

**ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES**

**Microbiological Quality and Safety of
Commercially Produced Sausages in
Addis Ababa**

**By
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*A Thesis Presented to the School of Graduate Studies of the Addis Ababa
University in Partial Fulfillment of the Requirements for the Degree of
Master of Science in Biology*

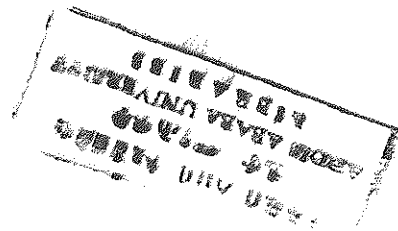
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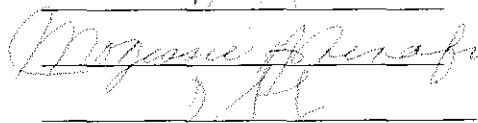
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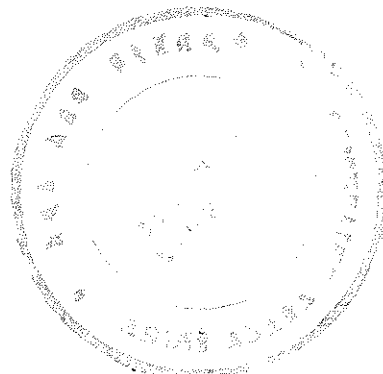
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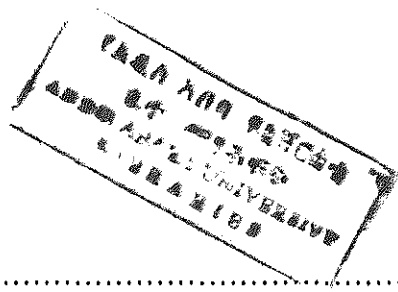
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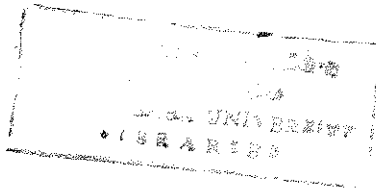


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ABBREVIATIONS AND SYMBOLS

ANOVA-Analysis of Variance

A_w-water activity

BPW-Buffered Peptone Water

CCP- Critical Control Point

CDC-Centers for Disease Control and Prevention

CFU- Colony Forming Unit

CV- Coefficient of Variation

DIM- Deterioration Index Method

DT 104-Definitive Type 104

DT 124- Definitive Type 124

FEHD-Food and Environmental Hygiene Department

FSIA- Food Safety Authority of Ireland

H₂O₂- Hydrogen per oxide

H₂S-Hydrogen Sulphide

HACCP- Hazard Analysis Critical Control Point

KOH- Potassium hydroxide

HUS- Hemolytic Uremic Syndrome

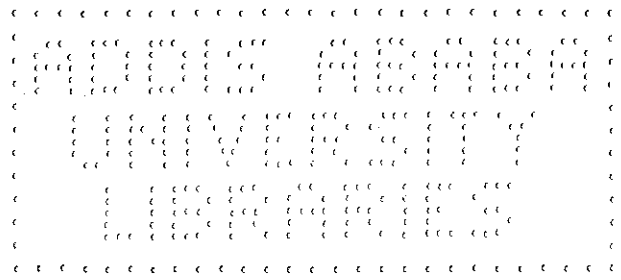
IOM- Institute of Medicine

LAB- Lactic acid bacteria

RTE- Ready to eat

RV broth -Rappaport Vassiliadis broth

SIM medium -Sulphide Indole Motility medium



SLT-Shiga Like Toxin

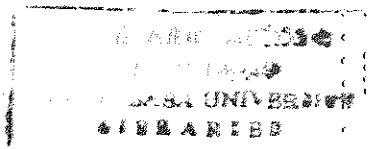
Spp. – Species

SS Agar -Salmonella-Shigella Agar

TSI Agar -Triple Sugar Iron Agar

WHO- World Health Organization

XLD medium -Xylose Lysine Desoxycholate medium



ABSTRACT

The microbiological quality and safety of emulsion type small diameter (Frankfurter) and large diameter (mortadella) sausages purchased from different supermarkets in Addis Ababa, Ethiopia, were investigated between November, 2004 and December, 2005. A total of 210 sausage samples consisting of 120 small diameter (30 each of pork, beef, veal and chicken) and 90 large diameter (30 each of pork, veal and chicken mortadella) sausage samples were included in the study. The majority of sausages had pH values above 6.00 with mean values ranging between 6.11 and 6.33. The moisture content values of small diameter sausages ranged between 32% and 46% and those of large diameter sausages ranged between 31% and 63%.

With regard to the microbial load of small diameter sausages, more than 53% of pork, 87% of beef, 57% of veal and 40% of chicken sausage samples had aerobic mesophilic counts $\geq \log 5$ cfu/g. Enterobacteriaceae were frequently encountered in these sausage samples and more than half of beef and veal sausage samples had Enterobacteriaceae counts $\geq \log 4$ cfu/g. The majority of pork, beef and veal sausage samples also harbored coliforms. Furthermore, more than 40% of veal sausage samples had coliform counts $\geq \log 4$ cfu/g. On the other hand, coliforms were rarely detected in chicken sausage samples. Enterococci were encountered in 60% of pork, 100% of beef and in more than 70% of veal and chicken sausage samples with counts ranging from log 2 to log 6 cfu/g. About 26% of pork, 60% of beef and 38% of veal sausage samples had *Staphylococcus* counts $\geq \log 5$ cfu/g. All sausage samples harbored LAB and more than 73% of pork, 60% of beef and about 40% of veal sausage samples had counts $\geq \log 7$ cfu/g. Over 86% of pork, 93% of beef, 38% of veal and 31% of chicken sausage samples had yeast counts $\geq \log 4$ cfu/g.

In the case of large diameter sausages or mortadellas, about 53% of pork, 48% of veal and 40% of chicken mortadella samples had aerobic mesophilic counts $\geq \log 5$ cfu/g, thus exceeded the typical aerobic mesophilic count value set for ready-to-eat foods. Enterobacteriaceae were encountered in more than 60% of pork, 50% of veal and 45% of chicken mortadella samples with counts between log 2 and log 4 cfu/g. More than 50% of pork and about 33% of veal and 40% of chicken mortadella samples had also coliform counts between log 2 and log 4 cfu/g. The majority of large diameter

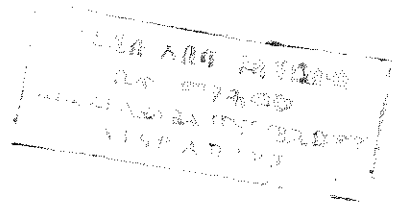
sausages harbored enterococci and staphylococci. LAB were encountered in all mortadella samples and 40% of pork and more than 30% of veal and chicken mortadella samples had counts $\geq \log 6$ cfu/g. The majority of mortadella samples contained yeasts and more than 45% of pork, 40% of veal and 30% of chicken mortadella samples had counts $\geq \log 4$ cfu/g.

The aerobic mesophilic flora of both small and large diameter sausages was dominated by Gram-positive organisms, with *Bacillus* being the most dominant species in both types of sausages. *Salmonella* was isolated from four (1.9%) samples consisting of two small diameter sausages and two mortadellas.

During aerobic storage of small diameter sausage samples and sliced mortadella samples at ambient temperature, both types of sausages spoiled within three to four days. At refrigeration storage, small diameter sausages spoiled within 12 to 16 days and mortadella samples spoiled within 12 to 20 days of storage. Spoilage in small diameter sausage samples was manifested as slime formation and off-odor development whereas spoilage in sliced mortadella samples was manifested as green discoloration and off-odor development.

In general, the majority of sausage samples investigated in this study had high microbial load. Time temperature abuse during processing or post-cooking contamination due to improper handling of the products or inadequate storage conditions or a combination of these factors may contribute to high microbial counts. Furthermore, the absence of microbiological control system of the end product, the raw material, or the other ingredients, at any stage of production and the poor sanitary condition of some processing plants revealed inadequacies concerning quality and safety of these products.

Key words/ phrases: Addis Ababa, Frankfurters, mortadella, quality, safety, *Salmonella*, sausage, supermarket



1. INTRODUCTION

Food is essential for survival. However, in spite of gaining good nutrition and satisfaction from eating food, occasionally human beings consume undesirable chemical and biological agents and toxins resulting in food borne illness. Consequently, in many countries food safety and quality is becoming a matter of increasing concern.

Food safety can be defined as food being free from chemical and biological danger or from any thing else which may generate adverse health effects (Unnevehr and Hirschhorn, 2000). Health hazards from food can arise from the raw materials used, from handling and through the other stages involved in the processing, transportation, storage and the sale of the food (Abalanka, 1999). Food quality has dimensions related to both production process and final product. Its determinants can be grouped in to four as hygienic properties, nutritional properties, functional properties, and organoleptic properties (Abalanka, 1999). Briefly, it can be defined as the subjective or objective valuation of food with respect to any or all of these four properties.

Food safety issues are becoming an increasingly serious threat to public health in developing countries. Lack of adequate regulations related to food safety as reflected in many unrecognized cases of food borne illnesses puts especially children and infants at high risk (Unnevehr and Hirschhorn, 2000). Biological contaminants, largely bacteria, viruses, and parasites constitute the major cause of food-borne diseases (Kaferstein, 2003). In developing countries, such contaminants are responsible for a wide range of diseases, including cholera, campylobacteriosis, *E. coli* gastroenteritis, salmonellosis, shigellosis, typhoid and paratyphoid fevers, brucellosis, amoebiasis, and poliomyelitis. According to a WHO study in 1993, 70% of the approximately 1.5 billion global episodes of diarrhea occurring annually, which resulted in 3 million deaths among children under five, had been estimated to be caused by biologically contaminated food (Motarjemi *et al.*, 1993). The same study also stated that contaminated food had been recognized as playing a major role in the epidemiology of cholera and other forms of epidemic diarrhea, substantially contributing to malnutrition.

Numerous food products are industrially processed for a longer shelf life than the original agricultural commodities they are made from (Jelen, 1985). For instance, red meats are usually further processed for the purpose of shelf life extension and also for flavor enhancement (Jay, 1996).

A variety of meat products are manufactured by the meat industry world wide. Sausages are popular and relished meat products world over (Sachindra *et al.*, 2005). They are usually defined as comminuted seasoned meats, stuffed in to casings (Savic, 1995). Growing consumer interest in foodstuffs of high nutritional value that are safe and hygienically prepared has prompted interest in sausages. On the other hand, these products, due to specific recipes of production, very short shelf life, storage conditions, and some times inappropriate management in the meat warehouse or shop, might be undesirable for consumption (Domanska and Rozanska, 2003).

Although particular problems arise with specific sausage products, in general, it may be said that for all sausages, three basic requirements must be fulfilled for a hygienically satisfactory product (Savic, 1985). These are a) conditions of sausage production must be such that should toxigenic organisms be present in or gain access to the product prior to, during or after processing, no bacterial toxins are formed; b) the final sausage product should not contain microorganisms likely to be pathogenic to man; and c) the total bacterial count of the sausage product should be reasonably low so that no decomposition or development of undesirable flavor occurs during the period of processing, distribution or storage.

The food industry in Ethiopia is at its young stage. Recently, sausages, locally produced on commercial scale, are being made available to the consumer. In Ethiopia, sausage production started in 1980's by one government owned meat processing factory which later shifted ownership to a private sausage manufacturer (Personal communication). During that time, sausages were produced mainly from pork and hence consumer demand was low due to religious taboos related to pork eating.



Currently, there are about five private sausage manufacturers. Some of the sausage manufacturers are located in Addis Ababa and others are found in Debre Zeit, which is found 47 km south of Addis Ababa. These manufacturers produce both small diameter (15-20mm) (Frankfurters) and large diameter (90-120 mm) sausages or mortadellas from meats of different animals such as veal, chicken, beef and pork. These meat products are available in various supermarkets in Addis Ababa. As most of the sausages are produced from veal, beef and chicken rather than from pork only, there is a growing consumer interest in these processed meat products. The initiation of processing meat products by the food industry in Ethiopia is a very commendable endeavor. Processing, in addition to improving the keeping quality of foods, avoids wastage of fresh meat parts which are usually not consumed by many people. Thus, the young food industry in Ethiopia should be encouraged and supported so that it can produce quality products with better keeping quality. Researchers are, therefore, expected to come up with scientific data that can help the production of safe and wholesome products. Ethiopian commercial sausage manufacturers thus should know the microbiological status of their products so that they could produce quality products that satisfy the local consumer and help them to go into the international market. However, there is no published information with regards to the microbiological quality and safety of these commercially produced sausages. Therefore, the present study was initiated with the objectives to:

1. determine the microbial load of different commercially produced sausages sold in various supermarkets in Addis Ababa,
2. identify and characterize the dominant isolates,
3. evaluate the bacteriological safety of sausages with regards to *Salmonella* and
4. assess the keeping quality of the sausages during storage at ambient and cold temperatures.

2. LITERATURE REVIEW

2.1. History of sausages

During their existence human beings have been confronted with the problem of limited keeping quality of animal and plant foods partly due to microbial activities. In response to this, during the last 5,000 to 10,000 years, a variety of techniques (such as drying, salting, fermentation, refrigeration, or freezing) evolved to increase the shelf life of foods of plant and animal origins (IOM, 1985).

Fresh meat is a highly perishable product because of its biochemical and biological composition. A number of interrelated factors influence the shelf life and keeping quality of meat, and these are holding temperature, atmospheric oxygen, indigenous enzymes, moisture, light and, most important, microorganisms (Serdengeci *et al.*, 2006). All of these factors can result in detrimental changes in the color, odor, texture and flavor of the meat. Sausage making, thus, evolved as an effort to economize and preserve meat that could not be consumed fresh at slaughter (Marchello and Robinson, 1998).

The process of preserving meats by stuffing salted, chopped meats flavored with spices into animal casings dates back thousands of years, to the ancient Greeks and Romans (Hudnall, 1999). The Romans made 'circelli', 'tomacinae', 'butuli' and other types of delicious sausage products, which were eaten during annual orgiastic festivals and sacrifices (Savic, 1985). Sausages and sausage products have since evolved into a wide variety of flavors, textures, and shapes resulting from variations in ingredients and manufacturing processes. The main ingredients used in these comminuted products are meat trimmings and cuts not marketed or consumed as fresh meat, lower grade carcasses, and certain specialized meat by-products such as tripe, liver, blood or blood plasma and non-meat ingredients called fillers or binders (Jelen, 1985). Food additives are used to accomplish certain functions such as coloring, antimicrobial, antioxidative, preservative, improved nutrition, increased emulsification and altered flavor (Ockerman and Sun, 1999).

2.2. Classification of sausages

In spite of their multiple varieties, sausages may be roughly divided into two general groups depending on their preparation, raw sausages (further divided as fresh and fermented) and heat-processed sausages (Savic, 1985; Marchello and Robinson, 1998).

Fresh sausages are made from fresh meats which are neither cured, smoked, fermented nor cooked (Savic, 1985). Fresh sausages are highly perishable products because of their characteristic pH and a_w . They have a pH value not lower than 5.5 and water activity (a_w) equal to or higher than 0.97 (Rantisiou *et al.*, 2005). They can be packaged in normal or modified atmosphere and stored at 4°C for a maximum period of 10 days (Cocolin *et al.*, 2003). Since no fermentation process is taking place during the storage at 4°C, the hygienic quality of the raw materials is the main factor affecting the final quality of the product (Cocolin *et al.*, 2003). The only hurdle to spoilage is represented by refrigeration temperature (Rantisiou *et al.*, 2005). They are either boiled or barbecued prior to consumption.

Fermented sausages are sausages consisting of a mixture of meat and fat particles, salt, curing agents, spices, etc., which have been stuffed into a casing, fermented (ripened) and dried (Fontana *et al.*, 2005). They can be either dry or semi dry (Jay, 1996). In general, dry sausages have a final pH of 5.0-5.3 and on average final moisture of <35%. Semi dry sausages have a final pH between 4.7-5.1 and a moisture content ranging from 45-50% (Getty, 2000). The sanitary condition of fermented meat products that do not receive any heat treatment is regulated only by the fermentation and drying they undergo (Tomicka *et al.*, 1997). Food fermentation is the oldest biotechnology. It is responsible for many properties of fermented foods such as flavour, shelf life, texture and health benefits (Giraffa, 2004).

According to Savic (1985), heat processed sausages are further classified into smoked precooked sausages, emulsion type sausages and cooked sausages. Smoked precooked sausages are mostly cured, non-fermented products. They are usually cooked before

consumption. Emulsion type sausages comprise ready-to-eat products made from comminuted and well-homogenized cured meats, fatty tissue, water and seasonings usually smoked and slightly cooked. Cooked sausages are cooked ready-to-serve products, basically made from previously cooked fresh or exceptionally cured raw materials, subjected to final cooking after stuffing, with or without additional smoking.

2.3. Sources of microbial contamination to sausages

Meat is an ideal growth medium for many organisms because it is high in moisture, rich in nitrogenous compounds (e.g. amino acids, peptides, proteins) and plentiful in minerals and accessory growth factors. Furthermore it has some fermentable carbohydrates, usually glycogen, and has favorable pH that allows the growth of most microorganisms (Frazier and Westhoff, 1978).

