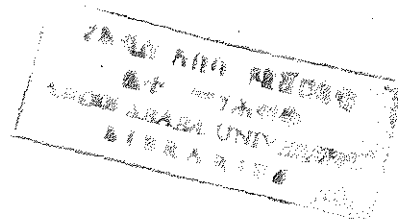


**ADDIS ABABA UNIVERSITY  
SCHOOL OF GRADUATE STUDIES**

**MICROBIOLOGICAL PROFILE AND SAFETY OF SOME STREET-  
VENDED FOODS IN ADDIS ABABA**

**BY**

**DIRIBA MULETA**



**A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES OF  
THE ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF  
THE DEGREE OF MASTER OF SCIENCE IN BIOLOGY (APPLIED  
MICROBIOLOGY)**

**JUNE, 1999**

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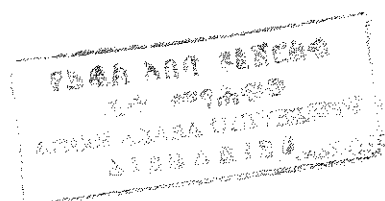
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## ABSTRACT

Microbiological profiles and safety of street-vended food collected from vendors in Addis Ababa, Ethiopia were determined in 1998 and 1999. The 150 samples of foods investigated consisted of *sambussa* (legume-based), cooked and sauced macaroni, lentil sandwich, *kitfo* (raw minced and spiced meat dish) and egg sandwich. All samples of *kitfo* and egg sandwich were held at temperatures that permit bacterial growth (15.5-24.5° C) and (15.5-34.5° C), respectively. Most samples of the other food products were stored at temperature range of 15.5-34.5° C. In general, *sambussa* had low counts of aerobic mesophilic bacteria and staphylococci. Aerobic bacterial spores, yeasts, members of Enterobacteriaceae, coliforms and *Bacillus cereus* were isolated rarely in *sambussa*. Highest aerobic mesophilic counts ( $> 10^8$  cfu/g) were recorded in egg sandwich. The other food products contained counts as high as  $10^7$  cfu/g. Presumptive *Bacillus cereus* attained greatest population level in macaroni samples ( $> 10^5$  cfu/g). In other dishes it had counts  $> 10^4$  cfu/g. Majority of the samples which were positive for aerobic bacterial spores, coliforms, Enterobacteriaceae, staphylococci and yeasts contained  $>10^4$ ,  $10^5$ ,  $10^5$ ,  $10^5$ , and  $10^4$  cfu/g, respectively. In the street foods considered in this study, moulds were either detected rarely or not at all. Variations in counts among the samples of the respective street foods were obvious (CV  $> 10\%$ ).

A total of 1552 bacterial isolates were screened from foods obtained from street vendors and were characterized into various genera and bacterial groups. Gram-positive organisms predominated (76.9%) along with *Staphylococcus* and *Micrococcus* species comprising 22.8% and 15% of aerobic microflora, respectively. From Gram-negative organisms, members of a family Enterobacteriaceae (14.5%) were frequently encountered. *Salmonella* was recovered from samples of egg sandwich and kitfo and macaroni yielded *Shigella*. Challenge studies showed that the test strains, *Salmonella typhimurium*, *Shigella flexneri* and *Staphylococcus aureus* grew in egg sandwich, *kitfo* and macaroni to markedly high levels. The faulty handling and serving operations, poor sanitation of the vending sites, time-temperature abuse during holding, addition of raw products either to cooked or uncooked dishes, the use of unclean multipurpose cloth and water of dubious quality (reused water) were deemed potentially hazardous. Thus, education of both vendors and consumers in basic principles of hygiene is an urgent issue by concerned bodies.

## 1. INTRODUCTION

In most countries the safety and quality of foods is becoming a matter of increasing concern. Man's food supply consists primarily of plants and animals and products derived from them. It is worthwhile to think of food safety as a commodity or service that can be purchased or sold. The inherent food safety problems are worsened by dependence on purchased foods of doubtful quality and safety and by increased population density.

Apparently, food themselves are frequently the source of contaminant microbes, as some foodstuffs naturally harbour notorious pathogens or may have been obtained from infected animals. Contaminated food and water are viewed as sources of various diseases in human societies since ancient times. Contaminants encompass a great variety of biological and chemical agents of disease. Thus bacteria, viruses, fungi, animal parasites and biotoxins are grouped under the former category. Microbes have highly diversified roles in foods. They are commonly encountered in foods principally as food-borne pathogens, bio-deteriorating, indicator organisms and normal contaminants (Aberra *et al.*, 1991). Nevertheless, the remarkable benefits obtained from some microbes cannot be overlooked.

At the very beginning, the number of microbes in food is often insufficient to pose food poisoning infection but the presence of adequate nutrients, moisture and appropriate temperature and other suitable conditions, together with longer holding time before consumption may increase their disease-producing potential (Roberts, 1982; Motarjemi and Nout, 1996).

In all countries, food-borne illness, particularly those caused by bacteria have deserved special attention. Evidently, such syndromes continue, more or less unnoticed, to pose morbidity, mortality and serious economic losses in both industrialized and economically disadvantaged countries (Abdulssalam and Kaferstein, 1994). However, the food-handling situation in developing countries is much more serious and threatens life because of poor sanitary conditions (WHO technical report series, 1984).

Cases of food-borne disease are well documented in the United States (Hedberg *et al.*, 1994). It has been estimated that number of cases of food-borne illness range from 6.5 to 81 million cases per year, with from 525 to greater than 7000 food-borne diseases associated with deaths per year. Moreover, economic losses to ill persons and other related problems cause the loss of national economy, which is estimated to be more than \$8 billion to \$23 billion annually. Similarly, in Africa, Asia (excluding China) and Latin America, about 1000 million cases of acute diarrhoea in children under the age of 5 years were reported (WHO technical

report series, 1984; Motarjemi and Nout, 1996), and over 3 million children died as a result. To date, contaminated foods (improperly handled infant formulas, in particular) are badly blamed for high incidence of diarrhoea in infants (Zelege and Haile Selassie, 1992; Wolde-Aregay and Mogessie, 1998).

Foods may come in contact with noxious pathogens from soil, water, domesticated animals, food handlers, food utensils, vermin, air and human and animal excreta in the environment (Frazier and Westhoff, 1978; Motarjemi and Nout, 1996). The marked health hazards that food-handlers pose vary widely. As a matter of fact, individuals to be in focus are people who handle food after pasteurization, cooking or processing. These include cooks and assistants in the kitchen, waiters, and vendors of unpacked or unwrapped commodities.

Street vendors are common in both developing and industrialized countries with a considerable expansion in the former. Urban markets have undergone expansion and diversification as social differences have increased and contributed to changes in consumption structure. To date, a significant proportion of individual food consumption occurs outside of the home, mainly with food provided by street vendors (Chauliac *et al.*, 1994; AgBendeche *et al.*, 1996; Oguntona *et al.*, 1998; Sobel *et al.*, 1998). Evidently, in large cities of developing countries, various food items of animal and plant origin are commonly vended at areas with busy economic activities and heavy movement of people (Bryan *et al.*, 1992a;

Mogessie, 1995; Roberts *et al.*, 1995; FAO, 1996, 1997; Van Kampen *et al.*, 1998). These areas include transportation centers, large construction sites, schools, factories, hospitals, churches, checkpoints and other similar business centers. Both male and female involve actively in the activities of street vending (FAO, 1990, 1996, 1997). Such informal type of economic sector provides considerable advantages both for vendors and consumers. However, the greatest impact it particularly has on formal economic sectors should not be undermined (FAO, 1996).

Multiple lines of evidence (WHO technical report series, 1984; Bryan *et al.*, 1992a; Mogessie, 1995) revealed that foods exposed for sale on roadsides may become contaminated by either spoilage or pathogenic microorganisms. Unequivocally, this constitutes a serious health hazard, particularly in economically disadvantaged countries where food surveillance centers are rudimentary or not there at all. Evidently, street vended foods have shown epidemiological links with illness (El-Sherbeeny *et al.*, 1985a; Saddik *et al.*, 1985; Abdussalam and Kaferstein 1993), and laboratory results have shown alarmingly high counts and presence of food-borne pathogens on street-vended foods (Arambulo *et al.*, 1994; Koo *et al.*, 1996; FAO, 1997; Freese *et al.*, 1998b). Furthermore, Faye and co-workers (1998) reported the transmission of intestinal parasites *via* street foods.

Studies made by FAO (1996) classified street foods into three groups. These encompass (1) foods prepared in small- or cottage-scale factories and brought to the street food stall for sale; (2) food prepared at the home of the vendor and brought to the street food stall for sale; and (3) foods prepared at the street food stall itself and sold. In Ethiopian cities and towns the first category includes mainly imported dry foods and packed beverages. The second and third categories are locally prepared following the traditional procedures and they are the focus of the present study.

In Ethiopia, various foods were reported to carry pathogens or allow the growth of pathogens (Mogessie, 1994; Mogessie and Yewelsew, 1996; Mezgebu and Mogessie, 1998; Wolde Aregay and Mogessie, 1998). Indeed, studies made by FAO (1997) in Africa, Asia and Latin America pointed out that the important aspect of street foods is their safety. The studies suggested that the problems associated with safety of street foods are real and need to be addressed to protect consumers. There is, however, limited information (Mogessie, 1995) on the microbial load and safety of street foods, in spite of the widespread use of such food items in Ethiopia. On the other hand, bacterial food-borne diseases pose considerable problems in countries like Ethiopia and thus call for a marked concern. Hence, there is strong need to generate data on the microbial load and safety of street foods in big cities such as Addis Ababa. Such information is useful to appreciate the safety problems related to street foods so that regulatory agencies

may take appropriate steps to improve safety and sanitation with respect to this economic sector. Therefore, the present study was initiated with the objectives to:

1. determine the microbial load of street foods,
2. identify and characterize the dominant isolates,
3. evaluate the bacteriological safety of food items sold by street vendors with regard to *Salmonella*, *Shigella*, *Bacillus cereus* and *Staphylococcus aureus*,
4. assess the growth potential of *Salmonella typhimurium*, *Shigella flexneri* and *Staphylococcus aureus* in some of these foods.

## **2. LITERATURE REVIEW**

### **2.1. Microbial activities in foods**

Food products serve not only as sources of nutrition for humans and other animals, but also as substrates for the growth of microorganisms. The uncontrolled growth of microorganisms in food causes spoilage, a serious problem accounting for sizable losses of food products. Spoilage microorganisms are those that can grow in food and cause undesirable changes in flavour consistency (body and texture), colour, or appearance (Hesseltine, 1983; Hayes, 1995). Also bacterial enzymes may effect drastic alterations that reduce the quality characteristics of foods and may render them ultimately unfit for human use. Ingram (1971) reported that some  $10^8$  bacterial cells per gram might be necessary to initiate detectable spoilage in foods over a number of days. Similarly, it has been suggested that spoilage produced in association with bacterial numbers substantially less than this figure is non-bacterial in origin.

Although microbial growth is a problem when it results in food spoilage, microorganisms are used beneficially for food production. The biochemical modification of primary products by active involvement of microorganisms and their enzymes is described as fermentation. Globally, fermented foods are essential components of diets (Hesseltine and Wang, 1980; Sanni, 1993). Likewise, a major portion of people's diet in Africa is obtained from fermented foods and beverages under the name of fermented traditional foods (Sanni, 1993). Many workers have

reported the enhanced nutritional value of fermented products as compared to the unfermented substances (Hesseltine and Wang, 1980; Hesseltine, 1983; Jay, 1992, 1996; Kelbessa and Keshava, 1998). The production and characteristics of enormous food products are based on fermentative roles of microorganisms. The intrinsic and extrinsic parameters largely determine the activities of fermenting organisms (Jay, 1992; 1996).

Fermentation is basically viewed as one of the most economical methods for human consumption (Motarjemi and Nout, 1996). Mixed-culture or spontaneous fermentation is the rule in African food fermentation, although it is not commonly recommendable (Sanni, 1993). Lactic acid bacteria (LAB) are considered to play a far-reaching role in various types of food fermentation under both industrial and traditional methods. Thus, lactic acid fermentation is a natural process brought about by the LAB naturally present in food or derived from a starter culture. Several investigators advocated the real benefits associated with Ethiopian food fermentation (Mogessie, 1993; Ayele *et al.*, 1997; Gulilat *et al.*, 1997; Fekadu *et al.*, 1998; Kelbessa and Keshava, 1998).

Some pathogenic microorganisms also contaminate foods and potable waters. Pathogenic microorganisms render foods harmful to humans by a variety of ways. According to one definition bacterial food poisoning refers to acute gastro-enteritis

resulting from the ingestion of food containing bacteria and/ or their toxins (Baird-Parker, 1971).

The current problem associated with spread of cholera in Latin America and Africa, and the difficulties encountered in its control, demonstrate very well the immensity of the task of combating food-borne and water-borne infections in economically disadvantaged countries (Abdussalam and Kaferstein, 1994). Epidemiological investigations have revealed that one of the main poses of food-borne diseases worldwide is time-temperature abuse during food preparation, leading to the survival and/or growth of contaminating pathogens or the production of toxin to disease causing levels (Motarjemi and Nout, 1996).

