Ethiop. J. Biol. Sci., 5(2): 189-245, 2006
 ISSN: 1819-8678

REVIEW ARTICLE

A REVIEW ON THE MICROBIOLOGY OF INDIGENOUS FERMENTED FOODS AND BEVERAGES OF ETHIOPIA

Mogessie Ashenafi[1]

INTRODUCTION

The interaction between microorganisms and human foods may be understood from three aspects. A desirable interaction is that microbial fermentation introduces desirable flavor and physical characteristics in many food products. Adversely, 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-flavor and physical defects in food products.

Microorganisms have been playing a pivotal role in the fermentation of human foods and beverages since the beginning of human civilisation. Fermented foods and beverages are defined as products obtained through desirable biochemical changes caused by the action of microorganisms or enzymes. In indigenous fermented foods, the microorganisms responsible for the fermentation are usually the microflora naturally present on the raw substrate. Fermentation is one of the oldest and most economical methods of producing and preserving food and is found to destroy undesirable components, to enhance the nutritive value, flavour and taste of the food, and to make the product safe from pathogenic microorgansisms.

Fermented foods are produced world-wide using various manufacturing techniques, raw materials and microorganisms. However, there are only four main fermentation processes: alcoholic, lactic acid, acetic acid and alkali fermentation. Alcohol fermentation results in the production of ethanol, and yeasts are the predominant organisms (e.g. wines and beers). Lactic acid fermentation (e.g. fermented milks and cereals) is mainly carried out by lactic acid bacteria. *Acetobacter* convert alcohol to acetic acid in aerobic conditions as in the production of vinegar. Alkali fermentation is basically a proteolytic type and often takes place during the fermentation of fish and

[1]Department of Biology, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia. E-mail: mogessie@gmail.com

seeds rich in proteins. These are popularly used as condiments.

Fermented plant products are among the most important sources of dietary proteins, carbohydrates, vitamins, minerals and fibre for many people in the developing world. However, due to the lower protein content and deficiency of certain essential amino acids, the nutritional and sensory qualities of these products are considered poor in comparison with foods of animal origin. Attempts to improve nutritional qualities of cereal products include genetic improvement and amino acid supplementation with protein concentrates or other protein-rich sources such as grain legumes. Additionally, several processing technologies, which include cooking, sprouting, milling and fermentation, have been put into practise to improve the nutritional properties of cereals (Mattila-Sandholm, 1998). Fermenting microorganisms can synthesize certain amino acids and improve protein quality and availability of B group vitamins. Fermentation also results in reduction in phytate, which may increase the amount of soluble iron, zinc and calcium several fold (Blandino *et al*., 2003).

The type of bacterial flora developed in each fermented food depends on intrinsic factors such as water activity, pH, salt concentration, availability of oxygen, composition of the food matrix, and extrinsic factors such as temperature, relative humidity and other parameters. Most fermented foods are dependent on lactic acid bacteria (LAB) to mediate the fermentation process, although yeasts are also involved in cereal fermentations. Lactic acid fermentation contributes towards the safety, nutritional value, shelf life and acceptability of a wide range of cereal-based foods.

According to Jay (1996), the term LAB is used to describe a broad group of Gram-positive, catalase-negative, non-sporing rods and cocci, usually non-motile, that utilize carbohydrates fermentatively and form lactic acid as the sole or major end product. According to the pathways by which hexoses are metabolised they are divided into two groups: homofermentative and heterofermentative. Homolactics, such as *Pediococcus*, *Lactococcus*, *Lactococcus* and some lactobacilli, produce lactic acid as the major or sole end product of glucose fermentation. Heterolactics, such as *Weisella* and *Leuconostoc* and some lactobacilli, produce equimolar amounts of lactate, CO_2 and ethanol from glucose. The preservative role of lactic fermentation in fermented products has been attributed to the production of acids, hydrogen peroxide and antibiotics. Another advantage of lactic acid fermentation is that fermented products involving LAB have viricidal (Esser *et al*., 1983) and antitumour effects (Jay, 1996).

Most of the indigenous fermented products are produced in Africa and Asia and a number of them utilize cereals in combination with legumes, thus improving the overall protein quality of the fermented product. Cereals are deficient in lysine, but are rich in cysteine and methionine. Legumes, on the other hand, are rich in lysine but deficient in sulphur-containing amino acids. Thus, by combining cereal with legumes, the overall protein quality is improved (Campbell-Platt, 1994).

Based on its rich cultural diversity, a wide variety of fermented foods and beverages are consumed in Ethiopia. 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.

Ethiopian indigenous fermented foods and beverages are products of acid-alcohol type of fermentation. The preparation of many indigenous or traditional fermented foods and beverages is still a household art. In Ethiopia, although some data were generated on the economic and nutritional implications of the indigenous fermented food in the 1970s, the involvement of Ethiopian researchers in studying the microbiology of traditional fermented foods started only in the 1980's and quite a number of publications have appeared during the last two decades. Considering the rich diversity in fermented food and beverage types 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 fermentation of milk and other dairy products, fermentation of '*enjerra*' and '*kotcho*' and other fermented legume and vegetable products, and beverages. Topics of concern in most of these works were basically microbial succession and accompanying changes, food safety, processing and spoilage of traditional fermented foods and beverages. The scope of this review is limited to microbiological studies made by various researchers on fermentation, microbial safety and spoilage of traditional fermented Ethiopian foods and beverages.

Traditional fermented dairy products

In Ethiopia, a considerable proportion of milk is consumed in the fermented form. The fermented product has different vernacular names such as *ergo*, *ititu*, *geinto* or *meomata* among the Amhara, Oromo, Sidama, or Wolayta people, respectively. The fermentation is usually natural, with no defined starter cultures used to initiate it. In most cases, this is made possible

through the proliferation of the initial milk flora, with microbial succession determined by ambient temperatures and chemical changes in the fermenting milk. In most urban homes, no attempt is 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 source of inoculum. Lactic acid bacteria also become established on the inner walls of the container and serve as starter culture. Incubation temperature does not usually vary significantly, particularly in the lowlands, and the taste of the fermented product may, in general, be more or less uniform. The fermented product may also be processed into traditional butter (*qibe*) and butter milk (*arrera*). The butter milk may further be processed into traditional cottage cheese (*ayib*) and whey (*aguat*) (Fig. 1).

Ergo (sour milk)

Almaz Gonfa *et al*. (1999) made a time course study on growth of microorganisms involved in the culturing of *ergo* and reported that *Lactococcus*, *Streptococcus*, *Leuconostoc* and *Lactobacillus* carried out the souring 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 at the end of the fermentation (Table 1). The aerobic mesophilic bacteria also had similar counts and the yeast population increased to 10^5 cfu/ml 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*.