Sausages, in addition to the meat components, have additional sources of organisms in the seasoning and formulation ingredients that are usually added in their production. Many spices and condiments have high microbial counts (Jay, 1996). Microorganisms may also gain access into sausages from environment, equipment and handlers, which all affect the microbiological status of the product. Comminuting also adds microbial contamination to sausages (Sachindra *et al.*, 2005). Even though processing conditions such as heat treatment reduce microbial levels, recontamination takes place during post processing, handling and storage of sausages. Thus, the microbial ecology of meat products will mainly depend on the environment, kind of meat and raw materials, equipment, handling practices, processing, packaging and storage temperature (Sachindra *et al.*, 2005).

2.4. Indicator microorganisms

To estimate food sanitary quality, the classic approach is based on the search for not only pathogenic microorganisms but also indicator microorganisms (Leclercq *et al.*, 2002). An indicator organism is a microorganism that indicates that a food has been exposed to

conditions that pose an increased risk, that the food may have been contaminated with a pathogen or held under conditions conducive to pathogen growth (Buchanan, 2000).

According to Tompkin (1983), the choice of an indicator is product and process specific, when evaluating the microbiological quality of food. Indicator organisms have been used in meat and poultry products to assess three factors: microbiological safety, hygiene during processing, and the keeping quality of the product.

The aerobic mesophilic count is among the more popularly used nonpathogenic microbiological indicators of food quality (Vandereit, 1985; FEHD, 2001; FSAI, 2001). It is generally used for descriptive evaluation of microorganisms on nonselective media under mesophilic and aerobic conditions of incubation (FEHD, 2001; FSAI, 2001). This plate method serves as an indicator of food quality and as a measure of the effectiveness and maintenance of procedural integrity of food preparation protocols (Shapton and Shapton, 1991). It is generally believed that high aerobic mesophilic counts in foods indicate greater risks of pathogens being present in consumable products, poor implementation of sanitation procedures or problems in process controls to which a test food item has been subjected (Miskimin *et al.*, 1976).

Another group utilized to indicate inadequate processing of foods is the family Enterobacteriaceae. The detection of any member of the Enterobacteriaceae family present in a meat product has been used to imply the presence of enteric pathogens. Beside this, high levels of Enterobacteriaceae on cooked meat products suggest post processing contamination or microbial proliferation due to inadequate storage conditions (Smith and Schaffner, 2004).

Coliforms are also one of the typical indicator organisms of food quality. Increased counts of coliform bacteria are indicative of failures in sanitation and very high counts can be dangerous to human health (Napravnikoval *et al.*, 2002). This does not mean, however, that all foods that are free from coliform bacteria are safe. According to Smith and Schaffner (2004), high numbers of coliforms in meat products may be representative of improper handling or storage which allows for the multiplication of any coliforms

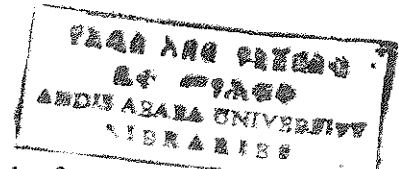
present. High counts of coliforms on cooked meat products would indicate post-processing contamination.

Enterococci may gain access into raw material and food products from primary habitats such as intestines of animals and humans, and from sources associated with unsanitary conditions of the production and handling of foods (Lukaova and Ustaakova, 2003). Due to their resistance to freezing, low pH, and moderate heat treatment, the enterococci have been suggested as indicators in some types of food products (Banwart, 1989). Barnes and Ingram (1955) reported that enterococci were recognized as important organisms in the flora of meats. These bacteria were isolated from spoiled canned hams in large numbers and, if these bacteria were initially present, they were able to multiply.

2.5. Spoilage microorganisms

Microbiological examination of foods is focused not only on causative agents of human diseases, but also on microorganisms causing spoilage and affecting shelf life (Napravnikova *et al.*, 2002). Food products serve as sources of nutrition for humans and other animals. But, they also are substrates for the growth of microorganisms. The uncontrolled growth of microorganisms in food causes spoilage, a serious problem accounting to sizable losses of food products. Spoilage microorganisms are those that can grow in food and cause undesirable changes in flavor, consistency (body and texture), color, or appearance (IOM, 1985; Hayes, 1995). Bacterial enzymes may also effect deterioration of frozen or dried foods during long time storage. These changes diminish the quality characteristics of foods and may render them ultimately unfit for human consumption. Most prone to spoilage are foods with high protein content, such as meat, poultry, fish, and milk, because they have a high dietetic value, neutral or slightly acid pH, and a high water content providing favorable conditions for bacterial growth (Huis and Veld, 1996).

Frankfurters (small diameter sausages) and mortadella have similarities to bologna and other emulsion-based sausages and luncheon meats prepared from a range of ingredients which contribute microorganisms to the final product (Abdullah, 2004). Bacteria, yeasts



and molds may be associated with processed meats, but the former two groups are most important in microbial spoilage (Jay, 1996). The bacterial flora of such products frequently includes staphylococci, micrococci and lactic acid bacteria (Harrigan and McCance, 1976). However these, like most of the vegetative bacteria which dominate raw meat e.g. *Pseudomonas* spp. and Enterobacteriaceae, are inactivated by cooking, but post-processing contamination can occur during slicing and skinning (Davies and Board, 1998).

The quality and shelf life of cooked meat foods are determined by the growth of microorganisms. Vacuum packaging has been shown to be very effective in extending the shelf life of perishable foods such as meat products (Church and Parsons, 1995). Under these conditions the oxygen supply will be restricted, the gas phase being determined by the rate of gas permeation through the film and the rate of oxygen consumption in the package, these changes having a selective effect on the microbial population (Labadie, 1999). Storage of meat products in gas-impermeable packs restricts the growth of *Pseudomonas* so that lactic acid bacteria (LAB), *Brochothrix thermosphacta* and Enterobacteriaceae become the major components of the spoilage microflora (Korkeala and Bjorkroth, 1997; Nychas and Drosinos, 2000). LAB were identified as the major spoilage population of vacuum-packaged emulsion-type sausages and other processed meats stored at refrigeration temperatures (Korkeala and Bjorkroth, 1997; Samelis *et al.*, 2000). These bacteria produce lactic acid, ethanol, acetic acid, hydrogen peroxide and carbon dioxide (Aymerich *et al.*, 2000). These same compounds that create favorable flavors in certain foods eventually increase in concentration, decreasing the sensory acceptability of the contaminated foods. Jay (1996) indicated that spoilage in these products is of three types: sliminess, discoloration and off-odor.

2.6. Pathogens

Ensuring the safety of food products depends on minimizing the initial contamination with pathogenic microorganisms and inhibiting their development during handling and storage (Stekelenburg, 2003). Pathogenic microorganisms can render foods harmful to

humans in a variety of ways. Foods may serve as vehicle of introduction of infectious microorganisms in to the gastrointestinal tract, e.g., *Salmonella* and *Shigella* (IOM, 1985). Multiplication of certain microorganisms in foods prior to consumption may result in production of toxins e.g. *Clostridium botulinum*, *Staphylococcus aureus* and *Bacillus cereus*. Microbial pathogens in food cause between 6.5 million and 33 million cases of human illness and up to 9,000 deaths in the United States each year, and the estimated annual cost of human illness caused by food-borne pathogens ranges from \$5.6 billion to \$9.4 billion (Buzby and Roberts, 1995).

Between 1969 and 1974, 3309 samples of pork sausages and sausage meat produced by two large and two medium sized manufacturers and several local butchers in Britain were examined for the presence of *Salmonella*. Of these, 786 samples were found to contain *Salmonella* (Roberts *et al.*, 1975). A study on the microbiological safety of sausages in England and Wales also detected *Salmonella* in 17% of uncooked fresh and frozen sausages (Nichols and de Louvois, 1995). The main serovar isolated was *S. Typhimurium*. Another study on the incidence and level of contamination of British fresh sausages and ingredients with *Salmonella* indicated an incidence of 65% contamination in pork and 55% in pork and beef sausages (Banks and Board, 1983). Mattick *et al.* (2002) studied the prevalence and number of *Salmonella* in uncooked economy catering sausages and found *Salmonella* spp. in 7.5% of frozen and 9.1% of chilled sausages.

Similarly, a microbiological examination of fresh pork sausages and fresh beef sausages from the point of sale in Manchester showed that 11.5% were contaminated with established food borne pathogens. The outer casing swabs of three sausages were positive for *Salmonella* (Williamson *et al.*, 2002). Moreover, it was found that pork sausages were more frequently contaminated with *Salmonella* (5%) than beef sausages (<1%). *S. Typhimurium* was the dominant serotype.

In their study on isolation of *E. coli* O157: H7 from foods in Greece, Dontourou *et al.* (2003) isolated in 1 out of 75 (1.3%) fresh sausages. A similar study on isolation of *E.*

coli O157:H7 from retail meats in Argentina reported an isolation rate of 4.8% from fresh sausages and 3.3% from dried sausages (Chinen *et al.*, 2001).

S. aureus is a ubiquitous organism, occurring in the skin and mucus membrane of most warm blooded animals and humans. It occurs naturally in a variety of foods of animal origin. In addition, food handlers are commonly implicated in the transmission of this pathogen to food (Jay, 1996). Staphylococcal food poisoning is caused by ingestion of a toxin formed by *S. aureus* in the food (Jay, 1996). The growth of *S. aureus* and the presence of enterotoxin in fermented sausages, particularly in Genoa and Italian type dry salami, have caused several outbreaks of food poisoning (Smith and Palumbo, 1980).

A survey on the bacteriological status of fresh and baked pork sausages in Ibadan, Nigeria showed that *Salmonella* spp. were present in 42% and 50%, *S. aureus* in 16% and 26%, and *E. coli* in 48% and 19% of fresh and baked pork sausage samples, respectively (Oyekole and Hassen, 1984).

2.7. Health hazards from sausages

In spite of their pleasant taste and high nutritional value, there are also several health hazards associated with sausages due to improper processing and handling.

An outbreak of *Salmonella* Typhimurium DT124 infection occurred in England between December, 1987 and January, 1988 which affected 101 people. The epidemiological and microbiological investigations identified small German salami sticks as vehicle of infection and the product was withdrawn from sale. The epidemiological investigation highlighted the occurrence of a long incubation period followed by bloody diarrhea (Cowden *et al.*, 1989). Similarly, between 1988 and 1994, there were 24 reported outbreaks of salmonellosis associated with sausages or sausage-meat in England and Wales, corresponding to more than 1000 cases of food poisoning (Nichols and de Louvois, 1995). Another case-control study of patients with gastroenteritis caused by *Salmonella enterica* serovar Typhimurium DT104 in England found a significant

association between human infection and the consumption of sausages (Wall *et al.*, 1994). In 1995, 93 persons became ill in New Mexico from beef jerky (a type of dry fermented sausage) that yielded three *Salmonella* serovars, *S. Montevideo*, *S. Kentucky* and *S. Typhimurium* (CDC, 1995). The product was produced in a commercial establishment, but it was unclear how the contamination occurred. In 2001, an outbreak of *Salmonella* Gold coast infections associated with the consumption of raw fermented sausage occurred in Germany (Bremer *et al.*, 2004).

In 1994, an outbreak of *Escherichia coli* O157:H7 infection was linked to a dry, fermented, pre-sliced pork and beef salami from delicatessen counters (CDC, 1995a). The salami outbreak involved 20 individuals in Washington and three individuals in California. In 1995, another outbreak of hemolytic uremic syndrome (HUS) in Australia was linked to semi-dry (uncooked) fermented sausage called mettwurst. This outbreak was attributed to *E. coli* O111:NM, a serotype that produces a Shiga-like toxin (SLT), and involved 23 individuals (CDC, 1995b). In addition, an outbreak of *E. coli* O157:H7 infection and recall was linked to a Hungarian fermented salami (Anonymous, 1999; Canadian Food Inspection Agency, 1999). These outbreaks could have been due to contamination of the raw meat mixes, inadequate process control to kill *E. coli* O157:H7, or cross-contamination of finished products (Bell and Kyriakides, 1998). An outbreak of trichinosis associated with the eating of improperly prepared homemade fermented sausage also occurred in Illinois. Of the 50 persons, who actually consumed the raw summer sausage, 23 became ill with trichinosis (Potter *et al.*, 1976). In late 1998, a *Listeria* outbreak from post-processed contaminated Frankfurters occurred in the USA that affected at least 50 people in 11 states (Anonymous, 1999a).

2.8. Types of sausages produced by Ethiopian commercial sausage manufacturers

Currently, Ethiopian commercial sausage manufacturers produce heat processed sausages, which may be grouped as emulsion type sausages. The products are small diameter (15-20mm) or large diameter (90-120mm) types. Small diameter sausages (Frankfurters) made from pork, beef, veal and chicken are made available to consumers. Each piece of

small diameter sausages is stuffed in natural casings and sold as a group of 9 or 10 pieces vacuum packed together in a plastic pouch. Frankfurter sausages are consumed worldwide (Luiz *et al.*, 2004). They are usually eaten after immersion in hot water for several minutes or grilling. The large diameter sausages produced by Ethiopian sausage manufacturers are commonly known as mortadella. Originally, mortadella was a traditional cured, cooked Italian sausage (Abdullah, 2004) but the version produced in Ethiopia is an emulsion type sausage. It is usually sold as vacuum-packed cylindrical rolls in casings or as slices. It can be consumed either cold or heated. Mortadella is an increasingly popular meat product in many countries because of its pleasant taste and texture, high nutritional value and ease of incorporation into sandwiches (Al-Shuibi, 1999 cited in Abdullah, 2004). Currently, mortadella made from pork, beef, veal and chicken is also available to consumers in different supermarkets in Addis Ababa.

Even though, there are minor differences, the production technology of all sausage manufacturers in Ethiopia is similar. To produce emulsion type sausage, frozen meat trimmings are first ground to permit easy mixing with additives. The ground meat is placed in a mixer with spices, preservatives (nitrates, sodium chloride, and polyphosphates), and other supplements such as oil, sugar, milk solids, wheat flour, blood and vine and mixed to form a sausage batter. Some manufacturers use only Ethiopian spices but there are also manufacturers who use imported ones. The mixture is transferred to a sausage-stuffer through which it is extruded into casings held over the stuffing horn of the sausage stuffer and vacuum stuffed according to the needed size of sausage. The vacuum is created by a vacuum pump, which constitutes part of the stuffing machine. For small diameter sausages, all producers use natural casings from small intestines of sheep or pork. For large diameter (90-120mm) sausages or mortadella, some producers use semi-synthetic casings and others use fully synthetic casings. The stuffed sausage is then placed into a smoking room. Immediately after smoking, the sausage is placed in hot water and cooked until its core temperature reaches 70-72⁰C. Heating and smoking of raw emulsion during Frankfurter processing serves several functions: It (i) sets the emulsion and forms the skin of skinless Frankfurters, (ii) kills the trichinae in the case of pork (iii) accelerates cured meat color development, (iv) imparts a desirable smoky flavor to the

Frankfurters, and (v) decreases the bacterial content of the Frankfurters (Palumbo *et al.*, 1974). Immediately, after cooking, cold water is made to flow over the sausage to lower its temperature. Each piece of small diameter sausage has on average about a length of 15-17cm, diameter of 15-20 mm, and weight of 20-40g. The sausages are kept in the refrigerator until shipped to different supermarkets.

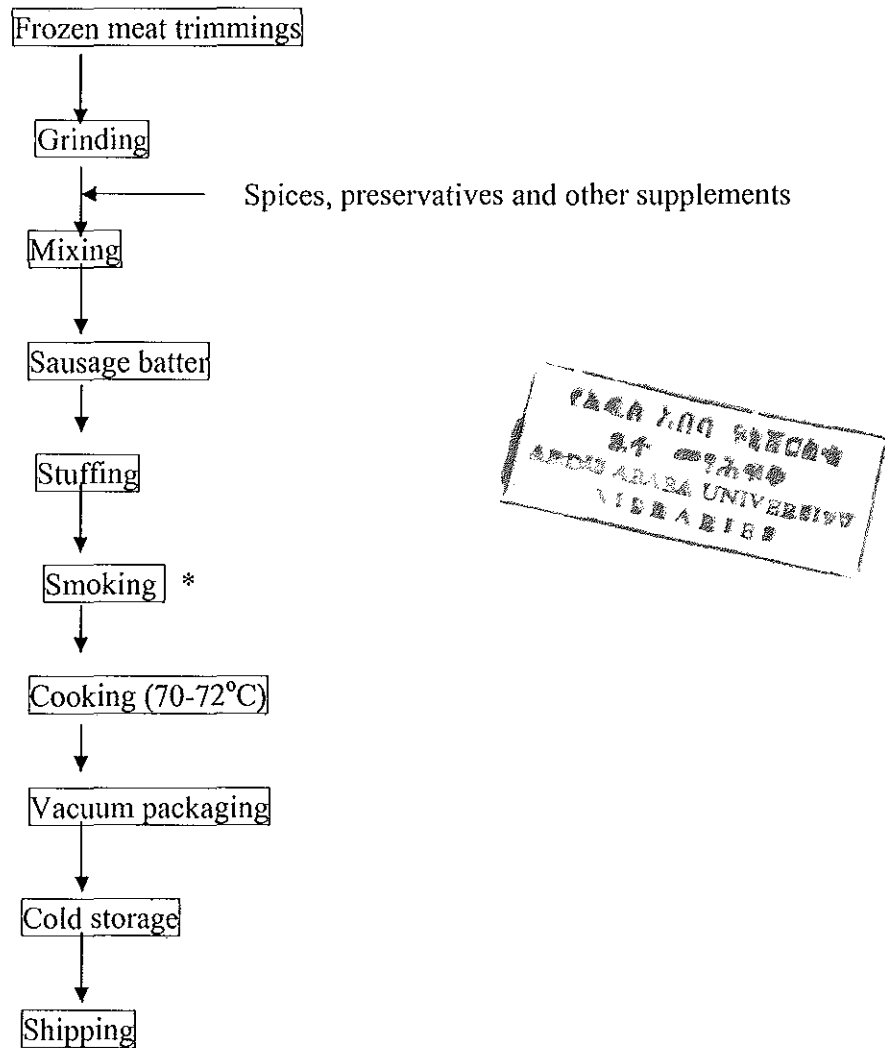


Fig.1.Flow diagram of a typical process used in the production of emulsion type sausages by Ethiopian sausage manufacturers.