## **2.2. Street foods**

Street foods are ready-to-eat foods and beverages prepared and/or sold by vendors/hawkers especially in streets and other public places (FAO, 1990). Types of vending sites encompass stalls, a variety of push-carts, roadside stands, hawkers with head-load and other arrangements depending upon the ingenuity of the individual, resources available, type of food sold, and the availability of other facilities.

For the reasons need no mention, street foods have invaded areas of busy economic activity and heavy population concentration. These areas are places of heavy labour work, schools, hospitals, railway stations, bus terminals and other similar places.

### **2.2.1. Socioeconomic and cultural aspects of street foods**

Preparation and sale of street foods is not a new activity and nearly common to developing countries, and is also present in developed countries although it may not play the same traditional role. As people migrate from different parts of a country, the town centres also become crowded with various ethnic elements bringing along with their own cultural and social background including their own dietary habits (FAO, 1990; Sujatha *et al.*, 1997).

Extensive rapid urbanization parallels the growth of the informal sectors of economy (AgBendeck *et al.*, 1996). Thus, there has been a proliferation of street food vendors; itinerant hawkers and merchants through the cities of the developing world (Arambulo *et al.*, 1994; Sobel *et al.*, 1998). The advantages provided by street foods are that they (1) are inexpensive, (2) include variety and traditional foods, (3) are quickly served, (4) are ready to eat immediately, (5) may provide nutritionally balanced meals, and (6) are often quite tasty (FAO, 1990; Moy *et al.*, 1997). In this context, definitely in the absence of this informal economic sector the lives of low-income urban workers will be difficult.

### **2.2.2. Nutritional value of street foods**

The popularity of street foods has reached a new dimension for various reasons. They are available at the places where they are required. With more people joining the labour force, and working away from home, street foods are the most accessible source of food intake (Sujatha *et al.*, 1997). Tremendous factors have contributed to the remarkable expansion of street vending operations (FAO, 1990). These include (1) shortage of housing, (2) problems associated with cooking and cooling facilities, (3) increasing costs, and (4) difficulty in procuring fuel. Ethnic groups can procure their favorite traditional food items from street foods. Street foods also offer variety. Additionally, development and expansion in tourism trade have further enhanced the need for foods served outside homes.

Millions of people depend upon street foods either as vendors or consumers (FAO, 1997). However, it would be wrong to assume that only poor or low-income people eat street foods. On the contrary, because of the variety of food items served, almost all segments of the society visit street food vendors (FAO, 1996). In general, the regular customers are low-income people, middle class social stratum, students, commuters/visitors and office workers. The proportions of population that depend on street foods are well documented (FAO, 1990). In Kuala Lumpur (Malaysia) and Iloilo (Philippines) nearly 25% and 30%, respectively of household expenditure goes for street foods.

The nutritional value of street foods depends upon the ingredients used and how they are prepared, stored and sold. Several investigators have reported that freshly cooked traditional street foods provide a considerable source of various nutrients (Oguntona and Kanye, 1995; Gerbouin-Rerolle, 1996; Moy *et al.*, 1997; Solomons, 1997; Oguntona *et al.*, 1998). It has been found out that an average of 500 g meal contained 20 to 30 g of protein, 12 to 15 g of fat and 174 to 183 g of carbohydrate and provided approximately 2000 kcal (FAO, 1996). Similarly, street foods offered as much as 40% of total energy intake for city residents, especially among young people in Thailand. Generally, street foods contributed nearly half the energy intake of adults under 40 years of age.

### **2.2.3. Street foods as means of employment potential**

Today, street foods are among the best economic sectors that can offer a great potential for employment. In many cases, the business can be commenced with non-recurring minimum capital and little expertise. Street food establishment provides employment to lower educated individuals who may otherwise not be able to find appropriate jobs (FAO, 1996). According to FAO (1990) it is estimated that 100,000 food vendors are employed in Malaysia and a million in China in various aspects of street food business. Likewise, the magnitudes in India, Indonesia, Nigeria, Thailand and Peru would be great. Additionally, 40,000 to 50,000 people were engaged in street food sector in Senegal in 1979 while modern agribusiness and industry provided jobs for 6,800 individuals (FAO, 1990).

#### **2.2.4. Street foods as income source**

The street food sector, as a source of nutrition, place of consumption and important provider of income for many urban-dwelling individuals and poor families in developing countries is commonly recognized as an inevitable phenomenon tied to urban growth.

In Singapore, 100,000 street food meals are purchased every day (FAO, 1990). The profit obtained by street vendors is more than the minimum wage earned in the formal sector. Employees in the street food sector receive daily or weekly wages, while employees in the formal sector receive monthly wages. Evidently, the profit earned by the street vendors is considerable and differs from country to country and depends on the initial invested capital (FAO, 1990, 1996).

#### **2.2.5. Role of women and children**

Basically, the participation of women highly out-weighs that of men in the involvement of street food establishments. In fact, street food vending offers a major economic independence to women and has several significant positive social implications (FAO, 1990). Their active involvement includes not only in preparation of food but also in its marketing and sale. This advantage is attained due to low start-up capital requirements (as equipment is available at home), the arrangement of

household duties with small scale trading and the possibility of feeding their families at lower cost.

Urban children, too, are involved in street food vending practices as (1) workers, preparing food (often in home), (2) seller of food at fixed stalls, hawkers in the street or worker for an employer, and (3) a robust consumer. Sizable number of school children largely depend on street foods as sources of their nutritional requirements (Chauliac *et al.*, 1994; Oguntona and Kanye, 1995; Gerbouin-Rerolle, 1996; Oguntona *et al.*, 1998; Chauliac *et al.*, 1998).

### **2.3. Health hazards**

In spite of numerous advantages offered by street foods there are also several health hazards associated with this sector of economy.

Apparently, high counts of *E. coli*, coliforms and aerobic mesophilic bacteria have been encountered in foods collected from street vendors (Tjoa *et al.*, 1977; Chen *et al.*, 1985; Wu *et al.*, 1985; FAO, 1996; van Kampen *et al.*, 1998; Sobel *et al.*, 1998). Similarly, a large number of pathogenic microorganisms such as *Staphylococcus aureus* and *Bacillus cereus* have been detected in various types of street foods (Chen *et al.*, 1985; El-Sherbeeney *et al.*, 1985a,b; Saddik *et al.*, 1985; Sokari, 1991; Bryan *et al.*, 1992c). The presence of these organisms in significant numbers undoubtedly poses potential health hazard. Several studies have

demonstrated the prevalence of *Listeria* species, particularly *Listeria monocytogenes* in various types of ready-to-eat food items (Pinner *et al.*, 1992; Ryu *et al.*, 1992; Arumugasamy *et al.*, 1994; Wilson 1995; Hao *et al.*, 1998).

Thus, unsatisfactory or potentially hazardous levels of pathogens associated mostly with cooked meat. Street foods also act as main vehicle for transmission of severe and fatal diseases. Moreover, *Salmonella* and *Shigella* species have considerably been recovered from various dishes obtained from street vendors (FAO, 1990; Bryan *et al.*, 1992a, c; Mogessie, 1995).

More recently, several workers from Latin America (Arambulo *et al.*, 1994), from Manila (Lim-Quizon *et al.*, 1994) and from Guatemala (Koo *et al.*, 1996) have reported the marginal problems associated with cases of aggressive and fatal disease, cholera, whose possible transmission route was undoubtedly traced to consumption of foods served by street vendors. This type of informal sector of economy is not only accused of bacterial putative pathogens but also seriously incriminated in harbouring excess mycotoxins (FAO, 1996).

Several investigators claimed that street vendors prepared food from raw materials of doubtful quality, cooked or stored food in cheap utensils, held food at a temperature that would permit bacterial growth, used water of questionable hygienic quality, used packing materials that were not of food-grade quality, had no facilities

for waste disposal, used unclean utensils and were unaware of the basic importance of personal cleanliness (WHO technical reports series, 1984; El-Sherbeeney *et al.*, 1985a; FAO, 1990; Mogessie, 1995). Most of the time their products are vulnerable to gross contamination by flies, insects, rodents, other filth and dust.

According to FAO (1990), one of the main contributors to bacterial contamination is the cooking of foods which are supposed to be served hot, but are made up in advance and stored for a long period of time before being served. When used, they are not reheated to sufficient temperature to kill harmful microbes. Apparently, this can lead to very high total plate counts, as well as elevated coliform and fecal coliform counts if exposed to these bacteria.

Evidently, such badly processed and handled foods may serve as one possible source of diarrhoeal illness worldwide where such sectors are in common practice (Ekanem *et al.*, 1994; van Kampen *et al.*, 1998).

Majority of the street vendors have no access to clean potable water. Being compelled by circumstances, they simply reuse their water. Reused water may have dissolved organic material in it to serve as a "culture medium" (FAO, 1990; Bryan *et al.*, 1992c). Presumably, such water can potentially support the proliferation of noxious pathogens. In Ibadan, Nigeria, water is considered to be the major source of food contamination (FAO, 1990). Similarly, water stored for consumers' use by a

number of street food vendors showed heavy bacteriological contamination of fecal origin in Pune (India). Within the same locality water collected from a water container of a vendor was found to be contaminated with *Salmonella newport*.

In general, personal hygiene is unsatisfactory among the vendors. In Peru, 76% of the vendors did not meet the national standards set for clean clothes (FAO, 1990). On the other hand, in Nepal, it had been noticed that street dogs had been allowed to lick the food plates before they were washed.

Unequivocally, such faulty practices may lead to some epidemiological evidence of certain disease outbreaks. Actually, some morbidity and mortality cases are well documented (FAO, 1990). In Malaysia, the consumption of *loh shee yun* (rice noodles) bought from different hawkers resulted in 14 deaths in 1988. In the same year, in Hongkong, 300 individuals became ill following consumption of *choi sum* (green vegetables) which reportedly was caused by excessive pesticide residue.

Sometimes, it appeared that hazardous chemicals and additives, namely, unauthorized colourants and preservatives, have been detected in street foods (FAO, 1990; Abdussalam and Kaferstein, 1993). These practices of adulteration and falsification are common in some developing countries.

Heavy metal contamination in samples of street foods is another health problem which many workers have seriously addressed (Auermann and Bortiz, 1977; FAO, 1990, 1996).

All street foods, however, do not equally allow bacterial proliferation (Abdussalam and Kaferstein, 1993). Indeed, dry bakery products, dried grains and adequately sugared and acidulated foods are less likely to transmit disease than gravies, cooked rice and low-acid dairy, egg and meat products all of which can support bacterial growth under conducive conditions. However, foods that are thoroughly cooked and consumed on the spot are safe while precooked foods held at ambient temperatures of 15-45° C for more than about four hours present a considerable risk. On the other hand, highly coloured foods and beverages are more likely to contain unpermitted additives than others.

In most cases, food safety and nutritional aspects are not the main concern for the majority of the regular consumers. Rather, it is the taste and variety, appearance and price that play the vital role in selection of food by purchasers. Furthermore, presence of food at required times and locations for the buyers are other crucial factors (FAO, 1990, 1997).

### **3. MATERIALS AND METHODS**

#### **3.1. Sample collection**

A total of 150 samples of street foods comprising 30 each of *"sambussa"*, *"macaroni"*, *"lentil sandwich"*, *"kitfo"* (raw minced meat) and *"egg sandwich"*, were collected from different sale outlets in Addis Ababa, Ethiopia between August, 1998 and April, 1999. Samples were collected in sterile aluminum plates using aseptic techniques. Samples of whole meals were purchased during late breakfast, lunch or supper. Holding temperatures of each sample were measured at point of collection by inserting a sensing bulb of a laboratory thermometer until the temperature of the food item was stabilized. During sample collection close observations were made on manner of food handling, hygienic condition of food holding utensils, sanitary condition of vending sites, cleanliness of the vendors and availability of water and the way it was used. Furthermore, number of consumers per given time, major ingredients (sauce), duration of cooking process and time between preparation and consumption were recorded. All samples were immediately brought to the laboratory and microbiological analysis was conducted within 1-3 hours of collection.

#### **3.2. Enumeration**

Mixed portions from the whole meals (sample units) were weighed and 20 g were added to 180 ml Buffered Peptone Water (BPW) (Oxoid) and homogenized for 1-

3 minutes using a stomacher lab blender (model 400, Seward JAC, London). Appropriate dilutions were spread-plated on various types of solid media for microbial counts. In case the microbial load appeared to be lower than  $<10^2$  cfu/g, pour plate from  $1/10^{\text{th}}$  dilutions were considered for counting. Solid media were employed for spread-plating.

### ***3.2.1. Aerobic Mesophilic counts (AMC)***

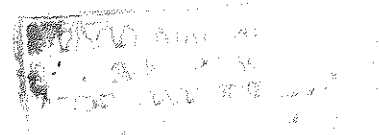
From appropriate dilutions 0.1 ml aliquots were spread plated in duplicates on pre-solidified surfaces of Nutrient Agar (NA) (Oxoid) plates.

### ***3.2.2. Aerobic bacterial spore count***

Homogenized samples were kept at  $80^{\circ}\text{C}$  for 10 minutes in a water bath to kill vegetative cells. Then 0.1 ml of appropriate dilutions were spread-plated in duplicates on pre-dried surfaces of Nutrient Agar (Oxoid) plates. The seeded culture plates were incubated at  $30-32^{\circ}\text{C}$  for 24-48 hours for colony counting.

### ***3.2.3. 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  $30-32^{\circ}\text{C}$  for 36 hours after which yellow colonies were counted as staphylococci.