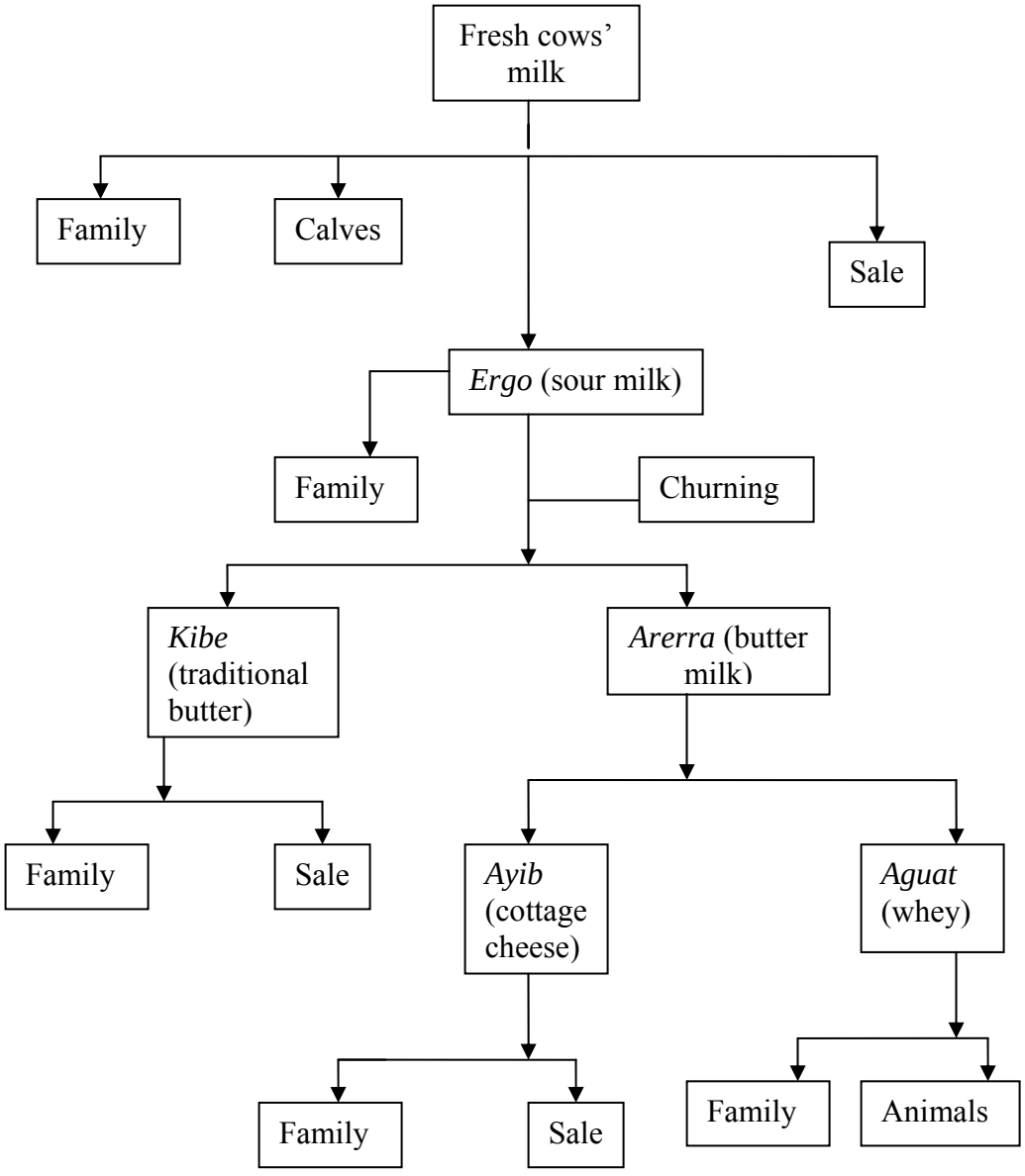

Fig. 1. Flow scheme for milk and milk products available to rural small-holder milk producers in Ethiopia (From Almaz Gonfa *et al*., 2001).

Table 1 Changes in lactic acid bacteria population (cfu/ml) during fermentation of raw milk to prepare *ergo*.

Fermentation Time, h	*Lactococcus remoris*	*Lactococcus lactis*	*Leuconostoc cremoris*	*Lactobacillus mesentroides*	*Streptococcus thermophillus*	*Micrococcus sp.*	*Lactobacillus deluburki*	*Lactobacilllus homi*
0	$1.9x10^3$	$3.9x10^5$	$5.4x10^5$	$8.8x10^5$	$4.4x10^5$	$6.6x10^3$	$2.0x10^3$	$8.0x10^3$
4	$5.4x10^5$	$3.8x10^6$	$6.2x10^6$	$7.1x10^5$	$3.0x10^7$	$1.8x10^4$	$2.5x10^4$	$5.0x10^3$
8	$4.0x10^6$	$1.2x10^8$	$7.1x10^7$	$3.5x10^7$	$1.5x10^8$	$2.1x10^5$	$1.1x10^5$	$4.0x10^4$
12	$6.2x10^6$	$7.0x10^8$	$1.1x10^8$	$5.3x10^7$	$2.0x10^8$	$5.1x10^5$	$2.6x10^5$	$1.2x10^5$
16	$5.7x10^7$	$2.4x10^8$	$8.4x10^8$	$3.9x10^8$	$1.9x10^9$	$5.8x10^5$	$5.7x10^5$	$2.3x10^5$
20	$5.0x10^7$	$8.0x10^8$	$9.0x10^8$	$1.2x10^9$	$4.0x10^8$	$8.0x10^5$	$7.5x10^5$	$3.0x10^5$
24	$6.1x10^7$	$1.8x10^9$	$2.8x10^9$	$1.2x10^9$	$6.2x10^9$	$2.2x10^6$	$4.4x10^6$	$5.0x10^5$

Source: Almaz Gonfa *et al.* (1999)

The development of microorganisms during *ergo* fermentation in raw milk collected from eight dairy farms in Awassa showed variations in various parameters (Mogessie Ashenafi, 1995). Initial aerobic mesophilic counts varied between 10^4 and 10^6 cfu/ml 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. Coliform counts, which were <10 - 10^4 cfu/ml at initiation of fermentation, reached 10^6 cfu/ml within 12 hours but this count decreased markedly thereafter. Lactic acid bacteria had initial counts of $<10^4$ - 10^6 cfu/ml, and maximum counts (10^7 - 10^9 cfu/ml) were attained at 24 hours (Table 2). Yeasts were at undetectable levels initially but reached counts of 10^5 cfu/ml 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.

Table 2 pH, titratable acidity (TA) and mean counts of aerobic mesophilic bacteria (AMB), coliforms and lactic acid bacteria (LAB) during the souring of *ergo*.

			Count (log cfu/ml)		
Time (h)	pH	% TA	AMB	Coliforms	LAB
0	6.6	0.18	5.6	3.0	5.9
6	6.5	0.20	7.4	5.0	7.1
12	6.1	0.36	8.1	5.9	8.0
24	4.2	0.81	9.0	5.3	8.2
36	4.1	0.72	8.8	4.1	8.1

Adapted from Mogessie Ashenafi (1995)

To study the effect of container smoking on the microbiological and biochemical qualities of fermenting *ergo* (Mogessie Ashenafi, 1996), raw milk was allowed to sour naturally at ambient temperatures (25-30^{o}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) 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 quality. 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, 1996). 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* flavor. 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 indicate 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 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 occur 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 with different strains.

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 monocytogenes* during the souring of milk into *ergo* (Mogessie Ashenafi, 1992; 1993; 1994c). All the test pathogens could grow to levels as high as 10^7 - 10^8 cfu/ml within 12 hours in fermenting 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 inhibition of *Salmonella typhimurium* and *Salmonella enteritidis* between 48 and 60 hours of fermentation of milk in non-smoked and smoked containers (Table 3). *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. *E. coli* O157:H7 inoculated in souring milk at initial levels of 10^3 cfu/ml grew to over 10^6 cfu/ml within 24 hours and counts at 72 hours were still at the level of 10^3 cfu/ml. Post-souring inoculation of the pathogen in *ergo*, however, resulted in complete elimination of the pathogen within 6 hours at ambient temperature storage, but it was recovered until 72 hours at refrigeration storage (Mekonnen Tsegaye and Mogessie Ashenafi, 2005).

Table 3 Mean pH and % titratable acidity (TA) values and counts of *S. enteritidis* in the absence and presence of LAB in fermenting milk in non-smoked and smoked containers.

	Time (h)	*S. enteritidis* alone			*S. enteritidis* with LAB		
		pH	%TA	Count (log)	pH	%TA	Count (log)
Non-smoked	0	6.4	0.17	3.77 ± 0.22	6.4	0.2	3.77 ± 0.2
	24	5.8	0.22	8.14 ± 0.84	3.9	0.6	6.05 ± 1.1
	48	5.5	0.28	8.36 ± 0.85	3.6	0.7	2.07 ± 3.1
	60	5.6	0.28	7.82 ± 1.15	3.6	0.7	1.72 ± 2.7
Smoked	0	6.6	0.17	3.77 ± 0.22	6.4	0.2	3.77 ± 0.8
	24	6.0	0.22	7.43 ± 1.13	4.0	0.6	4.89 ± 1.6
	48	5.8	0.29	8.89 ± 0.49	3.6	0.7	1.80 ± 2.0
	60	6.0	0.24	8.73 ± 0.16	3.6	0.7	0.40 ± 0.9

Source: Mogesssie Ashenafi (1993)

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 due to its good flavor. Longer keeping is not desirable because further drop in pH will 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 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*.

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 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 tested in controlled fermentation of pasteurized milk to *ergo*. The organoleptic quality of the product in relation to the various starter 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.

Ititu (concentrated sour milk)

The name *ititu* is used for a concentrated fermented milk prepared and consumed by the Borana tribes in southern Ethiopia. This pastoral/farmer community prepares *ititu* during the rainy season when milk is available in abundance for later consumption during the drier seasons when fresh milk supply is markedly scanty (Almaz Gonfa *et al*., 2001). The product has good keeping quality and remains acceptable for about two months at ambient temperature (25^oC-30^oC) and can be stored from about two months (Kassaye *et al*., 1991) to three months (Almaz Gonfa *et al*., 2001). The traditional processing and consumption pattern of *ititu* is well described by Almaz Gonfa *et al*. (2001). It is consumed as side dish with traditional porridge or thin-baked cereal chips. It can also be consumed as food or drink alone. It is considered as one of the special foods and served to very respected guests as well as to weaning-age children and the elderly.

During the traditional production of *ititu*, fresh milk is collected in a well-smoked fermenting vessel called *gorfa* (Kassaye *et al*., 1991). *Gorfa* is woven from fibers of selected plants into a lidded container with a capacity up to three liters. A new *gorfa* is washed with hot water, air dried, rinsed with fresh milk and smoked for a few minutes with splinters of *Acacia nilotica* or other plants. The lid of the *gorfa* is treated with leaves of *Ocimum basilicum* for cleaning and imparting desirable flavor to the product

(Kassaye *et al*., 1991; Almaz Gonfa *et al*., 2001). A small volume of milk (up to 300 ml) is added to the *gorfa* and is allowed to ferment naturally. When the milk coagulates, whey is removed by wooden pipette and an additional volume of fresh milk is added. The process of whey removal and addition of fresh milk is repeated several times until the product is concentrated enough and is ready for consumption (Fig. 2). Any mold growth on the surface of the curd is removed.