*Some manufacturers skip this step.

3. MATERIALS AND METHODS

3.1. Sample collection

A total of 210 commercially produced sausage samples were purchased from different supermarkets in Addis Ababa, Ethiopia between November, 2004-December, 2005. These consisted of 120 small diameter sausages (30 each of veal sausage, chicken sausage, pork sausage and beef sausage) and 90 large diameter sausages or mortadellas (30 each of veal mortadella, chicken mortadella and pork mortadella). Samples of small diameter sausages were purchased as packaged items (each weighing about 250g-300g) and mortadella samples were purchased as slices. Veal and chicken sausage products were made by one producer and small diameter beef and pork sausages were products of another producer. Pork mortadella products were produced by three different producers and samples were purchased in a manner to represent producers. All samples were collected aseptically and immediately brought to the laboratory for microbiological analysis. Microbiological analysis was conducted within 1 to 3 hours of sample purchase.

3.2. Microbiological analysis

For microbiological analysis, 25 g of sample was transferred aseptically to a sterile stomacher bag and 225 ml of sterile 0.1% (w/v) bacteriological peptone (Oxoid) water was added to it. This was homogenized for 1-3 minutes using a Stomacher lab blender (model 400, Seward JAC, London). Serial ten-fold dilutions were also prepared by transferring one ml of the homogenized sample to nine ml diluent.

3.2.1. Microbial enumeration

3.2.1.1. Aerobic mesophilic count

From appropriate dilutions, 0.1ml aliquots were spread-plated in duplicates on pre-dried surfaces of Plate Count Agar (Oxoid) plates. Colonies were counted after the culture media were incubated at 30-32°C for 48 hours.

3.2.1.2. *Counts of staphylococci*

From appropriate dilutions, 0.1 ml aliquots were spread-plated in duplicates on pre-dried surfaces of Mannitol Salt Agar (Oxoid) plates. The culture media were incubated at 30-32°C for 36 hours after which yellow colonies were counted as staphylococci.

3.2.1.3. *Counts of Enterobacteriaceae*

From appropriate dilutions, 0.1 ml aliquots were spread-plated in duplicates on pre-dried surfaces of Violet Red Bile Glucose Agar (Oxoid) plates. The seeded culture plates were incubated at 30-32 °C for 20-24 hours after which pink to red purple colonies with or without haloes of bile precipitation were enumerated as members of Enterobacteriaceae.

3.2.1.4. *Counts of coliforms*

From appropriate dilutions, 0.1 ml aliquots were spread-plated in duplicates on pre-dried surfaces of Violet Red Bile Agar (Oxoid) plates. The culture plates were incubated at 30-32°C for 24 hours after which purplish red colonies surrounded by reddish zone of precipitated bile were counted as coliforms.

3.2.1.5. *Counts of enterococci*

From appropriate dilutions, 0.1 ml aliquots were spread-plated in duplicates on pre-dried surfaces of Bile Aesculin Agar (ingredients: peptone, 8g; bile salts, 20g; ferric citrate, 0.5g; aesculin, 1g; agar, 15g; pH, 7.1±0.2). The seeded culture plates were incubated at 30-32°C for 24 hours. Colonies surrounded by blacken zone were counted as enterococci.

3.2.1.6. *Counts of lactic acid bacteria*

From appropriate dilutions, 0.1 ml aliquots were spread-plated in duplicates on pre-dried surfaces of MRS (De man, Rogasa, Sharpe) (Oxoid) agar plates. The inoculated plates were placed in anaerobic jar and incubated at 30–32°C for 48 hours. All colonies were counted as lactic acid bacteria.

3.2.1.7. *Counts of yeasts*

From appropriate dilutions, 0.1ml aliquots were spread-plated in duplicates on pre-dried surfaces of Chloramphenicol Bromophenol Blue Agar made from the following ingredients: yeast extract, 5g; dextrose, 20g; chloramphenicol, 0.1g; bromophenol blue, 0.01g; agar, 15g; distilled water, 1000ml; pH, 6-6.4. The seeded culture plates were incubated at 25-28°C for three to five days. Smooth (non-hairy) colonies without extension at periphery (margin) were counted as yeasts.

3.2.2. Flora analysis

After enumeration of aerobic mesophilic bacteria, about 10 to 20 colonies were picked randomly from countable plates and inoculated in to tubes containing about 5 ml Nutrient Broth No 2 (Oxoid). These were incubated at 30-32°C over-night. Cultures were purified by repeated plating and were characterized to the genus level and various bacterial groups (Aneja, 1993) using the following tests.

3.2.2.1. Cell morphology

From overnight pure broth culture, wet mount was prepared on a microscope slide. The preparation was observed under light microscope using oil immersion objective. The morphological criteria considered during the observation were:

Cell shape:	Regular: rods, cocci, coccoid forms Irregular: branched, coryneforms, pleomorph
Cell arrangement:	Singles, pairs, clusters, chains, and tetrads
Motility:	Motile, non-motile
Endospore:	Present, absent



3.2.2.2. KOH Test

This test was done according to Gregerson (1978). One or two drops of 3 % KOH solution were placed on a clean microscope slide. A colony was picked with a sterile bacteriological wire loop and stirred in the KOH solution for 10 seconds to 2 minutes and the inoculating loop was then raised slowly from the mass. When KOH solution became

viscous, the thread of slime followed the loop for 0.5 to 2 cm or more. Typically, this was observed in Gram-negative bacteria. In cases of no slime, the watery suspension did not follow the loop, the reaction was negative and this was seen in Gram-positive bacteria.

3.2.2.3. *Oxidation Fermentation (O/F) test*

The utilization of glucose by each isolate was assessed by O/F test as suggested by Hugh and Leifson (1953) to identify microorganisms that metabolize glucose fermentatively or oxidatively or that do not utilize glucose by either way.

Ingredients (g/l): Peptone, 2g; yeast extract, 1g; NaCl, 5g; K₂HPO₄, 0.2 g; glucose, 10g; bromophenol blue, 0.08g; agar, 2.5g; distilled water, 1000ml, pH, 7.10.

The freshly prepared medium (15ml amounts in 18 x 180 mm test tubes) was immediately cooled under tap water to avoid dissolution of oxygen in the medium. Broth culture was inoculated into the medium by stabbing with a sterile straight wire to the bottom. Acid formation and growth regions were interpreted after 2 and 5 days of incubation at 30-32°C.

3.2.2.4. *Catalase test*

Young colonies were flooded with a 3% solution of hydrogen peroxide (H₂O₂). The formation of bubbles indicated the presence of catalase.

3.2.2.5. *Cytochrome oxidase test*

This test was conducted following the method outlined by Kovacs (1956). Freshly prepared reagent A and B were mixed in the ratio of 2:3 immediately before use.

Reagents:

A) 1% - α -naphthol in absolute ethanol

B) 1% N, N- dimethyl -p-phenylenediammonium chloride in distilled water

After flooding the young colonies with the mixture on Nutrient Agar plates, appearance of a blue color on the colonies within 30 seconds to 2 minutes indicated a positive reaction. Any very weak or dubious reaction that occurred after 2 minutes was ignored.

3.3. Isolation and characterization of *Salmonella*

3.3.1. Primary enrichment

To test for the presence of *Salmonella*, 25g of sausage sample was mixed with 225ml Buffered Peptone Water (BPW), homogenized and incubated at 37°C for 18-24 hours for the metabolic recovery and proliferation of cells which could have been injured during processing or to bring the number of target organisms to a detectable level.

3.3.2. Secondary enrichment

The following broths were employed for secondary enrichment: Selenite Broth Base (SBB) (Oxoid) supplemented with sodium biselenite L121 (4g/l), Tetrathionate Broth Base (TBB) (Oxoid) supplemented with iodine solution (20ml/l), Rappaport – Vassiliadis (RV) broth (Merk), Mannitol Selenite Broth Base (MSBB) (Oxoid) supplemented with sodium biselenite L121 (4g/l), and Muller Kauffman Tetrathionate Broth Base (MKTBB) (Oxoid) supplemented with iodine (19ml/l) and 0.1% brilliant green (9.5ml/l) solutions. The selective property of these broths lies in their ability to inhibit non-targeted microorganisms like Gram-positive bacteria and coliforms and permit the rapid multiplication of *Salmonella*. After pre-enrichment in buffered peptone water, 1ml of culture was transferred into separate tubes each containing 10 ml of SBB, 10 ml of TBB, 10 ml of MSBB or 10 ml of MKTBB. Moreover, 0.1 ml of culture was also transferred in to a separate tube containing 10 ml of RV broth. SBB and MSBB were incubated at 37°C for 24 hours and TBB, RV and MKTBB were incubated at 43°C for 48 hours in water bath.

3.3.3. Solid media

MacConkey Agar No 3, Salmonella-Shigella (SS) Agar and Xylose Lysine Desoxycholate (XLD) medium (all from Oxoid) were used for plating purpose. A loopful of culture from each selective enrichment broth was streaked separately on to each of the



solid medium and incubated at 37 °C for 18-24 hours. Characteristic colonies from each selective medium were picked and further purified and tested biochemically. Uninoculated culture plates were incubated to check for sterility of the solid media.

3.3.4. Biochemical Identification

3.3.4.1. Triple Sugar Iron Agar (TSI) (Oxoid)

The butt was stabbed and the slant was streaked and incubated at 37°C for 24±2hrs to detect fermentation of glucose, sucrose and lactose as well as production of H₂S. The presence of alkaline (red) slant and acid (yellow) butt, with or without production of H₂S was considered as presumptive for *Salmonella*.

3.3.4.2. Lysine Iron Agar (LIA) (Oxoid)

The butt was stabbed and the slant was streaked and incubated at 37°C for 24±2hrs to assess decarboxylation of lysine. The presence of alkaline (purple) reaction through out the medium was considered presumptive for *Salmonella*.

3.3.4.3. Urea Agar (Oxoid)

The slant was streaked and the tube was incubated at 37°C for 24±2hrs to assess the hydrolysis of urea. No color change in the slant was considered as negative and thus presumptive for *Salmonella*.

3.3.4.4. Simmons Citrate Agar (Oxoid)

The slant was streaked and the tube was incubated at 37°C for 24±2hrs to investigate utilization of citrate as a sole source of carbon. The presence of growth and color change from green to blue was considered as presumptive for *Salmonella*.

3.3.4.5. SIM Medium (Oxoid)

This medium was stabbed to the bottom and incubated at 37°C for 18-24hrs for the determination of H₂S production and motility. Production of indole was also investigated by adding Kovac's reagent to growth in this culture medium. The non-utilization of indole

as a result of absence of deep red color at the surface of agar was considered as presumptive for *Salmonella*.

The ability of *Salmonella* to ferment mannitol, glucose or sucrose was assessed using a fermentation broth base with the following ingredients: Peptone, 10 g; NaCl, 5g; phenol red, 0.024g; distilled water, 1000ml, pH, 7.2. An amount of 10g each of mannitol, glucose or sucrose was separately added to the broth base. Fermentation tubes contained inverted Durham tubes to detect gas production. To ascertain sterility of the media for biochemical tests, all tubes with media were pre-incubated at 37°C for 18-24 hours. The broths were inoculated with young cultures of *Salmonella* and incubated at 37°C for 18-24 hours. *Salmonella* could ferment glucose and mannitol and produce gas.

After biochemical identification, presumptive *Salmonella* positive isolates were further confirmed by using API 20E identification system as described by the manufacturer (BioMerieux, France).

3.4. Determination of keeping quality of sausages during aerobic storage at ambient and cold temperatures

Sausage samples were purchased from supermarkets immediately after their arrival from factories. To determine the keeping quality of sausages after their vacuum package is opened, each sausage type was separately stored at ambient and refrigeration temperatures. The storage conditions were intended to reflect what normally would happen in routine food handling in home kitchen environments and food service establishments (opening the vacuum package, using some amount of sausage and storing the rest for another meal). Counts of aerobic mesophilic bacteria, Enterobacteriaceae, lactic acid bacteria and yeasts were monitored during the storage time. Counts were made at 8-hour intervals for sausages stored at ambient temperature and at 48-hour intervals for those stored at refrigeration temperature.

3.5. Measurement of pH and moisture content (%)

The pH value of each sausage sample was determined by blending 10 g sausage sample in a stomacher with 100 ml distilled water. The pH value of the homogenate was measured using a digital pH-meter. The moisture content of the sausages was determined by allowing the samples to dry to constant weight at 35°C.

3.6. Visual assessment of processing plants

Visual assessment of the production sites was done and the following observations were made:

1. The sanitary condition of the compound where the processing plant was established;
2. Disinfection of workers' shoes during movement in and out of the processing rooms;
3. The sanitary condition of processing rooms, equipments and workers;
4. Handling practices of materials by workers during processing;
5. Availability of veterinarians to inspect animals;
6. Implementation of microbiological control system of raw materials (meat and other ingredients) or final products;
7. Training level of employees working in food processing;
8. Inspection of processing plants by regulatory agencies.

3.7. Statistical analysis

To see if there was significant variation in counts within samples in each type of sausage, coefficient of variation (CV) was calculated. Difference in microbial counts among pork mortadella samples produced by three manufacturers was analyzed by analysis of variance (ANOVA) and means were separated by Tukey HSD test. Significance was determined at the 5% level.

4. RESULTS

Ethiopian sausage manufacturers produced only emulsion type sausages from pork, beef, veal, and chicken which could be of small diameter or large diameter in their size. This study considered small diameter sausages made from pork, beef, veal, and chicken and large diameter sausages or mortadella made from pork, veal, and chicken. All were made available to consumers in different supermarkets in Addis Ababa.

4.1. pH and moisture content (%) of sausages

The mean pH values of small diameter sausage samples were 6.27, 6.27, 6.27 and 6.11 for pork sausage, beef sausage, chicken sausage and veal sausage, respectively. Variations within samples of each sausage type were not significant ($CV < 10\%$) (Table 1). For mortadella samples, pH values were 6.18, 6.09 and 6.33 for pork, veal and chicken mortadella, respectively. Variations within samples in each type of mortadella were not also significant ($CV < 10\%$) (Table 2).

Table 1. The pH and moisture content (%) values of small diameter sausages.

Sample type	pH					Moisture content (%)				
	Min	Max	Mean	S.D.	%CV	Min	Max	Mean	S.D.	%CV
Pork sausage	5.88	6.52	6.27	0.15	2.39	40	46	43	1.9	4.40
Beef sausage	5.81	6.51	6.27	0.16	2.56	38	45	41	2.12	5.16
Veal sausage	5.77	6.39	6.11	0.13	2.13	32	37	35	1.40	4.06
Chicken sausage	5.36	6.62	6.27	0.25	3.98	32	40	36	2.68	7.55

The moisture content of small diameter sausages ranged from 40% to 46% for pork sausage, 38% to 45% for beef sausage, 32% to 37% for veal sausage and 32% to 40% for chicken sausage. Variations within samples of similar sausage types were, however, not significant ($CV < 10\%$) (Table 1). The moisture content of mortadella ranged from 31% to 63% for pork, 32% to 40% for veal and 32% to 47% for chicken mortadella. Variations in

moisture content were significant within samples of pork and chicken mortadella (CV>10%) (Table2).

Table 2. The pH and moisture content (%) values of large diameter sausages or mortadellas.

Sample type	pH					Moisture content (%)				
	Min	Max	Mean	S.D.	%CV	Min	Max	Mean	S.D.	%CV
Pork mortadella	5.72	6.59	6.18	0.26	4.25	31	63	41	6.44	15.8
Veal mortadella	5.21	6.52	6.09	0.32	5.26	32	40	35	2.55	7.22
Chicken mortadella	5.74	6.63	6.33	0.20	3.16	32	47	38	4.27	11.3

4.2. Microbial spectrum of sausages

The microbial load (aerobic mesophilic count) of small diameter sausages ranged from log 2 to log 8 cfu/g and those of large diameter sausages or mortadellas ranged from log 3 to log 7cfu/g. A total of 487 and 459 bacterial strains were isolated from small diameter and large diameter sausages, respectively and were characterized to various genera and bacterial groups. Different genera constituted the dominant microflora in both types of sausages.

Table 3. Frequency distribution (%) of dominant bacteria in small diameter sausages purchased from supermarkets in Addis Ababa.