#### ***3.2.4. Coliform counts***

From appropriate dilutions 0.1 ml aliquots were spread plated in duplicates on pre-solidified surfaces of Violet Red Bile Agar (Oxoid) plates. The seeded culture plates were incubated at 30-32° C for 20-24 hours after which purplish red colonies surrounded by reddish zone of precipitated bile were enumerated as coliforms.

#### ***3.2.5. Counts of Enterobacteriaceae***

From appropriate dilutions 0.1 ml aliquots were spread plated in duplicates on pre-solidified 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 precipitation were counted as members of a family Enterobacteriaceae.

#### ***3.2.6. Mould and yeast counts***

From appropriate dilutions 0.1 ml aliquots were spread plated in duplicates on pre-solidified Chloramphenicol Bromophenol Blue agar made from the following ingredients: yeast extract, 5 g; dextrose, 20 g; chloramphenicol, 0.1 g; bromophenol blue, 0.01 g; agar, 15 g; distilled water, 1000 ml; pH, 6-6.4). The seeded culture plates were incubated at 25-28° C for three to five days. During enumeration yeasts were distinguished from moulds by using stereoscopic microscope. Thus, smooth (non-hairy) colonies and without extension at periphery

(margin) were quantified as yeasts, whilst big, spreading and hairy colonies at margin and surface were counted as moulds.

### ***3.2.7. Bacillus cereus counts***

From appropriate dilutions 0.1 ml aliquots were spread plated in duplicates on pre-solidified surfaces of Phenol Red Egg Yolk Agar made from the following ingredients: peptone, 10 g; mannitol 10g; NaCl, 10 g; Lab Lemco, 1 g; phenol red, 0.025 g; agar, 15 g; egg yolk emulsion, 100 ml; distilled water 1000 ml, pH exactly 7.20 plates. To aseptically prepared egg yolk, equal amount of sterile physiological saline was added to constitute egg yolk emulsion. The seeded culture plates were incubated at 30-32° C for 24-48 hours after which rose colonies surrounded by zone of opacity counted as presumptive colonies of *Bacillus cereus*.

To check for the sterility, one plate was incubated at 37° C overnight from each batch of the solid medium used before inoculation was made.

### **3.3. Flora analysis**

After enumeration of aerobic mesophilic bacteria, about ten to twenty colonies were picked randomly from countable plates and inoculated into tubes containing about 5 ml Nutrient Broth No 2 (Oxoid). These were incubated at 37° C

overnight. Cultures were purified by repeated plating and were characterized to the genus level and various bacterial groups (Aneja, 1993) using the following tests.

### ***3.3.1. Cell morphology***

From overnight pure broth culture a hanging drop technique (Singleton, 1995) was employed. 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, tetrads
- Motility:            - Motile, non-motile

Spore staining was employed to check whether the isolate under question was endowed with endospore or not.

### ***3.3.2. KOH Test***

This test was performed as proposed by Gregersen (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 case of no slime and the watery suspension did not follow the loop, the reaction was negative and this was seen in Gram-positive bacteria.

### ***3.3.3. 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; K<sub>2</sub>HPO<sub>4</sub>, 0.2g; glucose, 10g; Bromothymol blue, 0.08g; agar, 2.5g; distilled water, 1000 ml, pH, 7.10.

The freshly prepared medium (15ml amounts in 18 x 180 mm test tubes) was immediately cooled under tap water and inoculated 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 37°C.

### ***3.3.4. Catalase test***

Young colonies were flooded with a 3% solution of H<sub>2</sub>O<sub>2</sub>. The formation of bubbles indicated the presence of catalase.

### ***3.3.5. 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%  $\alpha$ -naphthol in absolute ethanol
- B) 1% N,N-dimethyl-p-phenylene diammonium chloride in distilled water

After flooding the young (24 hours) 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.

## **3.4 Isolation and characterization of *Salmonella* and *Shigella* spp.**

### ***3.4.1. Primary enrichment***

To test for these genera Buffered Peptone Water was incubated at 37<sup>o</sup> C for 18-24 hours for the metabolic recovery and proliferation of cells in the food samples which could have been injured during cooking or to bring the number of target organisms to a detectable level.

### ***3.4.2. Secondary enrichment***

These broths were essentially important in that they inhibit non-targeted microorganisms like Gram-positive bacteria and coliforms and permit the rapid multiplication of *Salmonella* and *Shigella* spp. Selenite broth (SB) Tetrathionate

broth (TB) (both from Oxoid) and Rappaport-Vassiliadis (RVS) broth (Merk) were employed for this enrichment purpose. After pre-enrichment in BPW, 1 ml of culture was transformed into a tube containing 10 ml of selenite broth and into another tube containing an equal volume of TB. Moreover, 0.1 ml of BPW was also inoculated into a tube containing 10 ml of RVS broth. SB was incubated at 37° C and TB and RVS broth at 43° C for 48 hours in water both.

### **3.4.3. 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 selective enrichment broth was streaked separately on to each of the solid medium and incubated at 37° C for 18-24 hours.

Un-inoculated culture plates were incubated to check for sterility of the solid media. All colonies resembling *Salmonella* or *Shigella* were picked and further purified and a pure broth culture was used to inoculate the following biochemical tubes.

### **3.4.4. Biochemical Identification**

#### **3.4.4.1. Lysine Iron Agar (LIA) (Oxoid) slant**

The butt was stabbed and the slope was streaked to assess oxidative deamination of lysine on the slant and decarboxylation of lysine in the butt.

#### 3.4.4.2. Triple Sugar Iron Agar (TSIA) (Oxoid) slant

The butt was stabbed and the slant streaked to detect fermentation of glucose and lactose as well as production of H<sub>2</sub>S.

#### 3.4.4.3. Urea Agar (Oxoid) slant

The slant was heavily streaked to assess the hydrolysis of urea.

#### 3.4.4.4. Simmon's Citrate Agar (Oxoid) slant

The slant was streaked to investigate utilization of citrate as a sole source of carbon.

#### 3.4.4.5. SIM Medium (Oxoid)

This medium was stabbed for the determination of H<sub>2</sub>S production and motility. Production of indole was also investigated by adding Kovac's reagent to growth in this culture medium.

The following broths were inoculated to check for the utilization of mannitol, glucose or sucrose.

#### 3.4.4.6. Mannitol Broth (1%)

Ingredients: Peptone, 10g; mannitol, 10g; NaCl, 5 g; phenol red, 0.024g; distilled water, 1000ml, pH 7.2.

#### 3.4.4.7. Glucose Broth (1%)

Ingredients: glucose, 10g; peptone, 10g; NaCl, 5g; phenol red 0.024g; distilled water, 1000ml, pH 7.2.

#### 3.4.4.8. Sucrose Broth (1%)

Ingredients: sucrose, 10g; peptone, 10g; NaCl, 5g; phenol red 0.024g; distilled water, 1000ml, pH 7.2.

To detect fermentation and gas production from each biochemical sugar broth air negative inverted Durham tube was added before autoclaving and inoculation of the broth media in the tubes.

To ascertain sterility of the media for biochemical tests, all tubes with media were pre-incubated at 37<sup>o</sup> C for 18-24 hours. Biochemical tubes were incubated at 37<sup>o</sup> C for 18-24 hours. To check for the purity of broth inocula and reliability of biochemical tests, each broth inoculum was subcultured on MacConey Agar plates.

### **3.5. Determination of growth potential**

The growth dynamics of *Salmonella typhimurium*, *Shigella flexneri* and *Staphylococcus aureus* was assessed in "egg sandwich", "lentil sandwich" and "macaroni". These food items were pasteurized to kill any *Salmonella*, *Shigella*

and *Staphylococcus*, which might be present in the items. Then 100g of each street food was challenged separately with overnight culture of the test strains to give an inoculum level of  $10^2$  -  $10^3$  cfu/g. The challenged foods were mixed thoroughly and incubated at ambient temperature for 24 hours. To investigate the initial inoculum level, freshly inoculated foods (10g each) were homogenized separately in 90ml of BPW and 0.1ml of appropriate dilutions were spread plated on SS Agar (Oxoid) and Mannitol Salt Agar (Oxoid). SS Agar was used for counts of *Salmonella* and *Shigella* while Mannitol Salt Agar was for *S. aureus*. Portions of challenged food items (10g each) were further sampled aseptically at 4 hour intervals from 0-24 hours.

To see if there was variation in counts among samples, coefficient of variation (CV) was calculated by dividing the standard deviation by the mean.

## 4. RESULTS

### 4.1. The street vendors

The street vendors made various types of foods of animal and plant origin available to consumers. A total of 150 street vendors were considered in this study of which 80% were female (Table 1). The age of the vendors ranged from 9 to 49 years, (mean of  $28.48 \pm 12.49$ ). The younger females (age 5-15 years) sold mainly "*lentil sandwich*" samples and those above 36 years of age usually sold "*kitfo*". Those in between sold "*egg sandwich*" samples and "*macaroni*". The males consisted a fifth of the vendors and most sold "*sambussa*".

### 4.2. Description of street-vending practices

#### 4.2.1. "*Sambussa*"

"*Sambussa*" was prepared wrapping well cooked lentil, sliced green pepper, garlic and onion with dough and then frying it in small metal pan in oil. A piece weighed about 15.3 g. "*Sambussa*" was prepared at home and brought to vending site at around 10:00 a.m and kept there till sold. At about 4:00 p.m another new batch was added and displayed until sold or returned to the place of operation for over-night storage. The stalls were around "Markato" at the sidewalk of a main street where a large number of vehicles and people passed; inside temporarily constructed shelters which were located between a big mosque and church; and at centres with high concentration of people. The small temporary shelter was

constructed from cheap piece of polyethylene material, unclean sacks and other patched and worn out clothes.

**Table 1.** Age and sex distribution of street vendors

Type of food	Age (years)										
	5-15		16-25		26-35		36-45		46-55		
	M	F	M	F	M	F	M	F	M	F	
"lentil sandwich"	0	22	0	1	0	0	0	6	0	1	30
"egg sandwich"	0	4	0	18	1	7	0	0	0	0	30
"kitfo"	0	0	0	0	0	1	0	19	0	10	30
"sambussa"	2	1	7	7	2	1	7	0	3	0	30
"macaroni"	4	0	0	4	2	10	0	7	2	1	30
Total	6	27	7	30	5	19	7	32	5	12	150

M = Male  
F=Female

Some vendors displayed their food items on unclean oiled newspaper and bearing no cover for the purpose of attracting the attention of possible buyers. Mobile vendors held their sell items in side big baskets with paper cover. In most cases, "*sambussa*", once brought to the vending site, was displayed with no cover until the termination of the selling process. The vendors' hands and clothes were stained with frying oil. The majority of the vendors (93%) were serving their "*sambussa*" with their hands. Few used long pointed sticks. On the average about 23 consumers were each served with a whole meal weighing about 15.3 g per day per vendor. One "*sambussa*" meal was sold for 40 to 80 Ethiopian cents.

#### 4.2.2. "*Macaroni*"

Street vendors prepared "*macaroni*" by adding it to boiling water and cooking it for 10 to 15 minutes. During serving, it was mixed with tomato-based sauce. A single serving weighed about 270 g. Home cooked "*macaroni*" was brought to the vending station for sale where its sauce was prepared. The samples were collected from various transient vending operation around "Legehar", closest to a big garage where car washing activities by labourers was intensively taking place with water of questionable quality; thus, the site of vending was always wet and muddy, near a big grain store where a large number of trucks waited for loading and unloading; and at

the Foot Ball Stadium; and around "Piazza" at mini-bus terminals. At vending operation sites, "macaroni" and its sauce were separately held in a big plastic bucket and pan (both with lids), respectively. The food was transferred onto a small plastic tray by ladle and then sauce was added with a large spoon. The food was served to consumers and forks were supplied. The food items were made available to consumers as breakfast (6:00 - 10:00 a.m.), lunch (12:00-4:00 p.m.), and dinner (5:00-7:00 p.m.). The unsold left-overs were transferred to the next batch. Food serving utensils were soaked and rinsed in water, which had been used repeatedly for this purpose, before they were again washed for the next round of service. A large number of flies were landing on the basin containing cleaning water. On the average a vendor served about 23 consumers per day. Vendors' clothes, hands, food serving and holding utensils were of questionable hygienic quality. The price of a "maccaorni" meal varied between Birr 1.50 and 3.00.

#### 4.2.3. "Lentil sandwich"

These sandwiches were prepared by cooking whole lentils very well and mixing them with finely crushed garlic, ginger and salt. A sandwich normally weighed about 400g. The home prepared food was carried inside a large bowl and covered with a piece of polyethylene. It was frequently exposed during selling and buyers were allowed to choose for any particular sandwich of their liking. Vending sites and some other operations were shared with "kitfo" vendors. Around "Kotebe" and "Cherkos",

the street vendors moved from one place to another with their food items to search for customers. Some other vendors brought bread and hot spiced lentil (sauce) separately to the vending site ('*tej*' houses around "Cherkos"). The operation of stacking was performed upon request. Addition of onion (raw), slices of green pepper (raw) and chili was not uncommon. And yet others displayed their commodity at 'Karra' checkpoints where the food items were placed on serving plates with no cover to attract the attention of consumers. The majority of street vendors used neither spoon nor fork to serve their food items. After usage, utensils were simply wiped with a piece of unclean cloth and washing of food holding and serving utensils between each serving was not common among the vendors. About 21 individuals were served a whole meal in about 7 hours per day per vendor. The price of a sandwich varied between 50 cents and Birr 2.50, depending on size of meal and other factors.

