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Review article

THE MICROBIOLOGY OF ETHIOPIAN FOODS AND BEVERAGES: A REVIEW

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ABSTRACT: The paper reviews the available literature on the microbiology of traditional Ethiopian foods and beverages. The topic on milk and dairy products deals with the livestock resource of the country with respect to the microbiological quality of raw and processed milk, processing and microbial status of various fermented milks ('Ergo', 'Itutu'), traditional cottage cheese ('Ayib') and microbiological safety of milk and dairy products. Under traditional fermented foods, the microbiology of 'Enjerra' fermentation, 'Kotcho' fermentation and the fermentation of various condiments are discussed. Microbiological safety issues and spoilage of these products are also addressed. Discussion on the microbiology of traditional fermented beverages deals with popular products such as 'Tella', 'Borde' and 'Shamita'. Here, the nutritional and other chemical properties of the products are also presented. Some ready-to-eat foods are discussed with respect to their level of microbial contamination, spoilage and their importance in food-borne diseases. These include different traditional sauces, street foods and weaning foods. Finally, microbial load and safety considerations were addressed with respect to other food products such as beef, fish, vegetables, fruits and fruit juices. In conclusion, the review discusses the nature of food preparation in Ethiopia, food loss due to poor traditional household processing, the extent and limitations of scientific work done so far and suggests some recommendations to curb the problem.

Key words/phrases: Traditional food, beverages, fermentation, spoilage, safety

INTRODUCTION

Ethiopia is a country rich in cultural diversity. The variety of foods and beverages processed and consumed among the various ethnic groups are manifestations of this diversity. Although some of the food items may be consumed in their raw forms, processing of one type or another is usually a rule than an exception. This usually includes salting and drying, boiling, roasting, frying, baking, cooking, fermenting or various combinations of these.

Microorganisms are important in food products because they produce desirable flavor and physical characteristics in many food products during their fermentation; food products may become contaminated with pathogens or microbial toxins and thereby become vehicles for the transmission of disease to humans and other animals; and many microorganisms are capable of causing off-flavour and physical defects in food products.

Although the involvement of Ethiopian researchers in studying the microbiology of traditional foods started only in the 1980's, quite a number of publications have appeared since. Given the diversity in food and beverage items in the country however, the microbiology of a variety of Ethiopian foods still remains to be studied. Most of the works hitherto addressed microbiological issues on milk and other dairy products, 'Enjerra' and 'Kotcho', other legume and vegetable products, beverages, meat, poultry and other food products. Topics of concern in most of these works were basically fermentation and accompanying changes, food safety, processing and spoilage of traditional Ethiopian foods and beverages. The purpose of this paper is to review microbiological studies made by various researchers on fermentation, other processing methods, microbial safety and spoilage of traditional Ethiopian foods and beverages.

MILK AND OTHER DAIRY PRODUCTS

In Ethiopia, the population of cattle, sheep and goats is about 30, 23, and 17 million, respectively, with an estimated annual milk production of 800,000, 65,000 and 95,000 metric tones, respectively totaling to 960,000 metric tones (Azage Tegegne and Alemu Gebrewold, 1998). Almost all indigenous cattle are in the hands of the small-holder subsistence farmers, and milk produced is used for either family consumption or sold at local markets often processed into butter and 'Ayib' (Azage Tegegne and Alemu Gebrewold, 1998). Among the pastoralists in the lowlands, camel milk is the major source of nutrition. The estimated camel population in Ethiopia is 1.06 million (FAO-OIE-WHO, 1993) and the total annual production of camel milk in Ethiopia is 2442 kg per camel (Knoess, 1977). As 98% of the milk is produced in rural areas by subsistence farmers (Tsehay Reda, 1998), dairy processing is basically limited to household level. Large scale processing of dairy products is underdeveloped in the country. Although the livestock resource of Ethiopia is enormous, a lot more effort is required to enhance the productivity of Ethiopian livestock and to process the products in such a way that guarantees a better keeping quality and wholesomeness. To encourage the development of industrial dairy processing, generation of basic information on the microbiology of milk and its products is vital.

Raw milk

Milk is an ideal medium for the growth of microorganisms. Microorganisms in raw milk come from several sources. Organisms having access to the teat may invade the teat opening and migrate to the interior. Mastitis, an inflammatory disease of the mammary tissue, may lead to the development of a large number of the infectious organisms in milk from diseased quarters. Equipment generally contributes the largest proportion of microorganisms found in raw milk supplies. Although airborne microorganisms in the milking environment rarely contribute a significant number of the total microbial count of milk, extremely dusty conditions may increase the counts. Milk handling personnel may contribute various organisms, including pathogens, directly into milk (ICMSF, 1980).

Various undesirable and detectable organoleptic and physical changes in raw milk, such as souring, ropiness and slight coagulation, are caused by microorganisms. Prevention of microbial contamination of milk requires a combination of measures such as maintaining animals in a healthy condition, cleaning udders and rear quarters of the cow, cleaning milk contact surfaces and equipment, sanitary milk production practices by milk handling personnel and avoiding excessive airborne contamination.

An earlier study by Tadesse Mehari and Berhanu Abegaz Gashe (1990) on microorganisms in raw milk and sources of contamination at a processing plant showed that aerobic mesophilic count of raw milk ranged between 10^7 and 10^9 cfu(ml)$^{-1}$ and pasteurization brought the count down to 10^5 cfu(ml)$^{-1}$. The count later increased 2 to 4-fold due to subsequent contaminations. Possible sources of contamination to pasteurized milk were utensils for collecting milk from the pasteurizing unit and plastic sheets used to bag the pasteurized milk. Raw milk microflora was dominated by psychrophiles (98%), and the flora was still dominated by these groups (53%) after pasteurization in the processing plant. Laboratory pasteurization, on the other hand, eliminated all psychrophilic organisms (Tadesse Mehari and Berhanu Abegaz Gashe, 1990). This study has shown that pasteurizing milk, *per se*, does not guarantee shelf life improvement, unless aseptic conditions are adhered to after the pasteurization process.

Another study on a college dairy farm in Awassa compared the microbial load of milk directly obtained from the udder and fresh raw milk from milking utensils (Mogessie Ashenafi and Fekadu Beyene, 1994). Aerobic mesophilic counts for udder milk ranged between 10^3 and 10^4 cfu(ml)$^{-1}$, whereas raw milk from collecting utensils had counts as high as 10^6 cfu(ml)$^{-1}$. When raw milk was pasteurized in the laboratory, the count was reduced by 2 log units. However, maintaining the pasteurized milk at

ambient temperature brought the count back to 10^6 cfu(ml)$^{-1}$ in 12 hours. Pasteurization must be followed by immediate cooling to avoid multiplication of surviving microorganisms. Coliform counts in udder milk was about 10 cfu(ml)$^{-1}$, while this count in fresh raw milk collected from utensils was as high as 10^5 cfu(ml)$^{-1}$, indicating that major contamination occurred during the milk collection process. Laboratory pasteurized milk contained coliforms at a level less than 10 cfu(ml)$^{-1}$. In another study, aerobic mesophilic counts of milk collected from eight different small dairy farms in Awassa ranged between 10^4 and 10^7 cfu(ml)$^{-1}$, and coliform counts were 10^3-10^5 cfu(ml)$^{-1}$ (Mogessie Ashenafi, 1995a).

In a study on bacteriological quality of raw cow's milk obtained from four dairy farms and a milk collection center in and around Addis Ababa, Bisrat Godefay and Bayleyegn Molla (2000) reported that milk samples from the collecting buckets in the dairy farms had counts as high as 10^5 cfu(ml)$^{-1}$, those from storage containers (before cooling) had counts of 10^6 cfu(ml)$^{-1}$, and the count reached 10^8 cfu(ml)$^{-1}$ upon arrival at the processing plant. By this time, coliforms also reached 10^4 cfu(ml)$^{-1}$. Raw milk obtained from the collection center had even higher counts (10^7 cfu(ml)$^{-1}$). A similar observation was also made on milk from collection centers in Arsi highlands (Taye Tolemariam *et al.*, 1999).

A variety of microorganisms were isolated from raw milk by different workers. Tadesse Mehari and Berhanu Abegaz Gashe (1990) stated that the isolates mostly belonged to the genera *Bacillus, Streptococcus, Lactobacillus, Arthrobacter, Alcaligenes, Aeromonas* and *Pseudomonas*. The cocci were, however, more predominant than the rods. According to Mogessie Ashenafi and Fekadu Beyene (1994), the aerobic mesophilic bacteria of udder milk was dominated by cocci, namely *Streptococcus/Enterococcus, Staphylococcus* and *Micrococcus*, whereas fresh raw milk obtained from milking utensils was basically dominated by micrococci and coryneforms. Bisrat Godefay and Bayleyegn Molla (2000) reported that milk collected from udder mainly contained staphylococci and micrococci while that collected at later stages of processing yielded members of Enterobacteriaceae, in addition.

When raw milk was kept at ambient temperatures, aerobic mesophilic bacteria increased from 10^6 to 10^8 cfu(ml)$^{-1}$, coliforms from 10^5 to about 10^7 and yeasts from 10^3 to 10^6 cfu(ml)$^{-1}$ within 12 hours (Mogessie Ashenafi and Fekadu Beyene, 1994). Increase in counts of these microbial groups was also noted in laboratory-pasteurized milk but maximum counts reached were 10^6, 10^5 and 10^2 cfu(ml)$^{-1}$, respectively. Refrigeration improved the keeping quality of raw milk and count of aerobic mesophilic bacteria was 10^6 cfu(ml)$^{-1}$ until 24 hours. It took about 72 hours to reach

levels as high as 10^8 cfu(ml)$^{-1}$. Refrigerated pasteurized milk had aerobic mesophilic counts of less than 10^6 cfu(ml)$^{-1}$ at 72 hours. Coliforms and yeasts were not detected at this time.

These studies have demonstrated that contamination during milking and post-pasteurization handling are the most important factors that determine the keeping quality of raw milk. It is important to minimize contamination during milking and inhibit excessive proliferation of microorganisms until further processing. When initial counts in raw milk are high, pasteurization would not effectively reduce the microbial load because the degree of microbial heat resistance increases with increasing number of microorganisms in the medium (Jay, 1996). In their study on the keeping quality of raw milk, Mogessie Ashenafi and Fekadu Beyene (1993) reported that washing the udder with warm water or with 3.5% Savlon solution before milking slightly decreased the initial contaminating flora of raw milk, but did not improve the keeping quality of the milk. Cleaning storage containers with water alone or with detergent did not markedly affect the rate of proliferation of the initial flora, whereas the traditional method of smoking storage containers had significant inhibitory effect, particularly in the first 24 hours, thus improving the keeping quality of raw milk. This may be an effective way to preserve raw milk at household levels.

Bovine milk has a naturally occurring inhibitory system called the lactoperoxidase system. It consists of three components: lactoperoxidase, thiocyanate and H_2O_2. All three components are required for antimicrobial effects. However, the amount of thiocyanate and H_2O_2 in milk is lower than the required inhibitory level. The lactoperoxidase system can be activated by increasing the concentration of thiocyanate and H_2O_2 to the required level, which will result in a marked improvement in the keeping quality of raw milk. As the system is more effective at 30° C than at 4° C, it is recommended to be used to preserve raw milk in countries where refrigeration is uncommon (Jay, 1996). Taye Tolemariam *et al.* (1999) assessed the preservative effect of lactoperoxidase system for preservation of milk in Arsi highlands. Initiation of lactoperoxidase system preserved the milk for three hours longer than the untreated control and they recommended its use to preserve milk until delivery to the processing plants in the study area, which were three hours away from the collection sites.

Teshager Semereab and Bayleyegn Molla (2001) analyzed the bacteriological quality of camel's milk in the Afar Region and found that about 95% of the udder milk samples had total aerobic mesophilic counts between 10^4 and 10^5 cfu(ml)$^{-1}$ and coliform counts were less than 10 cfu(ml)$^{-1}$ in 57% of the samples. However, milk collected from milking utensil had aerobic

mesophilic counts of 10^6 cfu(ml)$^{-1}$ and coliform counts of 10^4 cfu(ml)$^{-1}$.. Udder milk mainly contained staphylococci and streptococci whereas milk from milking bowls was additionally contaminated with bacteria of environmental origin including Enterobacteriaceae.

'Ergo' or 'Ititu' (fermented milk)

Fermented milk is known to be more stable and advantageous than fresh milk. Fermentation preserves the high quality.of nutrients present in milk in a relatively more stable form. Fermented milks have also been prescribed for curing disorders of the stomach, intestine and other troubles (Fernandez *et al.*, 1987). There are a variety of fermented milk products in different parts of the world. Examples are Acidophilus milk (many countries), Bulgarian butter milk (Balkans), Kefir (Southwestern Asia), Kumiss (Russia), Taette (Scandinavia), Tarhana (Turkey), and Yogurt (world wide) (Jay, 1996).

In Ethiopia, a considerable proportion of milk is consumed in a fermented state as 'Ergo' or 'Ititu'. The fermentation is usually natural, with no defined starter cultures used to initiate it. This is made possible only through the proliferation of the initial milk flora, with microbial succession determined by chemical changes in the fermenting milk. In most urban homes no attempt seems to be made to control the fermentation. Raw milk is left either at ambient temperatures or kept in a warmer place to ferment. In rural areas, particularly among the pastoralists, raw milk is usually kept in a well-smoked container and milk from a previous fermentation serves as inoculum. Lactic acid bacteria also become established on the inner walls of the container and serve as starter cultures. Incubation temperature does not usually vary significantly and the taste of the fermented product may, in general, be more or less uniform.

Kassaye *et al.* (1991) studied chemical and microbiological characteristics of 'Ititu' randomly collected from individual households in Borana region. 'Ititu' had an average pH of 3.65, titratable acidity (as lactic acid) of 1.92%, fat and protein content of 9.05% and 7.17%, respectively. Most of these values varied markedly among samples, though. 'Ititu' was rich in amino acids such as glutamic acid, alanine, proline, leucine and serine. In a study on farm-made fermented milk in Southern Ethiopia, Fekadu Beyene and Abrahamsen (1997) reported that 'Ititu' had 3.3–3.7% fat, 3.3–3.6% protein and 3.3–3.5% lactose. These two studies showed distinct differences in protein and fat content of the fermented milk. Kassaye *et al.* (1991) further reported that the total bacterial count was 10^{12} cfu(g)$^{-1}$, mainly dominated by lactic acid bacteria. Yeast and mold counts were 10^8 cfu(g)$^{-1}$, and

coliforms were not detected. They identified the prevalent lactic acid bacteria as *Lactobacillus casei* and/or *Lactobacillus plantarum*.