Sausage type	No of isolates	<i>Bacillus</i>	<i>Micro-coccus</i>	<i>Staphylo-coccus</i>	<i>Strepto-coccus</i>	<i>Lacto-bacillus</i>	Enterobacteriaceae	Other Gram-positive rods
Pork sausage	107	40	13	18	10	8	4	7
Beef sausage	133	28	15	23	14	8	8	2
Veal sausage	129	25	21	18	13	7	9	7
Chicken sausage	118	57	20	12	7	5		
Total	487	37	17	18	11	7	5	5

Generally, 95% and 99.5% of small and large diameter sausages, respectively, were dominated by Gram-positive organisms. *Bacillus* spp. (37%) dominated the bacterial flora of small diameter sausages followed by *Staphylococcus* spp. (18%) and *Micrococcus* spp. (17%) (Table 3). The aerobic mesophilic bacterial flora of large diameter sausages was also dominated by *Bacillus* spp. (34%), followed by *Micrococcus* spp. (26%) and *Staphylococcus* spp. (17%) (Table 4). Streptococci were also frequently encountered in both small (11%) and large diameter sausages (12%).

Table 4. Frequency distribution (%) of dominant bacteria in large diameter sausages or mortadellas purchased from supermarkets in Addis Ababa.

Sausage type	No of isolates	<i>Bacillus</i>	<i>Micrococcus</i>	<i>Staphylococcus</i>	<i>Streptococcus</i>	<i>Lactobacillus</i>	Enterobacteriaceae	Other Gram-positive rods
Pork mortadella	181	28	25	20	12	9	1	4
Veal mortadella	137	38	26	16	12	7	—	0.7
Chicken mortadella	141	38	26	14	10	11	—	2
Total	459	34	26	17	12	9	0.4	2

4.2.1. Pork sausage

The aerobic mesophilic count of pork sausage samples ranged from log 3 to log 7 cfu/g and about 53% of the samples had counts \geq log 5 cfu/g (Fig. 2). The aerobic mesophilic bacterial flora was dominated by *Bacillus* spp. (40%) followed by *Staphylococcus* spp. (18%) and *Micrococcus* spp. (13%) (Table 3). Different microbial groups were found in all pork sausage samples at varying levels (Fig. 2). Enterobacteriaceae and coliforms were encountered in about 87% of the samples. Even though there were samples (40%), which had enterococci counts below detectable levels ($<$ log 2 cfu/g), samples which had counts as high as log 5 cfu/g were also encountered. About 73% of the samples had staphylococci counts ranging from log 4 to log 6 cfu/g. Counts of lactic acid bacteria were very high in all pork sausage samples ranging from log 6 to log 9 cfu/g and over 70% of the samples had counts \geq log 7 cfu/g. About 87% of the samples had yeast counts \geq log 4 cfu/g. Significant variations were noted in the counts of all microbial groups among pork sausage samples (CV=13.33%-63.34%) (Table 5).

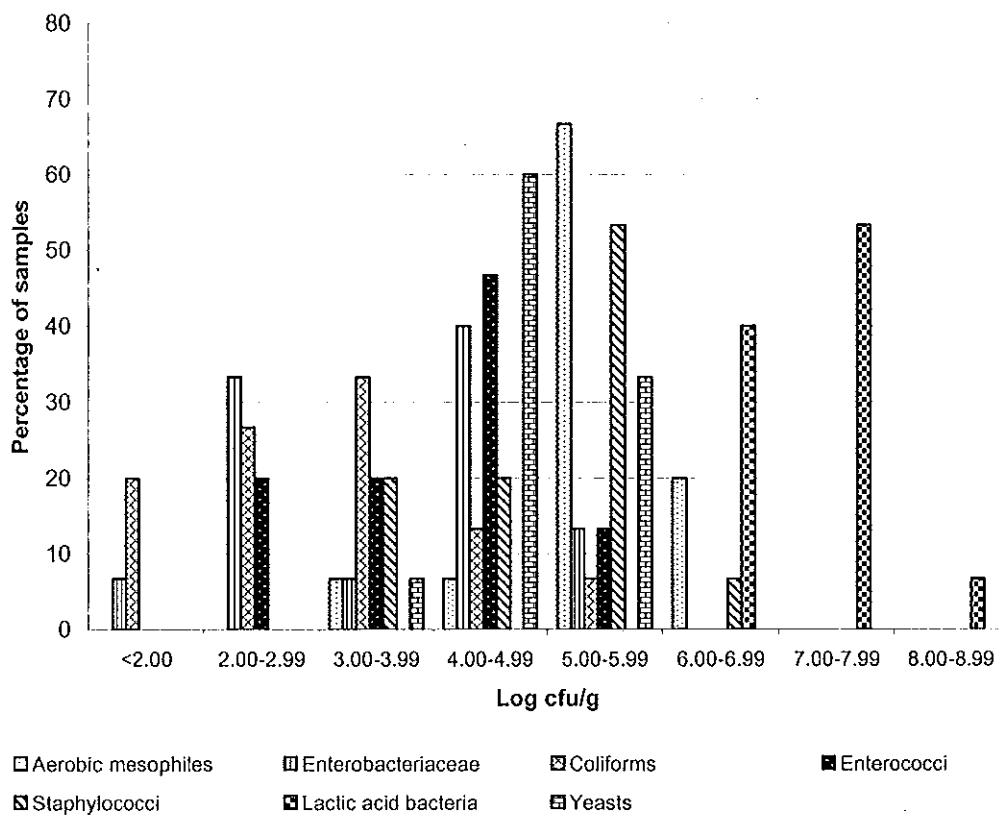


Fig.3. Distribution of microbial counts (log cfu/g) in beef sausage samples.

4.2.3. Veal Sausage

Over 50% of the veal sausage samples had aerobic mesophilic bacterial counts ranging from log 6 to log 9 cfu/g(Fig.4). About 40% also had lactic acid bacteria counts between log 7 and log 9 cfu/g. Over 30% of the samples had Enterobacteriaceae, coliforms, staphylococci and yeasts below detectable levels (<log 2 cfu/g) with the method used in this study. However, about 40% had staphylococci and 30% had coliforms and Enterobacteriaceae at levels > log 6 cfu/g (Fig. 4). Enterococci were encountered in about 30% of the samples at levels between log 5 and log 7 cfu/g. Significant variations were noted in counts of all microbial groups within veal sausage samples (CV=38.22%-80.35 %) (Table 5).

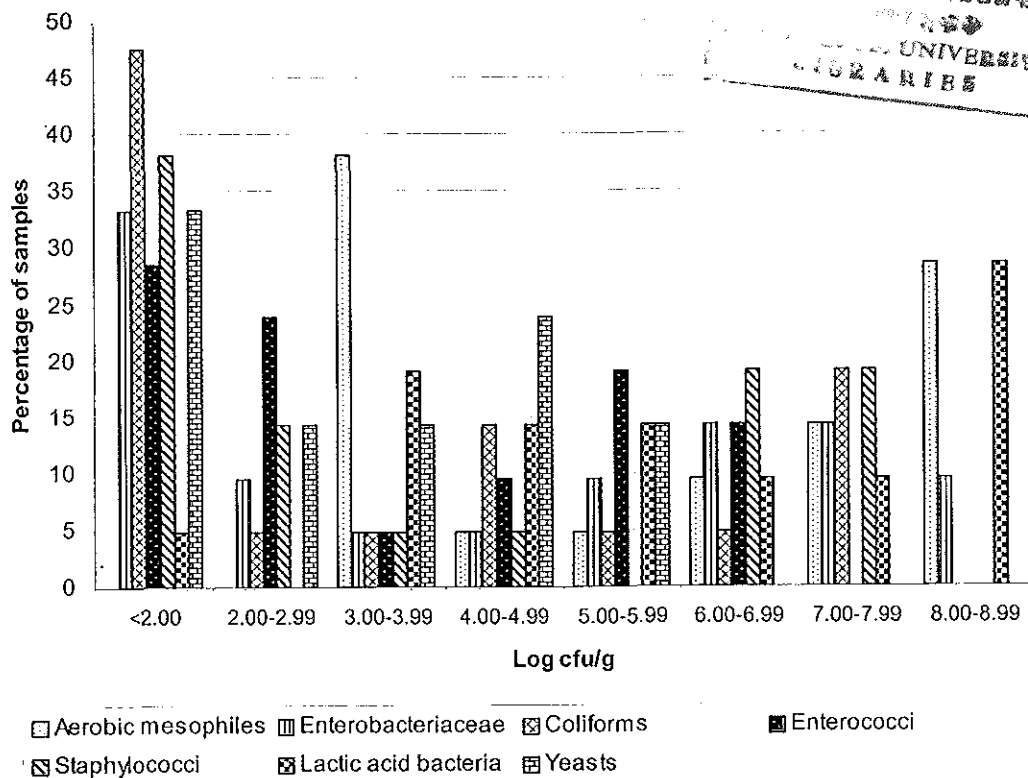


Fig.4. Distribution of microbial counts (log cfu/g) in veal sausage samples.

4.2.4. Chicken sausage

About 40% of the chicken sausage samples had aerobic mesophilic bacterial counts $\geq \log 5$ cfu/g (Fig. 5). The aerobic mesophilic bacteria was dominated by *Bacillus* spp. (57%), *Micrococcus* spp. (20%) and *Staphylococcus* spp. (12%) (Table 3). Over 50% of the samples harbored Enterobacteriaceae and staphylococci. However, coliforms were rarely encountered in chicken sausage samples as 95% of the samples had counts below detectable levels ($< \log 2$ cfu/g) (Fig. 5). Over 70% of the samples had enterococci counts $< \log 3$ cfu/g and lactic acid bacteria counts $> \log 4$ cfu/g. Counts of yeasts were below detectable limits in 45% of the samples. Variation in counts of all microbial groups within chicken sausage samples was significant (CV=26.76%-67.40%) (Table 5).

Table 5. Microbial counts (log cfu/g) of small diameter sausages purchased from supermarkets in Addis Ababa.

Bacterial groups	Pork sausage					Beef sausage					Veal sausage					Chicken sausage				
	Min	Max	Mean	S.D.	%CV	Min	Max	Mean	S.D.	%CV	Min	Max	Mean	S.D.	%CV	Min	Max	Mean	S.D.	%CV
Aerobic mesophiles	3.04	7.22	5.19	1.41	27.16	3.54	6.91	5.61	0.77	13.72	3.17	9.51	6.07	2.32	38.22	2.81	6.38	4.41	1.09	24.71
Enterobacteriaceae	<2.00	6.08	2.94	1.29	43.88	<2.00	5.44	3.46	1.40	40.46	<2.00	8.36	4.31	2.97	68.91	<2.00	4.00	2.02	1.12	55.51
Coliforms	<2.00	5.86	2.73	1.20	43.95	<2.00	5.17	2.98	1.43	47.98	<2.00	7.91	3.41	2.74	80.35	<2.00	2.30	1.06	0.29	27.36
Enterococci	<2.00	5.60	2.51	1.59	63.34	2.17	5.49	3.91	1.01	25.83	<2.00	6.93	3.46	2.19	63.21	<2.00	3.50	2.30	0.98	44.78
Staphylococci	<2.00	6.23	3.73	1.84	49.32	3.20	6.42	4.93	0.93	18.86	<2.00	7.66	3.82	2.79	73.03	<2.00	5.30	2.42	1.54	64.63
Lactic acid bacteria	6.10	9.74	7.50	1.00	13.33	6.21	9.87	7.30	0.96	13.15	<2.00	8.95	5.89	2.29	38.87	3.32	8.04	5.16	1.44	27.90
Yeasts	3.57	6.90	4.69	0.85	18.12	3.00	5.39	4.62	0.60	12.99	<2.00	5.90	3.00	1.77	59.00	<2.00	6.00	2.70	1.82	67.40

S.D.-standard deviation; %CV-percent coefficient of variation

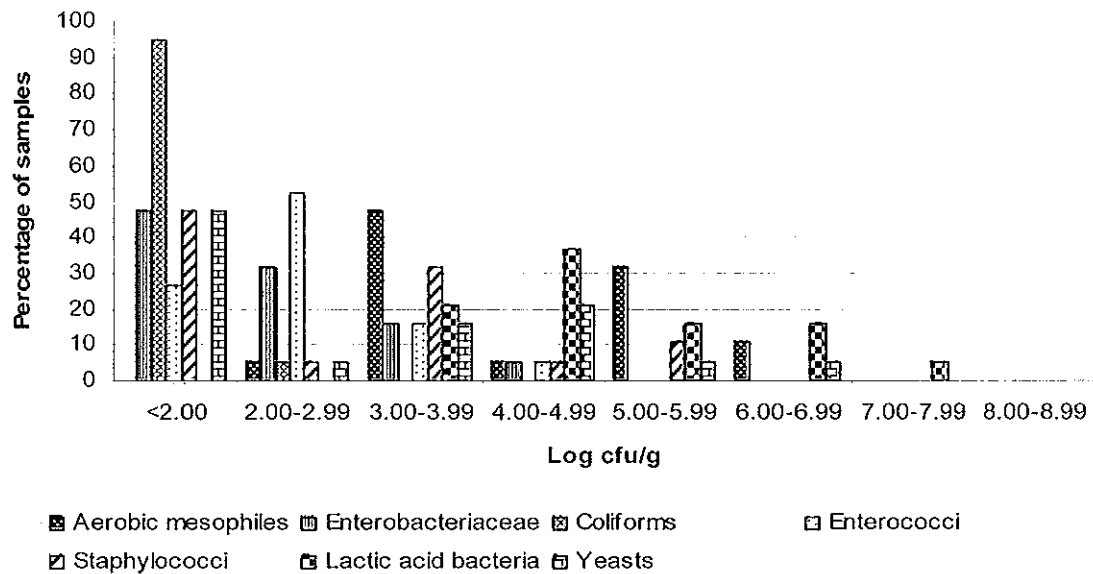
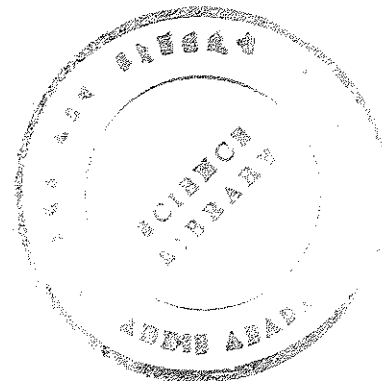


Fig.5. Distribution of microbial counts (log cfu/g) in chicken sausage samples.

4.2.5. Pork mortadella

The mean aerobic mesophilic count of pork mortadella samples was log 4.93 cfu/g (Table 6) and over 50% of the samples had counts \geq log 5 cfu/g (Fig. 6). The mesophilic bacterial flora of pork mortadella was dominated by *Bacillus* spp. (28%), *Micrococcus* spp. (25%) and *Staphylococcus* spp. (20%) (Table 4). Over half of the samples contained Enterobacteriaceae coliforms and enterococci at levels $>$ log 2 cfu/g. About 17% of the samples contained staphylococci at levels $>$ log 5 cfu/g. Counts of lactic acid bacteria ranged from log 3 to log 9 cfu/g and 40% of the samples had counts \geq log 6 cfu/g. Almost all pork mortadella samples harbored yeasts with counts between log 2 and log 5 cfu/g. For counts of all microbial groups, variation within pork mortadella samples was significant (CV= 21.09%-62.50%) (Table6).



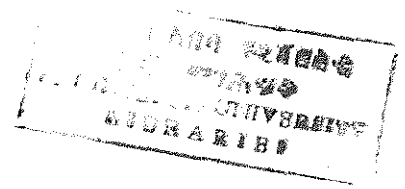


Table 6. Microbial counts (log cfu/g) of large diameter sausages or mortadellas.

Bacterial groups	Pork mortadella					Veal mortadella					Chicken mortadella				
	Min	Max	Mean	S.D.	%CV	Min	Max	Mean	S.D.	%CV	Min	Max	Mean	S.D.	%CV
Aerobic mesophilic bacteria	3.27	7.64	4.93	1.04	21.09	3.27	7.79	5.18	1.50	28.96	3.27	6.95	4.87	1.28	26.28
Enterobacteriaceae	<2.00	6.37	2.50	1.36	54.40	<2.00	4.49	2.07	1.21	58.45	<2.00	4.44	2.02	1.20	59.04
Coliforms	<2.00	6.30	2.24	1.40	62.50	<2.00	4.25	1.73	1.14	65.89	<2.00	4.31	1.87	1.13	60.42
Enterococci	<2.00	5.00	2.56	1.37	53.51	<2.00	5.66	3.04	1.76	57.89	<2.00	6.04	2.46	1.46	59.35
Staphylococci	<2.00	6.83	3.09	1.83	59.22	<2.00	6.31	3.67	1.56	42.50	<2.00	6.13	3.76	1.44	38.29
Lactic acid bacteria	3.17	9.79	5.68	1.65	29.04	<2.00	8.76	5.31	1.61	30.32	3.62	7.51	5.34	1.20	22.47
Yeasts	<2.00	5.62	3.87	1.00	25.84	<2.00	6.51	3.30	1.92	58.18	<2.00	7.54	3.28	2.21	67.38

S.D.-standard deviation; %CV-percent coefficient of variation

The pork mortadella samples from the three producers did not have significant difference in counts of aerobic mesophiles, staphylococci, LAB and yeasts ($P>0.05$) (Table 7). However, samples from producer 1 had significantly higher counts of Enterobacteriaceae than samples from producer 2 and also significantly higher counts of coliforms than samples from producer 2 and 3 ($p<0.05$). Similarly, the samples from producer 1 had significantly higher counts of enterococci than samples from producer 3 ($P<0.05$) (Table 7).