#### 4.2.4. "*kitfo*"

Street vendors prepared "*kitfo*" by chopping raw lean meat on a cutting board and adding powdered chili to which a variety of spices were added. Vendors did not add the usual spiced traditional butter to it. A single serving weighed about 220 g. "*Kitfo*" was prepared at home (12:00 a.m) and brought to stall outlet at 2:00 p.m and kept there till sold (8:00 p.m). The vending environment was at the entrance of a big '*tej*' house around 'Cherkos', a few meters away from a pit latrine. The room had

the capacity to accommodate about 200 customers at any one time, and was usually quite crowded. The food was held in side a large pan with lid and placed on wooden table for sale. The container was frequently exposed and sometimes left uncovered for about a quarter of an hour during serving period. Addition of pieces of raw onion, green pepper and dry powdered chili was common practice. *"kitfo"* was served to consumers on small plastic tray with spoon. The customers used either their hands (unwashed) or spoon to eat. After usage, food serving utensils were wiped with unclean piece of cloth now and then to make them ready for the next round. The utensils were visited by a dozen of flies. On an average, 20 customers were served with meal in 7 hours per day per vendor. The price of a *"kitfo"* meal varied between Birr 1.25 and 4.00.

#### 4.2.5. *"Egg sandwich"*

Street vendors prepared *"egg sandwich"* by mixing thoroughly contents of whole egg and adding salt, oil and some water before frying contents on a flat hot pan. A piece weighed about 11 grams. This food item was prepared at home (around 5:00 a.m) and brought to the stall for sale between 6:00 and 10:00 a.m. The vending operation site was at „Karra“check-point. *"Egg sandwich"* were held in small plastic trays and wrapped with reused polyethylene bags. They were displayed at the sidewalk of the main road to get the attention of customers. The fried eggs and sliced bread were carried separately. At the vending station, the fried eggs were inserted between slices

of bread by hand upon request for purchase. The vicinity of food vending was quite dusty and crowded due to the large number of buses and passengers at the checkpoint. Vendors' clothes, hands, food holding and serving materials were unhygienic. Most of the time customers were permitted to serve themselves. On the average a vendor sold about nine sandwiches in five hours every day. The price of one "egg sandwich" varied between Birr 1.50 and 2.50.

Additionally, none of the vendors had an appropriate waste disposal area, effective hair covering material, cooling facilities, municipal water from taps and other facilities at a point of vending operation. All the food items investigated were prepared following the traditional procedures.

#### **4.3. Storage temperature of street foods**

The various food items displayed were held at different temperatures. About 60% of the samples showed temperature range of 15.5<sup>o</sup> C to 24.5 <sup>o</sup> C, and about 30% showed temperature range of 25.5 <sup>o</sup> C to 34.5 <sup>o</sup> C. The remaining were between 35.5 <sup>o</sup>C and 54.5<sup>o</sup> C at the time of sampling (Table 2 ). Over 90% of these samples were stored at temperature range of 15.5 to 34.5<sup>o</sup> C.

#### **4.4. Microbial spectrum of street foods**

Over 61.3% of the street food samples had aerobic mesophilic bacteria counts higher than 10<sup>7</sup> cfu/g (Table 3). A total of 1552 bacterial isolates were isolated from street foods and were characterized to various genera and bacterial groups. Different genera

constituted the dominant microflora (Table 4). Gram-positive organisms dominated (76.9%), the majority being *Bacillus* spp. (29.1%) followed by *Staphylococcus* (22.8%) and *Micrococcus* spp. (15.4%). Among the Gram-negative organisms, members of the family Enterobacteriaceae (14.5%) were frequently encountered.

#### 4.4.1. "Sambussa"

The microbial load of "sambussa" was low and 27 of the 30 samples had counts lower than  $10^6$  cfu/g (Table 3) and 90% of these were held at temperatures ranging from 15.5° C to 34.5° C (Fig 1). Coliforms and Enterobacteriaceae had lower counts at these holding temperatures (Fig. 1). Twenty three and 21 of the samples yielded aerobic mesophilic bacteria and staphylococci, respectively (data not shown). Yeasts and bacterial spores were encountered in 1/3 of the samples.

Over 26 of the 30 samples had Gram negative rods below detectable levels ( $<10^2$  cfu/g). The mean counts of aerobic mesophilic bacteria did not exceed  $1.6 \times 10^5$  cfu/g (Table 5 and 6) and these were dominated by *Bacillus* spp. (43.8%) followed by other Gram positive rod (22.5%) (Table 4). Similarly, the count for staphylococci was limited to not more than  $2 \times 10^4$  cfu/g. For enumerated values, variation within „sambussa“ samples was significant (CV >10%) (Tables 5 and 6).

#### 4.4.2 "Macaroni"

The "macaroni" samples had high microbial load as 22 of the 30 samples yielded counts between  $10^7$  and  $10^8$  cfu/g (Table 3). Most of the samples with higher counts were held at a wider temperature range and total counts as well as counts of coliforms

and Enterobacteriaceae were  $>10^6$  cfu/g at these holding temperatures (Fig 2). The higher counts, including those of coliforms and Enterobacteriaceae were encountered in those samples stored between 15.5°C and 34.5°C (Fig 2). Twenty nine samples of "macaroni" contained high aerobic mesophilic counts ranging from  $3.2 \times 10^6$  to  $2.9 \times 10^9$  cfu/g. *B. cereus* counts were more than  $10^5$  cfu/g. Bacterial spores, coliforms, Enterobacteriaceae, staphylococci and yeasts each contained  $> 10^4$ ,  $10^5$ ,  $10^6$ ,  $10^5$ , and  $10^4$  cfu/g, respectively (Tables 5 and 6). Counts within "macaroni" samples revealed less variation (CV  $< 11\%$ ) for aerobic mesophilic bacteria and yeasts. Nevertheless, counts of *B. cereus*, bacterial spores, coliforms, Enterobacteriaceae and staphylococci showed significant variation among samples (CV  $> 12\%$ ) (Tables 5 and 6). The aerobic mesophilic flora of "macaroni" was mainly dominated by *Bacillus* spp. (54.8%) followed by members of the Enterobacteriaceae (17.6%) (Table 4). The holding temperature for the majority of the "macaroni" samples ranged from 15.5°C to 34.5°C and, at these holding temperatures, most samples had counts  $>10^6$  cfu/g (Fig 2).

Table 2. Holding temperatures of the various street foods.

Food Type	No samples	Temperature range ( °C)			
		15.5-24.5	25.5-34.5	35.5-44.5	45.5-54.5
"egg sandwich"	30	27	3	0	0
"kitfo"	30	30	0	0	0
"lentil sandwich"	30	6	17	6	1
"macaroni"	30	15	14	0	1
"sambussa"	30	14	13	0	3
Total	150	92	47	6	5

Table 3. Frequency distribution of microbial load in street foods. Numbers in the parentheses represent percentage

Food type	Number samples	Aerobic mesophilic count (cfu/g)						
		$<10^3$	$10^4 - 10^5$	$10^5 - 10^6$	$10^6 - 10^7$	$10^7 - 10^8$	$10^8 - 10^9$	$>10^9$
"sambussa"	30	7	11	9	1	1	1	-
"macaroni"	30	1	-	4	-	10	12	3
"lentil sandwich"	30	1	-	1	8	18	1	1
"kitfo"	30	-	-	-	9	9	8	4
"egg sandwich"	30	-	-	-	6	8	4	12
Total	150	9 (6)	11 (7.3)	14 (9.3)	24 (16)	46 (30.7)	26 (17.3)	20 (13.3)

#### 4.4.3 „Lentil sandwich“

The microbial load of „lentil sandwich“ was also high and 20 of the 30 samples had aerobic mesophilic bacterial counts higher than  $10^7$  cfu/g (Table 3). *B. cereus*, counts ranged from  $10^3$  to  $10^4$  cfu/g, coliforms and Enterobacteriaceae from  $10^4$  to  $10^7$  cfu/g, and staphylococci from  $10^4$  to  $10^6$  cfu/g (data not given).

Counts of aerobic mesophilic bacteria, coliforms and Enterobacteriaceae did not show significant variation among samples (CV < 10%) (Tables 5 and 6). Significant variations were, however, observed in counts of *B. cereus*, bacterial spores and yeasts (CV > 16%). Members of the family Enterobacteriaceae (28.4%) dominated the microflora of „lentil sandwich“ followed by *Bacillus* species (21.1%) and staphylococci (19.9%) (Table 4). Over 75% of these food products were stored within temperature range of 15.5 to 34.5°C (Fig 3).

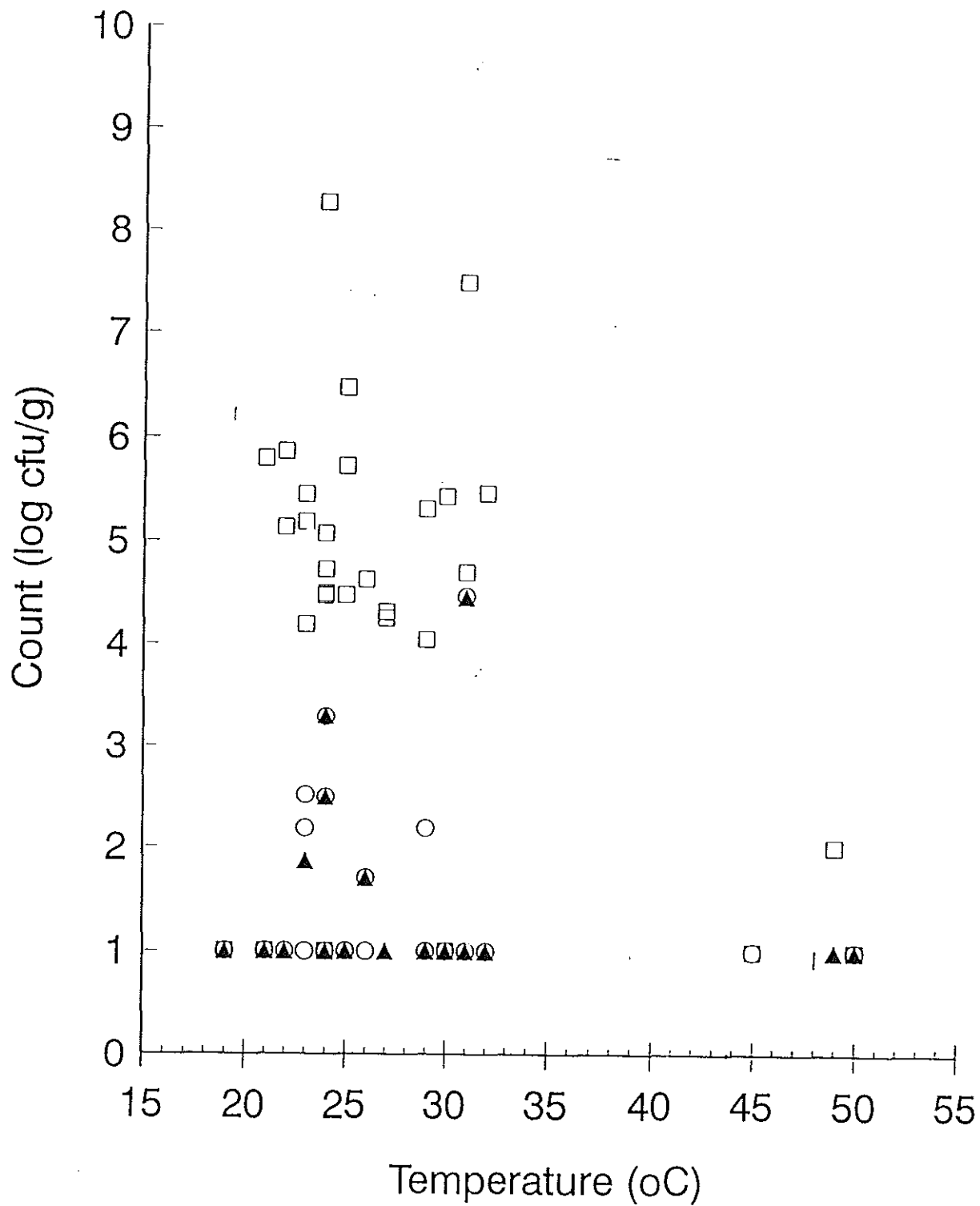


Fig 1. Holding temperature and microbial count relations in *Sambussa* samples (Aerobic mesophilic count (squares), coliforms (triangle) and Enterobacteriaceae (circle))

