Water	Bacterial counts (log_{10} cfu/ml) Mean ± SE			
	TVC	CC	ECC	FSC
Chilling water	2.54 ± 0.05	1.24 ± 0.28	ND	ND
Casing washing	2.74 ± 0.04	1.20 ± 0.01	ND	ND
Hand washing	4.61 ± 0.07	1.54 ± 0.06	ND	ND
Ice	2.65 ± 0.01	ND	ND	ND

TVC :total viable count, CC :coliform count, ECC :*Escherichia coli* count, FSC: faecal streptococcal count, ND:Not detected.

4.3.1.3 Equipment

The hygienic status of the working surfaces and equipment used in the processing plant were also evaluated. Bacterial counts of linking table surface, meat mincer, mixer, sausage filler and packaging material were estimated and the mean is given in table 77.

Table 77. Mean bacterial count of processing equipment in processing plant of beef frankfurter

Equipment	Bacterial counts (log_{10} cfu/cm^2) Mean ± SE			
	TVC	CC	ECC	FSC
Linking table	3.77 ± 0.00	ND	ND	1.00 ± 0.00
Meat mincer	4.30 ± 0.03	ND	ND	ND
Mixer	3.45 ± 0.04	ND	ND	1.00 ± 0.00
Sausage filler	4.93 ± 0.00	ND	ND	ND
Packaging material	2.45 ± 0.15	ND	ND	ND

TVC :total viable count, CC :coliform count, ECC :*Escherichia coli* count, FSC: faecal streptococcal count, ND:Not detected.

Among the equipment, sausage filler had the highest total viable count and the count was lowest in case of packaging materials. Coliforms and *E. coli* were not detected in any of the samples. Faecal streptococcal count at the level of 1 log_{10} cfu/g was observed in samples collected from linking table surface and mixer.

4.3.1.4 Raw Ingredients

Samples of raw materials such as beef, minced beef, spices and casings were subjected to the estimation of various bacterial counts (Table 78).

Table 78. Mean bacterial count of raw ingredients of beef frankfurter

Sample	Bacterial counts (log_{10} cfu/g) Mean ± SE			
	TVC	CC	ECC	FSC
Beef	5.66 ± 0.18	ND	ND	ND
Minced meat	5.39 ± 0.08	ND	ND	ND
Spices	4.42 ± 0.10	2.15 ± 0.03	1.30 ± 0.00	1.70 ± 0.00
Casings	4.70 ± 0.07	2.40 ± 0.25	ND	2.15 ± 0.15

TVC :total viable count, CC :coliform count, ECC :*Escherichia coli* count, FSC: faecal streptococcal count, ND:Not detected.

Beef samples revealed the highest mean total viable count, whereas spices had the lowest count. Coliforms were present at 2 log cfu/g level in samples of spices and casings. *E. coli* was detected only in samples of spices. Faecal streptococcal count was highest in samples of casings, whereas the organism could not be detected from beef and minced meat samples.

4.3.2 Chicken Pepperoni

4.3.2.1 Air

The mean microbial count of air samples obtained from the processing room before and after the production and packaging of chicken pepperoni are given in table 79.

Table 79. Mean microbial counts of air samples in chicken pepperoni processing room

Processing room	TVC (cfu/ft/min) Mean ± SE	YMC (cfu/ft/min) Mean ± SE
Before processing	1.20 ± 0.68	1.62 ± 0.56
After processing	1.63 ± 0.78	1.81 ± 0.58

TVC:total viable count, YMC:yeast and mold count

Mean total viable count of air samples collected from the processing room was higher after processing of the product than that before processing. Mean fungal count was also found to be considerably increased after processing.

4.3.2.2 Water

The mean total viable count (TVC), coliform count (CC), *Escherichia coli* count (ECC) and faecal streptococcal count (FSC) of water samples collected from the chilling tank, water used for washing the casings, hand washing samples of personnel engaged in processing line and samples of ice used in mixing with sausage batter are given in table 80.

Among the samples, hand washing samples had the highest mean total viable count whereas chilling water and ice samples had the lowest count. The highest coliform count was observed in hand washing samples. *Escherichia coli* and faecal streptococci were not detected. The organism could not be detected from ice samples.

Table 80. Mean bacterial count of water samples

Water sample	Bacterial counts (log_{10} cfu/ml) Mean ± SE			
	TVC	CC	ECC	FSC
Chilling water	2.47 ± 0.01	1.30 ± 0.00	ND	ND
Casing washing	2.65 ± 0.05	1.39 ± 0.08	ND	ND
Hand washing	4.61 ± 0.07	1.54 ± 0.06	ND	ND
Ice	2.47 ± 0.01	ND	ND	ND

TVC :total viable count, CC :coliform count, ECC :*Escherichia coli* count, FSC: faecal streptococcal count, ND:Not detected

4.3.2.3 Equipment

In order to identify the possible critical points of bacterial contamination during preparation of chicken pepperoni, the bacterial counts on linking table surface, meat mincer, mixer, sausage filler and packaging material were estimated and the mean counts are given in table 81.

Table 81. Mean bacterial count of processing equipment in processing plant of chicken pepperoni

Equipment	Bacterial counts (log_{10} cfu/cm^2) Mean ± SE			
	TVC	CC	ECC	FSC
Linking table	3.80 ± 0.00	ND	ND	ND
Meat mincer	4.22 ± 0.01	ND	ND	ND
Mixer	3.77 ± 0.00	ND	ND	ND
Sausage filler	4.86 ± 0.00	ND	ND	ND
Packaging material	2.00 ± 0.00	ND	ND	ND

TVC :total viable count, CC :coliform count, ECC :*Escherichia coli* count, FSC: faecal streptococcal count, ND:Not detected.

Among the equipments, sausage filler had the highest total viable count and lowest was recorded for packaging materials. Coliforms, *E. coli* and faecal streptococci were not detected from any of the samples analyzed.

4.3.2.4 Raw Ingredients

Samples of raw ingredients such as chicken, pork, minced meat, spices and casings were subjected to bacteriological examination and the results are given in table 82.

Table 82. Mean bacterial count of raw ingredients of chicken pepperoni

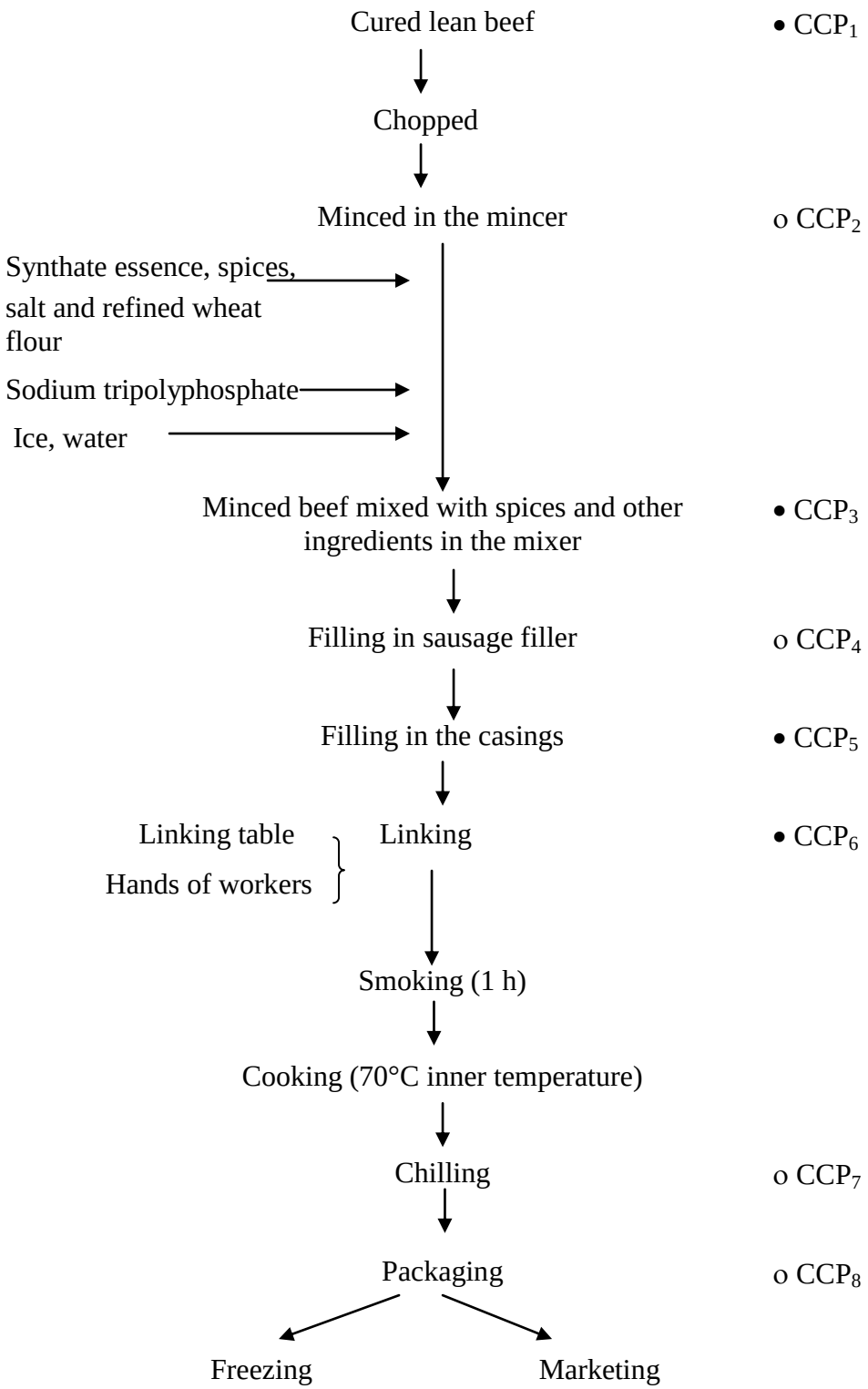
Sample	Bacterial counts (log_{10} cfu/cm^2) Mean ± SE			
	TVC	CC	ECC	FSC
Chicken	5.15 ± 0.15	2.73 ± 0.04	ND	2.95 ± 0.15
Pork	5.38 ± 0.09	ND	ND	2.38 ± 0.09
Minced meat	5.08 ± 0.09	1.58 ± 0.11	ND	1.63 ± 0.02
Spices	4.28 ± 0.21	2.07 ± 0.03	1.15 ± 0.15	2.71 ± 0.00
Casings	4.48 ± 0.41	2.18 ± 0.06	ND	2.95 ± 0.05

TVC :total viable count, CC :coliform count, ECC :*Escherichia coli* count, FSC: faecal streptococcal count, ND:Not detected.