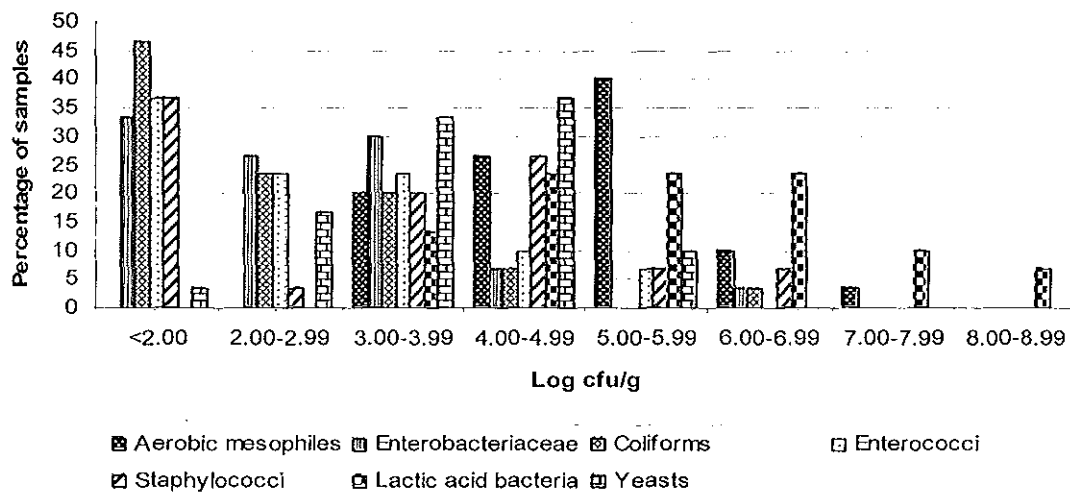


Fig.6. Distribution of microbial counts (log cfu/g) in pork mortadella samples.

Table 7. Microbial counts (cfu/g) of pork mortadella samples from three producers (n=30)

Microbial groups	Log cfu/g (Mean±S.D.)		
	Producer 1	Producer 2	Producer 3
Aerobic mesophiles	5.43±1.21a	4.82±0.83a	4.54±0.91a
Enterobacteriaceae	3.44±1.46a	1.89±1.01b	2.17±1.14ab
Coliforms	3.33±1.45a	1.56±0.92b	1.82±1.14b
Enterococci	3.53±1.22a	2.37±1.26ab	1.78±1.09b
Staphylococci	3.72±2.18a	2.89±1.69a	2.66±1.58a
Lactic acid bacteria	6.19±1.89a	5.98±1.59a	4.86±1.23a
Yeast	4.37±0.59a	3.91±0.78a	3.34±1.28a

*Averages in rows followed by the same letters are not significantly different ($P>0.05$).

4.2.6. Veal mortadella

The aerobic mesophilic count of veal mortadella samples ranged from log 3 to log 7 cfu/g and about 48% of the samples had counts \geq log 5 cfu/g (Fig. 7). The aerobic mesophilic bacterial flora of veal mortadella samples was dominated by *Bacillus* spp. (38%), *Micrococcus* spp. (26%) and *Staphylococcus* spp. (16%) (Table 4). Enterobacteriaceae and coliforms were encountered in about 52% and 33 % of the samples, respectively with counts $>$ log 2. Over 80% of the samples harbored staphylococci, some with counts as high as log 5 cfu/g. About 33% of the samples had LAB counts of \geq log 6 cfu/g. Over 60% of the samples contained yeasts and enterococci and in 19% of the samples the counts for both groups were \geq log 5 cfu/g. For all counts of microbial groups variation within veal mortadella samples was significant (CV=28.96%-65.89%) (Table 6).

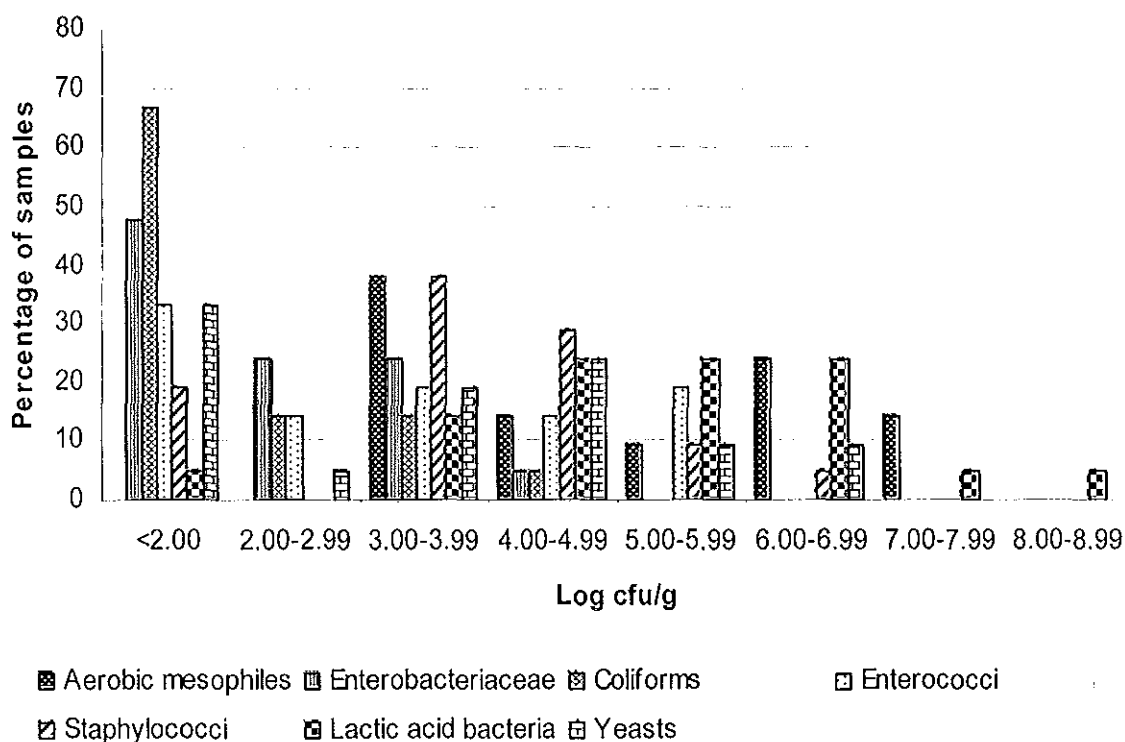


Fig.7. Distribution of microbial counts (log cfu/g) in veal mortadella samples

4.2.7. Chicken mortadella

The aerobic mesophilic count of chicken mortadella samples ranged from log 3 to log 6 cfu/g and about 40% of the samples had counts higher than log 5 cfu/g (Fig. 8). The aerobic flora was dominated by *Bacillus* spp. (38%), *Micrococcus* spp. (26%) and *Staphylococcus* spp. (14%) (Table 4). About 60% of the samples had Enterobacteriaceae and coliform counts below detectable levels with the methods used in this study. About 86% of the samples harbored staphylococci with counts ranging from log 3 to log 6 cfu/g. Enterococci were encountered in about 59% of the samples with counts between log 2 and log 6 cfu/g. All chicken mortadella samples yielded LAB with counts ranging from log 3 to log 7 cfu/g. Yeasts were below detectable levels in 27% of the samples. However, most samples contained yeasts with counts ranging from log 2 cfu/g to log 7 cfu/g. Significant variations

were noted for counts of all microbial groups within chicken mortadella samples (CV=22.47%-67.38%) (Table 6).

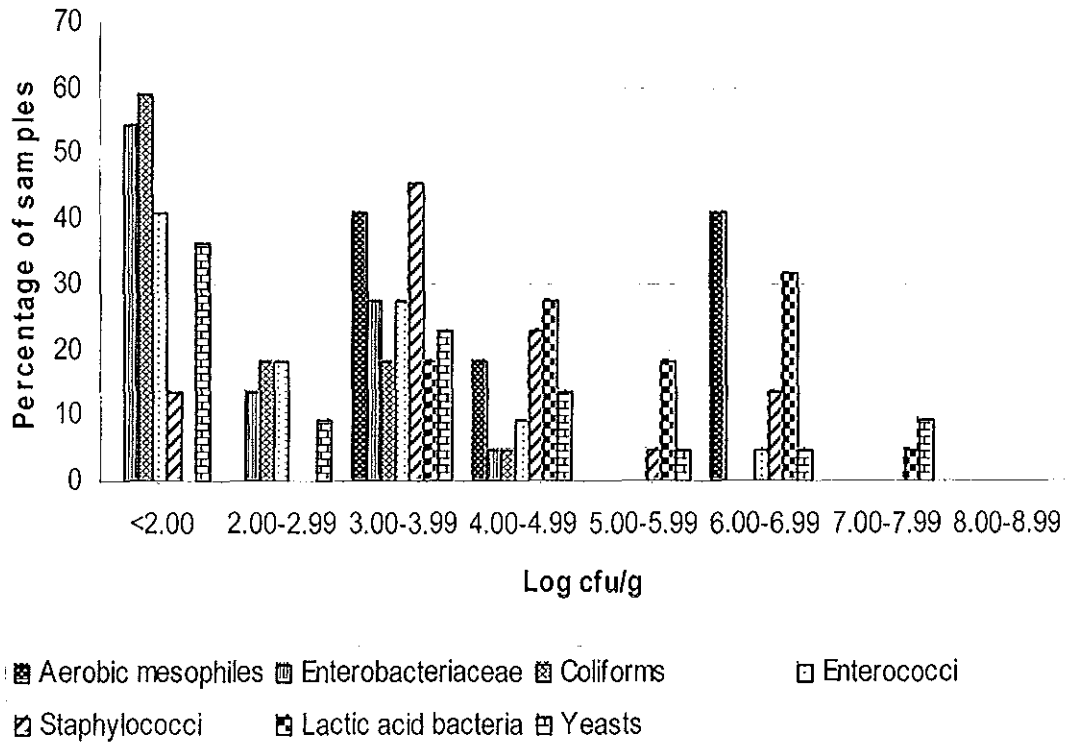


Fig.8. Distribution of microbial counts (log cfu/g) in chicken mortadella samples

4.3. Isolation and identification of *Salmonella* spp.

From a total of 210 sausage samples examined, four (1.9%) samples were found positive for *Salmonella*. *Salmonella* was isolated from two (1.7%) samples of small diameter sausages (chicken sausages) and two (2.2%) samples of large diameter sausages or mortadellas (1 chicken and 1 pork mortadella).

4.4. Microbial dynamics in sausages during aerobic storage at ambient and cold temperatures

4.4.1. Microbial dynamics in small diameter sausages (Frankfurters)

Initial counts of pork sausage sample on purchase day was around log 6 cfu/g for aerobic mesophilic bacteria, Enterobacteriaceae, lactic acid bacteria and yeasts. During aerobic storage at ambient temperature, all microbial groups showed a fast rate of growth until 24 h and the counts increased by 2 log units (Fig. 9). Thereafter, growth of LAB and yeasts was markedly low, whereas the counts of aerobic mesophilic bacteria and Enterobacteriaceae maintained the sharp increase and reached values as high as log 9 cfu/g at 72 h (3 days). At this time, the sample showed sign of spoilage. At refrigeration temperature, all microbial groups showed a steady growth throughout the duration of the experiment, but the LAB count leveled off after day 6 (Fig. 9). At day 12, increases by two or more log units were observed for aerobic mesophilic bacteria, Enterobacteriaceae and yeasts and the product showed signs of spoilage. Final counts at the time of spoilage were \geq log 8 cfu/g.

The beef sausage sample processed for storage studies initially had counts of aerobic mesophilic bacteria and LAB around log 6 cfu/g. Enterobacteriaceae were below detectable levels and yeast count was around log 4 cfu/g. During aerobic storage at ambient temperature, counts of aerobic mesophilic bacteria, LAB and yeasts increased by 2 log units or more until 24 h. Increase in count thereafter was slight (Fig. 10). The count of Enterobacteriaceae, on the other hand, remained below detectable levels until 24 h. However, it showed a sharp increase thereafter and the count increased by 5 log units until day 3. At the time of detection of spoilage (3 and 1/3 day), all counts were $>$ log 6 cfu/g and counts of aerobic mesophilic bacteria and LAB reached levels as high as log 9 and log 8 cfu/g, respectively. During refrigeration storage of beef sausage, all microbial groups grew steadily throughout the storage period at varying rates (Fig. 10). Aerobic mesophilic bacteria reached counts as high as log 10 cfu/g at time of detection of spoilage (day 12). LAB count at this time was around log 9 cfu/g. Although final counts of Enterobacteriaceae and yeasts at time of spoilage were relatively lower, their counts increased by about 5 log units in 12 days.

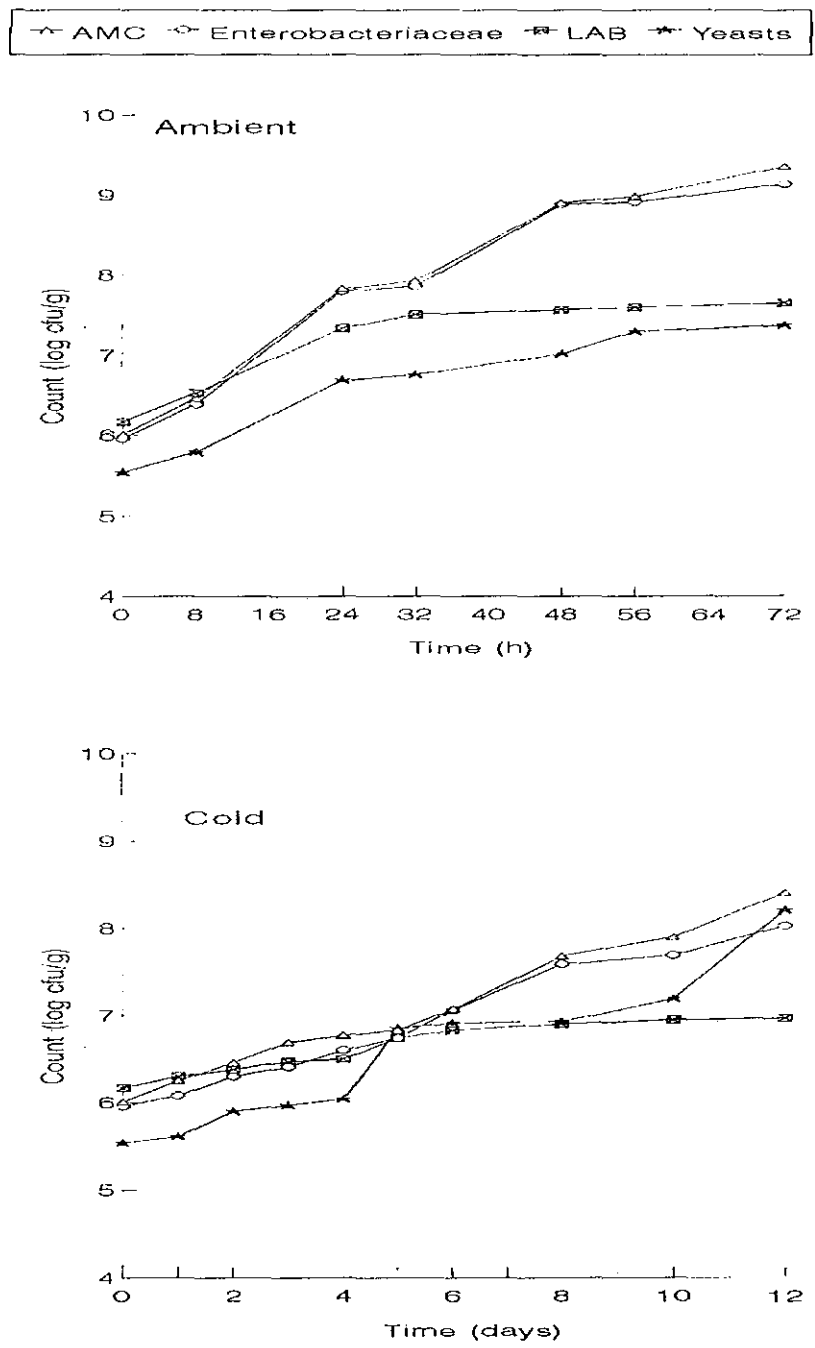


Fig.9. Dynamics of aerobic mesophilic bacteria, Enterobacteriaceae, lactic acid bacteria and yeasts during aerobic storage of pork sausage at ambient and refrigeration temperatures.