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* had increased contents of free and total amino acids when compared to fresh whole milk and was rich in amino acids such as glutamic acid, alanine, proline, leucine and serine (Kassaye *et al*., 1991) (Table 4). 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. Their *ititu* may only be the *ergo*-type rather than the concentrated sour milk. Kassaye *et al*. (1991) further reported that the total bacterial count was 10^{12} cfu/g, mainly dominated by lactic acid bacteria. Yeast and mold counts were 10^{8} cfu/g, and coliforms were not detected. They identified the prevalent lactic acid bacteria as *Lactobacillus casei* and/or *Lactobacillus plantarum*. The counts seem exaggerated, though, and more realistic values for lactic acid bacteria of about 10^{8} cfu/g were reported by Almaz Gonfa *et al*. (2001).

Table 4 Amino acid content (9 mg/g sample) of raw milk and *ititu*.

Amino acid	Day 0		Day 28	
	Free	Total	Free	Total
Alanine	0.29	9.06	1.74	12.65
Arginine	0.06	10.75	0.55	14.73
Aspartic	0.45	20.96	0.77	26.61
Cystine	0.00	1.81	0.00	2.16
Glutamine	1.23	67.96	2.45	85.27
Glycine	0.19	5.06	0.36	6.89
Histidine	0.06	8.22	0.16	10.85
Isoleucine	0.08	15.11	0.36	19.08
Leucine	0.22	28.45	1.29	40.33
Lysine	0.24	24.88	0.69	33.26
Methionine	0.05	8.85	0.18	11.90
Phenylalanine	0.14	14.67	0.64	19.48
Proline	0.08	29.81	1.66	36.64
Serine	0.24	15.46	0.97	20.64
Threonine	0.13	12.37	0.55	15.81
Tyrosine	0.09	14.87	0.57	21.60
Valine	0.13	17.51	0.54	22.18

Source: Kassaye *et al*., (1991)

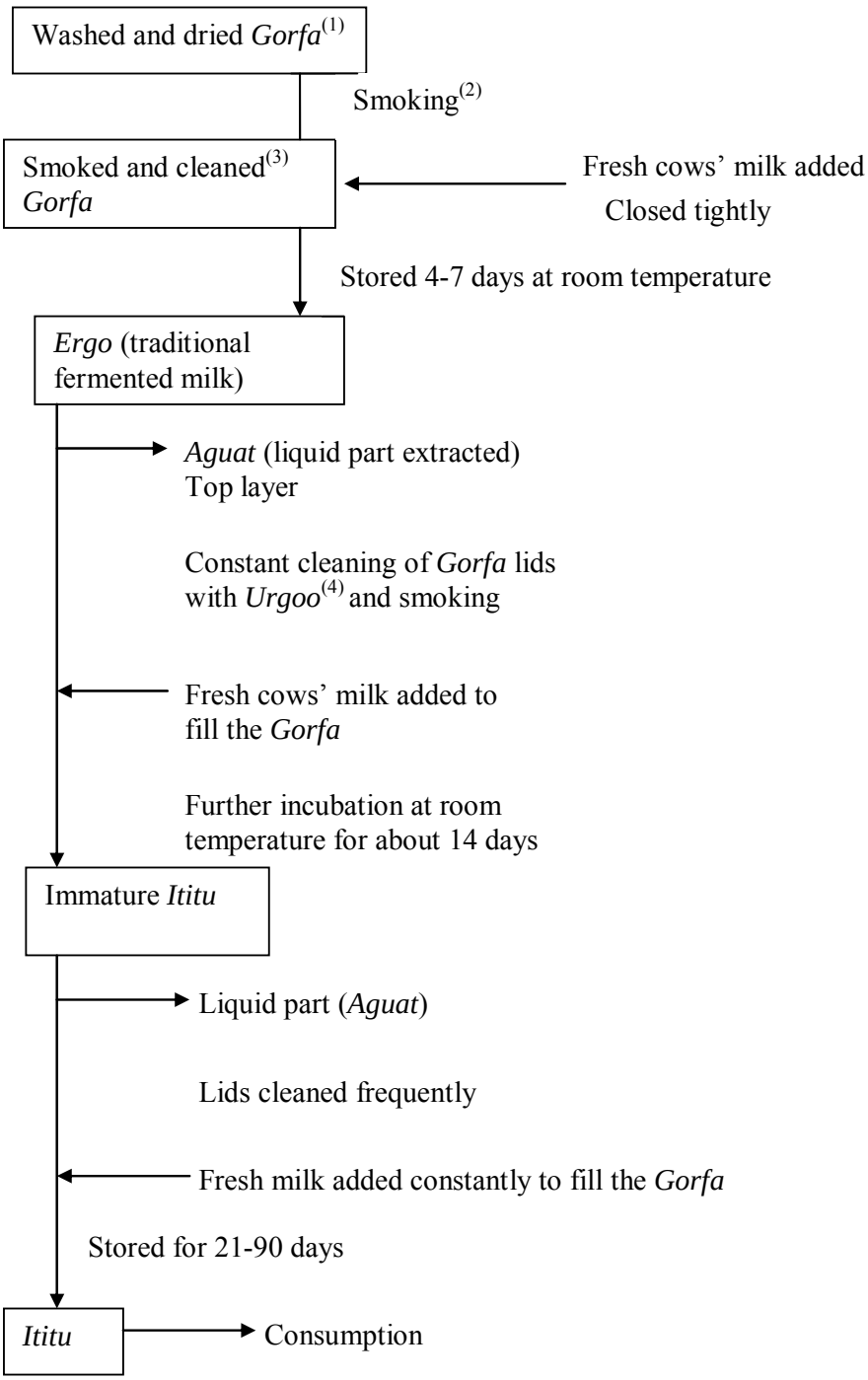

Fig. 2. Flow scheme for processing of *ititu* (From Almaz Gonfa *et al*., 2001).

Ayib (cottage cheese)

In Ethiopia, small-holder milk processing is based on sour milk mainly due to high ambient temperatures, consumer's preference and increased 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. It is an acidic product. *Ayib* is an important source of nutrients and serves as a staple diet. It may be consumed fresh as side dish, or it may be spiced with hot spices, salt and other herbs (Almaz Gonfa *et al*., 2001). Raw milk is collected in a clay pot and kept in a warm place (about 30^{o}C) for 24 to 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^{o}C until a distinct curd mass forms and floats over the whey. The whey is traditionally known as *aguat*. Temperature, however, can be varied between 40^{o}C and 70^{o}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), 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, respectively. Over 60% of the samples had psychrotrophic count of 10^6 cfu/g 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 levels (10^2 - 10^3 cfu/g). 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 increase in counts. 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* could 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 and *Lactobacillus fermenti* and *Lactobacillus plantarum* dominated the flora. The yeast flora consisted of nine different species belonging to the genera *Kluyveromyces* (46.8%), *Sporobolomyces* (31.5%), *Candida* (12.5%), *Torulopsis* (6%) and *Leucosporidium* (3.2%). Only *Kluyveromyces lactis*, *Kluyveromyces bulgaricus* and *Candida pseudotropicalis* could ferment lactose. One *Kluyveromyces* and one *Candida* species showed strong proteolytic activity. All yeast isolates were lipolytic (Mogessie Ashenafi, 1989).

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°C and 70°C (Table 5). 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. However, substantial number of yeasts, molds and lactic acid bacteria 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*.

Table 5 Microbial counts (log cfu/g) in raw milk, sour milk and *ayib* cooked at different temperatures.

Sample	pH	AMC	EC	EB	LAB	Staph	Y/M	ASF
Raw milk	6.7	6.73	4.20	4.54	5.63	4.30	3.70	3.53
Sour milk	4.0	9.26	6.34	6.26	8.76	4.36	5.82	3.30
Ayib:								
40°C/10h	3.8	8.62	4.85	3.94	5.58	<2	5.04	3.18
50°C/7h	4.0	7.79	<2	<2	4.81	<2	5.04	3.40
60°C/145 min	4.0	7.15	<2	<2	4.72	<2	3.04	3.11
70°C/55 min	4.0	6.60	<2	<2	3.60	<2	<2	3.43

AMC, aerobic mesophilic count; EC, enterococci; EB, enterobacteriaceae; LAB, lactic acid bacteria; Y/M, yeasts/molds; ASF, aerobic spore formers.
(adapted from Mogessie Ashenafi (1990b)

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. 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.

In a study on microbiological safety of *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) (Mogessie Ashenafi, 1990a). *Listeria monoctyogenes* was, however, not encountered in any of the samples. 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. Mekonnen Tsegaye and Mogessie Ashenafi (2005) showed that the count of *E. coli* O157:H7 increased during milk souring for *ayib* processing, but the count immediately after curd-cooking was below detectable limits, although the pathogen was recovered after enrichment. It was completely eliminated 24 hours after curd-cooking. When *E. coli* O157:H7 was inoculated into steam-treated *ayib* at low initial inoculation level and maintained at ambient temperatures, it was eliminated within a day. At higher initial inoculum level and ambient temperature storage, however, *E. coli* O157:H7 was detectable by enrichment until day 9 (Table 6). At refrigeration storage, decrease in counts was gradual and counts at day 9 were $>10^4$ cfu/ml.