Pork samples revealed the highest mean total viable count when compared to other ingredients and lowest was detected in spices. Coliforms were present at 2 log_{10} cfu/g level in samples of chicken, spices and casings, whereas coliforms were not detected in pork samples. *E. coli* was present only in spice samples. Faecal streptococci were present in all the raw ingredients analysed with the highest count in chicken meat samples and casings.

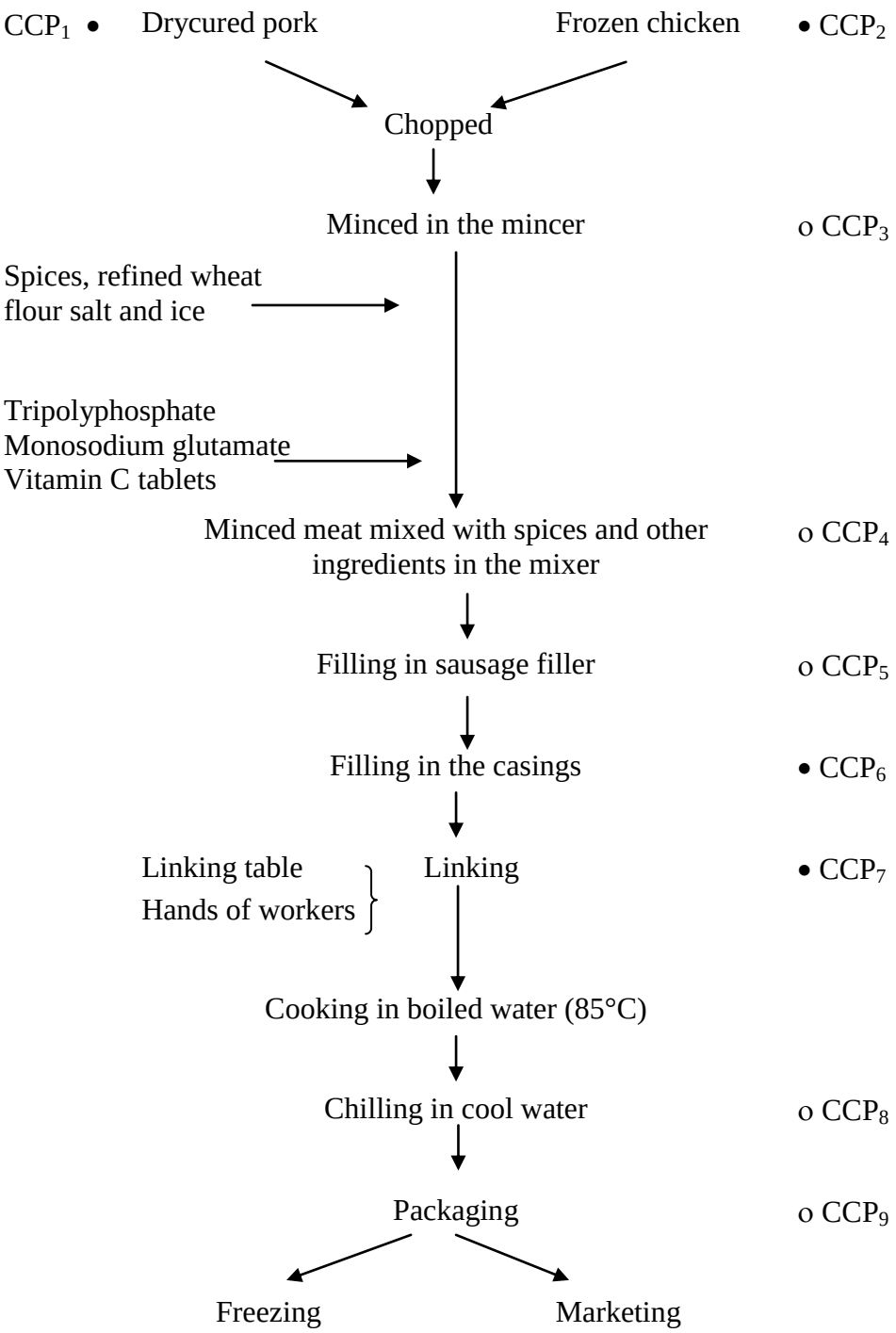
The critical control points of bacterial contamination during preparation of beef frankfurter and chicken pepperoni are given in Flow Charts 9 and 10 respectively.

Flow Chart 9. Critical control points in manufacture of Beef Frankfurter



• Point of major contamination
o Point of minor contamination

Flow Chart 10. Critical control points in manufacture of Chicken Pepperoni



o Point of minor contamination
• Point of major contamination

Discussion

5. DISCUSSION

The microbial, physico-chemical and organoleptic qualities of fresh, chilled and frozen samples of beef frankfurter and chicken pepperoni were evaluated during the investigation. The effect of chilling and freezing on these qualities was also studied. The microbial quality of the raw ingredients, processing equipment, packaging materials and environmental samples were evaluated to assess their role in bacterial contamination of the product.

5.1 BEEF FRANKFURTER

5.1.1 Fresh and Chilled Storage

5.1.1.1 Total Viable Count

Fresh beef frankfurter samples had a total viable count of 5.86 ± 0.15 $\log_{10}$ cfu/g. The count obtained was about 2 log higher than that reported by Krishnan and Sharma (1993) in case of buffalo beef sausages. Rao *et al.* (1999) and Samelis *et al.* (2000) also had reported lower total viable counts on day of preparation of smoked chicken sausages and frankfurters respectively.

A significant increase in total viable count was noticed throughout the period of chilled storage. On sixth day of storage there was about 1 log increase in the count compared to that on zero day. Count at the level of 10^7 cfu/g was observed in 41.67 per cent of the samples and initial spoilage changes were also noticed in 50 per cent of the samples on that day. After 10 days of chilled storage the count significantly increased to 7.25 ± 0.02 $\log_{10}$ cfu/g.

Only 50 per cent of the fresh samples studied, met the microbiological guideline prescribed for sausage at the point of sale of public health laboratory services (Gillbert *et al*., 2000), which indicate the count shall be within 10^4 to $<10^5$ cfu/g. Higher initial counts and early appearance of spoilage changes may

be attributed to high microbial load of raw ingredients, insufficient time-temperature maintenance during cooking as well as post processing contamination.

5.1.1.2 Coliform Count

Coliforms were not detected in fresh beef frankfurter samples. Anand *et al.* (1990) also reported a similar findings in case of moist cooked chicken sausages wherein they reported an initial count of 0.87 log/g in dry cooked chicken sausages. In case of vacuum packed frankfurters kept at 3°C the coliform count on zero day was 1.7 $\log_{10}$/g (Simard *et al.*, 1983).

During the chilled storage the count was increased by 1.41 log units than that on second day of storage and reached 2.82 ± 0.24 $\log_{10}$ cfu/g on tenth day. This count was significantly increased from second day onwards. On fourth, sixth and eighth day of storage all the samples had the count at 10^2 cfu/g level. On tenth day 25 per cent samples had the count at 10^3 cfu/g level. Increase in the level of coliform count during chilled storage was also reported by Anand *et al.* (1990).

Presence of coliforms in ready to cook meat products is indicative of the unsanitary conditions in the production plants. Coliforms have been reported to grow even at temperatures as low as –2°C and as high as 50°C (Jay, 1996). Frankfurters are smoked, semi-cooked sausages and presence of coliforms in the product suggests that organisms partially injured during processing might have adapted to proliferate at chilling temperature.

5.1.1.3 Escherichia coli Count

Escherichia coli was not detected in fresh samples and samples kept chilled for two days. This finding corroborate with that of Monge *et al.* (2000) in case of soybean sausages (50 per cent frankfurter and 50 per cent sausage mortadela).

Highest mean *E. coli* count was noticed on fourth day. However, no significant difference was noticed between the counts on fourth, sixth, eighth and tenth day. The count on all these days was at the level of 10^1 cfu/g. Prior and Casaleggio (1978) reported a count less than 3/g in bologna sausage during 12 days of storage at 5°C. Higher counts were reported by Palumbo *et al.* (1976) and Sharma *et al.* (1994) in pepperoni and pork sausage respectively.

Presence of *Escherichia coli* in food products is indicative of faecal contamination. Frankfurters being smoked, semi-cooked sausages, detection of *E. coli* in the product is suggestive of post processing contamination. Chilling water, packaging table and handling personnel may be the probable sources of recontamination of the product. The organism can remain viable for weeks to months in acidic foods like sausages at refrigeration temperature and improper cooking of such products can cause food- borne diseases in the consumers.