Almaz Gonfa *et al.* (1999) made a time course study on growth of microorganisms involved in the culturing of 'Ergo' ('Ititu') and reported that *Lactococcus, Streptococcus, Leuconostoc* and *Lactobacillus* carried out the culturing process. They also detected fairly high numbers of micrococci, spore formers and coliforms during the first 14 to 16 hours of fermentation. The lactococci, the most dominant group throughout the fermentation, reached counts as high as 10^9 cfu(ml)$^{-1}$ at the end of the fermentation. The aerobic mesophilic bacteria also had similar counts and the yeast population increased to 10^5 cfu(ml)-1 at 24 hours. Decrease in pH was noted and titratable acidity of the fermented product was 0.75%.

Yoneya *et al.* (1999) isolated lactic acid bacteria from three samples of 'Ergo' and found out that the lactococci produced L-lactic acid and were identified as *Lactococcus garvieae* and *Lactococcus lactis* subsp. *Lactis*. The lactobacilli produced D-lactic acid, and belonged to one species, but the strain appeared to be different from other species of the genus *Lactobacillus*.

The development of microorganisms during 'Ergo' fermentation in raw milk collected from eight dairy farms in Awassa showed variations in various parameters (Mogessie Ashenafi, 1995a). Initial aerobic mesophilic counts varied between 10^4 and 10^6 cfu(ml)$^{-1}$ among the various fermenting milk samples. In most cases 'Ergo' was formed at 24 hours and the average aerobic mesophilic count was >10^9 cfu(ml)$^{-1}$. Coliform counts, which were <10–10^4 cfu(ml)$^{-1}$ at initiation of fermentation, reached 10^6 cfu(ml)$^{-1}$ within 12 hours but this count decreased markedly thereafter. Lactic acid bacteria had initial counts of <10^4–10^6 cfu(ml)$^{-1}$, and maximum counts (10^7–10^9 cfu(ml)$^{-1}$) were attained at 24 hours. Yeasts were at undetectable levels initially but reached counts of 10^5 cfu(ml)$^{-1}$ at 24 hours. The drop in pH was gradual until 12 hours and fell sharply thereafter with most samples reaching values of 4.3 or below. Average initial titratable acidity (0.16%) increased to 0.88% at 'Ergo' formation. An interesting observation in this study was the high variability in microbial counts and other values among fermenting milk collected from different sources. The lactic microflora during the fermentation was dominated in most cases by cocco-bacillus shaped lactobacilli, but no further differentiation was made.

To study the effect of container smoking on the microbiological and biochemical qualities of fermenting 'Ergo' (Mogessie Ashenafi, 1996a), raw milk was allowed to sour naturally at ambient temperatures (25° C) in smoked or non-smoked containers. Milk in smoked containers had a lower rate of pH drop and the fermented product had good flavor for a longer

time after coagulation. The total count of non-lactic acid bacteria in milk in non-smoked containers reached a high count (>10^8 cfu(ml)$^{-1}$) within 12 hours, whereas milk in smoked containers required more than 24 hours to reach this level. Similarly, the growth of coliforms and lactic acid bacteria was slow in milk in smoked containers, thus assuring good and slow development of flavor components, safety of finished product and better keeping qualities. Lactobacilli dominated the flora of the fermented product in non-smoked containers, while lactococci were equally dominant in fermented milk in smoked containers.

Raw milk was also allowed to sour naturally at 20° C, 32° C, 37° C and 40°C (Mogessie Ashenafi, 1996a). At lower temperatures, the rate of acid formation was lower but the titratable acidity in the final products was higher. Milk incubated at lower temperatures had a better 'Ergo' taste. As the temperature of incubation was raised, the rate of pH drop was faster, and the time of coagulation became shorter. The rate of growth of the various groups of bacteria increased with an increase in incubation temperature. At lower incubation temperatures, lactococci dominated the lactic flora, while lactobacilli dominated at higher incubation temperatures. Smoking of containers may help to produce a safer and tastier 'Ergo' with better keeping quality at household level. In addition, lower incubation temperatures (around 20° C) may favor a gradual proliferation and succession of lactic acid bacteria and thus guarantee a desirable fermentation.

Findings from the various workers indicated that 'Ergo' is a product obtained by spontaneous fermentation and cannot be defined in terms of its microbiological or biochemical properties. It does not have any definite temperature and duration of incubation. Fermentation is carried out at ambient temperatures and precipitation of the casein is usually the sign of completion of fermentation. Consistency and flavor of 'Ergo' vary within or among households. Even in experimental controlled fermentations, variability in flavor components occurs with different strains of the same species. In their study on evaluation of lactic acid bacteria as starters, Fekadu Beyene *et al.* (1998) assessed *Lactococcus lactis* strains, previously isolated from naturally cultured milk products in Ethiopia, for milk coagulation time, organic acid metabolism and production of volatile flavor compounds such as acetaldehyde, diacetyl and acetoin. Evaluation of ten strains as single strain culture starters showed that the strains varied significantly in the production of volatile flavor compounds and metabolism of organic acids. The sensory properties of the cultured milk also differed when the different strains were used.

There may not be much to do about the microbiology of 'Ergo', if it remains to be produced on a household level. Use of 'Ergo' from a previous

fermentation as a starter for a boiled and cooled milk may help to produce a more or less uniform and safe product. But if 'Ergo' is to be produced on a large scale, some tasks have to be undertaken beforehand. It may be wise to start with isolating as many lactic acid bacteria as possible from 'Ergo' produced in the various ecological zones of the country. These cultures have to be identified and various combinations of them may be used for a controlled fermentation of 'Ergo'. The organoleptic quality of the product in relation to the various combinations may be determined. Those combinations having favorable organoleptic property may then be tested for their sensitivity to phages before they are used for large scale production. It may then be possible to define an 'Ergo' brand in terms of its microbiology and biochemistry.

'Ayib' (cottage cheese)

In Ethiopia, small holder milk processing is based on sour milk mainly due to high ambient temperatures, consumer's preference and increasing keeping quality of sour milk (O'Mahony, 1988). 'Ayib' is a traditional Ethiopian cottage cheese made from sour milk after the fat is removed by churning. Raw milk is collected in a clay pot and kept in a warm place (about 30° C) for 24–48 hours to sour spontaneously. The pH of sour milk is usually about 4. Churning of sour milk is carried out by slowly shaking the contents of the pot until the fat is separated. The fat is then removed and the defatted milk is heated to about 50° C until a distinct curd mass forms and floats over the whey. Temperature, however, can be varied between 40° C and 70° C without markedly affecting product composition and yield (O'Mahony, 1988). After gradual cooling, the curd is recovered from the whey. 'Ayib' comprises 79% water, 14.7% protein, 1.8% fat, 0.9% ash and 3.1% soluble milk constituents and the yield should be at least 1 kg of 'Ayib' from 8 liters of milk (12.5%) (O'Mahony, 1988). Fekadu Beyene and Abrahamsen (1997) analyzed various 'Ayib' samples produced by small-holders in three regions of southern Ethiopia and found out that the samples consisted of 80–81% moisture, 13.4–16% protein, 1.9–2.0% fat and 0.75–0.87% minerals.

In a study on the microbiological quality of 'Ayib' (Mogessie Ashenafi, 1990a), one hundred samples collected from an open market in Awassa had counts of mesophilic aerobic bacteria, yeasts and enterococci of 10^8, 10^7 and 10^7 cfu$(g)^{-1}$, respectively. Over 60% of the samples had psychrotrophic count of 10^6 cfu$(g)^{-1}$ and about 55% of the samples were positive for coliforms and fecal coliforms. *Bacillus cereus*, and *Staphylococcus aureus* were isolated at varying frequencies but at low numbers (10^2–10^3 cfu$(g)^{-1}$). The pH values of the samples varied between 3.3 and 4.6 with about 40% having pH lower than 3.7. In traditional 'Ayib' making, the milk itself may

have a high initial count of microorganisms and further processing may result in increasing numbers. However, since cooking of the curd is expected to decrease the count of microorganisms, 'Ayib' is supposed to have a lower microbial load after heating. Its low pH value should also assist in maintaining the low count for a certain period of time. The high microbial load in 'Ayib' would come from handlers and plant parts used for packaging and for imparting flavor.

Further analysis of 'Ayib' microflora showed that bacterial and yeast counts did not correlate with pH value of 'Ayib' samples (Mogessie Ashenafi, 1994a). However 'Ayib' samples with pH >4.0 contained more bacterial groups than those with pH <4.0. The Gram-positive rods dominated the aerobic mesophilic bacterial flora, *Microbacterium* and *Brevibacterium* spp. being the most abundant. Enterobacteriaceae and *Pseudomonas* spp. constituted the bulk of the Gram-negative rods. The count of lactic acid bacteria was around 10^6 cfu(g)$^{-1}$ and *Lactobacillus fermenti* and *Lactobacillus plantarum* dominated the flora. Nine different species constituted the yeast flora, but only *Kluyveromyces lactis*, *Kluyveromyces bulgaricus* and *Candida pseudotropicalis* could ferment lactose. One *Kluyveromyces* and one *Candida* species showed strong proteolytic activity. All yeasts isolates were lipolytic (Mogessie Ashenafi, 1989a).

In a study to determine the effect of curd-cooking temperature on the microbiological quality of 'Ayib' (Mogessie Ashenafi, 1990b), 'Ayib' was made by cooking defatted sour milk following traditional methods at temperatures between 40 and 70° C. Cooking at 40° C did not decrease the microbial load in general. At 50° C, counts of enterococci, Enterobacteriaceae and staphylococci decreased to <10^2 cfu(g)$^{-1}$. However, substantial number of yeasts, molds and lactic acid bacteria still remained in the product. Cooking at 60° C markedly decreased the number of most bacterial groups, yeasts and molds. Heat treatment at 70° C required a relatively shorter time for curd formation and achieved maximum reduction in number of the various microbial groups. Since temperatures higher than 80° C are reported to give the product a cooked flavor (O'Mahony, 1988), heat precipitation of curd at 70° C (pH, 4.0) was recommended as it resulted in a less contaminated and more wholesome 'Ayib'.

High counts of the different microbial groups observed in market 'Ayib' were basically results of improper handling after processing. Although the low pH of 'Ayib' prevents the growth of many food-borne pathogens, higher numbers of lactic acid bacteria and yeasts are not desirable in 'Ayib'. A much lower pH due to the activity of lactic acid bacteria may result in a too sour product with a low sensory quality. The proteolytic activity of

certain lactic acid bacteria and yeasts may also impart 'Ayib' with uncharacteristic flavors. In addition, oxidative yeasts can utilize lactic acid thus resulting in increase in pH, which allows the growth of the less acid-tolerant spoilage forms (ICMSF, 1980). Thus, appropriate temperature of curd cooking coupled with the low pH of the product should make 'Ayib' a safe and nutritious product with an improved keeping quality.

Safety considerations on milk and dairy products

Milk can easily be contaminated with food-borne pathogens of zoonotic importance Bagni *et al.*, (1998) isolated *Salmonella* from milk in Arsi region and isolation of *Mycobacterium bovis* from bovine milk was reported by Kinfe Getaneh and Eshetu Lemma (1987). Girma Tulu and Berhanu Abegaz Gashe (1992) also isolated seven species of streptococci and two species of staphylococci, including *Staphylococcus aureus,* from foremilk of 120 apparently healthy lactating cows in and around Addis Ababa. Considering the possibility of contamination of milk by food-borne pathogens from various sources, several studies were undertaken to determine the fate of *Salmonella* spp., *Bacillus cereus, Staphylococcus aureus* and *Listeria monocytogens* during the souring of milk into 'Ergo' (Mogessie Ashenafi, 1992, 1993b, 1994d). All the test pathogens could grow to levels as high as 10^7–10^8 cfu(ml)$^{-1}$ within 12 hours in milk. Smoking of containers significantly retarded the growth of the test pathogens, but only until 12 hours. Growth of lactic acid bacteria in souring milk resulted in complete inhibition of *Salmonella typhimurium* and *Salmonella enteritidis* between 48 and 60 hours of fermentation of milk in non-smoked and smoked containers. *Staphylococcus aureus* and *Bacillus cereus* were inhibited within 24 to 38 hours of fermentation in non-smoked containers and within 24 hours in smoked containers. *Listeria monocytogenes* in fermenting milk in non-smoked containers was inhibited after 48–60 hours, whereas inhibition was observed at 36 hours in smoked containers. It was suggested that the synergistic effect of pH, acids and container smoking were important in the complete inhibition of the test organisms.

In most cases, household preparation of 'Ergo' requires a one-day incubation at ambient temperatures. The milk coagulates within 24 hours and 'Ergo' is usually consumed preferably at this time because of its good flavor. In addition, increased drop in pH will also result in increased wheying off, which, in turn, results in loss of protein as whey. Observations in these studies have, however, indicated that *Salmonella* spp. and *Listeria monocytogenes* were not inactivated at 24 hours and the count, at this time, ranged between 10^5 and 10^6 cfu(ml)$^{-1}$ for *Salmonella* spp. and 10^3 and 10^4 for *Listeria monocytogenes*. In case of *Staphylococcus aureus* and *Bacillus cereus*, there was either a complete inhibition at 24 hours or the

number was below the level required to elucidate enough toxins to cause any gastroenteritis. Despite the general assumption that the low pH in 'Ergo' controls the proliferation of undesirable microorganisms, the dangers of listeriosis or salmonellosis from fresh 'Ergo' must not be underestimated. It was, thus, recommended to inoculate boiled milk with a three day old 'Ergo' to ensure the nutritious quality and wholesomeness of 'Ergo'.

Mohammed Abdella *et al.* (1996) studied survival and growth of *Salmonella* during the making of 'Ayib'. *Salmonella typhimurium, Salmonella enteritidis* and *Salmonella infantis* were able to grow when added to raw cheese milk, but none was able to survive the heating process at the prevailing low pH. When the *Salmonella* test strains were added after heating, they were able to survive for over 24 hours. They disappeared only after three days, by which time palatability had deteriorated. In another study on 'Ayib' sold in an open market, *Bacillus cereus* and *Staphylococcus aureus* were isolated at varying frequencies but at low numbers (10^2–10^3 cfu(g)$^{-1}$). *Listeria monoctyogenes* was, however, not encountered in any of the samples (Mogessie Ashenafi, 1990a).

TRADITIONAL FERMENTED PLANT FOODS

The use of microorganisms to process food goes back to ancient times. Fermented foods are essential parts of diets in all regions of the world. A number of food fermentation processes, including those that yield dairy products, sausages, pickles, sauerkraut and bread have been extensively investigated and documented. But many other foods prepared by the action of diverse species of fungi, bacteria and yeasts on plant materials are little known outside their native countries (Hesseltine and Wang, 1980). In Ethiopia, like in many developing countries, fermented food products constitute the major staple foods.

'Enjerra' fermentation

'Enjerra' is a fermented, sour, leavened pancake-like bread made from teff (*Eragrostis tef*), wheat, barley, sorghum or maize or a combination of some to these cereals. Teff 'Enjerra' is the most common and the main staple food in much of the central and northern highlands of Ethiopia.