Table 6 Counts (log cfu/g) of *Escherichia coli* O157 test strains during milk souring and *ayib* processing.

Strain	Milk souring						Defatted milk		After curd cooking			
	0h		24h		36h				0h		24h	
	pH	count	pH	count	pH	count	pH	count	pH	count	pH	count
9303	6.43	3.33	4.34	6.21	4.32	6.12	4.30	5.77	4.36	+	4.30	-
1847	6.46	3.65	4.43	6.51	4.35	6.00	4.31	5.57	4.31	+	4.30	-
4595	6.46	3.82	4.41	6.16	4.30	6.17	4.30	5.82	4.29	+	4.30	-
Mean	6.45	3.60	4.39	6.29	4.32	6.09	4.30	5.72	4.32		4.30	
S.D.	0.02	0.25	0.05	0.19	0.03	0.09	0.01	0.13	0.04		0	
% CV	0.3	7.0	1.1	3.0	0.6	1.4	0.2	2.3	0.8		0	

+, detectable only after enrichment; -, not detectable after enrichment; S.D., standard deviation; CV, Coefficient of variation. Source: Mekonnen Tsegaye and Mogessie Ashenafi (2005)

Qibe (traditional butter)

Qibe is a traditional Ethiopian butter which is made from *ergo* and not from cream (O'Connor *et al*., 1993). It has a white to yellowish color, depending on age, and is semi-solid at room temperature. It has a typical diacetyl taste and flavor when fresh, but extended storage at ambient temperatures results in putridity and rancidity. *Qibe*, without further processing, is used for hairdressing and as a skin cosmetic mainly by women. A small amount of the fresh form is traditionally fed to infants of weaning age. Generally, *qibe* is used in the diet after processing into 'nitir *qibe*' (traditional ghee), by heating it to boiling after selected types of spices are added to it. Nitir *qibe* is basically used for the preparation of stews made of legumes or meat, which are eaten with *enjerra*, a fermented pancake-like bread. In Addis Ababa, where consumption of *qibe* is believed to be high, over 54% of milk is converted to *qibe* (CSA, 1995).

Qibe is produced by churning *ergo* in traditional utensils with a volume of 20-25 liters. Milk for churning is accumulated over several days in the utensil and allowed to sour into *ergo*. Traditionally, *qibe* production is the responsibility of women and the processing of 20-25 liters of *ergo* needs 1 to 4 hours of churning time and about 1 kg of *qibe* is produced (O'Mahoney and Bekele, 1985). The curd is broken by agitation before churning starts. Agitation of churn is carried out by rocking the churn placed on the ground forwards and backwards, or by suspending it from a tripod or doorpost or shaking it on a person's lap (Almaz Gonfa *et al*., 2001; Coppock *et al*., 1991). This process results in the formation of fat granules which will coalesce into larger grains towards the end of the churning time. Final rotating of the churn on its base would lump the fat grains together into *qibe* which is then skimmed off. The *qibe* is kneaded in cold water to remove any residual buttermilk. *Qibe* has 17.2% moisture, 1.3% protein, 81.2% fat, 0.1% carbohydrate, 0.2% ash, 0.024% calcium and 0.0015% iron (EHNRI, 1997).

Arrera (defatted buttermilk)

Arrera is another byproduct of *ergo* obtained after removal of *qibe* after churning. It has a thin consistency and basically contains the casein portion of milk. Its taste and odor are similar to those of *ergo*. It is either consumed in that form or cooked to produce *ayib*. In contrast to other traditional dairy products, *arrera* has less calories. It contains 91.5% moisture, 3.1% protein, 1.4% fat, 3.4% carbohydrate, and 0.6% ash. A hundred grams of *arrera* give 95 mg calcium, 84 mg phosphorus, 1.0 mg iron, 0.03 mg thiamine, 0.21 mg

riboflavin and 0.10 mg niacin (EHNRI, 1997). It is, thus, used to supplement the diets of children and the elderly in rural areas. Surpluses are given to calves, lactating cows and dogs (Almaz Gonfa *et al*., 2001).

TRADITIONAL FERMENTED PLANT 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 of these cereals. *Enjerra* can be produced from any of the various cereals depending on availability and abundance of the cereals, which are cultivated in the agro-ecological zones suitable for their growth. Generally speaking, people on the highlands prepare *enjerra* from barley and wheat whereas those on the lowlands prepare it from maize, sorghum or millet. Wherever the soil type and rainfall patterns are suitable for cultivation of teff, *enjerra* from teff is more favored than that from the other cereals. Teff *enjerra* is the most common and the main staple food in much of the central and northern highlands of Ethiopia as well as among the urban community.

The various *enjerra* types produced from the different varieties of cereals do not have significant variation in their calorie, moisture, protein, carbohydrate or phosphorus contents. Significant variations are, however, observed in the other nutrients. The fermentation process results in significant reduction of most of the nutrients found in the cereal flour. However, in general, *enjerra* can be considered as good sources of energy, fiber, iron and vitamins (Table 7).

The preparation of teff *enjerra* consists of two stages of natural fermentation, which last for about 24 to 72 hours, depending on ambient temperatures. Temperature in the highlands of Ethiopia is generally between 17 and 25^{o}C. The only required ingredients are the teff flour and water. An appropriate amount of flour is mixed with twice its weight of water. This is kneaded thoroughly to produce a thick paste. 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 towards the final stage of a previous fermentation.

Table 7 Composition of various varieties of *enjerra* in terms of 100 grams of edible portion.

Enjerra ingredient	Energy (calories)	Moisture (%)	Nitrogen (g)	Protein (g)	Fat (g)	CHO (g)	Fiber (g)	Ash (g)	Ca (mg)	P (mg)	Fe (mg)	Thiamine (µg)	Riboflavin (µg)	Niacin (µg)
Barley (black)	124.90	68.30	0.54	3.40	0.10	27.60	0.80	0.60	34.00	96.00	3.60	0.13	0.07	1.00
Barley (white)	125.80	68.50	0.42	2.60	0.20	28.40	0.80	0.30	5.00	72.00	2.10	0.08	0.05	1.00
Maize (yellow)	161.20	60.80	0.55	3.40	1.20	34.20	0.80	0.40	4.00	86.00	2.10	0.14	0.07	0.40
Maize (white)	153.00	62.60	0.57	3.60	1.00	32.40	0.80	0.40	22.00	82.00	1.80	0.09	0.04	0.50
Millet (black)	156.10	60.50	0.53	2.90	0.50	35.00	2.90	1.10	1.29	88.00	16.80	0.09	0.04	0.20
Millet (mixed)	174.20	55.80	0.71	3.90	0.60	38.30	3.20	1.40	122.00	86.00	18.90	0.13	0.06	0.30
Sorghum (red)	136.10	66.10	0.42	2.30	0.50	30.60	0.70	0.50	10.00	90.00	1.70	0.16	0.27	0.60
Sorghum (mixed)	168.10	58.00	0.66	3.60	0.50	37.30	1.60	0.60	13.00	100.00	2.90	0.11	0.09	1.30
Teff (*Eragrostis tef*) (red)	155.90	60.20	0.58	3.40	0.70	34.00	1.80	1.70	50.00	115.00	14.70	0.09	1.16	0.60
Teff (*Eragrostis tef*) (white)	145.00	63.80	0.51	3.00	0.60	31.90	1.00	0.70	56.00	100.00	7.00	0.21	0.11	0.50
Teff (*Eragrostis tef*) (mixed)	150.20	62.20	0.64	3.80	0.60	32.40	1.40	1.00	68.00	105.00	13.80	0.21	0.17	0.50
Wheat (black)	147.70	63.20	0.87	4.90	0.50	30.90	1.10	0.50	17.00	91.00	2.20	0.16	0.19	1.00
Wheat (white)	145.60	63.40	0.55	3.10	0.40	32.40	1.10	0.70	21.00	147.00	4.40	0.15	0.17	1.00
Wheat (mixed)	157.40	60.80	0.65	3.40	0.60	34.60	1.20	0.60	25.00	104.00	1.80	0.16	0.17	1.30
Mean	148.66	62.44	0.59	3.38	0.57	32.86	1.37	0.75	32.02	97.29	6.7	0.14	0.19	0.73
SD	12.53	3.57	0.12	0.63	0.28	3.01	0.78	0.41	32.93	18.00	6.39	0.04	0.29	0.36
% CV	8.4	5.7	19.9	18.5	48.7	9.1	57.2	54.3	102.8	18.5	95.3	30.9	151.3	49.8

Compiled and modified from EHNRI (1997)

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 mixed with three parts of water and boiled for 2 to 5 minutes. This is called 'absit', a dough enhancer, and it is 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. At this stage the fermenting dough is thin enough to pour onto the hot flat pan, locally known as 'mitad' for steam-baking into *enjerra* (Fig. 3). The *enjerra* pan is made of clay and has a diameter of 45-60 cm. Baking is preceded by cleaning the heated pan with piece of cloth after greasing the pan with kale and cotton seeds. Pouring of dough starts from the outer part of the pan to the center moving clockwise direction. Bubbles start forming within seconds and the pan is then covered with a lid. *Enjerra* is thus baked on the bottom side and the upper side is baked by steam. The total baking time for one *enjerra* is 2 ½ - 3 ½ minutes. The temperature in the middle of the *enjerra* during the baking process would reach around 90^{o}C. The *enjerra* is removed from the baking pan with the help of a straw plate and allowed to cool down. The weight of one teff *enjerra* is 350-450 g. *Enjerra* can be kept for 3 to 4 days. Longer keeping results in drying and moldiness.