5.1.1.4 Faecal Streptococcal Count

In fresh beef frankfurter samples, the mean faecal streptococcal count was 2.02 ± 0.05 $\log_{10}$ cfu/g. Almost similar observation was recorded by Anand *et al.* (1990) in dry and moist cooked chicken sausages. It is also in agreement with findings of Samelis *et al.* (1998) in Greek Salami.

During chilled storage, there was significant increase in the count on all the days of storage except between fourth and sixth day and eighth and tenth day. About 1 log increase from the initial count was noted on eighth day. The mean count on tenth day was 3.30 ± 0.15 $\log_{10}$ cfu/g. Proliferation of streptococci in chilled chicken sausage samples was reported by Anand *et al.* (1990) also. The count on tenth day in chilled dry cooked chicken sausage was almost similar to that obtained in the present study.

Presence of faecal streptococci in large numbers is indicative of direct or indirect faecal contamination. Growth and multiplication of these organisms in foods is responsible for rapid development of spoilage changes. The classical

enterococci are considered as better indicators of food sanitary quality than coliforms, especially for frozen foods (Jay, 1996). This explains their ability to withstand low temperature.

5.1.1.5 Psychrotrophic Count

On the day of preparation, beef frankfurter samples had a mean count of 5.62 ± 0.02 $\log_{10}$ cfu/g. The count was 3 log higher than that reported by Anand *et al.* (1990) in raw chicken sausage and about 1.0 log higher than that described by Lee *et al.* (1997) and Yuste *et al.* (2000) respectively, in chicken breakfast sausage and frankfurters. During storage at chilling temperature a significant increase in count was noticed from sixth day onwards. After 10 days of chilled storage the count in 91.67 per cent of samples increased up to 10^6 cfu/g level. Lee *et al.* (1997) also reported proliferation of psychrotrophs during chilled storage of chicken breakfast sausage.

In case of refrigerated food products psychrotrophs constitute the spoilage microflora. Chilled storage of food permits the psychrotrophic population to reach millions per gram within few days, resulting in spoilage changes (ICMSF, 1980). Psychrotrophic spoilage bacteria increase markedly at refrigeration temperature (Khalafalla and Sherif, 1993).

5.1.1.6 Yeast and mold count

Initial mean count of beef frankfurter samples was 2.64 ± 0.06 $\log_{10}$ cfu/g, which was in accordance with reports of Simard *et al.* (1983) in frankfurter sausage. On the other hand, Samelis *et al.* (2000) had reported yeast count below 2 log cfu/g in frankfurters.

Statistical analysis revealed a significant increase in count throughout the period of chilled storage and the count was increased by 1.09 log units on tenth day of storage.

On sixth day cent per cent samples had the count at 10^3 cfu/g level and the count in 50 per cent samples was at 10^4 cfu/g on tenth day of storage. This is in agreement with findings of Adams *et al.* (1987), who reported a 2 log increase in yeast count of chilled British fresh sausage after 10 days of storage.

Yeast and molds are opportunistic spoilage organisms in meat products. Drying, salting or freezing reduces the water activity in meat products, which will suppress Gram-negative spoilage bacteria so that yeast and molds can proliferate and cause spoilage. Many of the food yeasts are psychrotrophic and so associated with spoilage of chilled meat products (Dillon, 1998).

5.1.1.7 Relationship between Various Microbial Counts of Fresh and Chilled Beef Frankfurter

Relationship between mean microbial counts of fresh and chilled beef frankfurter was studied by estimating the correlation coefficient.

Mean total viable count was positively and significantly ($P \leq 0.05$) associated with faecal streptococcal count, psychrotrophic count and yeast and mold count. Indicating that as total viable count increased faecal streptococcal count, psychrotrophic count and yeast and mold count also increased.

Mean coliform count showed a significant positive association with *E. coli* count. This indicates the possibility of faecal contamination. There was no positive relationship between coliform count and other mean counts.

E. coli count was positively and significantly correlated with psychrotrophic count also. Psychrotrophic count was also positively associated with faecal streptococcal count and yeast and mold count.

5.1.1.8 Isolation and Identification of Bacteria

Aeromonas

Aeromonas spp. was detected from fresh as well as chilled samples of beef frankfurters stored on all days. In the present study, the organism was detected in 23.61 per cent samples. Gobat and Jemmi (1993) detected the organism in 15.6 per cent of smoked cooked sausages.

Aeromonas isolated from the samples consisted of three major species viz., *A. hydrophila, A. sobria* and *A. caviae*. The isolation of these organisms from meat products had been reported by many workers (Okrend *et al.*, 1987; Gobat and Jemmi, 1993 and Singh, 1997).

During the investigation, *A. hydrophila* was isolated from 1.38 per cent of beef frankfurters, which was much lower than the rate of isolation of the organism (61.2 per cent) from smoked, cooked sausages (Gobat and Jemmi, 1993). Cent per cent of the isolates obtained during the study revealed hemolytic and hemagglutinating activity. Hemolytic activity of the organism is associated with the ability to cause cytotoxic effect (Yadav and Kumar, 2003) and haemagglutinating activity reflect the ability of the organism to produce enterotoxigenic effect in the consumers (Handfield *et al.*, 1996).

A. sobria was isolated from 11.11 per cent of the samples. However, Gobat and Jemmi (1993) reported the isolation of the organism from 22.5 per cent of the smoked, cooked sausage. Of the isolates, 75 per cent were hemolytic and 25 per cent had hemagglutinating character. However, Okrend *et al.* (1987) recorded that 69 per cent of the isolates revealed hemolytic activity.

A. caviae was isolated from 11.11 percent of the chilled beef frankfurter samples tested. Among the isolates, 75 per cent had hemolytic activity, which was much higher than the 18 per cent recorded by Okrend *et al.* (1987).

Hemagglutinating property was shown by 25 per cent of the isolates obtained in the study.

The high incidence of the organism in the fresh and chilled samples might be attributed to the contamination of the product from unwholesome water and also from contaminated work surface (Beuchat, 1991).

The virulence factors associated with *A. hydrophila*, *A. sobria* and *A. caviae* causes cholera-like illness and dysentery-like illness. Therefore, the detection of the organism in ready-to-cook meat product is of public health significance.

Escherichia coli

E. coli was not detected in fresh samples and chilled samples on day two of storage. The organism was isolated from 18.05 per cent of fresh and chilled beef frankfurter. Rate of isolation of the organism in the present study was much higher than that reported by Soriano *et al.* (2000) who had isolated the organism from 8.8 per cent of meat products.

The isolates were serotyped at National *Salmonella* and *Escherichia* Centre, Central Research Institute, Kasauli. Among these, 84.26 per cent of isolates belonged to the O2 serotype (Enterohemorrhagic *Escherichia coli*). These organisms produce shiga-like toxins (Verotoxin and Verocytotoxin). Hemolytic uremic syndrome and hemorrhagic colitis are caused by such strains of *E. coli*.

All the 13 isolates obtained from the chilled beef frankfurter samples were subjected to the congo red binding test, which revealed that 61.53 per cent had congo red binding property. This indicates the invasive property of the organism.

The characteristics of congo red binding constitutes a moderately stable, reproducible and easily distinguishable phenotypic marker (Rajil *et al.*, 2003). A

good correlation between pathogenic potential and congo red binding property had been reported by a number of workers (Yoder, 1989; Soni *et al.*, 2002).

The presence of this organism could be attributed to post processing contamination of the product and detection of this pathogenic and invasive organism is of great public health significance.

Salmonellae

None of the fresh and chilled samples had salmonellae, which is in agreement with the findings of Weissman and Carpenter (1969) and Samelis *et al.* (1998). However the isolation of the organism from fermented pork sausage had been reported by van Netten *et al.* (1986) and from uncooked economy or catering sausage by Mattick *et al.* (2002).

Pseudomonas aeruginosa

Pseudomonas aeruginosa could not be isolated from fresh samples of beef frankfurter. However, the organism was isolated from 5.56 per cent of chilled samples. Simard *et al.* (1983) reported the isolation of *Pseudomonas* from chilled frankfurter and Samelis *et al.* (1998) reported its presence in Greek day salami. However, Sherikar *et al.* (1979) reported that *P. aeruginosa* was absent in cocktail sausages and Oxford sausages.

Lactobacilli

Lactobacillus was not detected in any of the fresh samples. One sample on sixth day of storage and three samples on eighth day of storage revealed the presence of organism. Presence of lactobacilli in chilled frankfurter sausage had been reported by Blickstad and Molin (1983) and Simard *et al.* (1983).

In the present study, from chilled beef frankfurters two isolates of *L. brevis* and one isolate each of *L. curvatus* and *L. sake* were identified. Similarly isolation of *L. brevis* from fermented sausage had been reported by

Schillinger and Karllucke (1987). Hugas *et al.* (1993) and Korkeala and Bjorkroth (1997) had reported isolation of *L. curvatus* and *L. sake* from sausages.

Lactobacilli form a predominant part of spoilage microflora of refrigerated meat products. Production of acetoin, acetic acid and hydrogen sulphide by these organisms will result in development of off odors. Product discoloration is attributed to hydrogen sulphide and hydrogen peroxide production by these organisms. Many species of Lactobacilli had been isolated from ropy slime present on surface of spoiled sausages (Korkeala *et al.*, 1988). These organisms are found in the air and working surfaces of chilling and packaging rooms of meat processing plants. Sausages get contaminated with lactobacilli during handling, after preparation of the product.