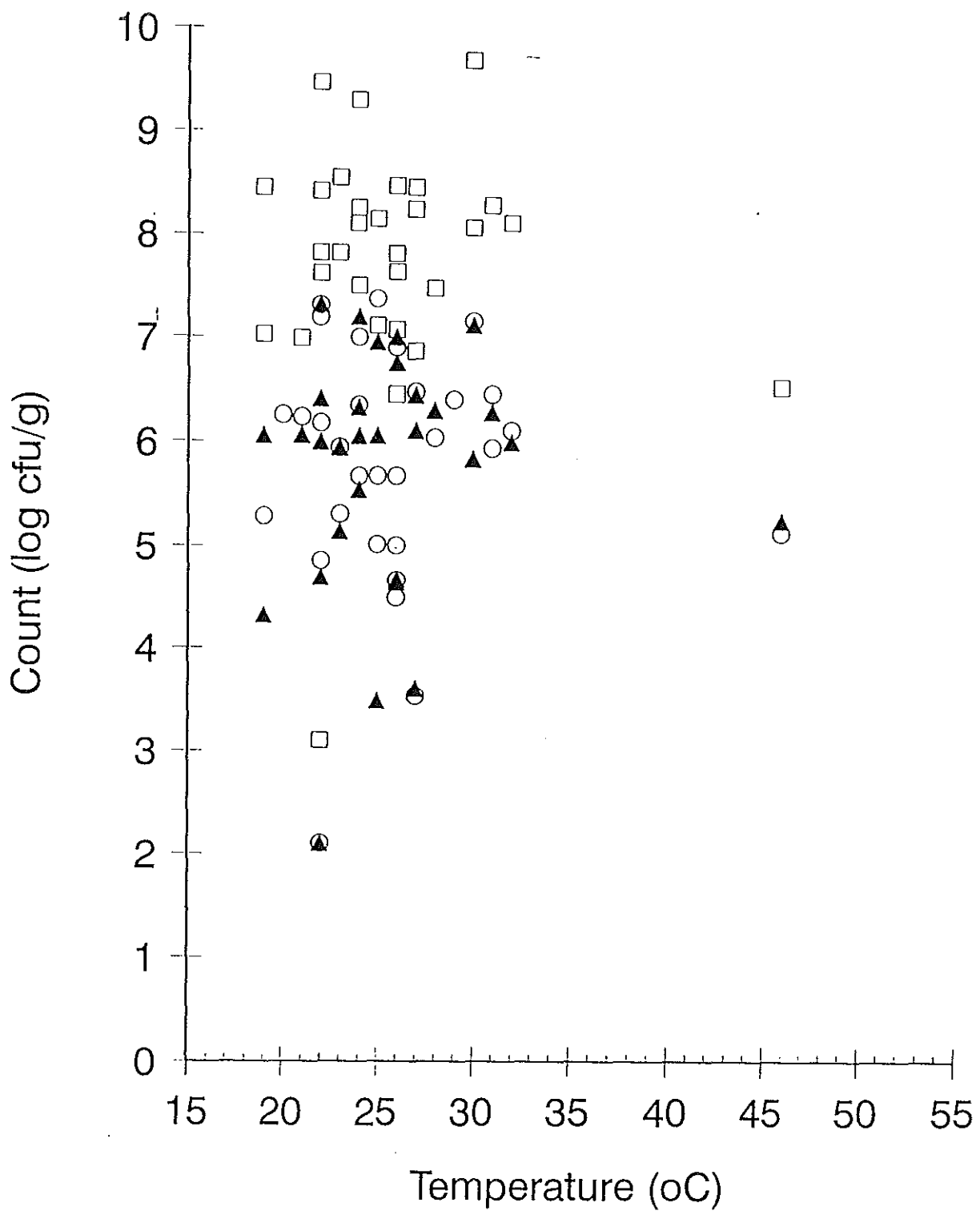


Fig 2. Relationship between holding temperatures and microbial counts in macaroni samples. (Aerobic mesophilic count (square), coliform (triangle) and Enterobacteriaceae (circle))

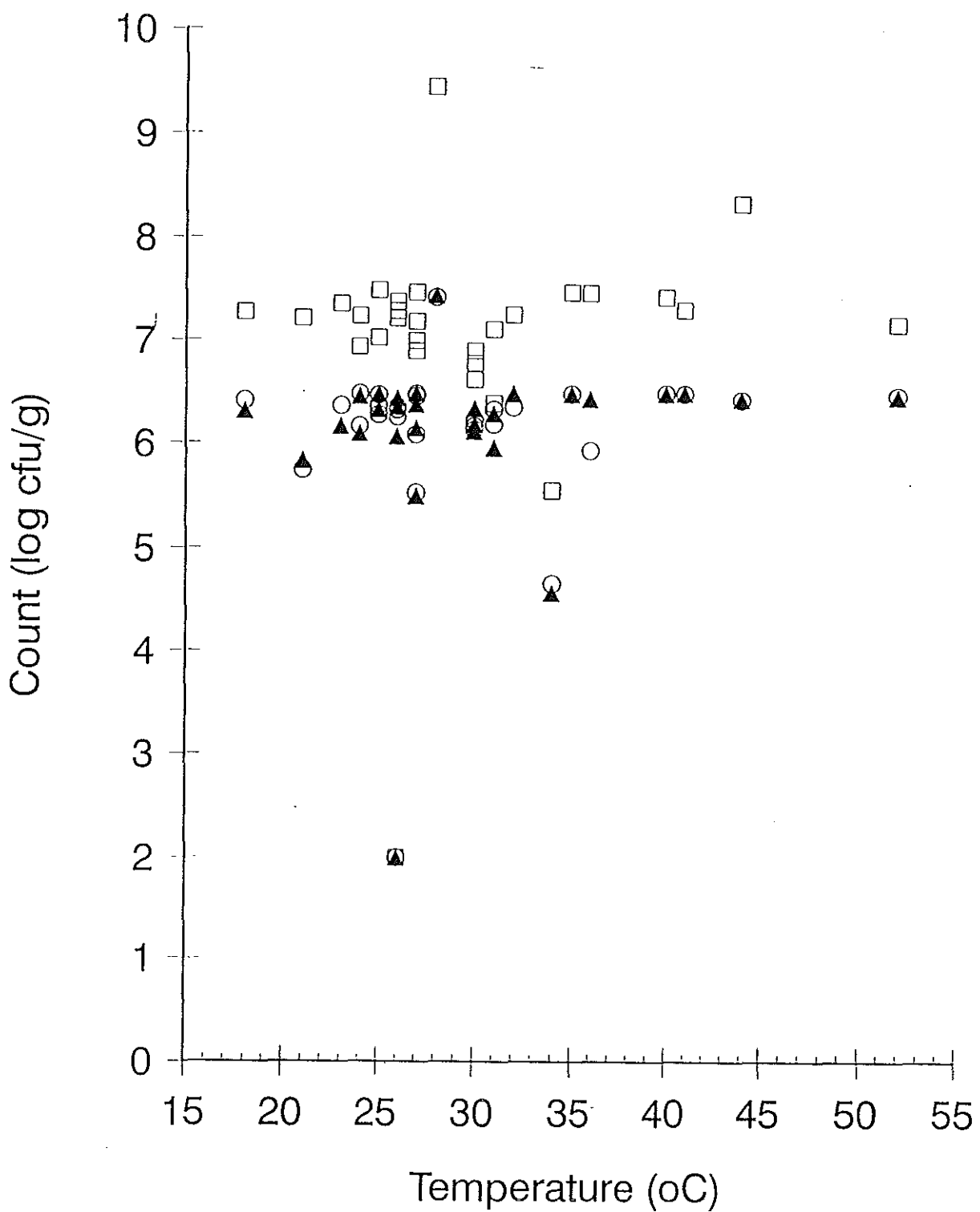


Fig 3. Holding temperature and microbial count relations in lentil sandwich samples (Aerobic mesophilic bacteria (square), coliforms (triangle) and Enterobacteriaceae (circle))

**Table 4.** Frequency distribution of dominant bacteria in foods collected from street vendors

Food type	Mean storage temp. (°C)±SD	No of isolates	<i>Bacillus</i>	<i>Staphylococcus</i>	<i>Micrococcus</i>	Enterobacteriaceae	Other gram +ve rods	<i>Pseudomonas</i>	<i>Alcaligenes spp.</i>	<i>Acinetobacter spp.</i>	<i>Aeromonas spp.</i>	<i>Flavobacterium spp.</i>
"sambussa"	27.8± 7.5	240	105(43.8)	41(17.1)	30(12.5)	2(0.8)	54(22.5)	2(0.8)	4(1.7)	-	2(0.8)	-
"macaroni"	24.9±5.1	312	171(54.8)	12(3.8)	6(1.9)	55(17.6)	37(11.9)	9(2.9)	7(2.0)	9(2.9)	6(1.9)	2(0.3)
"lentil sandwich"	29.7±7.3	331	70(21.1)	66(19.9)	50(15.1)	94(28.4)	2(0.6)	25(7.6)	13(3.9)	5(1.5)	3(0.9)	3(0.9)
"kitfo"	21.2±0.9	344	55(16)	132(38.4)	51(14.8)	55(16)	15(4.4)	10(2.9)	7(2)	20(2.9)	9(2.6)	-
"egg sandwich"	21.6±2.1	325	51(15.7)	103(31.7)	102(31.4)	19(5.8)	40(12.3)	-	10(3.1)	-	-	-
Total		1552	452(29.1)	354(22.8)	239(15.4)	225(14.5)	148(9.5)	46(3)	41(2.6)	24(1.5)	20 (1.3)	5(0.3)

Numbers in parentheses represent percentage of isolates

#### 4.4.4. "Kitfo"

All "kitfo" samples had counts higher than  $10^6$  cfu/g and 12 of the 30 samples had counts greater than  $10^8$  cfu/g (Table 3). The mean aerobic mesophilic counts of "kitfo" samples ranged between  $3.3 \times 10^6$  cfu/g and  $1.96 \times 10^9$  cfu/g. The mean counts of *B. cereus*, coliforms, Enterobacteriaceae, and staphylococci considerably exceeded  $10^4$ ,  $10^5$ ,  $10^6$ ,  $10^6$ , and cfu/g, respectively (Tables 5 and 6). Variation in coliform counts among "kitfo" samples was not significant (CV < 8%). Other counts, however, showed significant variations among samples (CV = 12-23%). The microflora of "kitfo" samples collected from street vendors were markedly dominated by various bacterial genera, *Staphylococcus* spp. being the most dominant (38.4%), followed by *Bacillus* spp. and members of the Enterobacteriaceae (16% each) (Table 4). The temperatures at which this food samples were held were quite different from the other food items. Twenty-eight of the thirty "kitfo" samples were held between 21°C and 30°C, and their counts at these holding temperatures ranged between  $10^6$  and  $10^9$  cfu/g (Table 7).

#### 4.4.5. "Egg sandwich"

All "egg sandwich" samples had counts  $>10^6$  cfu/g and 12 of the 30 samples had counts  $>10^9$  cfu/g (Table 3). Various types of microflora were detected with considerably high counts in this food product. The mean counts of aerobic mesophilic bacteria ranged between  $1.3 \times 10^6$  cfu/g and  $2.8 \times 10^{10}$  cfu/g. Notably

high counts were seen for coliforms ( $10^5$  cfu/g), members of the Enterobacteriaceae ( $10^5$  cfu/g) and staphylococci ( $10^6$  cfu/g). Members of the genus *Staphylococcus* (31.7%), *Micrococcus* (31.4%), *Bacillus* (15.7%) and other Gram-positive rods (12.3%) dominated the microflora in "egg sandwich" (Table 4). Significant variations were noted in the counts of all microbial groups among the samples of "egg sandwich" (CV = 15-26%) (Tables 5 and 6). "Egg sandwich" was held at temperatures below 30° C and, at this holding temperature, 19 of the 30 samples had counts higher than  $10^8$  cfu/g (Table 7).

Moulds were encountered only in 3 "egg sandwich", 11 "kitfo", 7 "lentil sandwich" and 2 "sambussa" samples at counts lower than  $10^4$  cfu/g (data not shown).

**Table 5.** Counts (log cfu/g) of Aerobic mesophilic bacteria (AMB), *Bacillus cereus* (BC), Bacterial spores (BS) and Coliforms (CF) in foods collected from street vendors in Addis Ababa

Food Type	No Sample	Aerobic mesophilic count			<i>Bacillus cereus</i>			Aerobic bacterial spores			Coliforms		
		$\bar{X}$	SD	%CV	$\bar{X}$	SD	%CV	$\bar{X}$	SD	%CV	$\bar{X}$	SD	%CV
"sambussa"	30	5.21	1.1	21.11	5.5			3.17	0.83	26.18	3.41	0.94	27.57
"macaroni"	30	7.93	0.80	10.09	5.3	0.84	15.85	4.4	0.71	16.14	5.85	1	17.09
"lentil sandwich"	30	7.20	0.63	8.79	4.17	0.73	17.51	4.29	0.87	20.28	6.25	0.45	7.26
"kitfo"	30	7.71	0.94	12.19	4.71	1.11	23.57	4.62	0.48	10.39	5.95	0.44	7.39
"egg sandwich"	30	8.38	1.31	15.58	4.57	1.15	26.16	4.57	1.05	22.98	5.43	1.18	21.73

$\bar{X}$  = mean value of counts, SD = standard deviation, %CV = percent coefficient of variation

**Table 6.** Counts (log cfu/g) of Enterobacteriaceae, Staphylococci, Yeasts, and incidence of *Salmonella* and *Shigella* in foods collected from street vendors in Addis Ababa

Food type	No sample	Enterobacteriaceae			Staphylococci			Yeasts			Number positive for	
		$\bar{X}$	SD	%CV	$\bar{X}$	SD	%CV	$\bar{X}$	SD	%CV	<i>Salmonella</i>	<i>Shigella</i>
"sambussa"	30	3.19	0.93	29.15	4.31	0.9	20.88	3.44	0.94	27.33		
"macaroni"	30	6.03	1.0	16.58	5.81	0.72	12.39	4.02	0.44	10.96	-	3
"lentil sandwich"	30	6.25	0.44	7.04	5.79	0.65	11.23	4.69	0.79	16.84	-	-
"kitfo"	30	6.09	0.47	7.72	6.52	0.70	10.74	4.90	0.81	16.53	9	-
"egg sandwich"	30	5.58	1.21	21.68	6.46	1.45	22.44	4.36	0.68	19.72	1	-

$\bar{X}$  = mean value of counts, SD = standard deviation, %CV = percent coefficient of variation

Table 7. Relationship between holding temperature and bacterial load in "kitfo" and "egg sandwich"

Temp. range (°C)	Aerobic mesophilic count (cfu/g)											
	$10^4 - 10^5$		$10^5 - 10^6$		$10^6 - 10^7$		$10^7 - 10^8$		$10^8 - 10^9$		$> 10^9$	
	"kitfo"	Egg	"kitfo"	Egg	"kitfo"	Egg	"kitfo"	Egg	"kitfo"	Egg	"kitfo"	Egg
41-55	-	-	-	-	-	-	-	-	-	-	-	-
31-40	-	-	-	-	-	-	-	-	-	-	-	-
21-30	-	-	-	-	9	5	8	4	7	5	4	7
15-20	-	-	-	-	-	1	1	1	1	2	-	5
Total	-	-	-	-	9	6	9	5	8	7	4	12

#### 4.5. Isolation and identification of *Salmonella* and *Shigella* spp.

*Salmonella* was isolated from 9 samples of "kitfo" and 1 sample of "egg sandwich" collected from street vendors. *Shigella flexineri* was encountered in three "macaroni" samples.