The preparation of teff 'Enjerra' consists of two stages of natural fermentation, which last for about 24 to 72 hours, depending on ambient temperatures. The only required ingredients are the teff flour and water. Inoculation is accomplished by consistently using partially cleaned

fermentation container and by adding some 'Ersho', the clear, yellow liquid that accumulates on the surface of the batter, from a previous fermentation. The fermentation process of teff 'Enjerra' is described by Berhanu Abegaz Gashe (1985). The initial 18 hours are characterized by vigorous evolution of gas and maximum dough-rising. This is followed by the appearance of an acidic yellowish liquid on the surface of the dough at about 30–33 hours of fermentation. Gas evolution decreases after the pH has fallen below 5.8 (31 hours). The liquid layer is discarded at the end of the first stage of fermentation. As soon as the liquid layer is poured off, about 10% of the fermenting dough is boiled for 2 to 5 minutes and then mixed with the rest in the fermentation vat. This process signals the initiation of the second stage of fermentation. By mixing the boiled dough with the rest in the vat, the dough-rising and gas formation processes are enhanced so they occur in a short time. Maximum dough-rising, which normally takes 30 minutes to 2 hours signals the termination of fermentation. This is baked into 'Enjerra' on an earthen pan.

A major source of inoculum for teff fermentation is the teff flour itself. The traditional threshing processes of teff would result in the contamination of the teff seeds with a wide variety of microorganisms of soil and fecal origin. Microbiological analysis of teff flour, collected from ten different households, showed that flour from seven households had mold count of 10^3 cfu(g)$^{-1}$, eight had Enterobacteriaceae count of 10^4 cfu(g)$^{-1}$ and all had aerobic mesophilic bacterial counts of $\geq 10^4$ cfu(g)$^{-1}$ (Mogessie Ashenafi, 1994b). Melaku Umeta and Faulks (1988), however, reported that yeasts were the major organisms in teff flour.

'Ersho' is supposed to be a starter for teff fermentation. A study on the microbial flora and chemical properties of 'Ersho' showed that 'Ersho' had a pH of 3.5 and titratable acidity of 4.46% (Mogessie Ashenafi, 1994b). It, thus, does not support the survival of various groups of microorganisms. The mean aerobic mesophilic bacterial count for 'Ersho' collected from different households was 10^6 cfu(ml)$^{-1}$ and this consisted of only *Bacillus* spores. Yeast counts ranged between 10^5 and 10^6 cfu(ml)$^{-1}$ and *Candida milleri, Rhodotorula mucilaginosa, Kluyveromyces marxianus* and *Pichia naganishii* were the major yeast species. *C. milleri* was found in over 80% of the 'Ersho' samples from every household. *R. mucilaginosa*, the second most abundant, was encountered only in <40% of the samples. Only the *Candida* and *Kluyveromyces* species were active gas producers from glucose, sucrose and a variety of other sugars. In addition, all isolates were known not to hydrolyze starch. Thus the yeasts in 'Ersho' may not be active in the fermentation of teff until fermentable sugars are available due to the degradation of teff starch. They may, however, be important in leavening the batter of teff and producing flavor compounds in the later stages of

fermentation. No study has so far presented a conclusive proof as to which groups of microorganisms are important in breaking down starch and producing enough fermentable sugars to initiate the fermentation.

In a study of the yeast flora of fermenting teff, Chaltu Gifawesen and Abraham Besrat (1982) consistently isolated two gross morphological types of yeasts, and one type by far dominated the other at the peak of the fermentation. They observed an average yeast count of $2x10^8$ cfu$(g)^{-1}$ of dough after 22–24 hours of fermentation. *Saccharomyces* and *Torulopsis* were the two physiological groups most commonly found during the prime of the fermentation. The yeasts most prevalent in the yellow fluid belonged to the genera *Candida* and *Pichia*, and these were discarded with the yellow fluid and *Saccharomyces* and *Torulopsis* were the dominating flora during the secondary fermentation. As their yeast isolates did not hydrolyze starch, they concluded that the yeasts could not be responsible for the primary breakdown of starch. In a previous study, Stewart and Getachew Asnake (1962), reported that *Candida guillermondii*, they isolated from fermenting teff, was responsible for starch hydrolysis and increase in concentration of reducing sugars in the early phase.

According to Berhanu Abegaz Gashe (1985), a complex group of microorganisms was involved in the fermentation and members of Enterobacteriaceae initiated the fermentation. These were active during the first 18 hours of fermentation and reduced the pH of the fermenting dough to about 5.8. At this stage *Leuconostoc mesenteroides* and *Enterococcus faecalis* took over. As the pH was further reduced to about 4.7, *Pediococcus cerevisieae, Lactobacillus brevis, Lactobacillus plantarum* and *Lactobacillus fermentum* became the dominant flora and remained so until fermentation was terminated at 72 hours. The lactic acid bacteria were responsible for the acidic characteristics of the dough. Yeasts only appeared in significant numbers at a latter stage of fermentation. In a previous study, Berhanu Abegaz Gashe *et al.* (1982) stated that initial fermentation activity was carried out by a group of Gram-negative aerogenic groups with the population increasing substantially during the first 36 hours. The activity of these groups resulted in excessive evolution of gas and 'dough rising'. Most of the bacteria they isolated were capable of hydrolyzing starch and suggested that increase in reducing sugars within 48 hours of fermentation could be due to amylase activity originating from flour and microorganisms. However, the resulting acidity (pH 5–5.5) reduced their population thereafter. The lactic acid bacteria carried the fermentation a step ahead reducing the pH further to 4.0. Yeasts became abundant only as the pH was reduced to below 5.0 and dominated the flora in the yellowish liquid layer after 50 hours of fermentation. Discarding the liquid layer resulted in loss of soluble compounds (amino acids, sugars and minerals) and a large portion of the microorganisms, which also removed 4–13% of

the nitrogen in dry weight basis depending on the duration of the fermentation.

Ayele Nigatu *et al.* (1997) isolated a significant population of Gram-positive, endospore forming rods from fermenting teff dough and reported that *Bacillus licheniformis* was the dominant species. Based on the biochemical features of the isolates, the authors suggested that *Bacillus* species might play active metabolic roles and enrich the substrate for succession and dominance by the lactic acid bacteria. The proliferation of lactic acid bacteria during fermentation not only produces the necessary metabolites for flavor and taste, but also inhibits the growth of undesirable microorganisms in the fermenting dough. Meaza Girma *et al.* (1989) reported that spoilage microorganisms were inhibited when the pH of the fermenting dough approached 5.0. Ayele Nigatu and Berhanu Abegaz Gashe (1994a) however stated that spoilage microorganisms could grow until the pH dropped to 4.7. Growth was inhibited and the microbial population decreased thereafter. Both groups argued that inhibition was not attributed to acidity alone and other metabolites produced by the lactic acid bacteria could play important roles in the inhibition of undesirable microorganisms. As observed by Meaza Girma *et al.* (1989), the baking process would eliminate vegetative forms of microorganisms and fresh baked 'Enjerra' should be free of microorganisms, except some *Bacillus* spores. Ayele Nigatu and Berhanu Abegaz Gashe (1998), however, reported that yeasts and fungi survived the baking temperature/time combination of 100°C/5min. The acidity of 'Enjerra' may, however, not favor the germination of most *Bacillus* spores, although fungal spores may germinate when the environment permits. The inclusion of sorbates or benzoates in the right proportion after completion of fermentation and immediately before baking may improve spoilage of 'Enjerra' due to molding.

No study has been conducted on rate and microbiology of fermentation in warmer regions, where fermentation time is markedly shorter. Similarly no such studies were made on the fermentation of other cereals used for 'Enjera' fermentation in the various parts of Ethiopia.

Microorganisms are able to produce various metabolites during the fermentation through their enzymatic action on the substrate. Melaku Umeta and Faulks (1988) studied the carbohydrate composition of flour milled from red- and white-seeded teff varieties and the changes in carbohydrate during fermentation. They reported that non-starch polysaccharides were largely unaffected by fermentation and baking. Starch content decreased by about 9%, indicating that it served as main source of energy for the fermenting microorganisms. Sucrose dominated

the free sugars in flour, but fructose was the dominating free sugar in fermenting dough and the baked product. The findings were similar for both varieties of tef.

The total iron content of teff is reported to be 0.0033% (Sufian and Pittwell, 1969) and 0.0036–0.0078% (Abraham Besrat *et al.*, 1980a). The effect of fermentation on the bio-availability of iron, phosphorus and zinc of teff and wheat was studied by Ramachandran and Getachew Bolodia (1984). After thoroughly cleaning and washing their samples, they followed up the fermentation by dialysis of the batter. They found out that fermentation increased the dialyzable portions of iron from 9% to 24%, phosphorus from 16% to 60% and zinc from 2% to 43%. They concluded that the increase in dialyzable iron might have a positive effect on its bioavailability, and might thus explain the rarity of iron-deficiency anemia among teff-consuming population of Ethiopia. Kelbessa Urga *et al.* (1997a) also studied the effect of fermentation on nutritional and anti-nutritional factors of teff. They reported that by the end of fermentation, protein content in dough decreased by 12% whereas non-protein nitrogen, free amino acids, free amino acid nitrogen, soluble protein and fat acidity increased by 6 to 10 folds. Iron, phosphorus and calcium decreased by 43%, 35% and 41%, respectively. Anti-nutritional factors such as phytic acids, tannins and trypsin inhibitors decreased by 72%, 55% and 69%, respectively. Total protein content of different cultivars of teff varied between 6.5% and 9.3% (Lester and Endashaw Bekele, 1981).

'Kotcho' fermentation

Almost 10 million people in Ethiopia are dependent on ensete (*Ensete ventricosum*), also known as 'false banana' (Pijls *et al.*, 1995). It is grown on 67,000 sq. Km in Ethiopia and 60 mature plants are estimated to provide sufficient food for 5–6 persons per year (Demeke, 1986). The plant does not produce edible fruit, but its corm and pseudo-stem are scraped to separate the starchy pulp from the fiber, and the pulp is made to ferment in earthen pits. According to Berhanu Abegaz Gashe (1987a), the scrapings from the leaf bases and the pulverized stems and corms are mixed and kneaded into mash known locally as 'kotcho'. The mash is rolled into a ball, covered with fresh ensete leaves and left at ambient temperature for 2–5 days. This is then mixed and kneaded and placed in a 1 m^3 pit lined with fresh ensete leaves. Four to eight mature ensete plants are required to obtain sufficient 'Kotcho' to fill a pit. The 'Kotcho' is then pressed by hands or feet, covered with fresh ensete leaves, and layered with discarded ensete parts. Heavy materials such as large stones are put on top of the layering to ensure the creation of airtight conditions in the pit. The length of fermentation time varies from a few weeks, to several months or years depending on ambient

temperatures of incubation. The yield of 'Kotcho' is about 34 Kg/plant or 9.5 tons/ha/year (Pijls *et al.*, 1995).

Berhanu Abegaz Gashe (1987a) studied and described the microbiology of 'Kotcho' fermentation. He reported that *Leuconostoc mesenteroides* initiated the fermentation and dominated the lactic flora with counts of 10^7 cfu(g)$^{-1}$ on day 8. The pH of the fermenting mass dropped from 6.5 to 5.6 in 8 days. *Lactobacillus coryneformis* and *Lactobacillus plantarum* dominated thereafter and further reduced the pH to 4.2 after 50 days. Spore formers were present at levels of $\leq 10^3$ cfu(g)$^{-1}$ during the first 15 days. Generally, the population of *Clostridium* spp. was two to five times more abundant than *Bacillus* spp. *Clostridium butyricum, Clostridium beijerinckii, Clostridium sticklandi, Bacillus subtilis, Bacillus megaterium, Bacillus licheniformis* and *Bacillus cereus* were among the spore-formers which appeared to show active growth in fermenting 'Kotcho'. Yeasts reached their highest counts (10^3 cfu(g)$^{-1}$) between 22 and 43 days and the yeast flora consisted of the *Trichosporon, Torulopsis, Rhodotorula* and *Candida.*

Abraham Besrat *et al.* (1980b) studied protein quality and quantity during fermentation of different varieties of enset. Fermentation reduced protein content of the relatively high-protein cultivars, but had no effect on, or in certain cases slightly increased, protein content of the relatively low-protein cultivars. They explained protein reduction to be due to leaching of the more soluble proteins and amino acids. They also stated that fermentation had the general effect of increasing the essential amino acid content of 'Kotcho'. Taye Bezuneh (1984) reported that fermentation reduced carbohydrate content of enset (50%) to 41% in 'Kotcho' and 54% in 'Bulla'. Enset protein (3.68%) was also reduced to 1.2% in 'Kotcho' and 0.25% in 'Bulla', and enset calcium (0.27%) to 0.195% in 'Kotcho' and 0.065% in 'Bulla'.

Kelbessa Urga *et al.* (1997b) determined changes in chemical composition during 'Kotcho' fermentation for seven weeks. Total protein, ash and total carbohydrates decreased by 15, 16 and 34%, respectively. Reduction in iron, phosphorus, and calcium was between 15–30%. Starch and available carbohydrates decreased by 51%, and soluble and reducing sugars decreased by over 80% during the fermentation. The pH of the fermenting mash decreased from 5.7 to 3.8 accompanied by a sharp increase in titratable acidity. The fermentation also reduced anti-nutritional factors such as tannins and trypsin inhibitors.

In his study on the microbial spoilage of 'Kotcho', Berhanu Abegaz Gashe (1987b) reported that 'Kotcho' became easily contaminated with microorganisms when removed from the fermenting pits and the major spoilage fungi belonged to *Penicillium, Trichoderma* and *Chaetomium* species. In addition, bacterial species belonging to *Leuconostoc,*

Pseudomonas, Bacillus and *Erwinia* were isolated from slimy 'Kotcho'. Microbial spoilage was manifested in the form of discoloration.

Mogessie Ashenafi and Yewelsew Abebe (1996a) studied the microbial load of market 'Kotcho' and 'Bulla' and found out that products brought to the Awassa open market for sale did not undergo appropriate fermentation and had pH values around neutral. 'Kotcho' and 'Bulla' had high counts of aerobic mesophilic bacteria and yeasts ($\geq 10^6$ cfu(g)$^{-1}$). Coliform counts were markedly higher in 'Bulla' (10^5 cfu(g)$^{-1}$) than in 'Kotcho' (10^3 cfu(g)$^{-1}$). Counts of enterococci, in both products, ranged between 10^4 and 10^5 cfu(g)$^{-1}$. Micrococci and *Bacillus* spp. dominated the aerobic bacterial flora. Among the yeast species, *Rhodotorula glutinis, Kluyveromyces marxianus* and *Pichia membranefaciens* were isolated from most samples. As 'Kotcho' and 'Bulla' appeared to be processed in unhygienic conditions, unfermented products are likely to spoil easily.