5.1.1.9 pH

Mean pH of fresh samples was 6.89 ± 0.02. Similar values were reported by Krishnan and Sharma (1993) in buffalo beef sausages and by Rao *et al.* (1999) in smoked chicken sausages.

A significant reduction in pH was observed throughout the storage, except between fourth and sixth day and eighth and tenth day. On day 10^{th} of storage the mean pH of the product reached 5.82 ± 0.02 from an initial value of 6.89 ± 0.02. Lowering of pH during chilled storage was observed by Borch *et al.* (1996), Rao *et al.* (1999) and Verma and Sahoo (2000) in meat products, smoked chicken sausages and chevon sausages respectively. Lowering of pH of the product during storage may be attributed to the development of lactic acid in the product (Borch *et al.*, 1996).

5.1.1.10 TBARS number

The mean TBARS number recorded in fresh beef frankfurter samples was 1.31 ± 0.04 mg malonaldehyde/kg. The value was higher in comparison with that of fresh buffalo beef sausages, (Krishnan and Sharma, 1993) chicken meat

sausage, (Reddy and Vijayalakshmi, 1998) and chevon sausages (Verma and Sahoo, 2000).

During the storage of beef frankfurter at 4-7°C the value was increased significantly and reached 2.14 ± 0.11 mg malonaldehyde/kg on tenth day of storage. Similar increase in TBARS number in chicken frankfurters, chevon sausage and Bologna sausage was recorded by Baker *et al.* (1972), Verma and Sahoo (2000) and Gines *et al.* (2003), respectively.

In food products hydroperoxides are formed due to auto-oxidation of unsaturated fatty acids. These products get decomposed into aldehydres, ketones, alcohols and lactones. Such products can impart off favors and rancid odor to the product. Analysis of many of these products has been used to measure the rate of lipid oxidation. The 2-TBA test measures the concentration of thiobarbituric acid reacting substances, mainly malonaldehyde, which can be correlated with development of rancidity.

5.1.1.11 Organoleptic Evaluation

Fresh and chilled samples up to second day of storage did not show any difference in color and odor scores. Statistical analysis revealed that the color and odor scores increased significantly during storage. On tenth day of storage the samples had slight discoloration and slight off odor. Development of surface sliminess started on fourth day of storage and cent per cent samples had surface slime by tenth day. Findings of the study indicate that the product had a shelf-life less than four days, which may be attributed to the lack of uniform exposure to smoke on all surfaces of sausage links.

The results of the present study differed from that of Anand *et al.* (1990) and Krishnan and Sharma (1993). The former researchers opined that raw chicken sausages had a shelf-life of seven days at 5 ± 1°C, mean while the latter workers reported that there was no significant change in color, odor and surface appearance in buffalo beef sausages up to seven days. Early onset of spoilage

could be attributed to the poor microbial quality of the product and improper smoking.

5.1.2 Frozen Storage

5.1.2.1 Total Viable Count

On the day of production the mean total viable count of sausage samples was 5.86 ± 0.15 $\log_{10}$ cfu/g. During frozen storage, there was no significant difference in mean total viable count when compared with that of fresh samples. Even then the count in frozen samples was 2 log higher than that reported by Krishnan and Sharma (1993) in buffalo beef sausage and Reddy and Vijayalakshmi (1998) in chicken meat sausage.

5.1.2.2 Psychrotrophic count

In case of fresh samples, the mean count was 5.62 ± 0.02 $\log_{10}$ cfu/g. Analysis revealed a significant ($P<0.05$) reduction in mean psychrotrophic count throughout the period of frozen storage when compared with that of the fresh samples. On 90^{th} day of storage the count was about 1 log lower than that of fresh sample. On perusal of literature it was found that studies on the product being tested were very few, so the results were compared with that of studies on other meat products. Bhoyar *et al.* (1998) had also reported slight lowering of the count in chicken steaks during frozen storage.

5.1.2.3 Relationship between Total Viable Count and Psychrotrophic Count of Frozen Beef Frankfurter

Total viable count and psychrotrophic counts were correlated with a high level of significance ($P\leq0.01$) on 30^{th} and 90^{th} day of storage. Highly significant relationship indicates that as total viable count increases psychrotrophic count also increases. From this observation it may be inferred that microflora of frozen meat products is dominated by psychrotrophic bacteria.

5.1.2.4 Isolation and Identification of Bacteria

Aeromonas

Aeromonas was isolated from 11.67 per cent of frozen beef frankfurter samples tested. The rate of isolation was much lower than that observed by Khalafalla and Sherif (1993) who had reported presence of this organism in cent per cent samples of frozen British beef sausage.

Mainly three species were isolated *A. hydrophila*, *A. sobria* and *A. caviae*. *A. hydrophila* was isolated from 2.78 per cent of samples tested. These isolates were non- hemolytic and non-hemagglutinating. This finding differed from that of Okrend *et al.* (1987) who reported that 93 per cent of *A. hydrophila* isolates from meat products were hemolytic.

A. sobria was isolated from 4.16 per cent of the frozen beef frankfurter samples. Among these isolates, 33.33 per cent were hemotytic and 66.67 per cent were haemagglutinating, whereas Okrend *et al.* (1987) had reported that 69 per cent of *A. sobria* isolates from meat products were hemolytic.

A. caviae was isolated from 2.78 per cent of frozen beef frankfurter samples. Fifty per cent isolates showed hemolytic and an equal per cent showed hemagglutinating activity. The proportion of hemolytic isolates was higher than the reports of Okrend *et al.* (1987).

Hemolytic activity is correlated with cytotoxic effects of the organism (Yadav and Kumar, 2003). The hemagglutinating property indicates the enterotoxigenic effect of the isolates (Handfield *et al.*, 1996).

Escherichia coli

E. coli was not present in any of the frozen samples of beef frankfurter. This finding can be explained by observations of Barnes (1976) who reported that *E. coli* could not multiply at storage temperatures below 5°C. Meanwhile

Khalafalla and Sherif (1993) had reported the isolation of this organism from frozen British beef sausages.

Salmonellae

None of the frozen samples revealed the presence of this organism, mean while the organism was detected in frozen economy or catering sausages by Mattick *et al.* (2002).

Pseudomonas aeruginosa

The organism was isolated from 4.16 per cent samples of frozen beef frankfurter. Presence of *Pseudomonas* in cent per cent samples of frozen beef sausage has been reported by Khalafalla and Sherif (1993).

Lactobacilli

Lacotbacilli were not detected from frozen beef frankfurter samples. This could be attributed to the lethal effect of freezing on lactobacilli (Hall *et al.*, 2001).

5.1.2.5 pH

pH of fresh samples (6.89 ± 0.02) was significantly higher than that of frozen samples. During frozen storage, there was no significant difference in pH of most of the frozen samples tested. Krishnan and Sharma (1993) and Reddy and Vijayalkshmi (1998) also reported that the difference in pH during frozen storage of sausages was negligible.

5.1.2.6 TBARS Number

A significant reduction in mean TBARS number was noticed for the frozen samples when compared to the fresh sample. Fresh samples had the highest value; 1.31 ± 0.04 mg malonaldehyde/kg. After 90 days of frozen storage the value was lowered to 1.04 ± 0.02 mg malonaldehyde/kg. Lowering of value

could be attributed to the formation of carbonyl addition products, which is associated with an apparent loss in malonaldehyde (Chang *et al*., 1961). Similar findings were reported by Krishnan and Sharma (1993) and Reddy and Vijayalakshmi (1998).

5.1.2.7 Organoleptic Evaluation

When compared with fresh samples there was no difference in color and odor scores of frozen samples. Surface slime formation was not observed in any of the samples. Krishnan and Sharma (1993) had also reported that there was no significant difference in color and odor scores of frozen buffalo beef sausages after eight weeks, whereas quality deterioration of frozen chicken meat sausages after 45 days had been recorded by Reddy and Vijayalakshmi (1998). So the study revealed that beef frankfurter can be stored at –20°C, for 90 days without spoilage.

5.2 CHICKEN PEPPERONI

5.2.1 Fresh and Chilled Storage

5.2.1.1 Total Viable Count

Fresh chicken pepperoni samples had a mean total viable count of 6.36 ± 0.13 $\log_{10}$ cfu/g. The count was almost similar to that observed in pepperoni samples collected from commercial sources (Palumbo *et al.*, 1976) and in Greek Salami by Samelis *et al.* (1998). However, the count of the sample in the present study was 2.8 log greater than that observed in moist cooked chicken sausage samples (Anand *et al.* 1990).

Only 33.33 per cent of the samples of the study met the microbial guideline prescribed for sausage at the point of sale by public health laboratory services (Gillbert *et al*., 2000), which indicate that the count shall be within 10^4 to $<10^5$ cfu/g. The high total viable count of the samples might be due to poor microbial quality of raw materials, insufficient time- temperature maintenance

during the processing and lack of hygiene during the various stages of production, processing and distribution.

In the present study after 10 days of chilled storage, the count increased to 7.17 ± 0.01 $\log_{10}$ cfu/g. On eighth day of storage the count in 83.33 per cent of the samples was at 10^7 cfu/g level. This finding are in accordance with that of Anand *et al.* (1990), who reported a total viable count of 7.08 log/g in chicken sausages stored for eight days at 5±1°C.