#### 4.6. Assessment of growth potential of test strains

*Salmonella typhimurium* reached counts  $\gg 10^8$  cfu/g within 24 hours in "egg sandwich", "macaroni" and "lentil sandwich". It increased by about 1 log unit in the first 4 hours and showed a steady growth thereafter. Its growth rate in "lentil sandwich" was relatively slower than in the other food items (Fig 4).

Growth of *Shigella flexineri* in the three food items was markedly fast in the first 4 hours. Final counts in the food items varied slightly and higher counts were noted in "macaroni" and "lentil sandwich". Growth rate in "lentil sandwich" was notably faster after 12 hours whereas growth in "macaroni" and "egg sandwich" was relatively steadier. Initial inoculum level of the test strain was much lower than that of the other test strains (Fig 5).

*Staphylococcus aureus* initially had relatively higher counts and increase by 2 log units was noted within 8 hours in all the three food samples. Growth rate in the first 8 hours was markedly high. Counts as high as  $10^5$  cfu/g were maintained after 8 hours, reaching final counts of  $>10^9$  cfu/g at 24 hours (Fig 6).

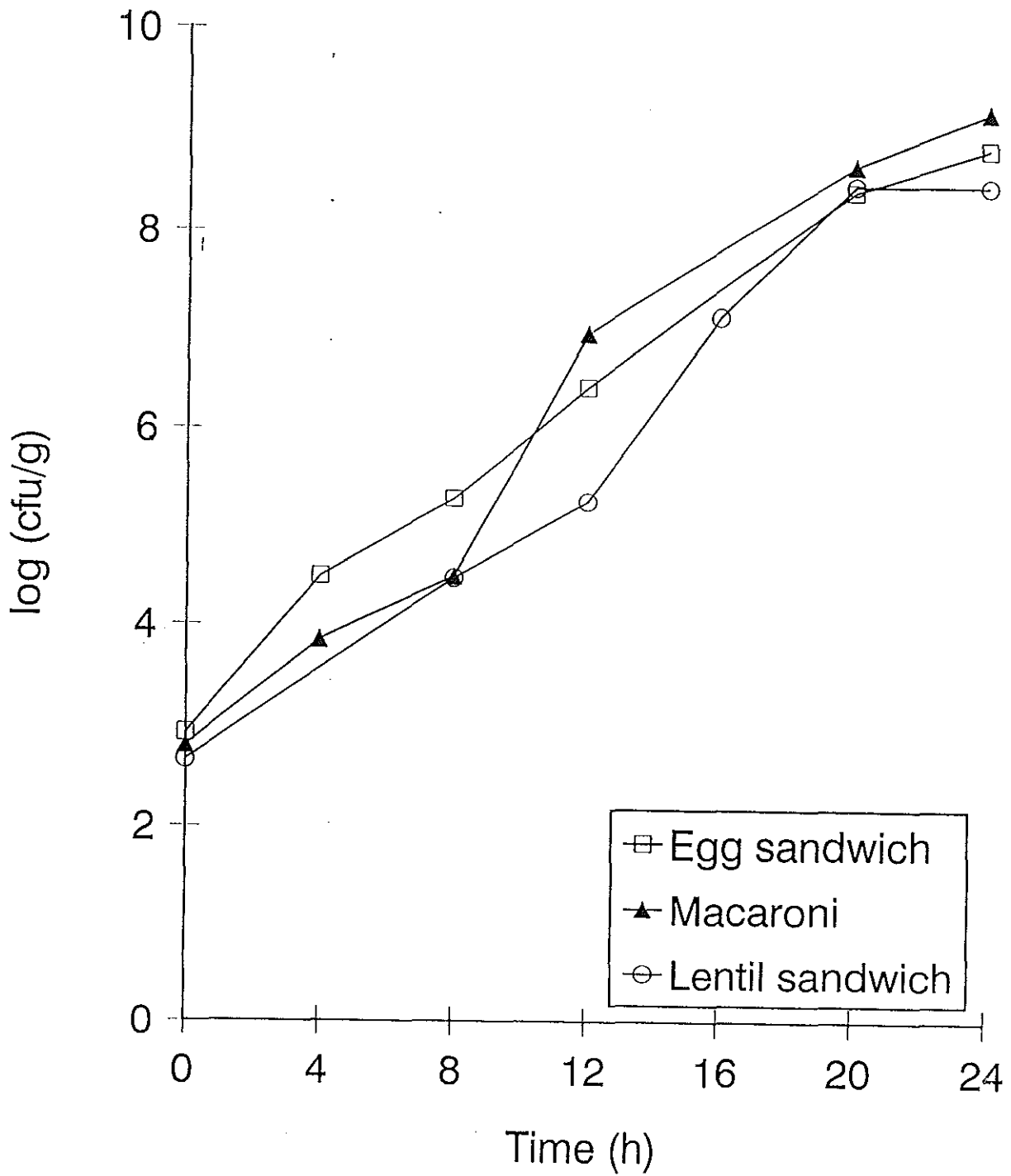


Fig 4. Growth pattern of *Salmonella typhimurium* in three street-vended foods.

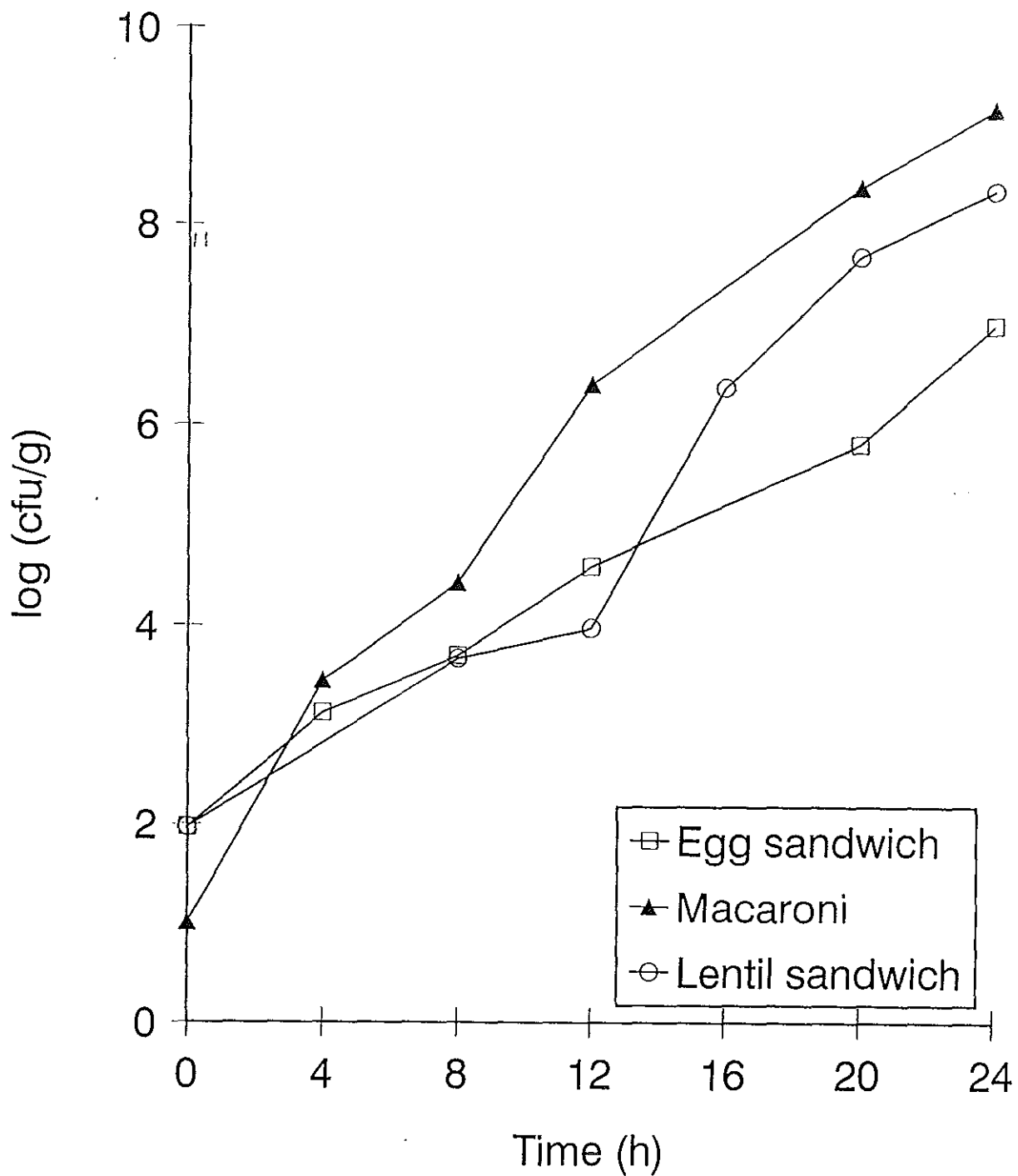


Fig 5. Growth pattern of *Shigella flexneri* in three street-vended foods

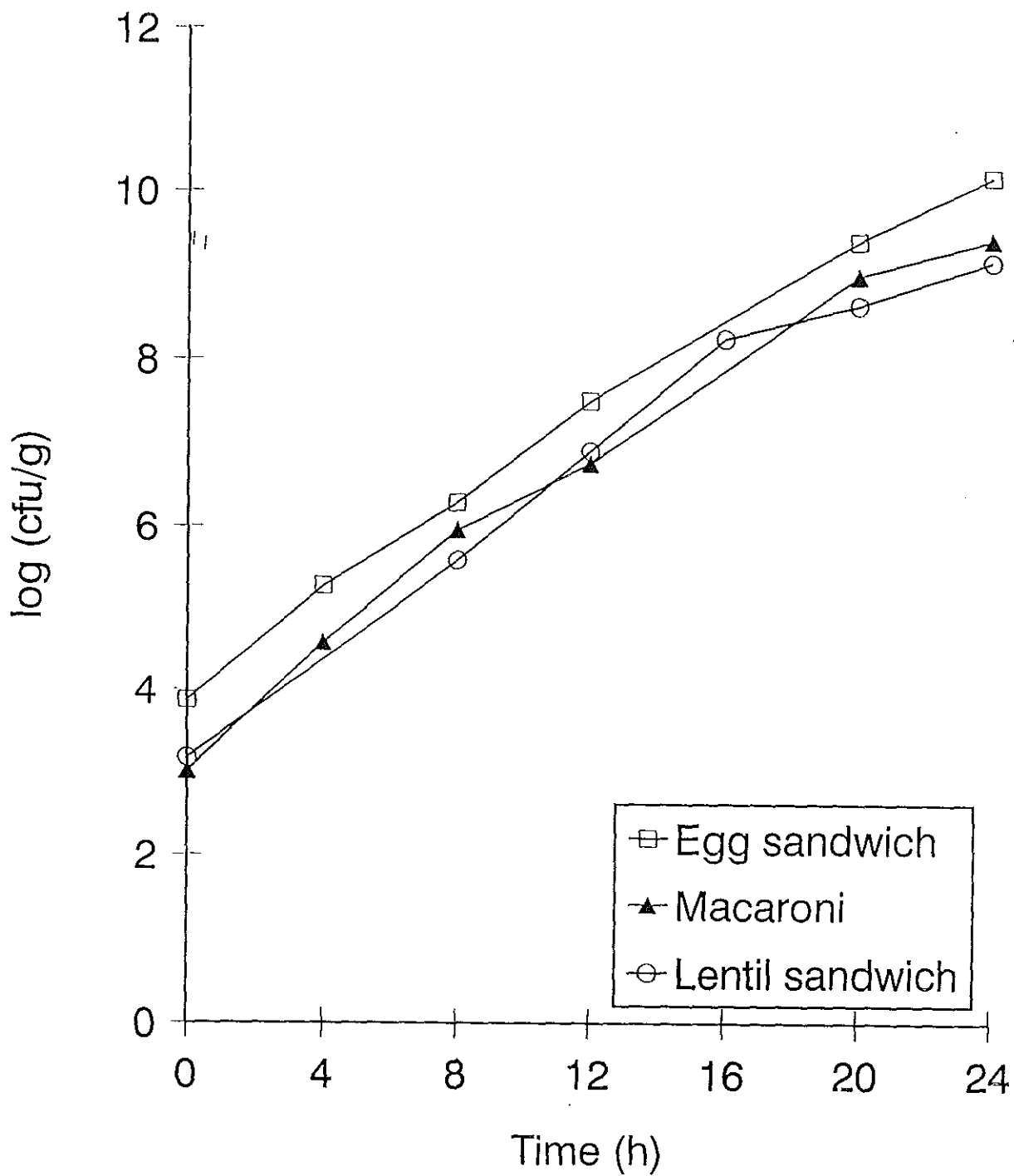


Fig 6. Growth pattern of *staphylococcus aureus* in three street-vended foods

## 5. DISCUSSION

Street vendors made various kinds of foods of plant and animal origin available to the consumers at affordable prices. For obvious reasons their vending sites were vastly inclined to areas with heavy population movement and hottest business activity centre. As in this study, many of the vendors in various parts of the world were females (FAO, 1997, Freese *et. al.*, 1998a).