When these products were stored at room temperature in a loosely wrapped condition, both products had undesirable odor, slimy surface and dark discoloration after eight days (Mogessie Ashenafi and Yewelsew Abebe, 1996b). Spoiled 'Kotcho' and 'Bulla' had very high counts of aerobic mesophilic bacteria (about 10^{10} cfu(g)$^{-1}$) and *Micrococcus* and *Bacillus* species dominated the spoilage flora. Psychrophilic microorganisms consisting of bacteria and molds were isolated at levels of $>10^4$ cfu(g)$^{-1}$ and mold spores caused dark discoloration. Microorganisms active in starch hydrolysis, proteolysis and lipolysis were encountered in the products at varying frequencies. Tightly wrapped samples, which served as control, did not show any detectable spoilage in terms of odor, consistency or color.

Fermented condiments

The majority of traditional fermentations are accompanied by certain biochemical changes of nutritional importance. Some fermented foods produce strong flavor such that the product is not consumed alone, but is added as a condiment to make the food more tasty and enjoyable (Hesseltine, 1983). Although in most parts of Africa, protein-rich food ingredients are often fermented to make condiments, low-protein ingredients are also fermented into condiments in Ethiopia.

A typical example of legume fermentation practiced in Ethiopia is 'Siljo' fermentation. 'Siljo' is a fermented product made from safflower (*Carthamus tinctorius*) extract and faba bean (*Vicia faba*) flour. It is a popular condiment during the long fasting period before Easter. Faba bean flower is thoroughly mixed with safflower extracts and cooked well to a gruel consistency. This is cooled to about 55° C and black mustard powder, homogenized in warm water, is added to it. After a thorough mixing, the gruel is left to ferment at ambient temperature. At around 32 hours of fermentation, peeled garlic and rue leaves are added to it. Its fermentation

is spontaneous and is usually ready for consumption after three days of fermentation. The fermented product is a gray gruel with a typical acidic and mustard flavor. It is consumed as a side dish to any one of the major legume-based sauces along with 'Enjerra'. 'Siljo' is believed to add some variety to the otherwise monotonous fasting dishes of the average highland Ethiopian. It is a household product, not produced in large amounts and whatever is produced is usually consumed within a few days.

Tetemke Mehari and Mogessie Ashenafi (1995) studied the microbiology of 'Siljo' fermentation and found out that as the major substrates were thoroughly cooked during initial preparation, the black mustard powder was the source of starter microorganisms. *Lactobacillus acidophilus, L. plantarum* and *L. delbruekii* initiated and later dominated the fermentation process. The pH of the fermenting mass dropped to 4.5 within 36 hours and reached 4.0 at day 7. Aerobic mesophilic and lactic acid bacteria were present at levels as high as 10^{10} cfu(ml)$^{-1}$ after 36 hours of fermentation, but Enterobacteriaceae were not detected. *Micrococcus, Bacillus* and *Lactobacillus* species dominated the flora. Crude protein, crude fat and ash increased slightly during the fermentation, with final values of around 28%, 25% and 7%, respectively. There was marked increase in protein availability and concentration during the fermentation. Senait Zewdie *et al.* (1995) reported that members of the genera *Enterococcus, Bacillus, Lactococcus, Lactobacillus* and yeasts were the dominant microorganisms in fermenting 'Siljo'. Enterococci showed decrease in number while lactococci, lactobacilli and yeasts increased during the fermentation period. The pH fell from an initial value of 6.1 to 4.2 at 96 h while the initial and final values of titratable acidity were 0.36 and 0.75, respectively.

'Awaze' and 'Datta' are other traditional fermented condiments and are consumed with other items on the basis of their desirable aromas and flavors. Both condiments result from the microbial fermentation of vegetable-spice mixtures. The major substrates in 'Awaze' are red sweet pepper (*Capsicum annum*), garlic (*Allium ursinum*), and ginger (*Zingber officinale*) to which some proportions of different spices are added. The major substrate in the making of 'Datta' is the small chili pepper (*Capsicum frutescence*) at its green stage. 'Awaze' is common in the north and central Ethiopia and is often used to flavor sliced raw or roasted meat and other traditional pancakes. 'Datta' is a condiment of similar use mainly in the southern part of the country.

Ahmed Idris *et al.* (2001) conducted microbiological and biochemical studies on the fermentation of 'Awaze' and 'Datta'. Ingredients for 'Awaze' preparation had a microbial load of 10^6 cfu(g)$^{-1}$ and the flora was dominated by *Bacillus* spp. The count of aerobic mesophilic bacteria decreased during the fermentation period. Lactic acid bacteria reached the maximum count of 10^9 cfu(g)$^{-1}$ at day 4 and the count remained >10^8 until

end of fermentation. The heterofermentative lactic acid bacteria dominated until day 3 and the homolactics took over thereafter. Yeasts also reached counts of 10^6 cfu(g)$^{-1}$ at end of fermentation on day 12. In 'Datta' fermentation, the count of aerobic mesophilic bacteria remained unchanged during the fermentation. Lactic acid bacteria initiated the fermentation at a level of 10^4 cfu(g)$^{-1}$ and reached 10^9 cfu(g)$^{-1}$ at end of fermentation on day 7. Contrary to 'Awaze' fermentation, the homofermentative lactic acid bacteria initiated and dominated 'Datta' fermentation for the first two days. The heterolactics dominated thereafter. Both fermentations were accompanied by declining pH and increasing titratable acidity. Both fermented condiments had low initial contents of available protein and reducing sugars and these did not show marked differences throughout the fermentation.

Safety considerations on fermented plant foods

Ayele Nigatu and Berhanu Abegaz Gashe (1994b) reported the antagonistic potential of fermented 'Kotcho' and aqueous extract of fermented 'Kotcho' against *Salmonella* spp., *Pseudomonas aeruginosa, Klebsiella* spp., *Bacillus cereus* and *Staphylococcus aureus*. Metabolites from the fermenting lactic acid bacteria were believed to prevent the survival and growth of the test organisms. Similarly, the growth of *Salmonella typhimurium, Staphylococcus aureus, Bacillus cereus* and *Pseudomonas aeruginosa* was inhibited when the pH of fermenting teff reached 5.0–5.5 (Meaza Girma *et al.*, 1989). As the test organisms grew in far more acidic conditions in broth than in fermenting tef, the authors suggested that the inhibition could be due to antimicrobial substance(s) produced by some of the fermenting lactic acid bacteria. The baking process of the fermenting dough, however, inactivated the vegetative forms of the test organisms. In another study on the effect of heat-treatment on the antimicrobial property of the fermented dough ('teff' and 'kotcho') and the baked products, Ayele Nigatu and Berhanu Abegaz Gashe (1998) observed that the test pathogens were more inhibited in high temperature treated fermented dough than that treated at lower temperatures. They, thus, concluded that if post-baking contamination was minimized or prevented, the products would be microbiologically safe, with respect to asporogenous pathogens, when served fresh. This was due to the increased inhibitory property of the baked products obtained through high temperature baking.

Gulelat Dessie *et al.* (1996) compared the fate of two *Salmonella* test strains in fermenting 'Siljo' and a control gruel which was not made to ferment. The test strains reached levels of 10^6 cfu(ml)$^{-1}$ within 24 hours in the control gruel. However, they could not grow in fermenting 'Siljo'. *S. enteritidis* and *S. typhimurium* were completely eliminated at 48 and 72 hours, respectively. In a similar study, *Staphylococcus aureus, Bacillus cereus* and *Listeria monocytogenes* grew luxuriously in control gruel, but were inhibited in fermenting 'Siljo' in 48, 24 and 48 hours, respectively (Gulelat Dessie *et*

al., 1997). Acid production by lactic acid bacteria and components of mustard power could have inhibited the test organisms. Challenge studies on 'Awaze' and 'Datta' fermentation with *Salmonella typhimurium* showed that the fermenting condiments had strong bactericidal property against the test strain. *S. typhimurium,* inoculated into the fermenting condiments at initial high levels (10^6 cfu$(ml)^{-1}$) was completely eliminated in fermenting 'Awaze' and 'Datta' in 56 and 32 hours, respectively (Ahmed Idris *et al.*, 2001).

TRADITIONAL FERMENTED BEVERAGES

Fermented beverages constitute a major part of the diet of traditional African rural homes serving as from inebriating drinks to weaning foods, in addition to their role in social functions such as marriage, naming and rain making ceremonies (Zvauya *et al.*, 1997). Traditional fermented beverages are those which are indigenous to a particular area and have been developed by people of that area themselves using age old techniques from locally available (mostly home-grown) raw materials. The traditionally fermented beverages are low-cost products in all aspects; they are manufactured using only rudimentary equipment such as earthen vessels and the handling and consumption often takes place under conditions of poor hygiene (Steinkraus, 1983). Fermented beverages produced from cereals are usually referred to as beers while those produced from fruits, milk sap, honey or molasses are classified as wines (Pederson, 1979).

Microorganisms of various groups appear to be involved in the fermentation of beverages indigenous to different parts of the world. The sources of these microorganisms are usually the raw ingredients and the traditional utensils used for fermentation processes. Initially, therefore, a wide variety of microorganisms are involved but most give way to more adaptive genera as the fermentation goes on. It may, thus, be said that the initiation of fermentation of most traditional fermented beverages may be undertaken by different groups of microorganisms as far as sufficient fermentable sugars are available in the substrate. As the fermentation proceeds and the environment becomes more and more acidic, yeasts and lactic acid bacteria dominate the fermentation. These two groups of microorganisms usually determine the alcohol content and flavor of the final product.

'Tella' fermentation

'Tella' has various vernaculars in the various regions and is a malt beverage based on substrates such as barley, wheat, maize, millet, sorghum, teff or other cereals. It is, by far, the most commonly consumed alcoholic beverage in Ethiopia. According to Samuel Sahle and Berhanu Abegaz Gashe (1991),

over 2 million hectoliters of 'Tella' is thought to be produced annually in households and 'Tella' vending houses in Addis Ababa. There are several recipes for making 'Tella' and every household appears to have its own version of recipe. Samuel Sahle and Berhanu Abegaz Gashe (1991) described the processes and microbiology of 'Tella' fermentation. Generally, earthen pots used for 'Tella' production are thoroughly cleaned with fresh leaves of *Vernonia amygdalina* and water and subsequently smoked in glowing splinters of *Olea europaes*. The fermentation is divided into four phases. During the first phase, powdered leaves of 'Gesho' (*Rhamnus prenoides*) are mixed with water in a small earthen pot and allowed to ferment for 4 days. The fermenting material is commonly called 'Tinsis'. This is transferred to a large earthen pot and the second stage begins by mixing it with barley malt, pounded stems of 'Gesho') and pieces of pan-cake like bread ('Kitta'). This is left to ferment for two more days. During the third stage more carbohydrate source and water are added to the container and the contents are mixed into a thick slurry called 'Difdif'. This is also allowed to ferment for two more days. At the final stage, the container is filled with water to the brim and the contents are again mixed thoroughly. The container is sealed to create anaerobic conditions and left to ferment for two more days. At the end of the fermentation, most suspended materials settle to the bottom of the container. Depending on the intensity of processing of the ingredients, the color may vary from light yellow to dark brown.

According to Samuel Sahle and Berhanu Abegaz Gashe (1991), the fermenting organisms were composed of *Saccharomyces* spp, (mostly *S. cerevisieae*) and *Lactobacillus* spp. The yeasts dominated the fermenting flora after the end of the first stage till the completion of fermentation. Increase in alcohol content was accompanied by yeast growth and decrease in reducing sugars and total carbohydrates. The pH and ethanol content are in the range of 4.5–4.8 and 2.8–5.0% (v/v) respectively, when 'Tella' is considered to be the most suitable for consumption. After ten days of fermentation, 'Tella' becomes too acidic to consume due to the growth of *Acetobacter* spp. which convert ethanol to acetic acid under aerobic conditions. According to Alemu Fite *et al.* (1991), 'Tella' collected from Debre Berhan, Ataye and Addis Ababa had alcohol content of 2.4–3.3%, 2.1–2.7% and 1.6–2.8%, respectively. Mean fusel oil content for the three places was 59 ppm, 59 ppm and 47 ppm, respectively and mean methanol content was 55 ppm, 27 ppm and 28 ppm, respectively. Belachew Desta (1977), in his survey of alcoholic content of some traditional beverages of Ethiopia, found that the ethanol content of 'Tella' ranged from 5.65% to 6.56%.

'Tej' fermentation

Honey wines are primitive types of wines that are not crystal clear products. Instead they are cloudy, effervescent containing residues of

substrates and fermenting yeasts and other microorganisms (Steinkraus, 1983). 'Tej' is a home processed, and commercially available honey wine. Often times, widely for commercial purposes, a mixture of honey and sugar may be used as major fermentable substrates. In cases where sugar is also used as substrate, coloring is added so that the beverage attains a yellow color similar to that made from honey. Some 'Tej' producers also include different concoctions such as barks or roots of some plants or herbal ingredients to improve flavor or potency of 'Tej'. According to Vogel and Abeba Gobezie (1983), during the preparation of 'Tej', the fermentation pot is seasoned by smoking over smoldering 'Gesho' (*Rhamnus prenoides*) and put back to the fermenting must. The pot is covered and fermented continuously for another five days, in warmer weather, or for 15–20 days, in colder cases. The mixture is stirred daily and finally filtered through cloth to remove sediment and *Rhamnus prenoides.*

Fermentation of 'Tej', like other traditionally fermented alcoholic beverages, relies on the microorganisms present in the substrates, fermentation vats and equipment. The lactic acid bacteria are known to produce a variety of chemical compounds relative to fermentation conditions. Their metabolic products contribute to the acidity and also add distinctive flavor and aroma to the fermenting material. Yeasts of the genus *Saccharomyces* were reported to be responsible generally for the conversion of sugars to ethanol in 'Tej' (Vogel and Abeba Gobezie, 1983). However, as 'Tej' fermentation is a natural fermentation, variability in lactic acid and yeast flora may result in variability in acidity, flavor and alcohol content of the product.

Bekele Bahiru *et al.* (2002) studied microbial variability in 'Tej' samples collected from various production units at different production times. They reported that mean counts of aerobic mesophilic bacteria and aerobic spores for the different production units were $<10^3$ cfu(ml)$^{-1}$. Coliforms and other members of Enterobacteriaceae were below detectable levels, basically due to the low pH and other inhibitory substances in 'Tej'. Yeasts were among the dominant groups of microorganisms in 'Tej' samples with mean counts $>10^6$ cfu(ml)$^{-1}$ for the different production units. Major yeast species in 'Tej' were *Saccharomyces cerevicieae, Kluyveromyces bulgaricus, Debaromyces phaffi* and *Kluyveromyces veronae*. Yeast counts showed significant variations within samples of a production unit. Lactic acid bacteria also had counts $>10^6$ cfu(ml)$^{-1}$ with significant variation within samples of the different production units. In most production units, heterofermentative lactic acid bacteria had higher counts than the homofermentative ones. The lactic flora consisted of *Lactobacillus, Streptococcus, Leuconostoc* and *Pediococcus* species. The lactobacilli were, however, the most frequently encountered groups. In most of the samples, the lactic flora was dominated by only two or three groups of lactic acid bacteria.