5.2.1.2 Coliform Count

All fresh chicken pepperoni samples were found free from coliforms. The findings of the study were in accordance with that reported by Anand *et al.* (1990). However, the organism was detected in chilled stored samples and the mean count reached up to the level of 3.84 ± 0.05 $\log_{10}$ cfu/g on day 10 of storage. On second and fourth day, the count was at 10^3 cfu/g level in 8.33 and 33.33 per cent of samples respectively. On sixth, eighth and tenth day, 16.67, 8.33 and 25 per cent of samples respectively had the count at 10^4 cfu/g level. Increase in the level of coliform count was also reported by Anand *et al.* (1990) in chicken sausages.

Coliforms have been reported to grow at temperature as low as –2°C and as high as 50°C (Jay, 1996). The product under study is a semi-cooked sausage. Multiplication of the organism in chilled product suggests that, partially injured coliforms might have adapted to the chilling temperature. Detection of these organisms in ready-to-cook chicken pepperoni reflects the unhygienic practices followed during its preparation. Improper handling and storage can allow the level to increase.

5.2.1.3 Escherichia Coli Count

E. coli were not detected in fresh and chilled samples of chicken pepperoni. Monge *et al.* (2000) also had reported absence of *E. coli* in cent per

cent of fresh sausage samples analysed. However, organism was detected in fresh pepperoni (Palumbo *et al.*, 1976) and in pork sausage (Sharma *et al.*, 1994).

Escherichia coli can remain viable for weeks to months in acidic foods like sausages at refrigeration temperature and improper cooking of such products can cause food-borne diseases in the consumers.

5.2.1.4 Faecal Streptococcal Count

Fresh chicken pepperoni samples had a mean faecal streptococcal count of 2.35 ± 0.18 $\log_{10}$ cfu/g. The findings of the study corroborates with the observations of Anand *et al.* (1990). During chilled storage, the count increased significantly up to sixth day. The mean count on tenth day of storage was 4.88 ± 0.03 $\log_{10}$ cfu/g. The count was 1.89 log higher than that of moist cooked chicken sausages on tenth day of storage (Anand *et al.*, 1990).

Presence of faecal streptococci in large numbers indicated direct or indirect faecal contamination. The organism at high numbers initially is responsible for rapid lowering of the pH of the product, discoloration and reflects poor curing.

5.2.1.5 Psychrotrophic Count

Initial psychrotrophic count was 5.66 ± 0.03 $\log_{10}$ cfu/g. In fresh chicken breakfast sausage the initial count was 1 log lower than the above result (Lee *et al.*, 1997). The count in fresh samples was about 3 log higher than that given by Anand *et al.* (1990) in case of raw chicken sausage.

During chilled storage, there was significant increase in the counts. It was in agreement with findings by Lee *et al.* (1997) who had reported an increase in psychrotrophic count during refrigerated storage of chicken breakfast sausage. This observation may be further confirmed by the ICMSF statement that, the refrigerated storage of foods permit the psychrotrophic population to reach millions per gram within few days, resulting in objectionable changes in odor,

taste and texture. Psychrotrophic bacteria constitute the most common cause of refrigerated food spoilage and many of these organisms indicate unsanitary conditions during manufacture and handling of meat products. Some of these organisms are pathogenic to man, producing necrotic and ulcerative lesions in the alimentary tract (Khalafalla and Sherif, 1993).

5.2.1.6 Yeast and Mold Count

Fresh sausage samples had a count of 3.66 ± 0.08 $\log_{10}$ cfu/g. The count obtained in the study was almost similar to that cited by Palumbo *et al.* (1976). But the count was 2 log higher than that reported by Anand *et al.* (1990) in case of chicken sausages.

In the present study from sixth day onwards all the samples had the count at 10^4 cfu/g level. On tenth day, the count increased significantly and reached 4.42 ± 0.05 $\log_{10}$ cfu/g. Gradual increase in count during chilled storage was also recorded by Anand *et al.* (1990). This count was 2 log higher than that reported by Anand *et al.* (1990).

Yeast and molds are important opportunistic spoilage organisms of meat products, but only cause concern when conditions are such that bacterial competition is reduced. In meat products, drying, salting or freezing reduces the water activity, which will suppress Gram negative spoilage bacteria and in turn will favour multiplication of yeast and mold. Yeast spoilage causes both off odor and slime formation on sausage surface. Many of the food yeasts are psychrotrophic and are therefore potential spoilage organisms of meat under chilled storage. Many of the food yeasts can survive temperature as low as –5°C (Dillon, 1998). High initial yeast and mold count and subsequent proliferation during chilled storage contribute to the development of spoilage changes in the product under study.

5.2.1.7 Relationship between Various Microbial Counts of Fresh and Chilled Chicken Pepperoni

Total viable count, coliform count and faecal streptococcal count were associated positively and significantly each other. Coliform count was associated positively with psychrotrophic count and yeast and mold count. Faecal streptococcal count showed significant relationship with psychrotrophic count and yeast and mold count.

5.2.1.8 Isolation and Identification of Bacteria

Aeromonas

Aeromonas could be isolated from 18.05 per cent samples of fresh and chilled chicken pepperoni. Gobat and Jemmi (1993) had reported isolation of this organism from 15.6 per cent of smoked cooked sausage samples.

Isolates of *Aeromonas* obtained belonged to three species viz., *A. hydrophila, A. caviae* and *A. sobria.* Isolation of there three species of *Aeromonas* from meat products had been reported by a number of workers. (Okrend *et al.*, 1987; Fricker and Tompsett, 1989; Gobat and Jemmi, 1993 and Singh, 1997).

A. hydrophila was isolated from 9.72 per cent of fresh and chilled chicken pepperoni samples, which is much lower than the rate of isolation (61.2 per cent) from smoked, cooked sausages (Gobat and Jemmi, 1993). Among these isolates 57.14 per cent of isolates were hemolytic and an equal per cent were hemagglutinating, whereas, according to Okrend *et al.* (1987) cent per cent of isolates of *A. hydrophila* from meat products were hemolytic.

A. sobria was isolated from 2.78 per cent of the samples. However Gobat and Jemmi (1993) had reported isolation of this organism from 22.5 per cent of smoked, cooked sausage samples. Cent per cent isolates obtained in the present study were hemolytic and 50 per cent isolates showed hemagglutinating ability.

Hemolytic activity in 69 per cent of *A. sobria* isolates from meat products had been reported by Okrend *et al.* (1987).

Among the 72 samples tested *A. caviae* was isolated from 5.56 per cent of samples. This finding differed from that of Gobat and Jemmi, (1993) who had isolated this organism from 16.3 per cent of the smoked, cooked sausage samples. In the present study 50 per cent of the *A. sobria* isolates from chilled chicken pepperoni had hemolytic ability whereas all of them were non-hemagglutinating. The proportion of isolates with hemolytic activity was much higher than that reported by Okrend *et al.* (1987).

Devlieghere *et al.* (2000) reported a high incidence of *Aeromonas* in foods. The presence of this organism in fresh and chilled samples of chicken pepperoni could be attributed to the contamination of product from contaminated water and work surfaces (Beuchat, 1991). The hemolytic activity of the isolates is associated with ability to produce cytotoxic effects (Yadav and Kumar, 2003) and the hemogglutinating property is indicative of the enterotoxigenic effect (Handfield *et al.,* 1996). Detection of this organism from ready to cook meat products is of great public health significance.

Escherichia coli

Escherichia coli were not detected from any of the fresh and chilled samples of chicken pepperoni tested. This finding is in agreement with that of Monge *et al.* (2000) who reported that *E. coli* was absent in 80 soyabean sausage samples tested. However, presence of this organism in sausages had been reported by Oyekole and Hassan (1984) and Timm *et al.* (1999).

Lactobacilli

Lactobacilli could not be detected in fresh samples and samples chilled for two and four days. Lactobacilli were detected from 11.66 per cent of chilled samples tested. Presence of Lactobacilli was reported by Korkeala and Lindroth

(1987), Korkeala and Bjorkroth (1997) and Samelis *et al.* (1998), in case of chilled ring sausages, cooked sausage and Salami sausage respectively.

Different species of Lactobacilli identified were *L. brevis, L. fermentum, L. curvatus* and *L. sake.* Borch *et al.* (1996), Korkeala and Bjorkroth (1997) and Hugas *et al.* (1993) also recorded isolation of these species of lactobacilli in sausage samples.

Lactobacilli are regarded as predominant bacteria associated with spoilage of refrigerated meat and meat products. Main defects developed by this species include off odors due to acetoin, acetic acid and hydrogen sulphide production, discoloration due to production of hydrogen sulphide or hydrogen peroxide and slime formation. The sausage surfaces become contaminated with lactic acid bacteria during handling after preparation of the product. These organisms are found in the air and on the working surfaces in the chilling and packing rooms of meat processing plants, suggesting that these can act as source of contamination.

Pseudomonas aeruginosa

In the study five isolates of *Pseudomonas aeruginosa* were obtained from the 72 samples analysed (6.94 per cent). Rate of isolation was much higher than that (1.2 per cent) reported by Sherikar *et al.* (1979) in case of pork products. Simard *et al.* (1983) and Samelis *et al.* (1998) had reported presence of *Pseudomonas* in sausages. These psychrotrophic organisms are spoilage agents as well as pathogens producing necrotic and ulcerative lesions in the human alimentary tract (Khalafalla and Sherif, 1993).

Salmonellae

None of the fresh and chilled samples yielded salmonellae. Weissman and Carpenter (1969) reported similar observation in case of smoked link sausage and frankfurters and Samelis *et al.* (1998) in case of Greek Salami sausage.

Meanwhile Banks and Board (1983), Herida *et al.* (2001) and Mattick *et al.* (2002) had isolated the organism from sausage samples.