In general, the majority of investigated food items yielded alarmingly high microbial load and, in some cases, even pathogens. The food handling and serving operations, cleanliness of the vendors and sanitary condition of the vending environment revealed inadequacies concerning hygiene. Furthermore, the lack of municipal (running) water and cooling facilities at point of vending sites worsened the situation.

Aerobic mesophilic counts from „*sambussa*“ samples were relatively lower whenever detected but slightly higher than what was reported in another study on *Shiro* sauce (Mogessie, 1995). Nevertheless, investigation made on legume based dishes revealed a significantly higher microbial load ranging from  $10^6$  to  $10^9$  cfu/g with prolonged holding at ambient temperature (Bryan *et al.*, 1992b).

Bacterial spores and yeasts were encountered only in ten samples and with counts as low as  $10^3$  cfu/g. Likewise, microbial groups such as Enterobacteriaceae, coliforms and *B. cereus* were encountered rarely. "Sambussa" was fried in oil at quite sufficient temperature, which could eliminate the viable cells. Its moisture content is significantly low. Moreover, this product was wrapped inside dough with which it was baked and this could confer protection to extraneous contaminants. Likely, the cumulative effect of these factors might have contributed to the reduced microbial load of the product. In few cases, however, it appeared that undercooking was noted, where the wrapped ingredients were observed to be poorly fried. On the other hand, this food item, once brought to point of vending, stayed there with no covering material till the termination of the selling process. The stalls, where it was vended, were highly dust-laden environments crowded with both people and vehicles. These factors jointly might explain the reason why *Bacillus* species (43.7%) dominated the microflora of "Sambussa." Evidently, the isolation of member 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).

Other Gram-positive rods also constituted the dominant flora along with *Bacillus* species. These organisms could have found entry to "sambussa" samples during faulty handling and serving activities. Their dominance, however, could be

accounted on reduced competition due to heat treatment during frying. By the same token, staphylococci appeared in 21 of 30 "*sambussa*" samples with counts of as low as  $2 \times 10^4$  cfu/g and constituted the third dominant group. Their presence could also have been due to faulty practices exercised by both vendors and consumers (self-serving), reduction of competition, time-temperature abuse and tolerance to various stresses. Indeed, 90% of the samples were stored at temperature ranges of (15.5-34.5°C) that could support the proliferation of most microbes.

Two critical practices are typically involved in disease causing time-temperature abuse: (1) preparation of food several hours before consumption and its holding at temperatures that favour the growth of pathogenic bacteria and/or the formation of toxins, and (2) inadequate cooking or re-heating of food to minimize or eliminate pathogens. It has been estimated that upto 70% of the episodes of diarrhoeal disease are food-borne in origin, i.e., caused by contaminated food, including drinking water and water used in food preparation (Motarjemi and Kaferstein 1993).

With respect to consumer's health, the frequent occurrence of *Bacillus* and *Staphylococcus* species could not be safe although the count made in this study was not so alarming. Neither *Salmonella* nor *Shigella* spp. was recovered from any of the "*sambussa*" samples. The absence of *Salmonella* and *Shigella*, as well as the marked absence of coliforms and Enterobacteriaceae is indicative of sufficient heat treatment during frying as Gram negative rods are relatively more sensitive to heat.

However, the mishandling and serving practices of this product after frying, coupled with ambient temperature for a long period of time, would significantly undermine the safety of the product.

Eighty six percent of cooked and sauced "*macaroni*" samples had aerobic mesophilic counts exceeding  $10^7$  cfu/g with mean count of  $8.5 \times 10^7$  cfu/g. Apparently, this mean value was by far higher than that reported for "*macaroni*" and spaghetti in Egypt (El-Sherbeeney *et al.*, 1985a) and in Awassa, Ethiopia (Mogessie, 1995). Further lower value was also reported by El-Sherbeeney *et al.* (1985b) from cereal and cereal containing dishes. Nevertheless, as in our study, similar counts of aerobic mesophilic bacteria were reported by Bryan *et al.* (1992a) from cereal based dish on lid of pan left overnight. A count higher than ours was also obtained from fried cereal dish left overnight. The mean counts of Enterobacteriaceae in this study were not low either. They were, in fact, higher than what was reported elsewhere (Mogessie, 1995). Counts of coliforms, *B. cereus* and staphylococci were markedly high and each had counts exceeding  $10^5$  cfu/g. At these levels, most food poisoning bacteria can elucidate sufficient toxins to cause food intoxication. The mean value of coliform counts recorded in this study were greater than that reported by (El-Sherbeeney *et al.*, 1985b) from cereal based dishes. Evidently, foods sold via vending machine were reported to harbour significant numbers of coliforms (Wu *et al.*, 1985).

El-Sherbeeny *et al.* (1985b) reported that higher counts of *B. cereus* ( $10^5$  - $10^9$  cfu/g) were isolated from remnants of cereal based meal implicated in outbreaks. Counts comparable to those obtained in our study were demonstrated elsewhere (Amodio-Cocchieri *et al.*, 1998) from various dishes. In other cases, counts as low as  $10^3$  cfu/g of viable cells of *B. cereus* were recovered from cereal dish (Bryan *et al.*, 1992a). Although foods may be heated adequately to eliminate vegetative microbes, spore-forming bacteria can be a major concern because ordinary cooking does not kill all spores. Thus, such large numbers of bacterial spores and *B. cereus* signify that their spores germinated and proliferated while the cooked foods were held on display for a long period of time (Bryan *et al.*, 1992a). Such significant number of *B. cereus* in food indicates a potential risk. Apparently, their resistant spores provide them unique privilege to be isolated from different environment.

Basically, high counts of aerobic mesophilic bacteria, Enterobacteriaceae, coliforms, and staphylococci were indicative of gross contamination after cooking while cooked "macaroni" samples were held at ambient temperature for a prolonged period of time. Longer storage at ambient temperatures was commonplace in the street vendors. Evidently, holding foods for more than four to six hours is one of the main contributing factors of high possible counts (EL-Sherbeeny *et al.*, 1985a, b; Bryan *et al.*, 1992a, b,c). In this study time-temperature abuse was by far deemed potentially hazardous as observed by other investigators (Motarjemi and Nout,

rice containing dishes obtained from street vendors. However, study made in Awassa (Mogessie, 1995) revealed isolation of *Salmonella spp.* with significant incidence rate of 40% from spaghetti.

Generally, *Bacillus spp.* dominated the microflora of street foods and were constantly isolated from all foods studied with considerable numbers. The vital role that spore formers play in those foods sold at road-sides is well documented (Bryan *et al.*, 1992a). Majority of food items that were sold at the stalls were of a nature that spore-forming bacteria will be the main concern. During ordinary cooking (a) all spores are not eradicated, (b) anaerobiosis is enhanced, and (c) spores are heat activated, which initiates them to germinate whenever environmental conditions become suitable for microbial growth. NNAS, RNAE and CIOM (1990) and Amodio-Cocchieri *et al.* (1998) described spices as possible sources of *Bacillus* species. Thus, in addition to the potential for spoilage when these spices are added to other foods, the potential health hazard of *Bacillus spp.* should not be ignored (Seenappa and Kempton 1981). Evidently, there is ample evidence relating cases of food poisoning to the presence of *B. cereus* in contaminated foods (Kaur, 1986; Kramer and Gilbert, 1989; Roberts *et al.*, 1995). Indeed, as far as their capital is concerned, the street vendors could hardly use complicated seasoning and spices that could be sources of pathogens. The frequently appearing non-spore forming dominant microbes from all street foods might have revealed either heavy

contamination and prolonged holding period or tolerance of some isolates to ordinary cooking temperature.

Sixty nine percent of "*lentil sandwich*" samples had aerobic (30EC) colony counts mostly greater than  $10^7$  cfu/g and counts ranged from  $>10^5$  to  $10^9$  cfu/g with mean counts of  $1.6 \times 10^7$  cfu/g. This mean count was significantly higher than that reported for legume-based dishes elsewhere (El-Sherbeeny *et al.*, 1985a; Bryan *et al.*, 1992a; Mogessie, 1995). Nevertheless, Bryan *et al.* (1992b) made a similar observation with greater counts of aerobic mesophilic bacteria from chickpea after cooking and stacking on trays. The mean counts of Enterobacteriaceae were substantially higher than what was observed elsewhere (Mogessie, 1995). Over 95% of "*lentil sandwich*" samples that were positive for Enterobacteriaceae and coliforms had counts significantly greater than  $10^5$  cfu/g. Perhaps this might have accounted for predominance of this group over other microflora of "*lentil sandwich*". Counts of *B. cereus* and staphylococci were also higher than that observed by other workers (Bryan *et al.*, 1992a, b, c). Higher counts of coliform bacteria were reported with a mean value as high as  $2.2 \times 10^8$  cfu/g from legume based dish after mixing and forming (Bryan *et al.*, 1992c). Samples that were positive for *B. cereus* had counts exceeding  $10^4$  cfu/g. Whilst samples that were positive for staphylococci contained counts greater than  $10^5$  cfu/g. These are the minimum numbers required to cause food poisoning in humans.

It is natural to obtain such high counts because several unsatisfactory sanitary operations were practiced mainly by the hawkers. Moreover, 96.7% of the samples were within 15.5 to 44.5° C at the time of sampling. Thus, this particular temperature range could promote rapid growth of common microorganisms and many target pathogens with prolonged holding periods as exercised by street vendors. Indeed, pathogenic bacteria will have limited opportunity to proliferate to large numbers, if the holding time is short.

Such large number of microflora indicate either possible gross contamination, prolonged holding time, under cooking or a combination of these (El-Sherbeeny *et al.*, 1985a). None of the samples of "lentil sandwich" yielded *Salmonella* spp. The potential for cross-contamination from hands of both hawkers and buyers, raw ingredients, unclean multipurpose cloth, unwashed utensils and flies that flected around from nearby toilet could substantially contribute to the poor microbiological status of "lentil sandwich". Similar observations were made by many workers (El-Sherbeeny *et al.*, 1985a). Bryan *et al.* (1992c) encountered *Salmonella* spp. on pulse patty leftover obtained from street vendors.

*Shigella* was not isolated from any samples of "lentil sandwich". This was in line with the observations of Mogessie (1995) where legume-based sauces did not yield any *Shigella*. However, El-Sherbeeny *et al.* (1985a) reported the recovery of

*Shigella* from legume-based food products in Egypt. Its absence in our study could be due to the adequate cooking temperature, although possibilities for post-cooking contamination were high.

The microflora of "*lentil sandwich*" was dominated by members of the family Enterobacteriaceae as witnessed by considerable counts of this group. This might be again an indication for poor handling and storage practice of this food commodity after cooking with long holding time at ambient temperature. The frequent appearance of *Bacillus* and *Staphylococcus* in food in significant number calls for marked concern. In fact, for cooked food mishandled in such a manner, encountering large number of staphylococci is not surprising enough, as staphylococci are normally inhabitants of hands and the skin.

The mean aerobic mesophilic count of raw minced meat ("*kitfo*") from street vendors in Addis Ababa was much lower than that reported for raw ground meat samples in Pakistan (Bryan *et al.*, 1992a). However, Saddik *et al.* (1985) made a comparable observation with greater counts of aerobic mesophilic bacteria from raw meat. About 70% of "*kitfo*" samples had aerobic mesophilic counts higher than  $10^7$  cfu/g. High counts of mesophilic aerobic microorganisms indicated bacterial growth due to high level of contamination. The mean counts of coliforms and *B. cereus* were exceedingly higher than what observed elsewhere (Bryan *et al.*, 1992a).