In a similar study, Bekele Bahiru *et al.* (2001) also showed that the pH values of 'Tej' samples varied between 3.02 and 4.90 and, at least 77% of the samples had pH values <4.0. The range of titratable acidity was 0.1g/100ml to 1.03g/100 ml and mean values for production units ranged between 0.34 and 0.6 g/100ml. About 65% of the 'Tej' samples had titratable acidity values of ≥4 g/100ml. Variation in pH and titratable acidity values were significant within samples of the same production unit. Mean total alcohol content for the production units was 6.95–10.9% and about 58% of the samples had alcohol content of 5–10%. Fusel oil content of samples ranged between 0.1 g/100L and 88 g/100L. Mean values for production units was 13.6–27.4 g/100L. About 50% of the samples had fusel oil contents of >20 g/100L. Mean values for total carbohydrate, total lipid, total protein and reducing sugars were 1.49–3.79 g/ml, <1.0 g/ml, 0.33–4.66 g/ml and 0.46–2.09 g/ml, respectively (Bekele Bahiru *et al.*, 2001). Variations in the various chemical and nutritional parameters were significant within samples of the same production unit.

According to Alemu Fite *et al.* (1991), 'Tej' collected from Gojam, Debre Berhan and Addis Ababa had alcohol content of 3.3–9.8%, 3.9–5.1% and 6.6–8.4%, respectively. Mean fusel oil content for the three places was 121 ppm, 44 ppm and 145 ppm, respectively while mean methanol content was 55 ppm, 40 ppm and 42 ppm, respectively. Belachew Desta (1977), in his survey of alcoholic content of some traditional beverages of Ethiopia, found that the ethanol content of 'Tej' ranged from 13–13.3%.

'Borde' fermentation

'Borde' is a traditional fermented beverage made from maize or wheat. It is a very popular meal replacement in southern Ethiopia and some other parts of the country. 'Borde' is prepared mainly from maize. Maize flour is soaked in excess water and then deeply roasted on a hot metal pan. After cooling, ground malt is thoroughly mixed into it, put into a large clay jar and further blended in boiling water. At this stage, ground barley whipped in hot water is added to it and allowed to ferment overnight. The fermenting mixture is filtered and served for consumption the next morning. 'Borde' is consumed while in an active stage of fermentation. It is usually consumed by low-income groups and, on the average, a laborer consumes about three liters of 'Borde' per day. Many factors could account for the role that many traditional fermented beverages play as meal replacements. The high carbohydrate content coupled with the small amount of alcohol serve as good source of energy. The high microbial count of yeasts and lactic acid bacteria qualify 'Borde' as good source of microbial protein. The relatively high lysine content of yeast protein would improve the nutritive value if added to grains such as maize, wheat, etc. It appears that the ingredients for 'Borde' fermentation vary among 'Borde' producing communities. Maize was reported to be the major ingredient in southern Ethiopia (Mogessie Ashenafi and Tetemke Mehari, 1995) whereas

wheat was the preferred ingredient in Addis Ababa (Ketema Bacha *et al.*, 1998). The processing steps, however, were not markedly different. 'Borde' has a short shelf life as it turns too sour to consume after about 16 hours of fermentation. It is, nevertheless, one of the important nutritious and low alcohol beverages in Ethiopia.

Mogessie Ashenafi and Tetemke Mehari (1995) studied microbiological and nutritional properties of ready-to-consume 'Borde' in Awassa town and reported that mean pH of the samples was 4.1. Counts of aerobic mesophilic bacteria and lactic acid bacteria were around 10^9 cfu(ml)$^{-1}$. Counts of Enterobacteriaceae was around 10^6 cfu(ml)$^{-1}$, and yeast count ranged between 10^7 and 10^8 cfu(ml)$^{-1}$. Variations in counts were markedly low among the samples. Total protein, soluble protein, fat and ash content of 'Borde' was 9.55%, 3.31%, 6.88% and 3.66%, respectively and, compared with the raw ingredient, fermentation resulted in increase in protein, fat and ash contents of the finished product.

Ketema Bacha *et al.* (1998) studied the microbial dynamics of 'Borde' fermentation and reported that the ingredients consisted of wheat flour and barley malt and the product was ready for consumption within 12 hours of fermentation. The malt contained a considerable number of aerobic mesophilic bacteria, lactic acid bacteria and yeasts. The aerobic mesophilic bacteria at the start of fermentation were dominated by micrococci, staphylococci, members of Enterobacteriaceae and *Bacillus* spp. The Gram-positive cocci and rods dominated after four hours and coliforms and Enterobacteriaceae disappeared thereafter. Lactic acid bacteria had initial counts of 10^5 cfu(ml)$^{-1}$ and reached counts as high as 10^9 cfu(ml)$^{-1}$ at 24 hours. Heterofermentative lactobacilli dominated the lactic flora throughout the fermentation and a steady increase in yeast count was observed as the fermentation proceeded. The pH of fermenting 'Borde' declined from 5.2 at the start to 3.8 at 12 hours.

'Shamita' fermentation

'Shamita' is a widely consumed low alcohol beverage with a thick consistency and is consumed as meal replacement by most people who cannot afford a reasonable meal. For 'Shamita' preparation, lightly roasted barley is ground to which salt, ground linseed and small amounts of spices are added to it. These are mixed with water, usually in the evenings, and the product is ready for consumption in the morning. Malt is not commonly used in 'Shamita' fermentation, although local 'Shamita' brewers in Addis Ababa use it frequently, and starch is the only principal fermentable carbohydrate. The microorganisms responsible for the fermentation come mostly from back-slopping using a small amount of 'Shamita' from a previous fermentation as well as from ingredients and equipment.

The pH of ready to consume 'Shamita' in Awassa town was reported to be 4.2 and the product had high microbial counts (10^6–10^7 cfu(ml)$^{-1}$) consisting of mainly lactic acid bacteria and yeasts (Mogessie Ashenafi and Tetemke Mehari, 1995). These microorganisms could make the product a good source of microbial protein. However, the product had poor keeping quality because of the high number of active microorganisms and became unacceptable about four hours after being ready for consumption. Compared to the major ingredient, barley, 'Shamita' had more total protein, soluble protein, fat and ash with values of 10.37%, 3.46% and 6.85%, respectively. In a microbiological study of 'Shamita' fermentation, Ketema Bacha *et al.* (1999) reported that all ingredients and the clay jar rinse water had large numbers of aerobic mesophilic bacteria (>10^4 cfu(ml)$^{-1}$) mainly consisting of *Bacillus* and *Micrococcus* spp. Barley malt contributed most of the lactic acid bacteria and yeasts, which were important to the fermentation. They dominated the fermentation flora reaching final counts of 10^9 and 10^7 cfu(ml)$^{-1}$, respectively. The dominant lactic flora consisted of both heterofermentative and homofermentative lactobacilli. The pH of fermenting 'Shamita' dropped from an initial value of 5.80 to 4.43 within 12 hours of fermentation. Coliforms and other members of Enterobacteriaceae as well as molds were eliminated after 16 hours of fermentation. Laboratory prepared 'Shamita' had comparable microbial counts with samples obtained from local 'Shamita' brewers in Addis Ababa (Ketema Bacha *et al.*, 1999).

The foods and beverages considered so far are preserved products in that their keeping quality is improved considerably over that of the raw materials from which they are made. This is particularly important for most house-holds because refrigeration is not affordable by many. Unfortunately, traditional processing does not have a mechanism to stop the fermentation at a stage where the quality of the product is at its best. Consequently, although other spoilage microorganisms or pathogenic ones may not grow in the products, the keeping quality of the fermented products is compromised because the same microorganisms responsible for the acceptable attributes of the products would make the products too sour to consume after a few days.

As can be observed hitherto, different workers reported different values for the parameters they measured during the fermentation process. Microbiological and chemical variability in the various products could be attributed to the spontaneous fermentation, as this depends on the microflora naturally present in the substrates, on utensils and equipment used. The different metabolic products of these randomized microflora at different stages, the physical and chemical environments and duration of fermentation have influence on the succession of microorganisms during fermentation and consequently result in microbiological and chemical variability of products at the time they are ready for consumption.

READY-TO-EAT FOODS

Microbiological studies on Ethiopian ready-to-eat foods or other food items are limited. There are a few reports on the microbiology of sauces, weaning foods, street foods, various fish, poultry and other meat products, vegetables and fruits.

Sauces

Various types of sauces are consumed in Ethiopia two to three times daily, along with traditional cereal pan-cakes. The sauces are often legume-based (made from roasted and ground faba bean or chick pea, split pea or split/whole lentil, etc), vegetable-based (cabbage, Ethiopian kale, potato, carrot, etc.) or meat-based (chicken, mutton, beef, etc.). Legume-based sauces are usually frequented in low-income families or during fasting periods, whereas meat-based sauces are luxuries for most families. In most cases dinning establishments and households with large families prepare their sauces in fairly large volumes early on the day and the sauces are usually kept at ambient temperatures for several hours until served with or without re-heating. Frequently these sauces are maintained at ambient temperatures overnight.

Although initial cooking (usually at above 85° C for 15–60 minutes) is supposed to eliminate most of the initial contaminants, the sauces are subjected to recontamination from equipment, utensil surfaces, food handlers, dust and airborne contaminants after cooking and during serving. Such practice could lead to spoilage and safety problems with possible public health implications. In addition, a variety of spices are added during the preparation of Ethiopian sauces. Spices are known to be heavily contaminated with microorganisms and they contribute micro-organisms including spoilage types to food products. Although spices are not produced in large quantities, their significance in Ethiopia can hardly be over-estimated (Jansen, 1981). Spices are needed every day in considerable amounts for the preparation of the main dish of the day (Goettsch, 1991). Ethiopian sauces are mostly hot-spiced made of a variety of spices. Different sauces have different flavors depending on the type and amount of spices and other constituents, the extent of cooking and other factors.

In a study of microbial spoilage of Ethiopian sauces at ambient temperatures (22–25° C), aerobic mesophilic bacteria, Enterobacteriaceae and yeasts were observed at levels of 10^2 cfu(ml)$^{-1}$ in fresh sauces obtained from different households (Mogessie Ashenafi, 1996b). Spoilage was noted at 48 hours in all legume-based sauces, most vegetable- and most meat-based sauces with counts as high as 10^7 to 10^8 cfu(ml)$^{-1}$. The spoilage flora

was dominated by *Bacillus* spp., Enterobacteriaceae, micrococci and staphylococci. Spoilage microflora of legume-based and vegetable-based sauces were dominated by *Bacillus* spp. whereas that of meat-based sauces was dominated by micrococci and staphylococci. It was assumed that some of the spoilage microorganisms might have survived cooking, while others were post-cooking contaminants.

A similar study was undertaken to assess the spoilage potential of selected bacterial isolates (Mogessie Ashenafi, 1997). A total of 56 test strains consisting of *Bacillus* spp., *Micrococcus* spp., Enterobacteriaceae, *Aeromonas* spp. and other Gram-positive rods, isolated from spoiled traditional Ethiopian sauces, were evaluated for their spoilage potential on legume-based, vegetable-based and meat-based sauces. All test strains spoiled legume-based sauces within 48 hours at ambient temperatures. Off-odor with or without gas production was detected at spoilage. Meat-based and vegetable-based sauces were spoiled only by 14 and 12 strains, respectively. Enterobacteriaceae were the major spoilers of most sauces in terms of off-odor and gas production. The two major spices which determine the color of sauces, 'berbere' and 'erd' (turmeric) showed some degree of retarding effect on spoilage microorganisms, with 'berbere' showing stronger inhibitory property until 12 hours of holding. In addition to the major component, *Capsicum annum*, 'berbere' consists of around ten other spices and its retarding effect may be due to the anti-microbial effect of some essential oils from the spices as observed by other workers (Aureli *et al*, 1992). However, as retardation was effective only for the first 12 hours, this ingredient may not be expected to improve keeping quality of sauces for a longer period. As it is not affordable to use cold storage in most households in Ethiopia, re-heating sauces satisfactorily immediately after each serving may help to improve the keeping quality of the sauces even when maintained at ambient temperatures.

Feleke Moges and Mogessie Ashenafi (2000) isolated and characterized *Bacillus* spp. from different spices commonly used to enhance the flavor of sauces. These included fenugreek, black cumin, Ethiopian caraway, ginger and cardamom. Spore counts ranged from $<10^2$ in cumin to 10^8 in ginger. Of the 781 *Bacillus* isolates obtained, the most frequently encountered species were *Bacillus pumilus* (43.7%) followed by *B. subtilis* (16.6%), *B. circulans* (11.2%), *B. licheniformis* (8.2%) and *B. cereus* (4.9%). The *B. pumilus* and *B. subtilis* isolates were active in proteolysis and lipolysis, whereas *B. cereus* isolates were more proteolytic and amylolytic. All test strains grew well in the three different types of sauces, but growth was markedly lower in vegetable-based sauces. Spoilage was manifested only in the form of foul odor, and was noted faster in legume-based sauces (24 hours) than in meat-based (25–36 hours) and vegetable-based (48–60 hours) sauces. The *Bacillus* spores would not be killed by the normal cooking temperatures of traditional sauces. The temperature may rather serve as a heat shock to

activate spores to germinate. Therefore, methods to avoid spoilage include adequate cooking followed by immediate consumption or continued cold storage. In case of absence of cold storage possibilities, longer keeping for more than 12 hours at ambient temperatures should be discouraged.

Street foods

Street foods are ready-to-eat foods and beverages prepared and/or sold by vendors/hawkers especially on streets and other public places. Types of vending sites encompass stalls, a variety of push-carts, road-side stands, hawkers with head-loads and other arrangements depending upon the ingenuity of the individual, resources available, type of food sold, and the availability of other facilities (FAO, 1990). Street foods have invaded areas of busy economic activity and heavy population concentration. In spite of the socio-economic advantages offered by street foods, there are also several health hazards associated with this sector of economy.

Mogessie Ashenafi (1995b) studied bacteriological profile of such foods sold in an open market in Awassa. The food items consisted of roasted offal, fish soup, cooked and sauced macaroni and spaghetti and traditional hot-spices legume sauce ("Shiro" sauce). Spaghetti and macaroni food items were cooked and mixed with sauce at home and brought to the market place for sale. They were held at ambient temperatures (20–30° C) until sold out. They had high aerobic mesophilic count ($>10^6$ cfu(g)$^{-1}$) and Enterobacteriaceae count ($>10^5$ cfu(g)$^{-1}$). Roasted offal, fish soup and "Shiro" sauce were cooked within the temporary shelters where the food was served and were held at relatively higher temperatures (>40° C). The aerobic mesophilic count in such cases was relatively lower ($<10^5$ cfu(g)$^{-1}$). Several bacterial general were isolated and *Bacillus* and *Micrococcus* spp. dominated the aerobic bacterial flora. The unhygienic conditions of the food service environment, possibility of cross contamination from utensils and keeping food items at ambient temperatures for several hours were considered to be critical points.