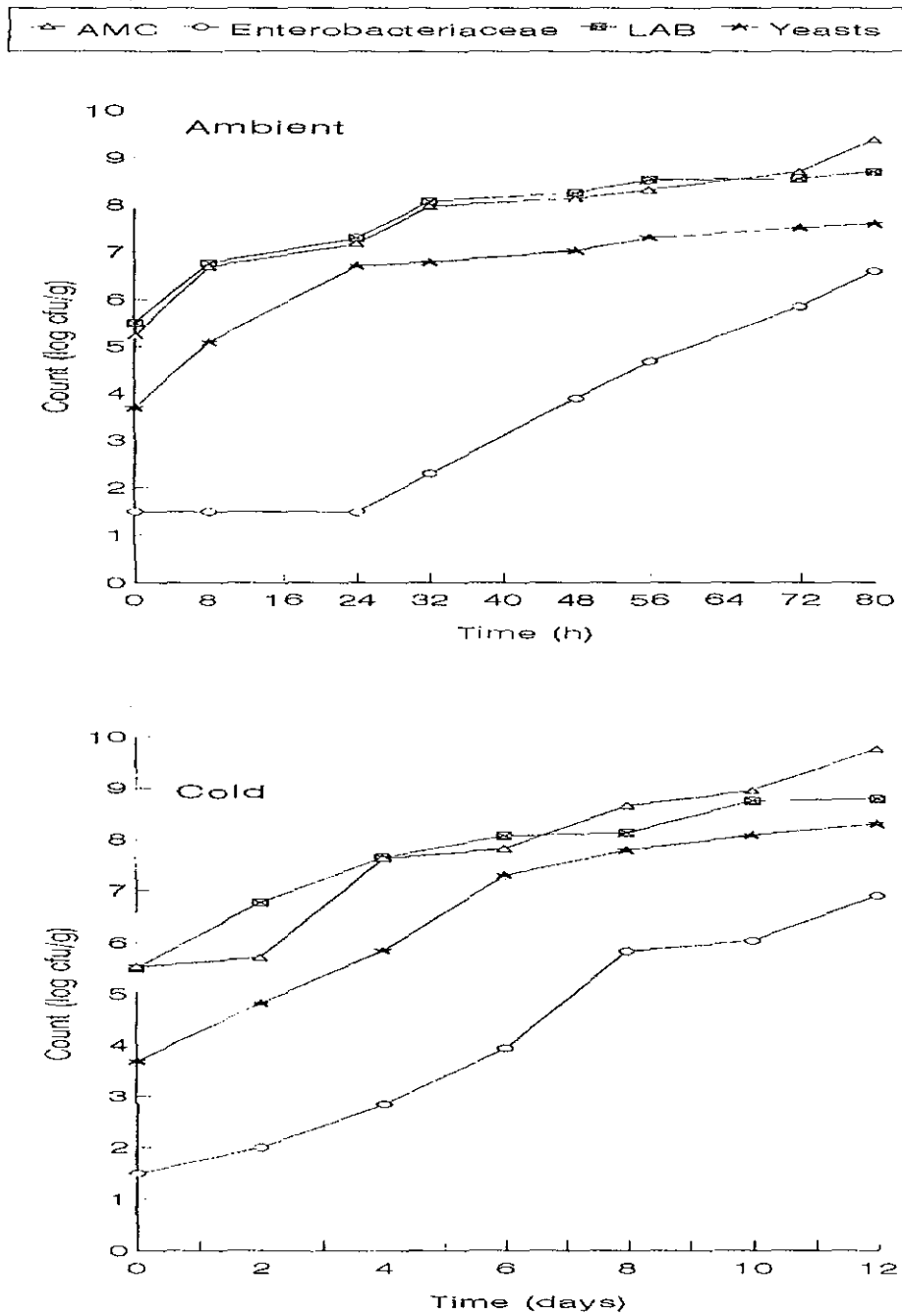
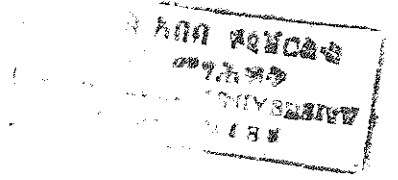


Fig.10. Dynamics of aerobic mesophilic bacteria, Enterobacteriaceae, lactic acid bacteria and yeasts during aerobic storage of beef sausage at ambient and refrigeration temperatures.



At the time of purchase, veal sausage had aerobic mesophilic and LAB counts around log 5 cfu/g. Counts of Enterobacteriaceae and yeasts were, however, below detectable levels (<log 2 cfu/g). During aerobic storage at ambient temperature, aerobic mesophilic bacteria and LAB grew steadily and their counts increased by 3 log units when spoilage was detected at 80 h (Fig. 11). Enterobacteriaceae showed a very sharp growth and increased by 5 log units until 32 h and reached final counts of log 8 cfu/g at 80 h. Yeasts also showed a sharp growth and increased by over 5 log units at 80 h. At refrigeration storage, the pattern of growth was similar to that of ambient storage. Aerobic mesophilic bacteria grew steadily and reached counts >log 8 cfu/g at time of spoilage (day 16) (Fig. 11). LAB increased by 2 log units until day 10, but the count leveled off thereafter. Counts of yeasts and Enterobacteriaceae increased by 2.5 log units within the first two days, and the counts at spoilage were log 7 and log 6 cfu/g, respectively.

On day of purchase, chicken sausage had aerobic mesophilic, LAB and yeast count ranging between log 3 and log 4 cfu/g. During aerobic storage at ambient temperature, growth of all microbial groups was steady and sharp (Fig. 12). Counts of aerobic mesophiles and Enterobacteriaceae increased by 3 log units within 24 hours. All groups reached counts around log 8 cfu/g within less than three days, and counts at time of spoilage (88 h) were >log 8 cfu/g. At refrigeration storage, a very sharp increase in count was observed until day 6, where the count of the various microbial groups increased by 5 to 6 log units (Fig. 12). Growth after day 6 was markedly low and counts of aerobic mesophilic bacteria and yeasts reached log 9 cfu/g at time of spoilage (day 14). Count of Enterobacteriaceae at time of spoilage was also high (log 8 cfu/g).

In all small diameter sausages, spoilage was manifested as formation of slime on the surface of the sausage and development of off-odor.

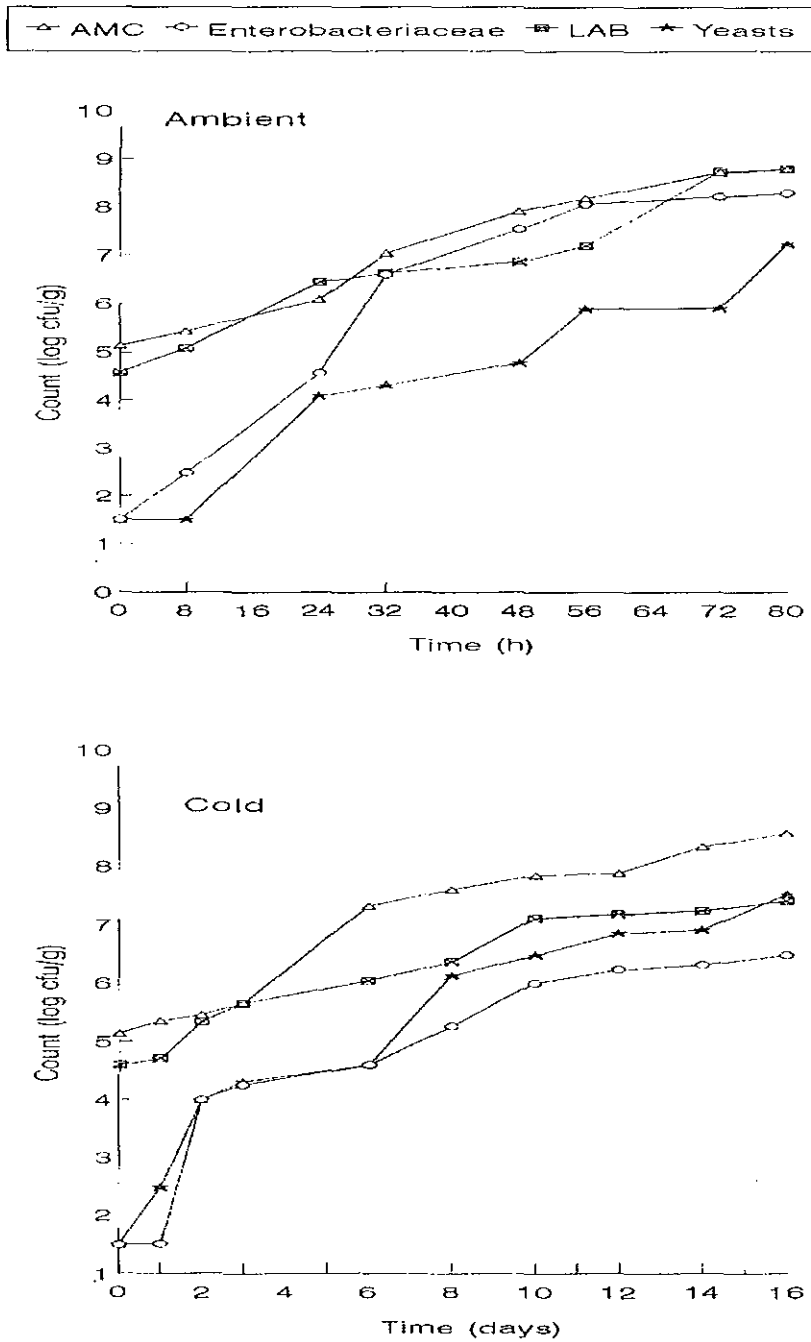


Fig. 11. Dynamics of aerobic mesophilic bacteria, Enterobacteriaceae, lactic acid bacteria and yeasts during aerobic storage of veal sausage at ambient and refrigeration temperatures.

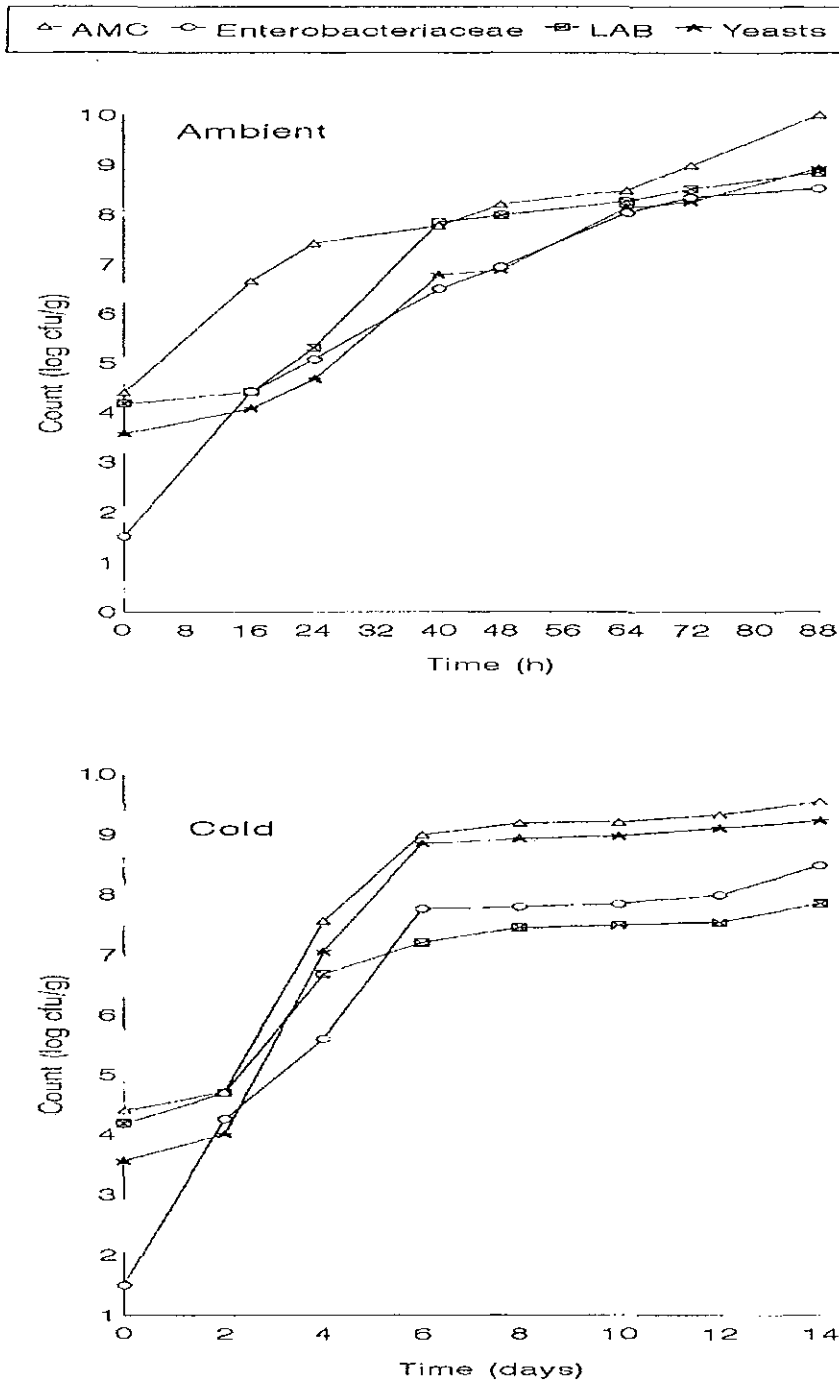


Fig.12. Microbial dynamics of aerobic mesophilic bacteria, Enterobacteriaceae, lactic acid bacteria and yeasts during aerobic storage of chicken sausage at ambient and refrigeration temperatures.

4.4.2. Microbial dynamics in large diameter sausages or mortadellas

Initial count of pork mortadella at time of purchase was log 6 cfu/g for LAB and log 4 cfu/g for aerobic mesophilic bacteria and yeasts. Enterobacteriaceae were below detectable levels (< log 2cfu/g). At ambient storage, all microbial groups grew steadily at varying rates (Fig. 13). Except the count of LAB which increased only by three log units at the time of spoilage (day 3); counts of yeasts, aerobic mesophilic bacteria and Enterobacteriaceae increased by 4, 5 and 6 log units, respectively at time of spoilage. At refrigeration storage, a gradual increase was noted in counts of aerobic mesophilic bacteria, LAB and yeasts and final counts of >log 8 cfu/g were reached at the time of spoilage (day 12). Enterobacteriaceae remained below detectable levels until day 4, but reached counts of about log 4 cfu/g at the time of spoilage.

On day of purchase, veal mortadella had aerobic mesophilic and LAB counts of around log 3 cfu/g and log 4 cfu/g, respectively. Counts of Enterobacteriaceae and yeasts were, however, below detectable levels (<log 2 cfu/g). At ambient temperature storage, aerobic mesophilic bacteria showed a steady growth all through the storage time (Fig. 14). Growth of LAB, yeasts and Enterobacteriaceae was delayed until 24 h. LAB showed the highest rate of growth after 40 h and reached counts > log 9 cfu/g at time of spoilage (3.5 days). Yeast and Enterobacteriaceae grew sharply after 24 h and increased by over 6 log units at time of spoilage. At refrigeration storage, LAB and aerobic mesophilic bacteria increased by 4 to 5 log units until day 6 and growth thereafter was markedly low (Fig. 14). Counts at time of spoilage (day 20) were around log 9 cfu/g. Growth of yeast and Enterobacteriaceae was delayed until day 8 and 10, respectively, but this was followed by a sharp increase in count. Yeast count reached log 6 cfu/g at time of spoilage and counts of Enterobacteriaceae remained around log 4 cfu/g between day 14 and time of spoilage.

▲ AMC ○ Enterobacteriaceae ■ LAB ★ Yeasts

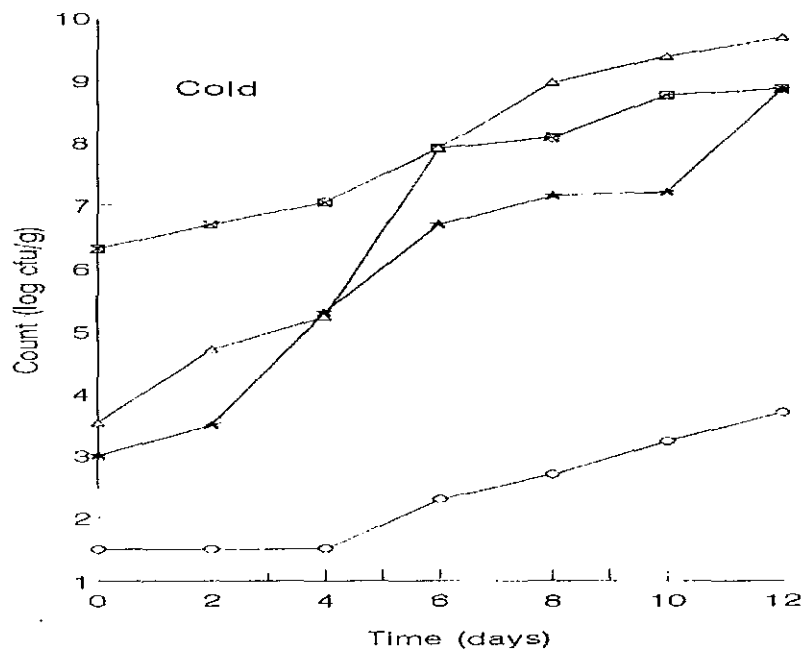
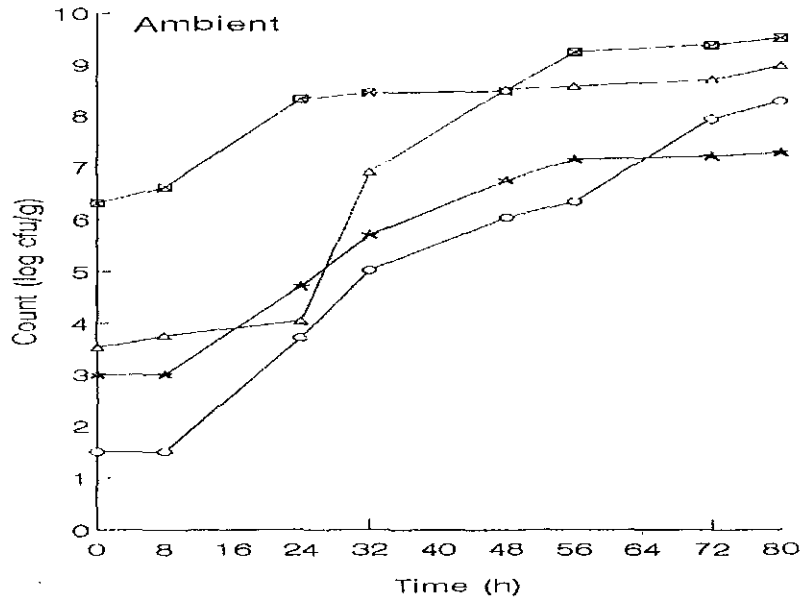
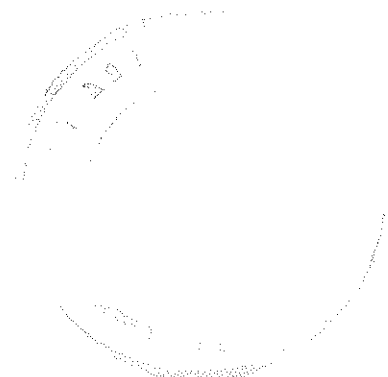


Fig.13. Dynamics of aerobic mesophilic bacteria, Enterobacteriaceae, lactic acid bacteria and yeasts during aerobic storage of pork mortadella at ambient and refrigeration temperatures.