'Absit' ensures that *enjerra* will have the proper texture and consistency. *Enjerra* baked without 'absit' or with less 'absit' than the required will have less amount of eyes on the upper surface. A higher number of larger eyes is a very desirable attribute of an attractive *enjerra*. It also tends to be brittle after few hours of baking. Too much 'absit' makes baking difficult. *Enjerra* baked at 24 hours or less is called 'aflegna *enjerra*' and has sweet taste. It is recommended for people suffering from gastritis and, thus, do not tolerate acidic foods.

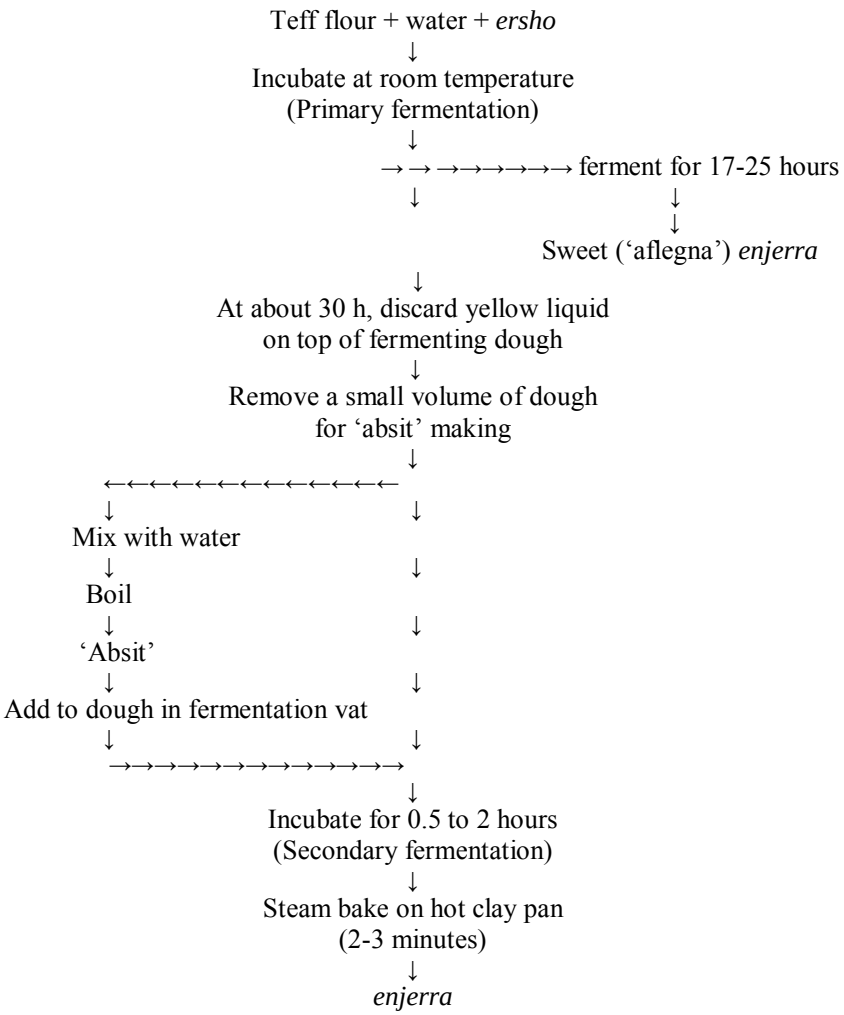

Fig. 3. Flow chart of enjerra production (adapted from Steinkraus, 1983).

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, eight had Enterobacteriaceae count of 10^4 cfu/g and all had aerobic mesophilic bacterial counts of $\geq 10^4$ cfu/g (Mogessie Ashenafi, 1994b). According to Berhanu Abegaz Gashe *et al*. (1982), total microbial count of teff flour was $>10^5$/g, mainly consisting of yeasts (10^4/g), fermentative Gram negative bacteria (10^4/g), aerobic spore formers (10^3/g) and lactic acid

bacteria (10^3/g). 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 (Table 8). The mean aerobic mesophilic bacterial count of *ersho* collected from different households was 10^6 cfu/ml and this consisted of only *Bacillus* spores. Yeast counts ranged between 10^5 and 10^6 cfu/ml 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 *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 latter 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 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 Asnake (1962), reported that *Candida guillermondii* isolated from fermenting teff, was responsible for starch hydrolysis and increase in concentration of reducing sugars in the early phase.

Table 8 Some chemical properties and microbial counts (log cfu/ml) of *ersho* collected from four households.

Household	pH	Titratable acidity (%)	Aerobic mesophilic bacteria	Yeasts
A	3.5±0.13	3.03±1.11	7.49±0.39	6.25±0.51
B	3.5±0.18	4.5±1.21	8.10±1.01	5.91±0.34
C	3.5±0.19	4.9±2.70	7.36±0.95	6.15±0.50
D	3.4±0.16	5.7±1.92	6.84±0.88	5.72±0.53
All	3.5±0.16	4.46±2.12	7.42±0.87	6.03±0.50

Adapted from Mogessie Ashenafi (1994b)

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 (Table 9). 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.

Table 9 Major reactions in the fermentation of teff.

Hrs	pH	Remarks on the fermenting dough	Predominant group of microorganisms
18	5.8	Dough rising completed; vigorous gas evolution	Members of the Enterobacteriaceae family
31	4.7	Liquid layer appeared, dough began to settle; gas evolution substantially reduced	*Leuconostoc mesenteroides* and *Streptococcus faecalis*
48	4.0	Liquid layer achieved maximum volume; dough settled completely; fair amount of gas evolution	*Pediococcus cerevisiae, Lactobacillus* sp. and yeasts
72	3.8	Confluent growth of film yeast on the liquid layer	Yeasts

Source: Berhanu Abegaz Gashe (1985)

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. 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). Because the test organisms grew in far more acidic conditions in broth than in fermenting teff, 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 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^{o}C/5 min. The acidity of *enjerra* may, however, not favor the germination of most *Bacillus* spores, although fungal spores may germinate when environmental conditions permits. The inclusion of sorbates or benzoates in the right proportion after completion of fermentation and immediately before baking may avoid 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 *enjerra* 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 content 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 teff.

Removal of the liquid layer (*ersho*) at the end of the primary fermentation removes soluble compounds. Berhanu Abegaz Gashe *et al*. (1982) reported that about 4-13% of teff nitrogen was lost depending on the stage of fermentation and suggested that this could be avoided by stopping the fermentation process before the liquid/solid separation.

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 dough. 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 fold. 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).

Although fermentation results in reduced nutrients in *enjerra*, it is seldom eaten alone. *Enjerra* is accompanied by one or more types of sauces (wot). A piece of *enjerra* is used to pick an amount of sauce and both are eaten together. A sauce may be hot-spiced, prepared with the addition of a red composite of spices called berbere, or mild-spiced prepared with the addition of turmeric. The sauces are basically legume-based, vegetable-based or meat-based. A combination of meat and vegetables or meat and legumes is also prepared in various households. The recipes and the preparation of the sauces vary from a household to another. Sauces are cooked with a good amount of vegetable oil or the more expensive *qibe* (traditional butter) depending on sauce type. Thus, the sauce ingredients are the major sources of nutrients in the diet. The importance of *enjerra* lies rather as a source of carbohydrate, iron and fiber. *Enjerra*, particularly from millet or red teff, has high iron content and in areas of the country where consumption of *enjerra* from red teff or millet is prevalent, people tend to have higher levels of hemoglobin and, thus, a decreased risk of anemia related to parasitic infection. Since the teff grain is too small to separate into germ, bran and endosperm, the flour has a much higher fiber content than other cereals. This is particularly important in dealing with diabetes and assisting with blood sugar control.