In a microbiological study of street foods in Addis Ababa, Dereba Muleta and Mogessie Ashenafi (2000) analyzed the microbiology of "Sambussa", macaroni, lentil sandwich, 'kitfo' and egg sandwich collected from different parts of the city. Of the 150 street vendors considered in the study, 80% were females and a vendor could sell over 20 servings of a particular food item in a day. All street food items were sold under unhygienic conditions in areas of high population movement. Except "Sambussa", which had aerobic mesophilic counts of $<10^6$ cfu(g)$^{-1}$, the counts were $>10^7$ cfu(g)$^{-1}$ in all the other food items. The other food items also had markedly high counts of bacterial spores, coliforms, other members of Enterobacteriaceae, staphylococci and yeasts. The street foods were also examined for the relation between their holding temperatures and bacteriological profile (Dereba Muleta and Mogessie Ashenafi, 2001a). 'Sambussa' samples were

held at 20–35° C and counts of coliforms and other members of Enterobacteriaceae were $<10^3$ cfu(g)$^{-1}$ in most samples. Macaroni samples were held at 10–35° C and these two groups of bacteria were above 10^6 cfu(g)$^{-1}$. Holding temperatures for lentil sandwiches were 18–45° C, and counts of coliforms and other members of Enterobacteriaceae were $>10^6$ cfu(g)$^{-1}$. Most of the 'kitfo' and egg sandwich samples were held at 21–30° C and their counts were $>10^8$ cfu(g)$^{-1}$.

Weaning foods

In Ethiopia, when an infant reaches 4 to 6 months of age, breast milk is usually supplemented, and later replaced by weaning foods. Infants are fed with various types of weaning foods depending on the income of the family. In a city-wide household survey in Addis Ababa, Tigist Ketsela and Dereje Kebede (1996) found that 44% of infants were bottle-fed. In South Ethiopia, 68% of the study child population were on weaning foods and among these, 40% had started on weaning foods at the age of 4–6 months (Abebe Bekele and Yemane Berhane, 1998). Common weaning foods among the various income groups include cow's milk, dehydrated milk, gruel made from cereal blend, gruel made from mixtures of milk and cereals, gruel made from cereal/legume combinations, adult food, and other items such as tea, fenugreek ('Abish'), water regained from rice, etc (Abebe Bekele and Yemane Berhane, 1998; Wolde-Aregay Erku and Mogessie Ashenafi, 1998a).

In order to improve the nutritional quality of weaning foods, Kelbessa Urga and Keshava (1998) studied the suitability of a fermented thin gruel of teff (teff 'Atmit') as a weaning food and reported that 'Atmit' fermented by a mixture of lactic acid bacteria, namely *Lactobacillus casei, Lactobacillus plantarum, Lactobacillus fermentum* and *Pediococcus pentosaceus* at 30° C for two days resulted in a significant reduction in phytic acid and increased availability of inorganic phosphorus, iron and zinc when compared to unfermented 'Atmit'. Fermentation by a combination of *Lactobacillus casei* and *Lactobacillus plantarum* resulted in a greater reduction of phytic acid and greater increase of inorganic phosphorus and zinc in comparison to other culture combinations.

One of the most common causes of child mortality in developing countries is diarrhea. Nearly all the pathogenic organisms that cause diarrhea get into the gastro-intestine of infants via the oral route. Weaning foods play an important role in acquisition of different groups of microorganisms including food-borne pathogens. According to the manner the weaning foods are handled after preparation, various types of microorganisms are likely to be introduced into them and further proliferate in them.

Zeleke Wolde-Tensay and Haile-Selassie Tesfaye (1992a&b) studied the bacteriological quality of infant feeding bottle-contents and teats in Addis Ababa. They reported that 59% of feeding bottle contents had bacterial

counts of 10^6 cfu(ml)$^{-1}$. The coliforms were the dominant bacterial isolates both from bottle contents and teat-swabs. The educational status of the nursing mothers or different handling of bottles did not have effect on the bacteriological quality of the weaning foods. They suggested that preparation of food in advance of need combined with improper storage and inadequate cooking, and poor hygiene could be factors that resulted in highly contaminated bottle contents. In a similar study of indigenous weaning foods in a rural Ethiopian setting in south west Ethiopia, Zeleke Wolde-Tensay and Aschalew Mengistu (1997) reported that the weaning foods were basically cereal-based and, in cases where cow's milk was used to supplement human milk, 59% was in the form of raw milk. Over 90 of milk and 70% of cereal-based weaning foods were grossly contaminated with aerobic mesophilic bacteria with counts of 10^5 cfu(ml)$^{-1}$. The coliforms and other Gram-positive cocci were among the dominant flora. They also noted that the households in the study community had poor sanitary conditions with cattle and pets living with humans under the same roof.

In a similar study on microbial load and microflora of weaning foods in Addis Ababa, Wolde-Aregay Erku and Mogessie Ashenafi (1998a). analyzed the microbiology of a variety of weaning foods from feeding bottles consisting of cow's milk, reconstituted powder milk, cooked cereal blend, a mixture of milk and cereal, and others. High levels of bacterial contamination were observed in all samples with counts ranging between 10^5 and 10^8 cfu(ml)$^{-1}$. Cow's milk and cereal blend were the most heavily contaminated ones. Although the bacterial isolates belonged to 12 different genera, the dominant isolates were coliforms (31%), staphylococci (30%), *Bacillus* spp (19%) and micrococci (14%). Analysis of deydrated factory-produced weaning foods showed that the contamination level was quite low. Possible sources of high contamination could be poorly cleaned and frequently reused utensils, people who prepare and handle weaning foods and contaminated feeding bottles.

Safety considerations on ready-to-eat foods

The various studies cited above have shown that sauces allowed the proliferation of a variety of food-borne microorganisms, although no pathogens were encountered in sauces collected from different households. Mogessie Ashenafi (1996c) studied the growth potential of *Salmonella typhimurium, Salmonella enteritidis, Staphylococcus aureus, Bacillus cereus* and *Listeria monocytogenes* in the various types of sauces. The *Salmonella* test strains increased by over 3 log units within 8 hours in legume-based sauces. The growth rate of the other test strains in legume-based sauces was higher than in meat-based or vegetable-based sauces. In vegetable-based sauces, the test strains increased only by one log unit in 24 hours.

The facts that the microbial load of most street foods is high, the time/temperature combination of holding these foods is favorable to bacterial proliferation, the hygienic condition of the food-serving environment is poor, and the presence of members of Enterobacteriaceae, staphylococci and *Bacillus* species in considerable numbers are all indicative of the safety risks associated with street foods.

Jiwa *et al.* (1981) obtained 33 enterotoxigenic isolates belonging to different bacterial genera isolated from vegetables, fish and meat products bought from stall owners at the Addis Ababa 'Markato' market and from city hawkers. The isolates consisted of a variety of Gram-negative rods including a strain of *Shigella dysenterieae.* All toxins were heat-labile. *Shigella* was also isolated from 8 of 20 spaghetti and 6 of 20 macaroni samples sold in stalls in an open market in Awassa (Mogessie Ashenafi, 1995b). In another study on foods sold by street vendors (Dereba Muleta and Mogessie Ashenafi, 2001b), *Salmonella* was isolated from 9 of 30 'kitfo' samples and *Shigella flexineri* from 3 of 30 macaroni samples. While the *Salmonella* isolates were sensitive to various antimicrobials, the *Shigella* isolates manifested multiple resistance to commonly used antimicrobials.

Zeleke Wolde-Tensay and Haile-Selassie Tesfaye (1992b) isolated enteropathogenic *E. coli* (EPEC), *Shigella* spp. and *Staphylococcus aureus* from infant feeding bottle contents in Addis Ababa. Although the rate of isolation of these pathogens was low, the authors suggested that the frequent isolation of coliforms from samples of bottle contents indicated that bottle feeding served as vehicle of transmission of bacterial pathogens among the studied population. Despite a higher count of members of Enterobacteriaceae in weaning foods from a rural setting, *Salmonella* was not encountered and *Shigella* was isolated only from a single sample (Zeleke Wolde-Tensay and Aschalew Mengistu, 1997). However, Wolde-Aregay Erku and Mogessie Ashenafi (1998b) encountered *Salmonella* in bottle contents made of cow's milk and gruel from cereal blend. Other pathogens, such as *Staphylococcus aureus* and *Bacillus cereus* were also isolated from these weaning foods in Addis Ababa. Although *Bacillus cereus* spores could survive the cooking temperature used to prepare the various weaning foods, *Salmonella, Shigella* and *Staphylococcus aureus* should be eliminated during the cooking process. Thus, post-cooking contamination was an important factor that played role in safety of the weaning foods considered in the study. Wolde-Aregay Erku and Mogessie Ashenafi (1998b) also showed that when *Salmonella* was inoculated into weaning foods, its count increased by 4 log units within 8 hours and reached counts as high as 10^8 cfu(ml)$^{-1}$ within 12 hours.

OTHER FOOD PRODUCTS

Microbial load and safety considerations

In all parts of the world, meat has long been regarded as a nutritious and highly desirable food. By its nature and origin, however, meat is not only highly susceptible to spoilage but is also frequently implicated in the spread of food-borne diseases. With the exception of the external surface and the gastrointestinal and respiratory tracts, the tissues of normal healthy animals contain few microorganisms. Meat, however, is contaminated by contact with the hide, skin or feet, stomach and intestine contents, plant and equipment, and even air in the processing and storage area (ICMSF, 1980). Contamination may take place during almost every operation of the slaughtering, cutting, processing, storage and distribution of meat.

Nyeleti *et al.* (2000) determined the prevalence and distribution of *Salmonella* in slaughter cattle, slaughterhouse personnel and minced beef samples in Addis Ababa. *Salmonella* was encountered in 9.8% of abdominal muscles and 11.9% of diaphragmatic muscles. About 8% of the minced meat samples from supermarkets were also positive for *Salmonella*. The *Salmonella* isolates belonged to six different serovars consisting of *S. dublin* (54.1%), *S. anatum* (27.6%), *S. saintpaul* (9.2%), *S. meleagridis* (5.1%), *S. roughform* (3.1%) and *S. muenchen* (1.0%). A study of microbial flora of fresh raw beef from butcher's shops revealed that mean aerobic mesophilic counts ranged between 10^5 and 10^8 cfu(g)$^{-1}$ and mean counts for Enterobacteriaceae ranged between 10^3–10^6 cfu(g)$^{-1}$ (Mogessie Ashenafi, 1994c). The aerobic mesophilic microflora was dominated by micrococci, coryneform bacteria and staphylococci. Considering the high level of bacteria contamination of raw beef, another study was made to assess the microbial load of 'kitfo' in its different processed forms (Mezgebe Tegegne and Mogessie Ashenafi, 1998). The mincing process in 'kitfo' preparation adds more microorganisms to the surfaces of the exposed tissue. Raw, medium-cooked and well-cooked 'kitfo' dishes, collected from different restaurants in Addis Ababa were analyzed for their microbial load. All raw 'kitfo' samples from all restaurants had high aerobic mesophilic bacterial counts of 10^7–10^8 cfu(g)$^{-1}$. Coliforms, staphylococci, lactic acid bacteria, yeasts and molds and aerobic spores had counts of >10^4, 10^6, 10^5, 10^4, and 10^3 cfu(g)$^{-1}$, respectively. The microbial load of medium cooked ('leb-leb') 'kitfo' was also high (10^6 cfu(g)$^{-1}$). Well-done 'kitfo' yielded no vegetative microorganisms. They concluded that holding minced 'kitfo' meat at room temperature for several hours should be avoided as meat supports proliferation of microorganisms.

Pegram *et al.* (1981) isolated *Salmonella* from samples obtained from farm livestock, an abattoir and a bone factory in Ethiopia. They detected 27 serotypes in 130 contaminated samples. The bone factory product was

heavily contaminated. In a study of incidence of food-borne pathogens in fresh raw beef, Mogessie Ashenafi (1994c) isolated *Salmonella* in 9% of the samples obtained from butcher's shops in Awassa. Bagni *et al.* (1998) also reported isolation of *Salmonella* from 26% of meat and milk examined in Arsi region.

Mezgebe Tegegne and Mogessie Ashenafi (1998) isolated *Salmonella* spp. from 21 of 50 raw 'kitfo' samples, but medium-cooked or well-cooked 'kitfo' samples were free from *Salmonella*. In a challenge study, they also observed that *Salmonella* test strains inoculated into 'kitfo' could grow to the level of 10^7 cfu(g)$^{-1}$ within 12 hours. In their study on occurrence of *Salmonella* in 205 retail foods in Addis Ababa, Bayleyegn Molla *et al.* (1999a) isolated 39 *Salmonella* strains from raw minced meat (20/50), chicken gizzard (8/28), chicken liver (7/31) and chicken heart (4/26). They did not encounter any *Salmonella* in 55 spice and 15 confectionery samples. Among the isolates, 34 were resistant to one or more antimicrobial agents (Bayleyegn Molla *et al.*, 1999b). They concluded that the high level of antibiotic resistance of food-borne salmonellae in the study area was an indication of indiscriminate and continuous use of sub-therapeutic doses of antibiotics in animals.

Girma Zewde (1999) studied the incidence of salmonellae in chicken carcasses and reported that about 62% of whole-carcass surface, 80% of wings, 60% of back and 50% of liver yielded salmonellae. The most frequent serotype was *Salmonella hadar* (47%), followed by *S. enteritidis* (23.5%), and *S. branderup* (11.8%). Other species consisting of *S. heidelberg*, *S. indiana* and *S. typhimurium* were also detected. The isolates showed some degree of resistance to four different antimicrobial agents. In a study of food poisoning outbreaks among college students in Gondar, Abreham Assefa *et al.* (1994) isolated *Salmonella newport* from the stool of six students and three food handlers. The implicated food was a breakfast of un-peeled, undercooked eggs, the only meal shared by all. There was, however, no report on the isolation of the etiologic agent from the implicated food.

The slime and intestinal flora of 'tilapia' fish from Lake Awassa consisted of twelve different genera of Gram-negative rods and those belonging to the coliforms dominated the slime flora, whereas *Aeromonas* spp. was the most dominant among the intestinal flora (Mogessie Ashenafi, 1993a). The microbial spoilage of this fish species was also evaluated by Mogessie Ashenafi *et al.* (1995). This is commercially the most important fish in Lake Awassa. Fishing at this lake is very unhygienic and the 3–5 hours exposure to temperatures of >25° C between capture and sale permits spoilage bacteria to multiply. Well cleaned fresh tilapia contained a bacterial count of 10^3 to 10^4 cfu(g)$^{-1}$, which was dominated by *Acinetobacter* and *Micrococcus* spp. At ambient temperature (25° C), spoilage occurred after 10 hours when the aerobic mesophilic count reached about 10^6 cfu(g)$^{-1}$. Colonies producing H_2S constituted 8%, 4% and 13% of the aerobic mesophilic

bacterial count for whole fish, gutted fish and fillets, respectively. At 4° C, spoilage occurred after 11 days when the aerobic mesophilic counts reached over 10^7 cfu(g)$^{-1}$. H_2S producers made up 1–10% of the total viable organisms. At ambient temperatures, *Aeromonas* was the major spoilage organism, whereas *Alteromonas* dominated the spoilage flora during cold storage.