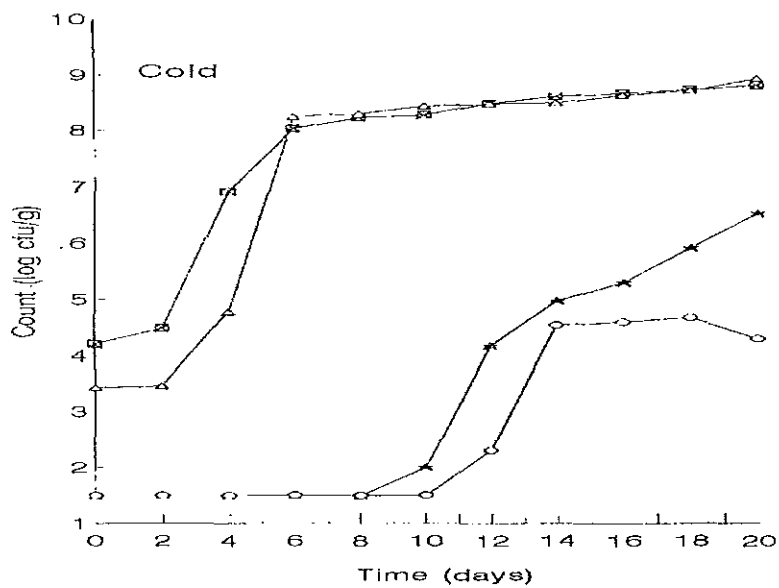
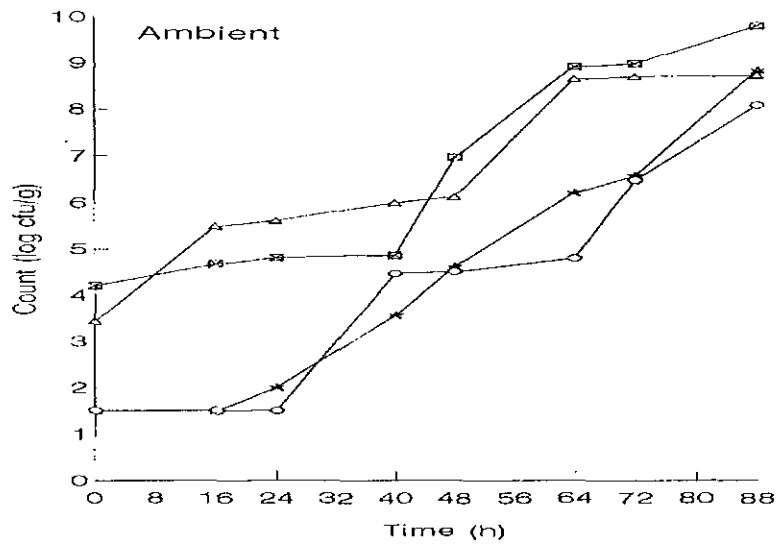
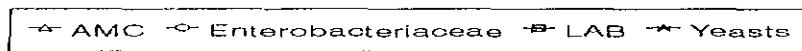
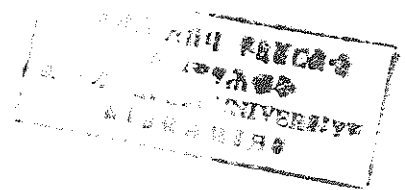


Fig. 14. Dynamics of aerobic mesophilic bacteria, Enterobacteriaceae, lactic acid bacteria and yeasts during aerobic storage of veal mortadella at ambient and refrigeration temperatures.



4.5. Results of visual assessment of processing plants

In most of the processing plants we visited, the compound was not asphalted. The sanitary condition of the various components of the processing plants (processing rooms, equipments) and sanitary practice of the workers could not be considered as satisfactory. In some processing plants, there was no mechanism to disinfect workers' shoes when they went in and out of the processing room. In some processing plants workers did not wear gloves and caps during processing. In most processing plants, the involvement of veterinarians to inspect their animals was almost none. For that matter, from the five sausage manufacturers, only one was monitored by Region 14 Bureau of Agriculture. This government-inspected processing plant was established in a compound where a vehicle maintenance workshop was located nearby. Trained workers were rare in each processing plant. Some processing plants stored final products with raw ingredients. Most of the processing plants did not have the set-up to monitor the microbiological quality of the raw materials and final products.

5. DISCUSSION

Sausages are popular meat products worldwide. Sausages, especially emulsion types, are also produced in Ethiopia on a commercial scale. Consumers are entitled to quality products, which are safe and wholesome. However, there is no published information regarding the quality and safety of sausage products in Ethiopia.

In general, the majority of sausages considered in this study had high microbial load and, in some cases, even pathogens were isolated. Time/temperature abuse during processing or post cooking contamination due to improper handling of the products or inadequate storage conditions or a combination of these factors may contribute to high microbial counts. Furthermore, the absence of microbiological control system of the end product, the raw material, or the other ingredients, at any stage of production and the poor sanitary condition of some of the processing plants revealed inadequacies concerning quality and safety of these products.

The majority of small diameter sausages (Frankfurters) analyzed in our study had pH values above 6.00. The variability in the pH values among samples in each type of small diameter sausage was low. Comparable results were reported for Polish edible offals taken from meat factories by Domanaska and Rozanska (2003) and cooked Frankfurter type sausage by Metaxopoulos *et al.* (2002). These pH values might make these products susceptible to bacteria as well as to mold and yeast spoilage (Jay, 1996) and could allow the multiplication of several bacterial pathogens (Ferrari and Torres, 2002). Mean pH value lower than ours (5.5) was also reported by El-Khateib *et al.* (1988) for Frankfurters collected from retail markets in Egypt. However, the moisture content values of the small diameter sausages obtained in our study were significantly lower than that reported for Frankfurters in other studies (El-Khateib *et al.*, 1988; Metaxopoulos *et al.*, 2002; Sabbag *et al.*, 2005, Sachindra *et al.*, 2005). All of them reported moisture content values of Frankfurters above 60%. Even though our sausage samples had low moisture content values than those usually Frankfurters do have ($\geq 60\%$), and fillers or binders such as wheat flour, salt, milk solids, oil and others

were added in their formulations, still they allowed the multiplication of many types of bacteria and yeasts.

The mean aerobic mesophilic counts of the various small diameter sausages in our study ranged between log 4 cfu/g and log 6 cfu/g. Except for chicken sausage samples, these mean values were relatively higher than that reported by Domanaska and Rozanska (2003) for Polish edible offals taken from meat factories. Further lower mean count of aerobic mesophiles (log 2 cfu/g) was also reported by Sabbag *et al.* (2005) for commercial Frankfurters in Argentina. El-Khateib *et al.* (1988) reported a mean aerobic mesophilic count of log 6 cfu/g for chicken Frankfurters collected from retail markets in Egypt. This mean value was markedly higher than the mean count value obtained in our chicken sausage samples. Although, there are no standards or guidelines regarding the microbial contamination of sausages in Ethiopia, Shapton and Shapton (1991) cited aerobic mesophilic count of < log 5 cfu/g for cooked sausages. Of our samples, 53% of pork, 87% of beef, 57% of veal and 40% of chicken sausage samples thus exceeded the typical aerobic mesophilic count value set for cooked sausages. A high aerobic mesophilic count is not in itself a health risk but in a cooked product it indicates an overall lack of hygiene (Little and de Louvois, 1998).

Long time storage of sausages either in factories or in shops may also contribute to an increase of aerobic mesophilic counts of sausages. It was reported that the mean aerobic plate count of vacuum packed cooked ring sausage one day after processing was log 3 cfu/g and it was log 5.6 cfu/g on the sale by date and log 5.84 cfu/g after 7 days after the sale by date (Korkeala *et al.*, 1985). Thus, high aerobic mesophilic count recorded in our sausage samples might not only reflect the sanitary quality but also continued growth of micro organisms that were already present in the products during storage in supermarkets.

The counts of Enterobacteriaceae obtained in the investigated small diameter sausages were also high especially in veal and beef sausage samples in which more than half of the samples had counts \geq log 4 cfu/g. Furthermore, veal sausage samples which had counts of Enterobacteriaceae as high as log 7 and log 8 cfu/g were also encountered. Coliforms were

also frequently encountered in the various sausage types at counts as high as log 3 and log 4 cfu/g. The mean values of counts of coliforms obtained from small diameter sausages in our study were higher than that reported by Domanaska and Rozanska (2003) for Polish edible offals which had mean count of coliforms as low as log 0.85 cfu/g.

Since these groups of bacteria (Enterobacteriaceae and/or coliforms) should be eliminated in the cooking process usually applied to Frankfurters, the presence of such high counts in some of the investigated sausage samples could indicate either insufficient heat treatment or post-cooking contamination during post heating process or inadequate storage conditions in supermarkets. These factors may allow the proliferation of microorganisms that survived heat treatment or the post treatment contaminants.

Relatively high counts of enterococci ($\geq \log 4$ cfu/g) were also encountered in small diameter sausages especially in beef and veal sausage samples. In processed meat products enterococci are not generally desirable because they cause spoilage (Giraffa, 2002). It is observed that enterococci can be found as spoilage contaminants in processed meats, either by surviving heat processing due to their thermoduric nature (Franz *et al.*, 1999), or by cross-contamination at the final stages of processing, such as slicing and packaging (Hugas *et al.*, 2003). Gordon and Ahmed (1991) stated that *E. faecium* can survive cooking to 68°C for 30 min during normal Frankfurter production.

The mean values of counts of staphylococci obtained from pork, beef, and veal sausage samples were around log 4 cfu/g. These mean values were greater than that reported for Polish edible offals purchased from meat factories (Domanaska and Rozanska, 2003). Moreover, sausage samples which had counts of staphylococci $\geq \log 5$ cfu/g were also frequently encountered in this study. Jensen *et al.* (1973) also reported the presence of log 3 cfu/g coagulase-positive staphylococci in Frankfurters. On the other hand, all 127 samples of cooked Frankfurters taken from establishments under federal inspection in Philadelphia were staphylococci-free after being processed and before being peeled (Surkiewicz *et al.*, 1976). Hill (1972) stated that cooked meat products such as bologna and Frankfurters should be staphylococci free. Any staphylococci found on cooked-meat products would result from

post-processing contamination. Staphylococci are common in unprocessed animal products and in products handled by hand. Heat processing, however, should reduce their numbers or eliminate them. One of the most important functions of the heating step of the Frankfurter process is the destruction or reduction of the bacterial populations, especially those of public health significance and those that limit shelf life of the finished Frankfurter. High counts of staphylococci ($>\log 5$ cfu/g) could result in staphylococcal food poisoning if the strains are toxin-producing types.

Counts of lactic acid bacteria recorded in small diameter sausages in our study were significantly high with high mean values. The dominance of LAB in the spoilage flora of emulsion sausages has been reported (Korkeala *et al.*, 1989). It was also reported that spoilage process started in cooked ring sausage packed in vacuum, when the lactobacilli count was greater than 10^7 cfu/g (Korkeala *et al.*, 1989). Over 70% of pork, 60% of beef and about 40% of veal sausage samples analyzed in our study had LAB counts $\geq 10^7$ cfu/g. However, there was not observable spoilage on the purchase day of the investigated sausages even if they had such high counts of LAB. In fact, Samelis and Georgriadou (2000) also reported that the shelf life of vacuum or modified atmosphere packed cooked meats should be preferably defined by an acceptable off odor /off flavor or appearance rather than a certain bacterial level. However, it can be suggested that high counts of LAB in our samples would contribute to spoilage of these products as observed by Korkeala *et al.* (1989).

Cooking of sausages during manufacturing destroys lactic acid bacteria on the surface of sausages. Sausages are recontaminated with spoilage lactic acid bacteria mainly during the processing stages after cooking (Korkeala and Bjoerkroth, 1997). Thus, the presence of such high counts of lactic acid bacteria in the sausages in our study might indicate the use of raw materials highly contaminated with LAB. Insufficient cooking process might not eliminate these bacteria. It was stated that an effective heat processing is essential during cooking to eliminate heat tolerant lactobacilli for enhancing the shelf life of sausages during storage at chill temperature. (Borch *et al.*, 1988). Ethiopian sausage manufacturers add milk solids as

fillers or binders in their sausage formulations. Jay (1996) described milk solids as possible sources of lactic acid bacteria to processed meat products.

The mean values of counts of yeasts obtained in small diameter sausages in our study were also high. Moreover, the majority of pork and beef sausage samples and more than 30% of veal and chicken sausage samples had high counts of yeasts. Jay (1996) described yeasts as one of the spoilage organisms of sausages which are usually contributed by milk solids added in sausage formulations. Palumbo *et al.* (1974) also reported that yeasts were the dominant spoilage flora of Frankfurters produced in their plant. Therefore, the presence of such high counts of yeasts in the sausages in our study might indicate a short shelf life these sausages would have either in shops or in consumers' home.

In general, high counts of aerobic mesophilic bacteria, Enterobacteriaceae, coliforms, staphylococci, enterococci, lactic acid bacteria and yeasts in small diameter sausages might indicate that the cooking process was inadequate since most of these microbial groups could be eliminated during cooking, that post cooking contamination had occurred, that the temperature of post cooking storage was inadequate to prevent bacterial growth, or that a combination of these factors was involved. In fact, inappropriate storage practices were observed in some supermarkets during displaying of the products for sale (displaying at cooled refrigeration displays rather than frozen), which may result in proliferation of the microorganisms present. It is, therefore, important to minimize post processing contamination as well as enforcing strict temperature control throughout storage and whilst on retail sale to minimize any proliferation of the organisms in food.

There was high variability in the counts of all microbial groups within the samples of each type of small diameter sausage. This shows the lack of quality control in processing, storage and handling of sausages. In fact, most of the processing plants had no system of microbiological control of the end product, the raw material or the other ingredients, at any stage of production. In addition to this, none of the processing plants stated the production and the sale by date of sausages. Thus, the variation in counts of the different microbial

groups among our sausages might arise from lack of control steps during processing and length of time the products were maintained in supermarkets.

Salmonella spp. was isolated from 2(1.7%) samples (chicken sausage) of small diameter sausages. *Salmonella* was not detected in any of cooked chicken Frankfurt sausage samples taken from a sausage factory found in the state of São Paulo, Brazil (Luiz *et al.*, 2004). Weissman and Carpenter (1969) also failed to isolate *Salmonella* from Frankfurters (n=8) purchased from retail markets in Georgia. It was stated that *Salmonella* are not usually found in Frankfurters because they cannot survive the heat process (Palumbo *et al.*, 1974). Palumbo *et al.* (1974) also indicated that the presence of salmonellae in finished Frankfurters would indicate either under-processing or recontamination after processing. *Salmonella* are usually killed by temperatures > 50°C, with the death rate increasing as the temperature increases (Doyle and Mazzotta, 2000). The detection of *Salmonella* in the investigated sausage samples in our study thus indicates the use of raw materials contaminated with *Salmonella*, accompanied by insufficient cooking process (under processing) or recontamination after cooking and prior to packaging. In fact, *Salmonella* are known to be present in the raw meat used in sausage manufacture (Hardy and Galton, 1955; Wilson *et al.*, 1961; Weissmann and Carpenter, 1969; Palumbo *et al.*, 1974). Luiz *et al.* (2004) also isolated *Salmonella* strains from four of the 30 samples of mechanically-deboned chicken meat used as a raw material in making a Frankfurt sausage and even in two of the 15 samples of sausage emulsion (13.33%) after the addition of preservatives. If sausages are cooked adequately prior to consumption, any *Salmonella* present would be killed and there would be no risk of food infection. Therefore, the delivery of adequate heat during the cooking of comminuted meat products is important to ensure food safety. However, Mattick *et al.* (2002) reported that *Salmonella* are not always killed during the cooking process (frying, grilling or barbecuing). The presence of *Salmonella* spp. in the sausage samples investigated in our study thus indicates the possible public health risk from eating sausages especially undercooked sausages. It also shows the need for close supervision of processing and sanitation practices.