Kotcho fermentation

Almost 10 million people in Ethiopia are dependent on enset (*Ensete ventricosum*), also known as 'false banana' (Pijls *et al*., 1995). Enset is the main source of food in the densely populated areas of central and southwestern Ethiopia. According to a description by Taye Bezuneh (1984), the enset plant grows tall and robust, ranging from 4 to 11 meters in height; its pseudostem dilates at the base to a circumference of 1.5-3.0 meters, and the more it is dilated at the base, the greater is its yield. The pseudostem length ranges from 2 to 5 meters depending on the clone and ecological condition of its cultivation. Its pseudostem and leaf mid-rib color vary considerably; some are purple to dark red but most are light green with variegated brown patches. Leaves are borne on the pseudostem almost from the same point and on short petioles, and are ca 5 meters long and 0.75-1.5 meters wide. The underground portion of the plant consists of a corm which is 0.70-1.8 meters long with a circumference of 1.5-2.5 meters at maturity. 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. The pseudostem is also excellent source of fiber used for making ropes, gunny bags, carpets and *kotcho* squeezing fiber. Enset leaves are used for many purposes: for lining fermentation pits and wrapping *kotcho* during baking; for making mattress and cushion; for animal feed and fuel (Mehtzun Tedla and Yewelsew Abebe, 1994). The plant is grown on a total of about 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 planting and transplanting of enset is carried out by men. Enset harvesting and processing is the responsibility of women. The harvesting and processing of enset for extracting edible and non-edible parts is probably one of the most laborious and cumbersome household responsibility of women in enset-culture areas. Mehtzun Tedla and Yewelsew Abebe (1994) described the process of traditional enset processing among the Wolayta and Sidama people (Fig. 4).

The job starts with the digging of a pit around the homestead. The inner part of the pit is lined with enset leaves to collect and prevent the juicy part from leaking into the ground. Pseudostem, removed from the enset plant, is tightly secured on a wooden pole and, using a bamboo scraper, the woman scrapes the fleshy part of the pseudostem down towards the pit. After a short while the juicy part sediments into a moist sticky product known as *bulla*. The clear solution over the *bulla* sediment is discarded. The remaining thick white *bulla* is thinly spread for dehydration, and a handful is then wrapped in fresh enset leaves, tightly tied with fiber and allowed to ferment. The fermenting *bulla* is protected from air and light to avoid undesirable color change, as white *bulla* has a higher market value. A serrated animal shoulder bone is usually used to pulverize the corm (root part) of the plant. The grated pieces of the corm are mixed with the fleshy scrapped pseudostem and buried in the pit. A pre-fermented *kotcho* is used as a starter to initiate the fermentation. According to Berhanu Abegaz Gashe (1987a), four to eight mature enset plants are required to obtain sufficient *kotcho* to fill a pit. The *kotcho* is then pressed by hands or feet, covered with fresh enset leaves, and layered with discarded enset parts. Heavy materials such as large stones are put on top of the layering to ensure the creation of airtight conditions in the pit.

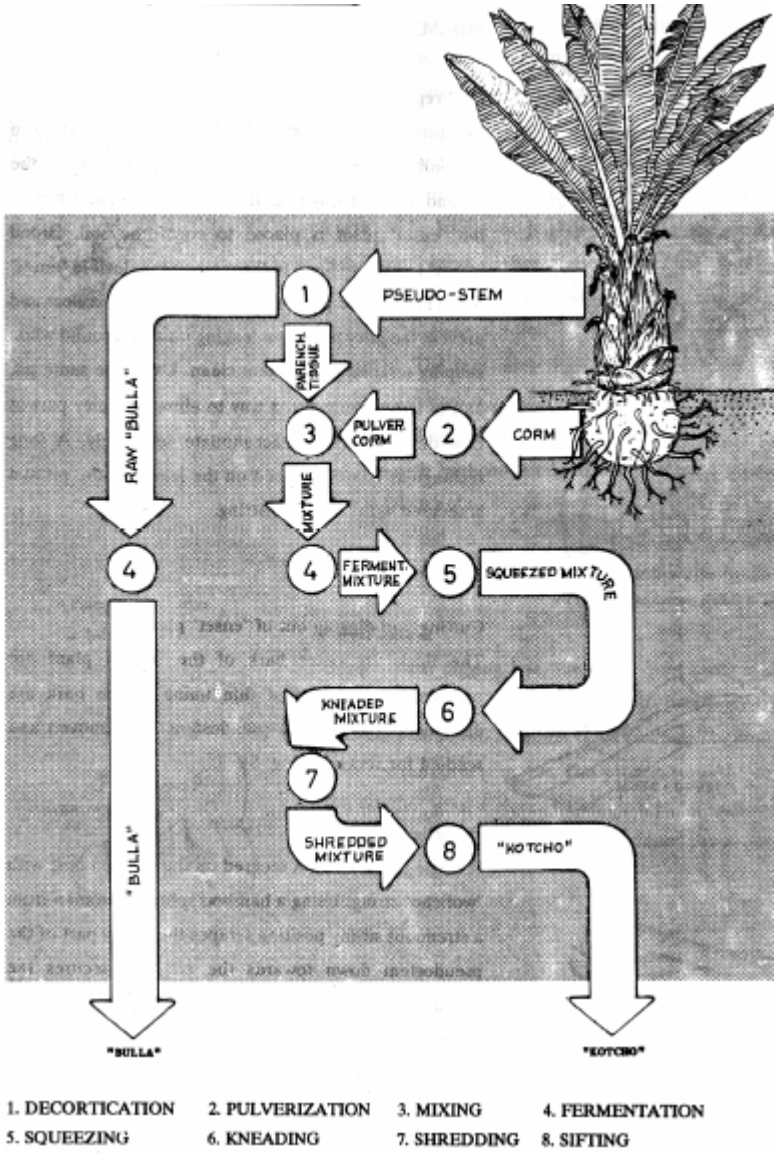

Fig. 4. Stages of enset processing (From Mehtzun Tedla and Yewelsew Abebe, 1994).

The length of fermentation time varies from a few weeks, to several months or years depending on ambient temperatures of incubation. In the cooler regions, it is kept in a pit for years and the quality is said to increase with increasing fermentation time. In warmer regions, fermentation is rapid and is therefore terminated within 3 to 6 months (Berhanu Abegaz Gashe, 1987a). After the fermentation is completed, a portion is removed from the

pit and the liquid is squeezed out of it resulting into a moist fibrous *kotcho*. This is kneaded and shredded. Final sifting removes the remaining fibers and gives a finely powdered substance, the *kotcho* powder. Taye Bizuneh (1984) compared the yield of the fermented product from three clones of enset and found out that only 25-33% of the fresh weight yield was retrieved as fermented product and yield of the fermented product was 18.5 to 29.8 kg per plant. Pijls *et al*. (1995), however, reported yield of *kotcho* to be about 34 kg/plant or 9.5 tons/ha/year.

Nutritional composition of the various enset products is given in Table 10. Enset foods are relatively high in carbohydrate and energy, but are poor in protein (EHNRI, 1997). Taye Bizuneh (1984) showed that the content of the various nutrients decreased during fermentation, possibly due to excessive leaching following the peak of microbial activity. Abraham Besrat *et al*. (1980b) also reported that fermentation resulted in a slight decrease of protein content but, at the same time, improved the quality of the protein as determined by amino acid profiles. They found the *kotcho* protein was generally higher in lysine than most cereals. Enset products contain more calcium than most cereals, tuber and root crops (Taye Bizuneh, 1984). This may be the reason why enset products are used for healing fractured bones (Taye Bizuneh, 1984; Mehtzun Tedla and Yewelsew Abebe, 1994). There are also other traditional medicinal values of the products (Mehtzun Tedla and Yewelsew Abebe, 1994): Gruel or porridge made of *bulla* is fed to new mothers to clean out the uterus, for milk production and for making them strong. *Bulla* gruel or porridge is given to newly circumcised children to speed up healing of the wound. Over-fermented *kotcho* in its dry form is also used to treat amoebiasis and cramp.

The fermented products of the enset plant are used to make different dishes such as thin, unleavened *kotcho* bread (simply called *kotcho*), bulla porridge (*genfo*), a thick cooked bulla gruel (*atmit*), and a shredded flake made of a mixture of *kotcho* and *bulla* (*firfir*). The fermented products of the enset plant are basically energy foods and are often blamed for causing protein deficiency disease when eaten alone as staple. However, it should be noted that dishes made of these products are traditionally served with other protein and vitamin sources. The various *kotcho* and bulla dishes are supplemented with milk, *qibe* (traditional butter), fenugreek, *ayib* (traditional cottage cheese), meat, kale or beans separately or in combination. Unfermented, fermenting or completely fermented *kotcho* is baked or cooked and eaten alone or in combination with various indigenous foods. Unfermented *kotcho* is consumed only when there is a shortage of the fermented or fermenting

material (Berhanu Abegaz Gashe, 1987a). The enset culture developed around this crop probably because it is a crop that provides energy foods that do not require constant attention. Enset competes well with other vegetation and is not seriously affected by droughts and pests.

Kotcho and the other enset food products are basically popular and staple foods among the Gurage and other ethnic groups in southern Ethiopia. Nowadays, enset foods are becoming increasingly popular among all ethnic groups in urban settings. It is now customary to serve Kitfo (spiced ground meat) with *kotcho* at holidays, weddings and in specialty restaurants.

Berhanu Abegaz Gashe (1987a) studied and described the microbiology of *kotcho* fermentation (Table 11). He reported that *Leuconostoc mesenteroides* initiated the fermentation and dominated the lactic flora with counts of 10^7 cfu/g 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 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 highest counts (10^3 cfu/g) 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 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*.

Table 10 Composition of various enset products in terms of 100 grams of edible portion.