5.2.1.9 pH

The mean pH of fresh chicken pepperoni samples was 6.28 ± 0.00. Similar values were recorded by Krishnan and Sharma (1993) for buffalo beef sausages, Samelis *et al.* (1998) for Greek Salami and Rao *et al.* (1999) in case of smoked chicken sausages.

During chilled storage, there was significant reduction in pH, reaching 5.41 ± 0.43 on tenth day. Borch *et al.* (1996) and Verma and Sahoo (2000) had also reported similar reduction in pH after chilled storage in case of meat and cured meat products. Findings of this study may be further confirmed by observations of Borch *et al.* (1996) who reported that pH of meat products may decrease from 6.0-6.5 to 5.0-5.3 during storage due to lactic acid production.

5.2.1.10 TBARS Number

The mean TBARS number of fresh sausage was 0.98 ± 0.01 mg malonaldehyde/kg. Slightly lower values were recorded by Krishnan and Sharma (1993), Reddy and Vijayalakshmi (1998) and Verma and Sahoo (2000) in case of buffalo beef sausage, chicken meat sausage and chevon sausage respectively.

During chilled storage TBARS number increased gradually and rose to 1.93 ± 0.05 mg malonaldehyde/kg on tenth day. This finding is in agreement with the observations of Baker *et al.* (1972) in chicken frankfurter, Verma and Sahoo (2000) in chevon sausages and Gines *et al.* (2003) in bologna sausage.

Auto-oxidation of specific fatty acids, mainly unsaturated fatty acids, produces short chain hydrocarbons and carbonyl compounds. Hyperoperoxides are the initial products of auto-oxidation, which tend to decompose to form aldehydes, ketones, alcohols and lactones. These products can impart off flavors and off odors to the product. Analysis of many such spoilage indicator

compounds has been used to follow the progress of lipid oxidation. The 2-Thiobarbituric acid test measures the concentration of thiobarbituric acid reacting substances, principle compound being malonaldehyde. Increase in TBARS number is correlated with development of off odor in spoiled rancid product as observed in the present study.

5.2.1.11 Organoleptic Evaluation

All the fresh samples and chilled samples on second day of storage had same color and odor scores. From fourth day onwards the color scores showed a significant increase and reached 4.5 ± 0.11 on tenth day of storage. A slight discoloration and off odor was noticed on 10^{th} day of storage.

Sliminess appeared in chilled samples from sixth day onwards and all samples developed sliminess on surface on tenth day. The observations indicate that the product had a shelf-life of less than six days.

The observations of the present investigation do not conform the findings of Adams *et al.* (1987) and Krishnan and Sharma (1993). The former worker reported the development of grey surface discoloration, stale yeasty odor and visible slime formation after nine days of chilled storage of British fresh sausages, whereas the latter researchers opined that there was no significant change in color, odor and surface appearance in sausage during chilled storage for seven days. Early development of spoilage changes could be due to high initial microbial load.

5.2.2 Frozen Storage

5.2.2.1 Total Viable Count

Fresh chicken pepperoni samples had a mean total viable count of 6.36 ± 0.13 $\log_{10}$ cfu/g. On 15^{th} day of frozen storage, count was reduced by 0.8 log. Krishnan and Sharma (1993) recorded a similar lowering of total viable count of buffalo beef sausage after one week of frozen storage.

All the frozen samples tested had count significantly lower than that of fresh samples. The reduction in the total viable count might be attributed to the fact that a proportion of the microbial population is killed or sub lethally injured during the freezing process (Cox *et al*.1998), and also freezing causes an intra and extra cellular osmotic gradient which can result in cellular disruption (Mazur, 1984).

5.2.2.2 Psychrotrophic Count

The mean psychrotrophic count of the fresh samples was 5.86 ± 0.15 $\log_{10}$ cfu/g. The count was almost similar to that reported by Khalafalla and Sherif (1993). During storage at –20°C there was significant lowering in count when compared with fresh samples. The count in samples on 90th day was about 1.4 log lower than that of fresh samples. This observation corroborates with that of Bhoyar *et al.* (1998).

5.2.2.3 Relationship between Total Viable Count and Psychrotrophic Count of Frozen Chicken Pepperoni

Total viable count and psychrotrophic counts were related with a high significance positively on 15th day of storage. During frozen storage on all other days the relationship was positive but nonsignificant. Highly significant association revealed that as total count increases the psychrotrophic count also increases. This indicates that psychrotrophic bacteria form the major part of microflora during the freezer storage of sausages.

5.2.2.4 Isolation and Identification of Bacteria

Aeromonas

Aeromonas was isolated from 15.27 per cent of the frozen chicken pepperoni. The rate of isolation of the organism from samples in the present study was much lower than that reported by Khalaffalla and Sherif (1993) who

reported the presence of the organism in cent per cent of the frozen beef sausage samples.

A. hydrophila was isolated from 5.56 per cent of the samples tested. The isolation of this organism from frozen product might be attributed to the fact that the organism can survive at –72°C for 18 months (Abeyta *et al.*, 1986). *A. sobria* was isolated from 2.78 per cent and *A. caviae* was isolated form 6.94 per cent of samples.

Cent per cent of *A. hydrophila* isolates were hemolytic and 50 per cent had hemagglutinating ability.. The former property of the organism is associated with the ability to cause cytotoxic effects (Yadav and Kumar, 2003) and the latter property indicates the ability to produce enterotoxigenic effect in the consumers (Handfield *et al.*, 1996).

Cent per cent of *A. sobria* isolates showed hemolytic activity and 50 per cent of isolates were hemagglutinating. The proportion of isolates with hemolytic activity was much higher than that recorded by Okrend *et al.* (1987) who reported that only 69 per cent of the isolates had this characteristic.

Among *A. caviae* isolates 60 per cent were hemolytic and all isolates were hemagglutinating. However, the former characteristic was shown by only 18 per cent of organism isolated by Okrend *et al.* (1987).

Escherichia coli

E. coli was not detected from any of the frozen samples of chicken pepperoni. This might be attributed to the fact that the organisms do not multiply at a temperature below 5°C (Barnes, 1976). However, presence of this organism in frozen beef sausages had been reported by Khalafalla and Sherif (1993).

Salmonellae

None of the frozen samples revealed the presence of salmonellae. However presence of this organism in frozen sausages had been reported by Mattick *et al.* (2002).

Pseudomonas aeruginosa

Pseudomonas aeruginosa was isolated from 9.72 per cent of frozen chicken pepperoni samples. Khalafalla and Sherif (1993) reported presence of *Pseudomonas* in cent per cent samples of frozen beef sausage. The organism is associated with spoilage and capable of producing necrotic and ulcerative lesions in human alimentary tract. Thus, the presence of the organism in ready-to-cook food is of great public health significance.

5.2.2.5 pH

The mean pH of fresh samples was significantly higher than that of frozen samples. In frozen samples the pH was in the range of 6.14 – 6.22 during 90 days of storage. This finding corroborates with that of Krishnan and Sharma (1993) and Reddy and Vijayalakshmi (1995).

5.2.2.6 TBARS Number

When compared with fresh samples a significant reduction in TBARS number was noticed in frozen samples. Fresh samples had the highest value (0.98 mg malonaldehyde/kg) and frozen samples on 75^{th} day had the lowest value (0.84 mg malonaldehyde/kg). This lowering of value might be attributed to the formation of carbonyl addition products that would account for the apparent loss in malonaldehyde during freezing (Chang *et al.* 1961). Loss of malonaldehyde can also occur through its reaction with guanidine to form 2-aminopyrimidine (Brown, 1962) or with urea to form 2-hydroxy pyrimidine. Similar observations

were reported by Krishnan and Sharma (1993) and Reddy and Vijayalakshmi (1998).

5.2.2.7 Organoleptic evaluation

Fresh samples and frozen samples had similar mean color and odor scores. This is in accordance with findings of Krishnan and Sharma (1993), who reported that there was no significant difference in color and odor scores of frozen buffalo beef sausage up to eight weeks. However results of the present study differed from that in case of frozen chicken meat sausages by Reddy and Vijayalakshmi (1998). The study revealed that chicken pepperoni could be stored frozen for 90 days without any change in color and odor and slime formation.

5.3 ASSESSMENT OF CRITICAL CONTROL POINTS

In order to identify various critical points of microbial contamination during preparation of beef frankfurter and chicken pepperoni, samples of air, water, equipment and raw ingredients were analysed for microbial quality.

5.3.1 Air

The mean total viable count of air samples in the processing room was 1.20 ± 0.68 cfu/ft/min before processing and after processing the count was 1.63 ± 0.78 cfu/ft/min. Eisel *et al.* (1997) had reported a total aerobic count of 0.6 cfu/m^3 in the red meat processing plant. The study revealed that microflora of air may contribute to the contamination of product in the processing room. Kaloianov *et al.* (1987) also opined that there was considerable increase in the number of indicative organisms during work in meat dressing plants. The mean yeast and mold count of the processing room before processing was 1.62 ± 0.56 cfu/ft/min and after processing, it was increased to 1.81 ± 0.58 cfu/ft/min. Yeasts and molds are ubiquitous in the environment and many are psychrotrophic in nature and are therefore potential spoilage organism of refrigerated meat and

meat products (Dillon, 1998). Contamination of processed meat products with these organisms may result in early spoilage.