Consumption of raw food items of plant or animal origin is a wide-spread practice in Ethiopia. It is common practice in most parts of the country, and by all age groups to 'drink' raw egg yolk as traditional medicine for respiratory and other ailments. The widespread consumption of untreated, raw, green peppers and tomato slices for their appetizing and seasoning effects is not an uncommon practice in all income groups. Raw fish is widely consumed in areas around the Rift Valley lakes in southern Ethiopia. Analysis of these food items showed that raw tomato and green pepper had aerobic mesosphilic bacterial counts of 10^6 cfu(g)$^{-1}$ and raw fish had 10^6 cfu/cm^2. Egg yolk had much lower counts (10^2 cfu(ml)$^{-1}$). Coliforms constituted about 10% of the counts in all food items (Mogessie Ashenafi, 1989b). *Staphylococcus aureus* was isolated from 4%–8% of tomato, green pepper, eggs and fish. The toxingenicity of the isolates was, however, not determined. *Shigella* was encountered in 3.6% of tomato samples.

Aberra Geyid *et al.* (1991) studied the microflora of some fruits and vegetables consisting of avocado, banana, grape fruit, guava, mango, orange, papaya, pineapple, tomato, carrot, cauliflower, garlic, onion, kidney bean and potato. The isolates were made up of indicators (3%), spoilage types (60%), food-borne pathogens (20%) and normal contaminants (8%). *E. coli* type 1 dominated the indicators, bacilli, molds and *Enterobacter* spp. dominated the spoilage types and the pathogenic group was dominated by *Staphylococcus aureus, Salmonella typhimurium* and *Bacillus cereus.* Preservation of fruits and vegetables in the form of jams or jelly, squash or juice, or by keeping them in 2% salt brine packed in tight glass jar reduced the microbial content by >94%, >85% and 100% for indicators, spoilage forms and pathogens, respectively.

Despite the growing consumption of fruit juices prepared by the various fruit juice vendors in urban areas in Ethiopia, scientific information on the microbial status of fruit juices is scanty. In a study of the fate of *Salmonella* and *E. coli* test strains in fresh prepared fruit juices, Beteseb Yigeremu *et al.* (2001) reported that papaya and avocado juice (pH>5.7) allowed test strains to reach numbers >10^7 cfu(ml)$^{-1}$ within 16 hours at ambient temperatures. In pineapple juice (pH, 3.8), *E. coli* test strains were eliminated whereas *Salmonella* increased slightly at ambient temperatures within 16 hours. Refrigeration temperature did not allow growth of test strains in fruit juices. Orange juice (pH, 3.1) did not allow the survival of the test strains at both holding temperatures. Although pasteurization may

not be practical in the Ethiopian fruit juice market, it was recommended to require fruit juice vendors to have procedures in place to reduce the number of disease-causing microorganisms to the same level achievable by pasteurization.

CONCLUSION

Food preparation is predominantly a house-hold phenomenon in Ethiopia, although dining establishments are available in urban and semi-urban areas. The food industry in the country is not well developed. Every household appears to process food starting from raw ingredients to the final products. In cases where fermentation is important to obtain a certain product, the microorganisms naturally present on the raw ingredients or in the containers spontaneously take care of the process. The creation of the right environment for the microorganisms to result in a desirable product is based on women's indigenous knowledge, which has improved itself through generations.

Most of the microbiological studies conducted so far have concentrated on those traditional foods and beverages popular among the people inhabiting the central and northern highlands of the country. Such studies must be extended to other less known indigenous foods and beverages the popularity of which is limited only to the areas of origin. This may help to come across novel microorganisms with novel metabolites, which subsequently may have industrial importance.

So far, most of the fermentation studies attempted to describe the microbiological successions and the accompanying chemical changes during the fermentation process. Keeping in mind that such studies should continue on indigenous foods and beverages not yet described, attempts should also be made to undertake controlled fermentation studies with selected mixed culture starters and to optimize the process conditions. This would result in products which are consistent and definable in their flavours and other biochemical parameters, have good keeping quality and are, in general, wholesome. This may pave the way for large scale commercial production. Large scale production has the advantage of reducing wastage during processing, which is significant at house-hold level, in addition to improving the keeping quality of the products.

Food safety studies should be done extensively to determine the important food-borne pathogens associated to specific food items. Challenge studies, in this respect, would help to elucidate the behaviour of food-borne pathogens during processing, preparation or storage of the various foods, and eventually help to recommend measures for control of food-borne infections at house-hold level. It would be useful to see the relations

between prevalence of food-borne diseases and the food preparation practice and environment, particularly among low-income families, to improve the health status of family members in such groups. More microbiological studies are also required on food establishments, slaughter houses and the food industry to generate enough data for the implementation of Hazard Analysis and Critical Control Point (HACCP) procedures that will guarantee the production and distribution of safe food to the public.

Quite a substantial amount of food is lost at house-hold level due to two major reasons. The first is direct loss due to microbial spoilage. One can imagine how much "Enjera" is lost to molding at the house-hold level per baking cycle. If one calculates the amount lost annually and extrapolate this to loss at national level, the figure will be appalling. Such losses apply to the various sauces and beverages, too. The second type of loss is rather indirect. In many instances, people tend to consume more than they require because they know the food will spoil if made to stay longer. This is the case in most of the Ethiopian house-holds because they cannot afford cooling devices. Food items, which could reasonably last much longer under proper processing or storage, are consumed, thus 'lost', in much shorter period of time without the physiological need to do so. Microbiological studies to improve the keeping quality of indigenous foods through microbial processing, use of food preservatives or combination of both would significantly contribute to curb problems of food shortage at house-hold level. The dictum is "think globally, act locally".

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REFERENCES

1. Abebe Bekele and Yemane Berhane (1998). Weaning in Butajira, south Ethiopia: a study on mothers' knowledge and practice. *Ethiop. Med. J.* **36**:37–45.
2. Aberra Geyid, Frew Tekabe, Asmamaw Tigre, Mulu Girma and Sisaynesh Assefa (1991). A preliminary study of the microflora level of some fruits and vegetables: pre- and post-preservation. *Ethiop. J. Health Dev.* **5**:57–65.
3. Abraham Besrat, Atnafseged Admasu and Mebrahtu Ogbai (1980a). Critical study of the iron content of teff (*Eragrostis tef*). *Ethiop. Med. J.* **18**: 45–52.

4. Abraham Besrat, Haile Mehansho and Taye Bezuneh (1980b). Effect of varietal difference and fermentation on protein quantity and quality of ensete. *Nut. Rep. Int.* **20**:245–250.

5. Abreham Assefa, Getahun Mengistu and Moges Tiruneh (1994). *Salmonella newport*: outbreak of food poisoning among college students due to contaminated undercooked eggs. *Ethiop. Med. J.* **32**:1–6.

6. Ahmed Idris, Tetemke Mehari and Mogessie Ashenafi (2001). Some microbiological and biochemical studies on the fermentation of "Awaze" and "Datta", traditional Ethiopian condiments. *Int. J. Food Sci. Nutr.* **52**:5–14.

7. Alemu Fite, Amhaselassie Tadesse, Kelbessa Urga and Elias Seyoum (1991). Methanol, fusel oil and ethanol contents of some Ethiopian traditional alcoholic beverages. *SINET: Ethiop. J. Sci.* **14**:19–27.

8. Almaz Gonfa, Alemu Fite, Kelbessa Urga and Berhanu Abegaz Gashe (1999). Microbiological aspects of 'Ergo' ('Ititu') fermentation. *SINET: Ethiop. J. Sci.* **22**:283–289.

9. Aureli, P., Costantine, A. and Zolea, S. (1992). Antimicrobial activity of some plant essential oils against *Listeria monocytogenes*. *J. Food Protect.* **55**:344–348.

10. Ayele Nigatu and Berhanu Abegaz Gashe (1994a). Inhibition of spoilage and food-borne pathogens by lactic acid bacteria isolated from fermenting teff (*Eragrostis tef*) dough. *Ethiop. Med. J.* **32**:223–229.

11. Ayele Nigatu and Berhanu Abegaz Gashe (1994b). Survival and growth of selected pathogens in fermented kocho (*Enstete ventricosum*). *East Afr. Med. J.* **71**:514–518.

12. Ayele Nigatu and Berhanu Abegaz Gashe (1998). Effect of heat treatment on the antimicrobial properties of tef dough, injerra, kocho and aradisame and the fate of selected pathogens. *World J. Microbiol. Biotechnol.* **14**:63–69.

13. Ayele Nigatu, Berhanu Abegaz Gashe and Tarekegn Ayele (1997). *Bacillus* spp. from fermented tef dough and kocho: identity and role in the two Ethiopian fermented foods. *SINET: Ethiop. J. Sci.* **20**:101–114.

14. Azage Tegene and Alemu Gebre-wold (1998). Prospects for peri-urban dairy development in Ethiopia. **In:** *National Conference of Ethiopian Society of Animal Production*, pp. 28–39, ESAP.

15. Bagni, M., Cuteri, V. and Mekonnen, M. (1998). *Salmonella* contamination of food of animal origin in Ethiopia. *Ob. Doc. Vet.* **19**:55–58.

16. Bayleyegn Molla, Kleer, J. and Sinell, H.J. (1999a). Occurrence, distribution and level of *Salmonella* in selected food items in Addis Ababa (Ethiopia). *Fleischwirtsch. Int.* **4**:37–39.

17. Bayleyegn Molla, Kleer, J. and Sinell, H.J. (1999b). Antibiotic resistance pattern of foodborne *Salmonella* isolates in Addis Ababa (Ethiopia). *Berl. Munch. Tierarztl. Wochenschr.* **112**:41–43.

18. Bekele Bahiru, Tetemke Mehari and Mogessie Ashenafi (2001). Chemical and nutritional properties of "Tej", an indigenous Ethiopian honey wine:

variations within and between production units. *J. Food Technol. Afr.* 6:104–108.

19. Bekele Bahiru, Tetemke Mehari and Mogessie Ashenafi (2002). Yeast and lactic acid flora of "Tej", an indigenous Ethiopian honey wine: variations within and between production units. *SINET: Ethiop. J. Sci.* (Accepted)
20. Belachew Desta (1977). A survey of the alcoholic content of traditional beverages. *Ethiop. Med. J.* **15**:65–68.
21. Berhanu Abegaz Gashe (1985). Involvement of lactic acid bacteria in the fermentation of tef (*Eragrostis tef*), an Ethiopian fermented food. *J. Food Sci.* **50**:800–801.
22. Berhanu Abegaz Gashe (1987a). Kocho fermentation. *J. Appl. Bacteriol.* **62**:473–477.
23. Berhanu Abegaz Gashe (1987b). Spoilage organisms of kocho. *MIRCEN J. Appl. Microbiol. Biotechnol.* **3**:67–74.
24. Berhanu Abegaz Gashe, Meaza Girma and Abraham Bisrat (1982). Tef Fermentation. 1. The role of microorganisms in fermentation and their effect on the nitrogen content of tef. *SINET: Ethiop. J. Sci.* **5**:69–76.
25. Beteseb Yigeremu, Mulugeta Bogale and Mogessie Ashenafi (2001). Fate of *Salmonella* spp and *E. coli* in fresh-prepared orange, avocado, papaya and pine apple juices. *Ethiop. J. Health Sci.* **11**:89–95.
26. Bisrat Godefay and Bayleyegn Molla (2000). Bacteriological quality of raw cow's milk from four dairy farms and a milk collection center in and around Addis Ababa. *Berl. Munch. Tierarztl. Wochenschr.* **113**:276–278.
27. Chaltu Gifawesen and Abraham Besrat (1982). Yeast flora of fermenting tef (*Eragrostis tef*) dough. *SINET: Ethiop. J. Sci.* **5**:21–25.
28. Demeke, T. (1986). Is Ethiopia's *Ensete ventricosum* crop her greatest potential food? *Agric. Int.* **12**:362–365.
29. Dereba Muleta and Mogessie Ashenafi (2000). Some street-vended foods from Addis Ababa: microbiological and socio-economical considerations. *Ethiop. J. Health Sci.* **10**:89–100.
30. Dereba Muleta and Mogessie Ashenafi (2001a). Bacteriological profile and holding temperatures of street-vended foods from Addis Ababa. *Int. J. Environ. Health Res.* **11**:95–105.
31. Dereba Muleta and Mogessie Ashenafi (2001b). *Salmonella, Shigella* and growth potential of other food-born pathogens in Ethiopian street-vended foods. *East Afr. Med. J.* **78**:576–580.
32. FAO (1990). *Street Foods.* Report of an FAO Expert Consultation. *FAO Food Nutrition Paper* **46**:3–30.
33. FAO-OIE-WHO (1993). *Animal Health Year Book.* P. 122.
34. Fekadu Beyene and Abrahamsen, R.K. (1997). Farm-made fermented milk and cottage cheese in southern Ethiopia. *Trop. Sci.* **37**:75–79.
35. Fekadu Beyene, Narvhus, J. and Abrahamsen, R.K. (1998). Evaluation of new isolates of lactic acid bacteria as a starter for cultured milk production. *SINET: Ethiop. J. Sci.* **21**:67–80.

36. Feleke Moges and Mogessie Ashenafi (2000). Characterization of *Bacillus* spp. from spices and assessment of their spoilage potential in various traditional Ethiopian sauces. *SINET: Ethiop. J. Sci.* **23**:87–101.

37. Fernandez, C.F., Shahani, K.M. and Amer, M.A. (1987). Therapeutic role of dietary lactobacilli and lactobacilli fermented dairy products. *FEMS Microbiol. Rev.* **46**:347–356.

38. Girma Tulu and Berhanu Abegaz Gashe (1992). Prevalence of mastitic streptococci and staphylococci in the foremilk of lactating cows in the vicinity of Addis Ababa. *Ethiop. J. Agric. Sci.* **13**:75–81.

39. Girma Zewde (1999). Productivity of three brands of modified semi-solid Rappaport-Vassiliadis (MSRV) medium and isolation of *Salmonella* from chicken carcasses and chicken pieces. *Fleischwirtsch.* **79**:71–73.

40. Goettsch, E. (1991). *Plant Genetic Resources of Ethiopia.* Cambridge University Press, Great Britain.

41. Gulelat Dessie, Kebede Abegaz and Mogessie Ashenafi (1996). Fate of *Salmonella enteritidis* and *Salmonella typhimurium* during the fermentation of 'siljo', a traditional Ethiopian fermented product. *East Afr. Med. J.* **73**:432–434.