Enset (*Ensete ventricosum*) product	Energy (calories)	Moisture (%)	Nitrogen (g)	Protein (g)	Fat (g)	CHO (g)	Fiber (g)	Ash (g)	Ca (mg)	P (mg)	Fe (mg)	Thiamine (µg)	Riboflavin (µg)	Niacin (µg)
Enset powder	196.00	49.70	0.30	0.90	0.20	47.70	1.20	1.60	77.00	60.00	10.10	0.04	0.05	0.00
Bulla powder	180.50	54.90	0.03	0.20	0.10	44.70	0.30	0.10	41.00	20.00	2.60	0.01	0.00	0.10
Bulla bread	186.10	53.30	0.03	0.30	0.10	46.00	0.30	0.30	45.00	18.00	4.60	0.01	0.00	0.10
Bulla porridge	80.30	81.00	0.03	0.20	1.10	174.00	0.50	0.30	30.00	10.00	2.60	0.01	0.00	0.10
Kotcho powder	211.10	46.70	0.20	0.60	0.30	51.50	1.20	0.90	32.00	36.00	3.70	0.03	0.04	0.30
Kotcho bread	219.40	43.70	0.20	1.00	0.20	53.40	1.30	1.70	93.00	43.00	2.40	-	0.10	0.20
Kotcho porridge	90.60	78.90	0.11	0.70	2.20	17.00	1.30	1.20	50.00	21.00	4.90	0.01	0.03	0.10

Compiled from EHNRI (1997)

Table 11 Estimated number of organisms/g dry weight of fermented *kotcho*.

Fermentation (days)	pH	Moisture (%)	Temp. of *kotcho* (°C)	Spore-formers	*Leuconostoc* spp.	*Streptococcus faecalis*	*Pediococcus cerenisiae*	*Lactobacillus* spp.*	Yeast	Total count†
0	6.5	84	15	-	-	-	-	-	$1x10^1$	$1x10^1$
2	6.3	75	17	-	$3x10^1$	$7x10^1$	-	-	$3x10^2$	$4x10^2$
4	6.0	72	16	-	$1x10^4$	$2x10^2$	-	$1.5x10^2$	$6x10^1$	$1.04x10^4$
6	5.8	69	15	$3x10^2$	$4x10^6$	$4x10^3$	-	$9x10^2$	$2x10^2$	$4.01x10^6$
8	5.6	66	15	$2x10^3$	$3x10^7$	$9x10^2$	$1x10^1$	$3.3x10^6$	$4x10^2$	$3.33x10^7$
15	4.9	63	17	$1x10^3$	$3.6x10^9$	$9x10^2$	$4x10^3$	$5.4x10^9$	$9x10^2$	$9x10^9$
22	4.5	60	18	$2x10^2$	$1.3x10^7$	-	$6x10^3$	$1.67x10^{10}$	$2x10^3$	$1.67x10^{10}$
29	4.4	60	18	$8x10^2$	$4x10^6$	-	$4x10^3$	$2.1x10^9$	$3x10^3$	$2.1x10^9$
36	4.4	59	15	$3x10^2$	$2x10^6$	-	-	$7.4x10^7$	$4x10^2$	$7.6x10^7$
43	4.3	51	18	$1x10^1$	$5x10^4$	-	-	$1.1x10^7$	$9x10^3$	$1.11x10^7$
50	4.2	60	16	$8x10^1$	$1x10^3$	-	-	$6x10^5$	$8x10^1$	$6x10^5$
64	4.2	60	17	$1x10^2$	$6x10^2$	-	-	$1.2x10^5$	$9x10^1$	$1.21x10^5$
79	4.2	60	18	$6x10^1$	$1x10^2$	-	-	$1.25x10^4$	$1x10^1$	$1.27x10^4$

- Populations less than 10/g dry weight of fermenting *kotcho*.

* Values represent the sum of the *Lact. coryneformis* subsp. *coryneformis* and *Lact. planiarum* populations.

† Count represents the sum of the microorganisms present at any one of the fermentation periods.

(Source: Berhanu Abegaz Gashe (1987a)

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). Coliform counts were markedly higher in *bulla* (10^5 cfu/g) than in *kotcho* (10^3 cfu/g). Counts of enterococci, in both products, ranged between 10^4 and 10^5 cfu/g. 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) 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 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 did not show any detectable spoilage

in terms of odor, consistency or color.

Ayele Nigatu and Berhanu Abegaz Gashe (1994b) showed the antagonistic potential of 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. In another study on the effect of heat treatment on the antimicrobial property of the fermented *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.

Traditional fermented condiments

Siljo fermentation

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). 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 sufflower extracts and cooked well to a gruel consistency. This is cooled to about 55^{o}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 48 hours of fermentation, peeled garlic, rue leaves and other spices are added to it. Its fermentation is spontaneous and the product is usually ready for consumption after three to five days of fermentation (Fig. 5). 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 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 because the major substrates were thoroughly cooked during initial preparation, the black mustard powder was the source of starter microorganisms. It contained *Lactobacillus acidophilus*, *L. plantarum* and *L. delbruekii* and the yeasts *Saccharomyces cerevisieae*, *Rhodotorula glutinis*, *Yarrowia lipolytica* and *Saccharomyces rouxii*. The fermentation was, however, initiated and later dominated by *L. plantarum* and *L. acidophilus*.

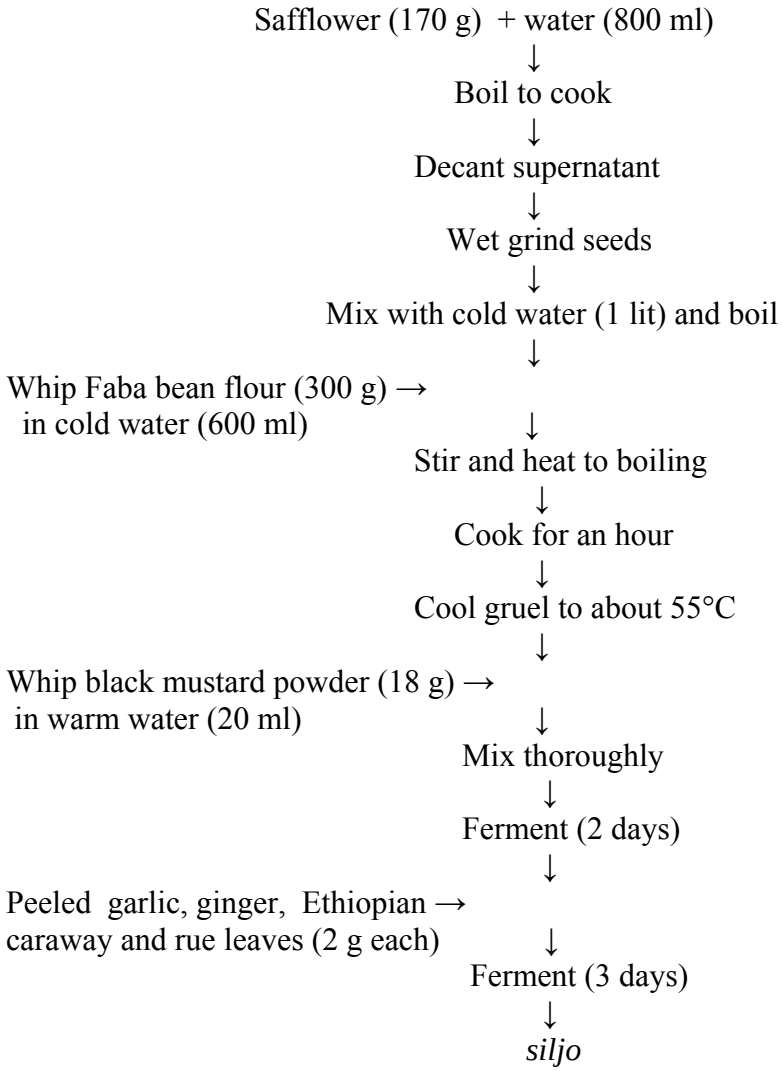

Fig. 5. Flow chart of *siljo* fermentation.

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 after 36 hours of fermentation, but Enterobacteriaceae were not detected. *Micrococcus, Bacillus* and *Lactobacillus* species dominated the flora (Table 12). 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 and the initial and final values of titratable acidity were 0.36 and 0.75, respectively.

Table 12 Microbial counts of fermenting *siljo.*

Time (h)	Counts (log cfu/ml)			No. of individual isolates from AMC plates*				
	pH	AMC	LAB	M	L	B	Ac	Al
0	6.0	4.77	1.78	28	-	4	8	-
12	6.0	6.46	5.34	10	6	14	6	2
24	5.5	8.32	7.15					
36	4.5	9.42	9.31	5	22	13	-	-
48	4.5	10.08	9.98	3	26	11	-	-
60	4.2	10.34	10.26	3	26	11	-	-
72	4.2	10.58	10.53	2	25	13	-	-
96	4.2	10.72	10.69	3	25	12	-	-
120	4.1	10.88	10.82	8	19	13	-	-
144	4.1	10.53	10.50	10	20	6	4	-
168	4.0	10.04	9.91	13	16	7	4	-
192	3.9	9.23	9.16	10	14	8	-	8

* 40 isolates were taken from each plate: M-*Micrococcus*; L-*Lactobacillus*; B-*Bacillus*; Ac-*Acintobacter*; Al-*Alcaligenes*; AMC- Aerobic mesophilic count; LAB-Lactic acid bacteria.
Source: Tetemke Mehari and Mogessie Ashenafi (1995)

The moisture content of *siljo* was about 86%. A slight increase in crude protein, crude fat and ash was observed 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 (Table 13).