Sausages spoiled within three to four days during aerobic storage at ambient temperature. At refrigeration temperature, the sausage samples spoiled after 12-16 days of storage. Temperature is a major factor in food deteriorative reactions; especially in microbial spoilage since specific growth rate and lag phase are highly temperature-dependent (Cayre *et al.*, 2003). All microbial groups (aerobic mesophilic bacteria, Enterobacteriaceae, LAB and yeasts) reached high counts on the day spoilage was detected. Bayne and Michener (1975) indicated that Frankfurters could be spoiled by non-hazardous spoilage microorganisms if they are subjected to temperature abuse. These organisms can survive the heat treatment in moderate numbers, and they can also be present as a result of post processing contamination.

In this study, even microbial groups (Enterobacteriaceae and yeasts) which were in low counts ($<2 \log \text{ cfu/g}$) on the purchase day of some sausages grew faster and reached high counts on the day spoilage was detected. In fact, the significant contribution of yeasts to sausage spoilage under aerobic and temperature-abused conditions was also observed by Samelis and Georgriadou (2000). LAB also grew faster and reached high counts in samples stored at ambient and refrigeration temperatures. The good growth and dominance of LAB in cooked meat emulsions stored under vacuum in high oxygen permeability films both at low and high temperatures was also observed by Cayre *et al.* (2005). Cayre *et al.* (2005) reported that independent of temperature and oxygen permeability, LAB counts increased from $\log 2$ to $\log 8 \text{ cfu/g}$ in cooked meat emulsions. Spoilage in our sausage samples was manifested as slime formation on the surface of casings and development of off-odor by the products. Jay (1996) indicated that from the slimy material of Frankfurters, microorganisms such as yeasts and lactic acid bacteria could be isolated. Comparable result was noted in taverna sausage sample stored in air, which showed green discoloration after 12 days of storage at 10°C (Samelis and Georgriadou, 2000). Sabbag *et al.* (2005) also reported that the maximum storage time of Frankfurters, determined with Deterioration Index Method (DIM) or sensory evaluation (odor, hardness, cohesiveness and springiness) and considering a microbial count of $4\text{-}5 \log \text{ cfu/g}$ as a limit of shelf life of Frankfurters, was 11–12 days either at 10 or 15°C . Off odor development was also noticed in emulsion type buffalo sausage

packed without any modification of atmosphere inside the pouches after 16 days of storage at 4°C (Sachindra *et al.*, 2005).

Our sausage samples were in edible condition for about ten days of storage at refrigeration temperature. Finnish Frankfurter sausages which were also stored aerobically at 5°C remained in edible condition for an average of 10 days (Pohja *et al.*, 1964). On the other hand, Marchello and Robinson (1998) stated that Frankfurters should be consumed within 7 days after opening the vacuum package. Data indicated that the shelf life of Frankfurter-type sausages under vacuum packaging was about 35–42 days at a storage temperature of 4°C (Blickstad and Molin, 1983).

The majority of large diameter sausage or mortadella samples in our study had pH values above 6.00. Mortadella samples which had pH values as high as 6.5 were also frequently encountered. Comparable pH values were reported by Abdullah (2004) for beef and lamb mortadella samples. Bologna type sausages sold in Turkey had also a mean pH value of 6.4 (Apaydin *et al.*, 2003). The pH values of such products can be expected to be relatively high, attributable to addition of sodium polyphosphate (Abdullah, 2004). This might make these products to be susceptible for microbial spoilage as well as pathogen growth as these pH values could allow the multiplication of bacteria, yeasts and molds. However, the moisture content values of the mortadella samples obtained in our study were markedly lower than moisture content values obtained in beef and lamb mortadella samples which had mean values above 60% (Abdullah, 2004).

The mean aerobic mesophilic counts of the different large diameter sausages or mortadellas were around log 5 cfu/g. The recommended reference value for the aerobic mesophilic count of ready-to-eat (RTE) foods was cited in several studies to be < 10⁵ cfu/g (Solberg *et al.*, 1990; Shapton and Shapton, 1991; Jay, 1996). The Japanese local prefectural government similarly defined RTE foods with < 5.0 log cfu/g as safe (Kaneko *et al.*, 1999). Thus, about 53% of pork, 48% of veal and 40% of chicken mortadella samples exceeded the typical aerobic mesophilic count value set for RTE food products. In contrast, 90% (n= 30) of the

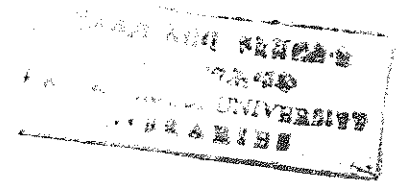
samples of bologna type sausage sold in Erzurum markets in Turkey had aerobic mesophilic counts $< \log 5$ cfu/g (Apaydin *et al.*, 2003).

The mean values of Enterobacteriaceae and coliform counts recorded for mortadella samples were relatively low but coliform counts were still higher than what was reported by Apaydin *et al.* (2003) for bologna type sausages sold in Turkey in which all of samples had coliform counts under detectable levels ($< \log 2$ cfu/g). In fact, heat treatment should eliminate all Enterobacteriaceae and coliforms during processing. Therefore, the presence of these microbial groups in our mortadella samples is indicative of either heat treatment below the appropriate temperature, or contamination thereafter.

Enterococci were also encountered in more than 60% of the different mortadella products, and at counts $> \log 4$ cfu/g in some samples. On the other hand, enterococci were not detected in any of the samples of bologna type sausages (n=30) sold in Turkey (Apaydin *et al.*, 2003).

More than 80% of pork, veal and chicken mortadella samples had staphylococci counts $\leq \log 4$ cfu/g. Thus, it may not be hazardous because counts $> \log 5$ cfu/g are required to elucidate enough enterotoxin to cause food poisoning. But if storage temperature is abused, this could reach to counts high enough to cause food poisoning. Actually, mortadella samples which had staphylococci counts $\geq \log 5$ cfu/g were also encountered. Since mortadella usually does not require further cooking for consumption, the presence of such high counts of staphylococci in some mortadella samples can be a risk factor for the consumers in terms of staphylococcal food poisoning.

The large diameter sausages investigated in our study harbored high counts of lactic acid bacteria with mean values as high as $\log 5$ cfu/g. These mean values were exceedingly higher than those reported by Apaydin *et al.* (2003) for bologna type sausage samples sold in Turkey in which 80% of the samples had counts $< 2 \log$ cfu/g. Although there was not observable spoilage on the purchase day of these sausages, such high counts of lactic acid bacteria may limit their shelf life. It is reported that, to increase the shelf life of vacuum or



modified atmosphere packed cooked meats, the first measure is to reduce post-heating contamination, mainly with LAB, as much as possible (Holley, 1997). The presence of such high counts of lactic acid bacteria in large diameter sausages might indicate insufficient cooking, since these bacteria could be inactivated by cooking. It might also indicate improper handling after processing or proliferation of the microorganism due to inadequate storage conditions.

The majority of pork and more than 60% of veal and chicken mortadella samples had yeast counts with mean values as high as $\log 3$ cfu/g. Furthermore, mortadella samples which had yeast counts $\geq \log 4$ cfu/g were also encountered very frequently. Yeasts are spoilage organisms in cooked meat products. Hence, the presence of such high counts of yeasts in our samples may shorten the shelf life of these products. In a study elsewhere, 67% of bologna type sausage samples had yeast and mould counts $< \log 2$ cfu/g (Apaydin *et al.*, 2003). This shows that, by employing the appropriate processing and storage conditions, yeast counts can be maintained at low levels.

With regard to the microbial quality of pork mortadella samples from the three producers, the mean values of counts obtained in this study indicated that there was no significant difference in the counts of aerobic mesophiles, staphylococci, LAB and yeasts among the products of the three producers considered. However, significant differences were detected in the counts of Enterobacteriaceae, coliforms and enterococci. Relatively, high counts of these microbial groups were obtained in samples from producer 1. This might indicate inadequate heat-processing or post-cooking contamination due to improper handling or storage practices in producer 1. In fact, the hygiene and sanitary conditions of the production site of producer 1, as observed during the visiting period, were far from satisfactory.

Mortadella products are usually sold sliced. Slicing might pose a microbiological risk because of the potential for recontamination via the slicing blade. It is reported that cooked meat slicing machines, if inappropriately cleaned, could be sources of contamination and cross-contamination (Little and de Louvois, 1998; Elson *et al.*, 2004).

Sliced mortadella samples spoiled within short period of time (within three to four days) during aerobic storage at ambient temperature. At refrigeration temperature, a pork mortadella sample spoiled after 12 days and a veal mortadella sample after 20 days of storage. Spoilage in these products was expressed as green discoloration and development of off-odor (especially buttery odor in the case of veal mortadella). It is observed that greening generally appears when an anaerobically stored meat product is exposed to air (Grant *et al.*, 1988). Both pork and veal mortadella samples had lower counts of aerobic mesophilic bacteria and Enterobacteriaceae ($< \log 2 \text{ cfu/g}$) on the purchase day. But the pork mortadella sample had very high counts of LAB ($\log 6.31 \text{ cfu/g}$) and yeast counts higher than the veal mortadella sample on the purchase day. Not considering other physicochemical characteristics, the presence of high initial count of LAB and yeasts in the pork mortadella sample may contribute to its faster spoilage than the veal mortadella sample. In fact, green discoloration in the pork mortadella sample was noticed on the 10th day of storage. Green discoloration can be caused by different members of LAB and enterococci (Borch *et al.*, 1988; Grant *et al.*, 1988). Jay (1996) stated that, in spite of the discoloration, the green product is not known to be harmful when eaten. On the 12th day, there was also an appearance of surface growing yeasts on the pork mortadella sample and, at this stage, the sausage was no more consumable. Usually mortadella slices are consumed immediately after purchase. They are not kept at ambient temperature for three or more days. Thus, microbial spoilage may not be a serious problem.

Salmonella spp. was isolated from two of the 90 mortadella samples (2.2%). This was much higher than that reported by Little and de Louvois (1998) in which *Salmonella* was isolated in two of 1488 (0.1%) samples of cooked sliced meats purchased from butchers' premises in the United Kingdom. Tompkin (1983) indicated that the presence of salmonellae in ready-to-eat meat or poultry products is potentially hazardous. To inactivate bacterial pathogens, it is recommended that a temperature of 70°C is achieved in all parts of the sausage for a minimum of 2 min or the equivalent (Gaze *et al.*, 1989). Thus, the detection of *Salmonella* spp. in the mortadella samples investigated in our study could indicate insufficient heat processing or post cooking contamination due to improper handling. Contamination may also occur in supermarkets via slicing blades. Therefore, the presence of *Salmonella* in

mortadella could indicate the possible public health risk from eating mortadella since this product is usually consumed with out further heating.

Generally, *Bacillus* spp. dominated the microflora of both small diameter and large diameter sausage or mortadella samples. The isolation of members of this genus from several raw and processed foods all over the world is attributed to their virtue of having resistant endospores that confer tolerance to adverse conditions and various stresses (Kramer and Gilbert, 1989). Palumbo *et al.* (1974) also indicated the dominance of Gram-positive spore forming rods in commercially processed Frankfurters. IOM (1985) described spices as possible sources of large numbers of aerobic spore forming bacteria to the sausage or luncheon meat emulsions. Micrococci constituted the second dominant group in all large diameter sausage samples and small diameter veal and chicken sausage samples. The dominance of micrococci in these sausages might be due to their heat resistance nature. Palumbo *et al.* (1974) indicated that the predominant organisms surviving the heating step were micrococci in Frankfurters produced in their plant which increased to substantial numbers in the product during storage at 5 °C. Micrococci have been shown to cause spoilage in packaged Frankfurters (Ayres, 1951).

Staphylococci were the second dominant group in small diameter pork and beef sausage samples and constituted the third dominant group in large diameter sausages. Staphylococci are the normal flora of many meat animals and they are also part of the normal flora of man, residing in nasal passage, throat, and skin (Baird-Parker, 1974 cited in Palumbo *et al.*, 1977). Because of this ubiquitous occurrence in nature, they are often found in various raw meats (Jay, 1962). The presence of staphylococci in raw meat and their known heat resistance suggest that they could be a problem in heat-processed meat products (Angelotti, 1961). It is reported that other microflora present in meat have an adverse effect on the growth of staphylococci and that staphylococci grow better in cooked meat and in fresh meat treated with salt. The later suppresses the growth of saprophytic microflora normally present in meat (Altabari, 1984).

Microorganisms such as streptococci, lactobacilli and Gram-positive non-spore forming rods were also detected in our sausage samples. IOM (1985) indicated that thermophilic non-spore forming bacteria such as Group D streptococci and some lactobacilli may survive the heat process. Generally, the microflora of both large diameter and small diameter sausages was dominated by Gram-positive organisms. In their study on the microflora of packaged Frankfurters and their radiation resistance, Drake *et al.* (1958) observed the dominance of Gram-positive organisms in Frankfurters. It may be difficult to have products with out Gram-positive contaminants, as most are abundant in the environment. But high counts are usually indicative of post processing contamination and inappropriate storage conditions.

The following could be said regarding the processing plants considered in this study. The compounds of the processing plants could be source of air-borne contaminants which could reach products carried by suspended soil particles. Apparently, managers and workers of the processing plants did not have some idea about microbial contamination of products and how the contamination could occur. Lack of sanitary maintenance of processing rooms and equipments, absence of precautions (such as wearing gloves and head covers, disinfecting shoes during stepping into production areas) in handling products could explain part of the problems related to high levels of contamination of the various products. Based on the observations done in this study, in order to improve the microbiological quality of the products, processing plant personnel should understand the following critical control points. i) raw materials can be excessively contaminated ii) additives such as spices may contain excessive numbers of aerobic spore forming bacteria that survive normal heat processing. Therefore, iii) appropriate time/temperature combination should be determined and strictly adhered to during processing (cooking, cooling, and storage) iv) processing equipments should be made to work in aseptic conditions and v) employees should follow strict sanitary practices during processing.

6. CONCLUSION AND RECOMMENDATIONS

Based on the findings obtained in this study, the following conclusions and recommendations are forwarded:

1. The majority of the samples we analyzed showed the presence of high microbial load, which indicated high contamination during or after processing of the products. High variability in the counts of all microbial groups within the sample of each sausage type might also indicate the lack of constant processing parameters and quality control during manufacturing and/or post-production handling of sausages.
2. This study also pointed out a possible microbial safety problem from eating certain sausages because they contained target pathogens. Chicken and pork mortadella samples were especially the products of concern as these products are usually consumed without cooking. Concerning small diameter sausages, effective cooking prior to consumption should be practiced to eliminate suspected pathogens.
3. Microflora of sausages was dominated by Gram-positive organisms in which *Bacillus* was the dominant species followed by *Micrococcus* and *Staphylococcus* spp. Although these genera are abundant in livestock environment, appropriate heating and storage conditions could markedly reduce them.
4. Although sausages are being produced on a commercial scale in Ethiopia, there are no standards established with respect to production methods and technology, food additives and their quantities, ingredients and microbiological quality. As Ethiopia is rich in livestock resources, this economic sector could be strengthened through using these resources effectively. Thus, standards and quality control systems should be developed by regulatory agencies to improve the food quality and safety to satisfy the local consumer as well as to develop products that meet export standards.

5. Occasional microbiological analysis should be done at each step of processing, namely, surfaces of equipment and utensils, hands of operators, condiment mixture, minced meat, sausage batter, stuffed sausage, and cooked sausage in order to set Critical Control Points (CCPs) in the sausage production system. Thus, HACCP method could be implemented to get safe products with good keeping quality.
6. None of the processing plants recommended the production date, the expiry date (sell-by -date) and the storage temperature for sausages. Some manufacturers did not even state the name of the specific sausage product. The consumer should be informed and instructed to follow the basic instructions regarding holding temperatures.
7. In general, the hygiene and sanitary conditions observed in the sausage factories were not very satisfactory. Most of the factories had no system of microbiological control of the end product, the raw material, or the other ingredients, at any stage of production. Moreover, in most factories the handling of products at all stages was carried out by people who did not follow basic sanitary precautions. Thus, factories should improve the sanitary conditions in sausage production process.
8. With regard to supermarkets, prevention of cross contamination (especially for large diameter sausages or mortadellas as these products were sold sliced) and proper cold storage practices are also essential.
9. The manufacturers should be monitored by regulatory agencies, regarding the sanitary condition of their processing and the quality of their products. In fact, it was observed that out of the five sausage manufacturers, only one was monitored by Region 14 Bureau of Agriculture.
10. There is a need to educate factory workers, supermarket employees, consumers, food handlers and all others who have access to the products about the importance of hygienic handling and appropriate storage systems of these products.



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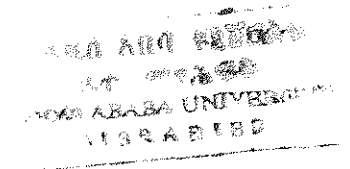
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