42. Gulelat Dessie, Kebede Abegaz and Mogessie Ashenafi (1997). Effect of 'siljo' fermentation on growth of *Staphylococcus aureus, Bacillus cereus* and *Listeria monoctyogenes. Ethiop. Med. J.* **35**:215–223.

43. Hesseltine, C.W. (1983). The future of fermented foods. *Nutr. Rev.* **41**:293–301.

44. Hesseltine, C.W. and Wang, H.L. (1980). The importance of traditional fermented foods. *Biosci.* **30**:402–404.

45. ICMSF (1980). *Microbial Ecology of Foods*, Vol II. Academic Press. New York.

46. Jansen, P.C.M. (1981). *Spices, Condiments and Medicinal Plants in Ethiopia, their Taxonomy and Agricultural Significance.* Center for Agricultural Publishing and Documentation. Wageningen, The Netherlands.

47. Jay, J.M. (1996). *Modern Food Microbiology.* 5th edn. Chapman and Hall. New York.

48. Jiwa, S.F.H., Krovacek, K. and Wadstrom, T. (1981). Enterotoxigenic bacteria in food and water from an Ethiopian community. *Appl. Environ. Microbiol.* **41**:1010–1019.

49. Kassaye, T., Simpson, B.K., Smith, J.P. and O'Connor, C.B. (1991). Chemical and microbiological characteristics of 'Ititu'. *Milchwissensch.* **46**:649–653.

50. Kelbessa Urga and Kesheva, N. (1998). Effect of fermentation by mixed cultures of lactic acid bacteria on the HCl-extractability of some minerals from tef (*Eragrostis tef*) atmit. *SINET: Ethiop. J. Sci.* **21**:183–194.

51. Kelbessa Urga, Alemu Fite and Eskinder Biratu (1997a). Effect of natural fermentation on nutritional and anti-nutritional factors of tef (*Eragrostis tef*). *Ethiop. J. Health Dev.* **11**:61–66.

52. Kelbessa Urga, Alemu Fite and Eskinder Biratu (1997b). Natural fermentation of enset (*Ensete ventricosum*) for the production of kocho. *Ethiop. J. Health Dev.* **11**:75–81.

53. Ketema Bacha, Tetemke Mehari and Mogessie Ashenafi (1998). The microbial dynamics of 'Borde' fermentation, a traditional Ethiopian fermented beverage. *SINET: Ethiop. J. Sci.* **21**:195–205.

54. Ketema Bacha, Tetemke Mehari and Mogessie Ashenafi (1999). Microbiology of the fermentation of 'Shamita', a traditional Ethiopian fermented beverage. *SINET: Ethiop. J. Sci.* **22**:89–102.

55. Kinfe Getaneh and Eshetu Lemma (1987). Isolation of *Mycobacterium bovis* from bovine milk and tissue: implications for public health and animal production. **In:** *National Livestock Improvement Conference*, pp. 107–110. Institute of Agricultural Research, Addis Ababa, Ethiopia.

56. Knoess, K.H. (1977). The camel as a milk and meat animal. *World Anim. Rev.* **22**:39–44.

57. Lester, R.N. and Endashaw Bekele (1981). Amino acid composition of the cereal tef and related species of *Eragrostis* (Gramineae). *Cereal Chem.* **58**:113–115.

58. Meaza Girma, Berhanu Abegaz Gashe and Lakew, B. (1989). The effect of fermentation on the growth and survival of *Salmonella typhimurium, Staphylococcus aureus, Bacillus cereus* and *Pseudomonas aeruginosa* in fermenting tef (*Eragrostis tef*). *MIRCEN J. Appl. Microbiol. Biotechnol.* **5**:61–66

59. Melaku Umeta, M. and Faulks, R.M. (1988). The effect of fermentation on the carbohydrates in tef (*Eragrostis tef*). *Food Chem.* **27**:181–189.

60. Mezgebe Tegegne and Mogessie Ashenafi (1998). Microbial load and incidence of *Salmonella* spp. in 'kitfo', a traditional Ethiopian spiced, minced meat dish. *Ethiop. J. Health Dev.* **12**:135–140.

61. Mogessie Ashenafi (1989a). Proteolytic, lipolytic and fermentative properties of yeasts isolated from 'Ayib', a traditional Ethiopian cottage cheese. *SINET: Ethiop. J. Sci.* **12**:131–139.

62. Mogessie Ashenafi (1989b). Microbial load, incidence and antibiograms of some disease causing microorganisms on raw food items consumed in Ethiopia. *MIRCEN J. Appl. Microbiol.Biotechnol.* **5**:313–319.

63. Mogessie Ashenafi (1990a). Microbiological quality of 'Ayib', a traditional Ethiopian cottage cheese. *Int. J. Food Microbiol.* **10**:263–268.

64. Mogessie Ashenafi (1990b). Effect of curd cooking temperature on the microbiological quality of 'Ayib', a traditional Ethiopian cottage cheese. *World J.Microbiol. Biotechnol.* **6**:159–162.

65. Mogessie Ashenafi (1992). Growth potential and inhibition of *Bacillus cereus* and *Staphylococcus aureus* during the souring of 'Ergo', a traditional Ethiopian fermented milk. *Ethiop. J. Health Dev.* **6**:23–30.

66. Mogessie Ashenafi (1993a). Slime and intestinal aerobic microflora of *Tilapia nilotica* from Lake Awassa, Ethiopia. *East Afr. Agric.Forest. J.* **59**:171–175.

67. Mogessie Ashenafi (1993b). Fate of *Salmonella enteritidis* and *Salmonella typhimurium* during the fermentation of 'Ergo', a traditional Ethiopian sour milk. *Ethiop. Med. J.* **31**:91–98.

68. Mogessie Ashenafi and Fekadu Beyene (1993). Effect of container smoking and udder cleaning on the microflora and keeping quality of raw milk from a dairy farm in Awassa. *Trop. Sci.* **33**:368–376.

69. Mogessie Ashenafi (1994a). The aerobic microflora and lactic acid bacteria of market 'Ayib'. *Ethiop. J. Agric. Sci.* **14**:104–111.

70. Mogessie Ashenafi (1994b). Microbial flora and some chemical properties of 'Ersho', a starter for teff (*Eragrostis tef)* fermentation. *World J. Microbiol. Biotechnol.* **10**:69–73.

71. Mogessie Ashenafi (1994c). Microbial flora and incidence of some food-borne pathogens on fresh raw beef from butcher's shops in Awassa, Ethiopia. *Bull. Anim. Health Prod. Afr.* **42**:273–277.

72. Mogessie Ashenafi (1994d). Fate of *Listeria monocytogenes* during the fermentation of 'Ergo', a traditional Ethiopan sour milk. *J. Dairy Sci.* **77**:696–702.

73. Mogessie Ashenafi and Fekadu Beyene (1994). Microbial load, microflora and keeping quality of raw and pasteurized milk from a dairy farm. *Bull. Anim. Health Prod. Afr.* **42**:55–59.

74. Mogessie Ashenafi (1995a). Microbial development and some chemical changes during the making of 'Ergo', a traditional Ethiopian fermented milk. *Bull. Anim.Health Prod. Afr.* **43**:171–176.

75. Mogessie Ashenafi (1995b). Bacteriological profile and holding temperatures of ready-to-serve food items in an open market in Awassa, Ethiopia. *Trop. Geog. Med.* **47**:244–247.

76. Mogessie Ashenafi and Tetemke Mehari (1995). Some microbiological and nutritional properties of "Borde" and "Shamita", traditional Ethiopian fermented beverages. *Ethiop. J. Health Dev.* **9**:105–110.

77. Mogessie Ashenafi, Yewelsew Abebe and Elias Dadebo (1995). Microbial spoilage of fresh water fish (*Oreochromis niloticus*) at low (4° C) and ambient (25° C) temperatures. *Trop. Sci.* **35**:395–400.

78. Mogessie Ashenafi (1996a). Effect of container smoking and incubation temperature on the microbiological and some biochemical qualities of fermenting 'Ergo', a traditional Ethiopian sour milk. *Int. Dairy J.* **6**:95–104.

79. Mogessie Ashenafi (1996b). Microbial spoilage of some traditional Ethiopian sauces at ambient temperature. *SINET: Ethiop. J. Sci.* **19**:207–216.

80. Mogessie Ashenafi (1996c). Growth potential of some food-borne pathogens in various traditional Ethiopian sauces. *Ethiop. J. Health Dev.* **10**:41–45.

81. Mogessie Ashenafi and Yewelsew Abebe (1996a). Microbial load and incidence of *Staphylococcus aureus* in market "Bulla" and "Kotcho", traditional Ethiopian processed food products from enset (*Ensete ventricosum*). *Ethiop. J. Health Dev.* **10**:117–122.

82. Mogessie Ashenafi and Yewelsew Abebe (1996b). Microbial spoilage of market "Bulla" and "Kotcho", traditional Ethiopian processed food products from enset (*Ensete ventricosum*). *Ethiop. J. Agric. Sci.* **15**:121–130.

83. Mogessie Ashenafi (1997). Evaluation of the spoilage potential of selected bacterial isolates on Ethiopian sauces and effect of two major sauce spices on spoilage microflora. *SINET: Ethiop. J. Sci.* **20**:91–99.

84. Mohammed Abdella, Becker, H. and Terplan, G. (1996). Comparative studies on the detection of salmonellae in Ethiopian cottage cheese (ayib) using different culture methods. *Arch. Lebensmittelhyg.* **47**:83–90.

85. Nyeleti, C., Bayleyegn Molla, Goetz, H. and Kleer, J. (2000). Prevalence and distribution of salmonellae in slaughter cattle, slaughterhouse personnel and minced beef in Addis Ababa (Ethiopia). *Bull. Anim. Hlth. Prod. Afr.* **48**:19–24.

86. O'Mahony, F. (1988). *Rural Dairy Technology - Experience in Ethiopia.* ILCA Manual No 4. International Livestock Center for Africa, Addis Ababa.

87. Pederson, S.C. (1979). *Microbiology of Fermentation.* 2nd edn. AVI Publishing Co., Inc.West Port, Connecticut.

88. Pegram, R.G., Roeder, P.L., Hall, M.L.M. and Rowe, B. (1981). *Salmonella* in livestock and animal products in Ethiopia. *Trop. Anim. Health Prod.* **13**:203–207.

89. Pijls, L.T.J., Timmer, A.A.M., Wolde-Gebriel, Z. and West, C.E. (1995). Cultivation, preparation and consumption of ensete (*Ensete ventricosum*) in Ethiopia. *J. Sci. Food Agric.* **67**:1–11.

90. Ramachandran, K. and Getachew Bolodia (1984). The effect of fermentation on the iron, phosphorus and zinc content of tef (*Eragrostis tef*). *Ethiop. Med. J.* **22**:45–48.

91. Samuel Sahle and Berhanu Abegaz Gashe (1991). The microbiology of Tella fermentation. *SINET: Eth. J. Sci.* **42**:81–92.

92. Senait Zewdie, Kelbessa Urga and Ayele Nigatu (1995). Microbiology of siljo fermentation. *SINET: Ethiop. J. Sci.* **18**:139–142.

93. Steinkraus, K.H. (1983). *Handbook of Indigenous Fermented Foods.* Marcel Dekker, Inc. New York.

94. Stewart, B.R. and Getachew Asnake (1962). Investigation of the nature of injera. *Econ. Bot.* **16**:127–130.

95. Sufian, S. and Pittwell, L.R. (1969). Iron content of teff (*Eragrostis abyssinica*). *J. Sci. Food Agric.* **19**: 439.

96. Tadesse Mehari and Berhanu Abegaz Gashe (1990). A survey of the microflora of raw and pasteurized milk and the sources of contamination in a milk processing plant in Addis Ababa, Ethiopia. *J. Dairy Res.* **57**:233–238.

97. Taye Bezuneh (1984). Evaluation of some *Ensete ventricosum* clones for food yield with emphasis on the effect of length of fermentation on carbohydrate and calcium content. *Trop. Agric.* **61**:111–116.

98. Taye Tolemariam, Baars, R.M.T. and Fekadu Beyene (1999). Evaluation of initiation of lactoperoxidase system for preservation of milk in Arsi highlands. *J. Agric. Environ. Int. Dev.* **93**:25–33.

99. Teshager Semereab and Bayleyegn Molla (2001). Bacteriological quality of raw camel (*Camelus dromedarius*) milk in Afar region (Ethiopia). *J. Camel Practice Res.* **51**:51–54.

100. Tetemke Mehari and Mogessie Ashenafi (1995). Microbiology of "Siljo", a traditional Ethiopian fermented legume product. *World J. Microbiol. Biotechnol.* **11**:338–342.

101. Tigist Ketsela and Dereje Kebede (1996). Pattern of feeding of infants in Addis Ababa, Ethiopia. *Ethiop. J. Health Dev.* **10**:57–65.

102. Tsehay Reda (1998). Milk processing and marketing options for rural small scale producers. **In:** *National Conference of Ethiopian Society of Animal Production*, pp. 61–67. ESAP.

103. Vogel, A. and Abeba Gobezie (1983). Ethiopian "Tej". In: *Handbook of Indigenous Fermented Foods*. Steinkraus, K.H. (ed). Marcel Dekker, Inc. New York.

104. Wolde-Aregay Erku and Mogessie Ashenafi (1998a). Microbial load and microflora of weaning foods obtained from pediatric outpatients in Addis Ababa. *Ethiop. J. Health Dev.* **12**:141–147.

105. Wolde-Aregay Erku and Mogessie Ashenafi (1998b). Prevalence of some food-borne pathogens and growth potential of *Salmonella* spp. in weaning foods from Addis Ababa, Ethiopia. *East Afr. Med. J.* **75**:215–218

106. Yoneya, T., Nakajima, H., Shimizu, K., Miyamoto, T. and Kataoka, K. (1999). Isolation and characterization of lactic acid bacteria from 'Ergo', a traditional Ethiopian fermented milk. *Milk Sci.* **48**:65–71.

107. Zeleke Wolde-Tensay and Aschalew Mengistu (1997). Bacterial isolates from indigenous weaning foods in rural Ethiopian setting, Jimma Zone, south west Ethiopia. *Ethiop. Med. J.* **35**:93–102.

108. Zeleke Wolde-Tensay and Haile-Selassie Tesfaye (1992a). Bacteriological quality of infant feeding bottle contents and teats in Addis Ababa, Ethiopia. *Ethiop. Med. J.* **30**:79–88.

109. Zeleke Wolde-Tensay and Haile-Selassie Tesfaye (1992b). Isolation of enteric pathogens and coliform bacteria from infant feeding bottle content in Addis Ababa, Ethiopia. *Ethiop. J. Health Dev.* **6**:1–4.

110. Zvauya, R., Mygochi, T. and Parawira, W. (1997). Microbial and biochemical changes occurring during the production of manvusu and mangisi, traditional Zimbabwean beverages. *Plant Foods Human Nutr.* **51**:43–51.