5.3.2 Water

Samples of chilling water, water used for washing casings, hand washings and ice used in preparation of sausage batter were tested for bacterial quality. Study revealed that hand washing samples had the highest total viable count (4.61 ± 0.07 $\log_{10}$ cfu/ml). Unhygienic handling during packaging may cause contamination of the semi-cooked sausages. Thus, it can be assumed that personnel working in the plant may contribute to the total bacterial load of the final product. This is in agreement with findings of Korkeala and Bjorkroth (1997). Chilling water, water used for washing, casings and ice can contribute to the bacterial load of the final product. Faecal streptococci and *E. coli* were not present in any of the water samples analyzed.

Highest coliform count was also seen in hand washings (1.54 ± 0.06 $\log_{10}$ cfu/ml). The contamination of hands could be attributed to poor working practices (Tebutt, 1986). Sausages get contaminated with coliforms during post processing handling.

5.3.3 Equipment

The bacterial counts on linking table surface, meat mincer, mixer, sausage filler and packaging material were estimated to assess their extent of involvement in contamination of the chicken pepperoni and beef frankfurter. Highest mean total viable count was obtained for sausage filler and lowest was reported for the packaging materials.

Among the equipments involved in production of sausages, the sausage filler had a significant role in contributing to the total viable count of the final product. The count obtained in the present study was higher than that reported by

Eisel *et al.* (1997) who showed that processing equipment had a total viable count of only 1 cfu/cm^2.

E. coli and faecal streptocci were not detected in samples collected from equipment in the processing line of chicken pepperoni, whereas, faecal streptococci at the level of one log_{10} cfu/cm^2 was observed in samples collected from linking table surface and mixer in the processing line of the frankfurter. Presence of faecal streptococci on the working surfaces indicates the necessity of proper cleaning, washing and disinfection practices in the sausage processing plant (Kaloianov *et al.*, 1987).

5.3.4 Raw Ingredients

Samples of raw ingredients used in preparation of beef frankfurter and chicken pepperoni were subjected to bacteriological analysis.

Samples of beef, chicken, pork and minced meat showed high total viable count. Highest mean count (5.66 ± 0.18 log_{10} cfu/g) was recorded for beef samples. Casings were also found to contribute to the total microbial load of the sausages prepared. Total viable count of minced meat samples and casings reported in the present study was 1 log higher than that reported by Anand *et al.* (1990).

Coliforms were present at 2 log cfu/g level in chicken meat samples and samples of spices and casings. Anand *et al.* (1990) had also reported presence of coliforms in raw ingredients used in preparation of chicken sausages. Among the ingredients, chicken meat showed highest coliform count (2.73 ± 0.04 log_{10} cfu/g), followed by casings (2.40 ± 0.06 log_{10} cfu/g). *E. coli* was noticed only in the spices samples.

All the raw ingredients used in the preparation of chicken pepperoni had faecal streptococcal count at $10^1 – 10^2$ cfu/g level. Samples of spices and casings used in preparation of beef frankfurter also were found to contain faecal

streptococci. Therefore, the study revealed that all the raw ingredients played an important role in the contamination of the product with faecal streptococci. A mean faecal streptococcal count of 2.62 log/g in minced meat had been reported by Anand *et al.* (1990).

The present study highlights the effect of refrigeration on microbial, physico-chemical and sensory qualities of beef frankfurter and chicken pepperoni. Shelf-life under refrigerated storage was less than four and six days respectively, for beef frankfurter and chicken pepperoni. Frozen samples developed no change in organoleptic qualities up to 90 days. Early spoilage under chilled storage may be attributed to the high initial microbial load and the contamination from the processing environment. Unhygienic handling of the product can also cause contamination of the final product. The study reflects the importance of implementing principles of Hazard Analysis Critical Control Programme, which, helps to prioritize process steps focusing on hazards that can be reduced or eliminated.

Summary

6. SUMMARY

Sausages are comminuted meat products, which are modified by various processing methods to yield desirable organoleptic and keeping qualities. The quality assurance of these products is important as the survival of microorganisms can render these foods hazardous and can reduce shelf life.

The present investigation was aimed at assessing the microbial, physico chemical and organoleptic quality changes in beef frankfurter and chicken pepperoni sausage during chilled and frozen storage. All the samples were tested for the isolation of certain pathogenic and spoilage organisms. Environmental samples, hand washing of personnel engaged in processing line, equipment and packaging materials were also studied to assess their role in contamination of the final product.

Sausage samples were collected from six batches. From each batch 24 samples, each consisting of 250 g, were collected on the day of production. Samples were randomly divided into two groups, one consisted of 12 samples, and the other consisted of 10 samples. The Former group was stored at –20 ºC for 90 days and latter at 4-7 º C for 10 days. Two samples were tested on the day of production. Chilled samples were analysed at two days interval and frozen samples at 15 days interval.

On the day of preparation the mean total viable count of beef frankfurter was 5.86± 0.15 $\log_{10}$ cfu/g, and that of chicken pepperoni was 6.36 ± 0.13 $\log_{10}$ cfu/g. Significant increase in the total viable count was noticed throughout the period of chilled storage of the products.

Coliforms were not present in fresh samples of both the products. From second day onwards the count increased significantly and reached 2.82 ± 0.24

$\log_{10}$ cfu/g and 3.84 ± 0.05 $\log_{10}$ cfu/g on tenth day in beef frankfurter and chicken pepperoni, respectively.

E. coli were detected from fourth day onwards in chilled beef frankfurter and the count remained at 10^1 cfu/g level throughout the chilled storage. The organism could not be detected from samples of chicken pepperoni.

The mean faecal streptococcal count of fresh beef frankfurter samples was 2.02 ± 0.05, which increased to 3.30 ± 0.15 $\log_{10}$ cfu/g after 10 days of storage. In chicken pepperoni initial count was 2.35 ± 0.18 $\log_{10}$ cfu/g and there was about 2.5 log increase in mean count by 10^{th} day of chilled storage.

Mean psychrotrophic counts of fresh sausage samples were 5.62 ± 0.02 and 5.66 ± 0.03 $\log_{10}$ cfu/g, respectively, for beef frankfurter and chicken pepperoni. A significant increase in count was noticed during the chilled storage.

Yeast and mold count on the day of preparation was 2.64 ± 0.06 and 3.66 ± 0.08 $\log_{10}$ cfu/g in beef frankfurter and chicken pepperoni, respectively. After 10 days of chilled storage there was more than one log increase in counts.

Aeromonas hydrophila was detected from 1.38 per cent samples of fresh and chilled beef frankfurter and 9.72 per cent samples of fresh and chilled chicken pepperoni. Cent per cent of the isolates obtained from beef frankfurter and 57.14 per cent isolates obtained from chicken pepperoni were hemolytic and cent per cent and 57.14 per cent of the isolates, respectively, were hemagglutinating.

Aeromonas sobria was isolated from 11.11 per cent samples of fresh, chilled beef frankfurter, and 2.78 per cent samples of fresh and chilled chicken pepperoni. Among these, 75 per cent of the isolates from beef frankfurter and cent per cent isolates from chicken pepperoni had hemolytic activity and 25 per cent and 50 per cent of the isolates respectively were hemagglutinating.

Among 72 fresh and chilled samples tested, 11.11 per cent samples of beef frankfurter and 5.56 per cent samples of chicken pepperoni revealed the presence of *Aeromonas caviae.* Seventy five per cent of the isolates obtained from beef frankfurter and 50 per cent isolates obtained from chicken pepperoni showed hemolytic property and 25 per cent isolates from beef frankfurter were hemagglutinating. None of the isolates from chicken pepperoni were hemagglutinating. Hemolytic and hemagglutinating abilities are associated with enteropathogenic effects of these isolates.

Escherichia coli could be isolated from 18.05 per cent samples of chilled beef frankfurter. Isolates were serotyped at National *salmonella* and *Escherichia* center, Central Research Institute, Kasauli. It was found that 84.62 percent of these isolates belonged to serotype O2 (Enterohaemorrhagic E. *coli*.) and 15.38 percent were rough and untypable. All the isolates were tested for congo red binding ability in a medium containing bile salt and 61.53 per cent were positive and produced brick red colonies. This ability indicates the invasive nature of *E. coli* isolates. None of the chicken pepperoni samples revealed the presence of *E. coli.*

None of the samples revealed the presence of Salmonellae. *Pseudomonas aeruginosa* was isolated from 5.56 percent samples of fresh and chilled beef frankfurter and 6.9 per cent samples of fresh and chilled chicken pepperoni.

Lactobacillus brevis was detected from 2.78 per cent samples of fresh and chilled beef frankfurter and 4.16 per cent samples of fresh and chilled chicken pepperoni. *Lactobacillus curvatus* was isolated from 1.38 per cent samples each of fresh and chilled beef frankfurter and chicken pepperoni. *Lactobacillus fermentum* was obtained from 2.78 per cent samples of fresh and chilled chicken pepperoni. *Lactobacillus sake* was detected from 1.38 per cent samples of both the sausages.

The mean pH of fresh beef frankfurter samples was 6.89 ± 0.02 and that of fresh chicken pepperoni samples was 6.28 ± 0.00. Statistical analysis revealed that during chilled storage there was gradual lowering of pH. In beef frankfurter pH was reduced by 1.07 units and in chicken pepperoni it was reduced by 0.87 units during 10 days of storage.

The TBARS number is a measure of the rancidity in the product. It was observed that chilled storage significantly increased the value. The TBARS number of fresh beef frankfurter samples was 1.31 ± 0.04 mg malonaldehyde/kg and that of fresh chicken pepperoni samples was 0.98 ± 0.01 mg malonaldehyde/kg. After 10 days the mean values rose to 2.14 ± 0.11 and 1.93 ± 0.05 mg malonaldehyde/kg respectively.