Comparably, Van kampen *et al.* (1998) encountered high counts of coliforms in meat dishes. Over 50% of "kitfo" samples that were positive for Enterobacteriaceae had counts  $> 10^6$  cfu/g. This may suggest possible cross-contamination between the intestinal contents and raw meat. Added to this was a prolonged period of holding at ambient temperature. About 80% of "kitfo" samples that were positive for staphylococci had aerobic mesophilic counts exceeding  $10^7$  cfu/g. Seemingly, such large numbers of staphylococci are usually introduced into raw minced product either during chopping operation or other similar processes. At this level they can pose serious health hazard, particularly food poisoning syndrome. Sixty percent of "kitfo" samples that were positive for *B. cereus* had counts exceedingly greater than  $10^4$  cfu/g. Various spices and meat products harbour *B. cereus* (Mantynen and Lindstrom, 1998). The presence of this organism in food with significant number can pose serious health hazard. Thus, ingestion of food containing  $10^6$  -  $10^8$  cfu/g of viable cells and preformed toxin (notoriously termed as emetic type) can result in food poisoning (Roberts *et al.*, 1995).

The microflora of "kitfo" was dominated by *Staphylococcus*, *Bacillus* and members of Enterobacteriaceae and *Micrococcus* spp. Indeed, the heterogeneous flora on lean meat could come likely from the contamination of animal and/or the processing environment either during slaughtering process or rearing period or other similar operations. During sampling time, 100% of "kitfo" samples were within temperature

range of 15.5-24.5<sup>o</sup> C. The often lengthy exposure to ambient temperature permits spoilage bacteria to multiply. Apparently, meat carcass and meat joints stored at temperatures over 20<sup>o</sup> C or above will inevitably undergo spoilage mostly by genera *Escherichia*, *Aeromonas*, *Proteus*, *Staphylococcus*, *Micrococcus* and *Bacillus* (Hayes, 1995).

Samples of "kitfo" did not yield *Shigella*. Similar observations were made in another study (El-Sherbeeney *et al.*, 1985a; Saddik *et al.*, 1985; Bryan *et al.*, 1992a). However, the possibilities exist for potential cross-contamination and, as indicated earlier, can be diverse.

*Salmonella* was isolated from samples of "kitfo". Similarly, Bryan *et al.* (1992a, c) reported the isolation of this pathogen from ground raw meat. Nonetheless, in Egypt, samples of fish and other sea foods obtained from street vendors did not harbour *Salmonella* (Saddik *et al.*, 1985). Salmonellae could have come from various sources. *Salmonella* is common in raw foods of animal origin. In addition, the frequent inclusion of raw vegetables (sliced onion and green pepper) into raw "kitfo" could possibly introduce these organisms in to "kitfo". It has been claimed that consumption of raw vegetables can potentially constitute a serious health hazard (Mogessie, 1989; van Kampen, 1998). This organism could be acquired from hands of the vendors during the mincing process and during "kitfo" preparation, which

guaranteed contamination of sterile internal tissues or hands of the consumers. Microbial contamination of hands is obvious when an individual makes hand contact with one's own excreta, environmental surfaces contaminated with substances like mucus, blood, soil, urine, feces or even food (Almeida *et al.*, 1995; Paulson, 1997). Cutting boards, knives, unclean multipurpose cloth (used to rub food serving trays and spoons after each consumption) and storage pans could serve as possible sources of contamination. *Salmonella* has been recovered from chopping board (Bryan *et al.*, 1992c).

The over-crowdedness at the vending site and flies from nearby latrine could help the likely entry of *Salmonella* into the food commodity. Additionally, carrier animals or food handlers are good sources. There is ample evidence to incriminate domestic food animals as important sources of *Salmonella* spp (Kirkpatrick and Benson, 1987). Evidently, animals that recovered from attack of *Salmonella* still shed the organisms to farmland and act as a continual source of infection for other healthy animals (Hinton *et al.*, 1986; Linton and Hinton, 1988). Furthermore, *Salmonella typhi* and *Shigella* species are aggressive pathogens that cause diseases after an infected person handles food (Paulson, 1997; Paugliese and Favero, 1997). A couple of decade back, food obtained from a factory caused serious outbreaks of food poisoning where the stool examination of employees revealed 136 excreters of *Salmonella* spp. (Hayes, 1995). Actually, several investigators (Mogessie, 1989, 1994; Roels *et al.*, 1997; Little and de Louvois, 1998; Mezegbu and Mogessie,

1998), have seriously addressed the health problem associated with consumption of raw meat as observed in the present study.

Fried "egg sandwich" samples harboured high counts of aerobic mesophilic bacteria with mean value of around  $10^8$  cfu/g. This mean value is significantly higher than what was reported elsewhere from raw egg shell (Mogessie, 1989) and cooked foods of animal origin (Bryan *et al.*, 1992a, c). Interestingly, 63.3% of "egg sandwich" samples contained counts  $> 10^8$  cfu/g. Such extreme bioburden considerably can suggest inadequate frying, unhygienic handling and serving added to long holding time at bacterial growth temperature.

Members of Enterobacteriaceae and coliform had mean counts of  $3.8 \times 10^5$  and  $2.7 \times 10^5$  cfu/g, respectively. These high counts again revealed gross contamination after heat treatment. Bacterial spores and presumptive *B. cereus* were constantly encountered in samples of "egg sandwich". Their isolation with high quantities disclose either inadequate frying temperature or crowdedness around vending areas that could soil the product leading to spore germination of presumptive *B. cereus*.

Members of the genus *Staphylococcus* (31.7%), *Micrococcus* and other Gram-positive rods were isolated frequently from samples of this food product. Enterobacteriaceae appeared infrequently. Noticeably, the predominance of

staphylococci paralleled their viable cell counts with mean value of  $2.9 \times 10^6$  cfu/g. Thus, 60% of the samples had cell counts exceeding  $10^6$  cfu/g. All "egg sandwich" samples were held at a temperature range of 15.5-34.5° C during collection. This temperature could support most microorganisms that got access to this food item. Perhaps the frying operation might reduce the competing microbes thus facilitating proliferation of staphylococci. A mixture of saprophytic and psychrophilic bacterial species pose definite repressive effects on the growth of staphylococcal population (Peterson *et al.*, 1962). Effective heat shock of these mixtures drastically reduces their population. Thus, staphylococci subsequently inoculated during faulty operations are left without intensive competition. Peterson *et al.* (1962) further elaborated that foods which selectively favour the growth of staphylococci by sharply inhibiting the growth of other genera, always involve in staphylococcal food poisoning. Furthermore, foods which have a protective action on staphylococci through the action of substances such as eggs, starches and lipids were also implicated in food intoxication syndrome. Additionally, their ability of tolerance to high salt concentration (up to 10%) can confer them special advantage over others as revealed in "kitfo" thus making them dominant among the microflora.

Although the vending operations were extremely inviting as was seen in other food items for serious contamination, samples of the "egg sandwich" did not contain *Shigella* as also was demonstrated by another study (Mogessie, 1989).

*Salmonella* was detected in one sample of "egg sandwich". Previously, it has been encountered in samples of egg shell and cooked chicken (Bryan et al., 1992c). The trend of contamination in all investigated foods in the present study was almost alike. This organism could have been acquired from hands of workers (carrier / normal) or utensils during preparation of raw liquid egg. Because of its extraordinarily adhesive characteristics this product was commonly vulnerable to heavy contamination with a wide variety of different organisms. The water that vendors added to unpasteurized liquid egg might contribute for further contamination. From the observation and temperature record made at point of collection, it appeared that the frying temperature was not adequate enough to eliminate pathogens. Thus, *Salmonella* could survive if it were there. Possibly, this target pathogen could have come from infected hens. Egg shell may be contaminated during its passage down the oviduct or from the environment after laying (Burow, 1993; Roberts *et al.*, 1995). Additionally, *Salmonella* could have been introduced due to poor sanitary practices exercised by street vendors and the consumer (self service).

In all street foods considered in this study, moulds were either detected rarely or not at all. Moulds and yeasts can get an access into foods through either unhygienic equipment or as air-borne contaminants. Such contamination routes were typical features of all the investigated vending sites. These organisms prefer foods with low

water activity, low pH, and high salt or sugar contents. All samples of street foods with exception of 1 "*lentil sandwich*", 1 "*macaroni*" and 20 "sambussa" samples were positive for yeasts with considerable counts. During the investigation period "*kitfo*" and many samples of "*lentil sandwich*" were collected from vending sites, which had closest proximity to `tej' house. This area was flooded with residues of `tej' or the product itself creating sugary environment.

Challenge studies showed that *Salmonella* test strain grew to population levels of  $>10^6$  cfu/g in "*egg sandwich*" and "*macaroni*" within 12 hours but the level obtained in "*lentil sandwich*" was  $>10^5$  cfu/g within the same period of time. These were, however, lower than that obtained in fermenting *ergo*, a traditional sour milk (Mogessie, 1993) and that in legume-based traditional sauces (Mogessie, 1996). Still these levels were slightly lower than what was reported for "*kitfo*", a traditional Ethiopian spiced, minced meat dish (Mezgebu and Mogessie, 1998). In all the three food items, *Salmonella typhimurium* could attain the infectious dose level within 4 to 8 hours, where fewer than 100 viable cells are needed to be ingested to produce the disease syndrome.

*Shigella flexneri* attained a level of  $> 10^4$ ,  $10^6$  and  $10^3$  cfu/g in "*egg sandwich*", "*macaroni*" and "*lentil sandwich*", respectively, within 12 hours. There has not been report on growth dynamics of *Shigella* in traditional foods in Ethiopia. Hence,

comparison of our results with previous studies was not possible. This test strain steadily grew to a level of infective doses within 4 to 8 hours. At this cell number *Shigella* could initiate a successful infection. Although *Shigella* showed a steady type of growth between 0 and 8 hours, particularly in "lentil sandwich", the rate increased markedly from 12 hours onwards and reached a comparable count with *Salmonella* at 24 hours.

The growth of *S. aureus* was enhanced considerably in the three food types and reached a count greater than  $10^6$  cfu/g within 12 hours. Its growth was remarkably fast and attained population level exceeding  $10^9$  cfu/g within 24 hours. A count less than this level was found in other studies in imitation cheeses (Bennett and Amos, 1983), in a wild edible mushroom (Lindroth *et al.*, 1983) and in gruel (Gulillat *et al.*, 1997). Its growth was uniquely luxurious in "egg sandwich" likely due to nutritional differences of these food items.

At a level exceeding  $5.6 \times 10^5$  cfu/g, *S. aureus* was reported to secrete staphylococcal enterotoxin type A in food samples of potatoes obtained from street vendors in Pakistan (Bryan *et al.*, 1992b). In our study this population level was attained at an early growth period (4 hours) in "egg sandwich" and at 8 hours in "macaroni" and "lentil sandwich". Thus, most likely our test strain commenced to secrete its toxins, termed as enterotoxins, between 4 and 8 hours. This time interval exactly fits the holding period of many street vended foods investigated. Evidently, it has been reported that *S. aureus* counts ranging from  $10^5$  to  $> 10^9$  cfu/g are recovered from

foods implicated in food poisoning outbreaks (Harvey *et al.*, 1982; Nunez *et al.*, 1988). In our case, this population level was reached between 4 and 24 hours.

As far as the growth dynamics of these test strains is concerned our results indicated serious problems associated with long holding period of food items at ambient temperatures given the various possibilities for pathogens to be introduced in to foods.

## 6. Conclusion and Recommendation

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

- (1) The study of microbiological profile and safety of street foods should be further continued with multivariate parameters. This economic sector has reached a new dimension in most Ethiopian towns in general, Addis Ababa, in particular. Thus, studies covering these areas in the country are much important to see the real picture of the problem.
- (2) Anaerobic organisms have greatest role in various food poisoning outbreaks. To that effect, the real contribution of anaerobic organisms to food safety should also be evaluated along with other bacterial pathogens like *Listeria*, *Campylobacter*, *Yersinia*, *Vibrio* and *Aeromonas* species.
- (3) The samples we collected from this city proved the presence of extremely high microbial load with the exception of "sambussa" indicating heavy contamination of the products.

- (4) Microflora of street foods is quite heterogeneous. Both Gram-positive and -negative organisms were frequently encountered amongst which Gram-positive bacteria appeared to be the most dominant microflora. This investigation undoubtedly points out high risks of eating certain street vended foods ("*egg sandwich*", "*kitfo*" and "*macaroni*") for they harboured target pathogens. Thus, "*egg sandwich*", "*kitfo*" and "*macaroni*" are the products of concern. "*Sambussa*", however, if properly handled, seems safe. The single most effective way of combating microbial hazards in food is to cook it thoroughly and eat it promptly.
- (5) Street foods are highly exposed to numerous extraneous matter. To that effect, analysis of food for the presence of soil, road-dust, insects and rodents is required.
- (6) This study indicated that street vendors and consumers require education in hygienic handling of foods with respect to post-cooking contamination, relationship between holding temperatures and time, environmental sanitation, site selection and proper waste disposal. Regular medical follow-up for food handlers to minimize risks associated with chronic carriers is another important point to be focused. Other factors to be avoided are self-service, reusing water, close proximity of food vending site to toilet, use of unclean multi-purpose cloth to wipe food utensils dry, addition of raw

products into cooked foods and holding raw minced meat at bacterial growth temperature for extended period of time. Thus, to serve wholesome product, hygienic operation in the food processing chain is vitally important. Additionally, regulatory agencies need to be aware of the shortcomings of street-vending operations and take appropriate steps to improve food safety and sanitation.

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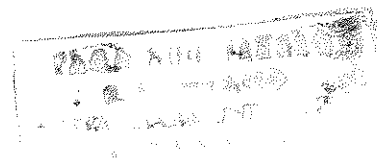
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