Sausage samples were evaluated for organoleptic qualities to monitor the development of spoilage changes. Slight off odor and slight discoloration was developed in cent per cent samples of both products by 10^{th} day of chilled storage. In case of beef frankfurter samples a slight sliminess was noticed on the less smoked inner surface of the sausage links on fourth day. Surface sliminess was observed from sixth day onwards in chilled chicken pepperoni. So the study revealed that the shelf-life of beef frankfurter at chilled storage was four days and that of chicken pepperoni was less than six days.

Frozen beef frankfurter and chicken pepperoni samples were subjected to estimation of various bacterial counts. Mean total viable counts of fresh and frozen samples of beef frankfurter did not differ significantly ($P \leq 0.05$.). The mean total viable count of fresh chicken pepperoni samples was significantly ($P < 0.05$) higher than the count on all other days during frozen storage.

The analysis revealed a significant ($P \leq 0.05$) reduction in mean psychrotrophic count of both the products throughout the period of frozen storage, when compared to fresh samples.

All the samples were tested to isolate certain pathogenic and spoilage organisms.

Aeromonas hydrophila was detected from 2.78 per cent samples of frozen beef frankfurter and 5.56 per cent samples of frozen chicken pepperoni. *Aeromonas hydrophila* isolates from frozen beef frankfurter were non-hemolytic and non- hemagglutinating. Cent percent isolates obtained from chicken pepperoni were hemolytic and 50 per cent of the isolates were hemagglutinating.

Aeromonas sobria was isolated from 4.16 per cent samples of frozen beef frankfurter and 2.78 per cent samples of frozen chicken pepperoni. Among the isolates 33.33 per cent obtained from beef frankfurter and cent per cent isolates obtained from chicken pepperoni had hemolytic activity and 66.67 per cent and 50 per cent of the isolates respectively were hemagglutinating.

Among frozen samples tested 2.78 per cent samples of beef frankfurter and 6.94 per cent samples of chicken pepperoni revealed the presence of *Aeromnas caviae*. Hemolytic activity was shown by 33.33 per cent of the isolates from beef frankfurter and 60 per cent isolates from chicken pepperoni and 50 per cent and cent per cent of the isolates respectively were hemagglutinating.

Salmonellae, *Escherichia coli* and lactobacilli were not detected from frozen sausage samples.

Pseudomonas aeruginosa was isolated from 4.16 per cent samples of frozen beef frankfurter and 9.72 per cent samples of frozen chicken pepperoni.

Student's 't' test revealed that pH of fresh samples was significantly ($P<0.05$) higher than that of the frozen samples of both the products.

A significant ($P\leq0.05$) reduction in mean TBARS number was noticed for the frozen samples, when compared to the fresh samples. The mean TBARS number was reduced by 0.27 units after 90 days of frozen storage of beef

frankfurter. In case of frozen chicken pepperoni the lowest value recorded was 0.84±0.01 mg malonaldehyde/ kg on 75^{th} day.

Fresh and frozen samples of both the products were having similar color and odor scores throughout the frozen storage. Surface slime formation was not observed in frozen samples. Study revealed that both products could be stored frozen up to 90 days without any change in color odor and without slime formation.

Air samples collected from the processing room had higher mean total viable count after processing than that before processing of both the products. (1.20 ± 0.68 and 1.62 ± 0.56 $\log_{10}$ cfu/min respectively). Mean yeast and mold count also was found to be considerably increased from 1.63 ± 0.78 before processing to 1.81 ± 0.58 $\log_{10}$ cfu/min after processing.

The mean total viable count, coliform count, *Escherichia coli* count, and faecal streptococcal count of water samples collected from the chilling tank, water used for washing the casings, hand washing samples of personnel engaged in processing line and samples of ice used in mixing were also estimated. Among the samples, hand washings had the highest mean total viable count (4.61 ± 0.07 $\log_{10}$ cfu/ml) and coliform count (1.54 ± 0.06 $\log_{10}$ cfu/ml). *Escherichia coli* and faecal streptococci were not detected in any of the water samples and samples of ice.

The hygienic status of the linking table surface, meat mincer, mixer, sausage filler and packaging material were also assessed. Among the equipment, sausage filler had the highest total viable count; 4.93 ± 0.00 $\log_{10}$ cfu/cm^2 and the count was lowest in case of packaging materials. Coliforms and *E. coli* were not detected in any of the samples. Faecal streptococcal count at the level of 1 $\log_{10}$ cfu/g was observed in samples collected from linking table surface and mixer in the processing line of beef frankfurter.

Among the ingredients used in preparation of beef frankfurter, samples of beef had (5.66 ± 0.18 log_{10} cfu/g) the highest total viable count. Pork samples used in the preparation of chicken pepperoni revealed the highest mean total viable count (5.38±0.15 log_{10} cfu/g) when compared to other ingredients. Samples of spices had the lowest count. Coliforms were present at 2 log cfu/g level in samples of chicken, spices and casings. *E. coli* was detected only in samples of spices. Faecal streptococcal count was highest in samples of chicken and casings, whereas the organism could not be detected from beef samples.

From the above study, it was observed that about 50 per cent of fresh beef frankfurter samples and 33.33 per cent of fresh chicken pepperoni samples satisfied the microbiological guideline prescribed by public health laboratory services. Shelf life of beef frankfurters was found to be four days and that of chicken pepperoni was less than six days under chilled storage. Frozen storage considerably increased the shelf life. It was revealed that frozen samples had a shelf life more than 90 days. The study gives an insight to the necessity of strict observance of hygienic precautions at each and every step in the preparation of ready-to-cook meat products.

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EFFECT OF REFRIGERATION ON THE QUALITY OF BEEF FRANKFURTER AND CHICKEN PEPPERONI

AMBILI V. S.

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Faculty of Veterinary and Animal Sciences
Kerala Agricultural University, Thrissur

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Department of Veterinary Public Health
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR - 680 651
KERALA, INDIA

ABSTRACT

The present study was conducted to assess the microbial, physico-chemical and organoleptic qualities of beef frankfurter and chicken pepperoni at chilled and frozen storage. Effect of storage on these products was studied by estimating various microbial counts, assessing the presence of certain pathogenic and spoilage bacteria and studying the changes in pH and TBARS number and organoleptic qualities like color, odor and presence of sliminess.

In chilled samples (4-7°C) the mean total viable count, faecal streptococcal count, psychrotrophic count and yeast and mold count were found to increase significantly as storage period progressed. In both the products, coliforms reached a detectable level by second day of chilled storage and thereafter the count increased. *E. coli* were detected only from samples of chilled beef frankfurter from fourth day onwards and the count remained at 10^1 cfu/g level.

Aeromonas hydrophila, A. sobria and *A. caviae* were the three species of *Aeromonas* isolated from the chilled samples of both the products. Hemolytic and hemagglutination assays of these isolates were also carried out which is indicative of enteropathogenic effects.

Escherichia coli were isolated from beef frankfurter samples. Among the isolates 84.62 per cent belonged to the serotype O2 (Enterohaemorrhagic *E. coli*.). Salomonellae could not be detected from any of the samples.

A number of samples revealed the presence of *Pseudomonas aeruginosa*. Important species of lactobacilli isolated were *Lactobacillus brevis, Lactobacillus curvatus, Lactobacillus fermentum* and *Lactobacillus sake.* The mean pH and TBARS values were increased during chilled storage, indicating the progress of development of acidity and rancidity. Color and odor scores gradually increased

from fourth day onwards and slight discoloration was noticed on 10th day in both the products. Surface slime was developed on fourth day in beef frankfurter samples and from sixth day onwards in chicken pepperoni samples. Thus, the shelf life was found to be four days for beef frankfurter and less than six days for chicken pepperoni stored at 4-7° C.

When samples were stored at –20°C, it was observed that mean total viable counts of fresh and frozen beef frankfurter samples did not differ significantly. Frozen samples of chicken pepperoni had the total viable count significantly ($P \leq 0.05$) lower than that of fresh samples.

Aeromonas hydrophila, *A. sobria* and *A. caviae* were isolated from frozen samples of both the products. Many of the isolates were hemolytic and hemagglutinating.

Pseudomonas aeruginosa were also detected. *E. coli* , salmonellae, and lactobacilli were not isolated from any of the frozen samples.

Frozen sausage samples had lower mean pH values when compared to fresh samples and mean TBARS values were found to decrease gradually during the frozen storage.

Color and odor scores remained the same during frozen storage and slime formation was not observed in frozen samples. Study revealed that frozen samples of both products had a shelf-life of 90 days.

In order to identify various critical points of bacterial contamination, samples of air, water, rinse samples from equipment, hand washing of personnel in the processing line and packaging material were examined for their hygienic quality.

The mean total viable count and yeast and mold count of air samples were found to increase after processing. Among the water samples, the high microbial count was recorded for hand washings, reflecting unsanitary working practices.

Among the equipment, sausage filler was found to contribute maximum to the total microbial load of the product.

Among the raw ingredients, samples of beef used for preparation of beef frankfurter and samples of pork used for chicken pepperoni were found to possess high bacterial load. Coliforms were present at 2 log cfu/g level in all the ingredients. Faecal streptococci were detected in all the ingredients except beef. *E. coli* were present only in samples of spices.

Study reflects the importance of quality assurance during every step of preparation of ready-to-cook meat products to avoid the early spoilage and to safe guard consumer health. Presence of pathogenic organisms in these products is of great public health significance as improper cooking can cause outbreaks of food borne diseases.