Table 13 Changes in some nutritional properties of fermenting *siljo.*

Time (h)	Crude protein (%)	Crude fat (%)	Ash (%)	Protein availability (%)			Protein concentration (%)
				With papain	Without papain	Difference	
0	27	21	7	1.36	0.25	1.10	1.34
24	27	20	7	1.80	0.40	1.40	1.47
48	28	23	7	2.25	0.65	1.60	1.72
72	28	25	7	4.60	1.62	2.98	3.01
96	28	25	7	4.91	1.50	3.41	3.22

Source: Tetemke Mehari and Mogessie Ashenafi (1995)

Gulilat 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 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* at 48, 24 and 48 hours, respectively (Gulilat Dessie *et al*., 1997). Acid production by lactic acid bacteria and components of mustard power could have inhibited the test organisms.

Eden Ephraim and Mogessie Ashenafi (2005) evaluated the fate of *Salmonella typhimurium* DT 104 during the fermentation of *siljo* at ambient and cold temperatures. *Siljo* was made to ferment naturally and the count of lactic acid bacteria reached 9.9 log cfu/ml on day 5. The pH dropped from an initial value of 5.8 to 4.65 during this time. The lactic acid flora was dominated by *Leuconostoc* spp. At ambient temperature storage (18–22^{o}C), the product spoiled on day 16. The spoilage was caused by *Bacillus* spp. At refrigerated storage (4^{o}C), however, the count of *Bacillus* spp. was below detectable limits (<1 log cfu/ml) until the end of the experiment on day 16. When *Salmonella typhimurium* DT 104 was inoculated into the fermenting gruel at low initial levels (2.8 log cfu/ml), the count decreased steadily and the test strain was not detected by enrichment on day 5. At higher initial inoculum level (5.5 log cfu/ml), complete elimination was observed on day 7. In a non-fermenting control gruel, count of the test strain increased by about 3 log units on day 7. In another experiment, the fermented product was inoculated with *Salmonella typhimurium* DT 104 at low or high inoculum levels and stored at ambient and refrigeration temperatures. The results indicated that, at normal contamination levels in the kitchen environment, fermentation for five days would completely eliminate *Salmonella typhimurium* DT 104 from the fermenting gruel; ambient temperature storage of the product would completely eliminate the pathogen after three days; and its survival is markedly prolonged by cold storage.

Awaze

Awaze is a traditional fermented condiment and is consumed with other items on the basis of its desirable aroma and flavor. It is the product of the microbial fermentation of vegetable-spice mixtures. *Awaze* is common in the north and central Ethiopia and is often used to flavor sliced raw or roasted meat and other traditional pancakes. The major ingredient for *awaze* preparation is red sweet pepper (*Capsicum annum)*. The spices added to it include garlic (*Allium sativum*), ginger (*Zingiber officinale*), sweet basil

(*Ocimum sanctum*), rue (*Ruta chalepensis*), cinnamon *(Cinamommum zylanicum*), clove (*Eugenia caryophyla*), Ethiopian caraway (*Trachyspermum copticum*), Ethiopian cardamom (*Aframomum anguistifolium*), and salt. The preparation of the ingredients is as follows. All the seeds of the sweet pepper are first discarded and the fruits are thoroughly washed with tap water and sun-dried. The dried seedless fruits are gently pulverized with a wooden mortar and pestle. Fresh ginger and garlic are peeled, washed and mixed together with small proportions of fresh sweet basil and seeds of rue. These are then mixed with the already dried and pulverized sweet pepper and kneaded together. The whole kneaded mixture is left in a container overnight to form a moist solid mash. The mash is sun-dried by thinly spreading on a clean surface. Small proportions of the dry spices including clove, cinnamon, Ethiopian caraway, Ethiopian cardamom and sweet basil together with certain amount of salt are gently heat-treated separately on a hot metal pan. The heat-treated spice mixture is mixed with the kneaded and dried pepper-spice mixture and is dry-milled. This is finally sieved through a fine wire mesh. The fermentation of *awaze* starts by whipping a portion of the ground pepper-spice ingredient with warm water until it acquires a thick consistency. This is then left to ferment at ambient temperatures.

Ahmed Idris *et al*. (2001) studied the fermentation of *Awaze*. Ingredients for *awaze* preparation had a microbial load of 10^6 cfu/g 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 at day 4 and the count remained $>10^8$ until end of fermentation (Table 14). The heterofermentative lactic acid bacteria dominated until day 3 and the homolactics took over thereafter. Yeasts also reached counts of 10^6 cfu/g at the end of fermentation on day 12. A steady decline in pH was observed in the course of the fermentation and the major drop in pH was noted between 24 and 48 hours. This was also accompanied by increase in titratable acidity. At day 14, pH was around 3.7 and titratable acidity was 0.38%. *Awaze* can be consumed at early or later stages of fermentation. Inoculation of *Salmonella typhimurium* in fermenting *awaze* resulted in a fast elimination of the pathogen (Ahmed Idris *et al*., 2001). The fermented product also inhibited survival of *E. coli* O157:H7 when stored at ambient temperature, whereas cold storage extended the survival to over 7 days (Mekonnen Tsegaye *et al*., 2004).

Datta (Qotchqotcha)

Datta (also known as *qotchqotcha*) is a condiment of similar use as that of *awaze* mainly in the southern part of the country. The major substrate in the making of *datta* is the small chili pepper (*Capsicum frutescence*) at its green stage. The green pepper, together with the seeds, is thoroughly washed and cut into pieces. Garlic and ginger, in small proportions, are peeled, washed and cut into small pieces. The pepper, garlic and ginger are mixed with small amounts of fresh sweet basil and seeds of rue. The mixed ingredients are manually wet-milled on a flat traditional stone-mill into a greenish paste. This is transferred into a container, tightly closed and left to ferment at ambient temperatures.

In *datta* fermentation, the count of aerobic mesophilic bacteria remained unchanged during the fermentation (Ahmed Idris *et al*., 2001). Lactic acid bacteria initiated the fermentation at a level of 10^4 cfu/g and reached 10^9 cfu/g at end of fermentation on day 7 (Table 14). Contrary to *awaze* fermentation, the homofermentative lactic acid bacteria initiated and dominated *datta* fermentation for the first two days. The heterolactics dominated thereafter.

Challenge studies on *datta* fermentation with *Salmonella typhimurium* (Ahmed Idris *et al*., 2001) and *E. coli* O157:H7 (Mekonnen Tsegaye *et al*., 2004) showed that the fermenting condiments had strong bactericidal property against the test strains. The fermenting product, when stored at ambient temperature, also had a fast inhibitory property against *E. coli* O157:H7, although the pathogen survived for more than seven days at refrigeration storage (Mekonnen Tsegaye *et al*., 2004).

The fermentation of *awaze* and *datta* 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 (Ahmed Idris *et al*., 2001).

Table 14 Changes in counts (log cfu/g) of lactic acid bacteria during *awaze* and *datta* fermentation.

Time (h)	*Awaze*			*Datta*		
	pH	Homofermentative	Heterofermentative	pH	Homofermentative	Heterofermentative
0	5.6	<4	6.0	5.56	4.85	<3
24	5.3	<5	7.43	5.18	6.5	<5
48	4.5	<7	9.11	4.93	<5	7.91
72	4.43	9.14	9.5	4.71	<5	7.78
96	4.36	9.0	<5	4.69	<5	7.83
120	4.31	9.14	<5	4.63	<5	9.11
144	4.09	8.74	<5	4.61	<5	8.93
168	3.94	8.69	<5	4.61	<5	9.03
192	3.92	8.85	<5	END	END	END
216	3.86	8.49	<5			
241	3.86	8.56	<5			
264	3.82	8.53	<5			
288	3.78	8.99	<5			
296	END	END	END			

Source: Ahmed Idris *et al*. (2001)

TRADITIONAL FERMENTED ALCOHOLIC BEVERAGES

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.

The way of preparing *tella* differs between the ethnic groups and depends on tradition and the economic situation. Although the basic processing steps are similar, every *tella*-maker seems to have her own recipe. The clay container (*insera*) is washed with water and fresh leaves of *grawa* (*Vernonia amygdalina)* several times. The well-cleaned container is then inverted over smoking splinters of *weyra* (*Olea europaea*) for about 10 minutes. This will eliminate microorganisms sensitive to antimicrobial components of wood smoke. It also contributes to the desirable flavor of the fermented product. To make *bikil* (malt), grains of barley or wheat are moistened while in a container and left to germinate for about three days. And this is finally sun-dried. *Bikil* is the source of amylase for the fermenting cereals used in *tella* preparation. The *gesho* plant (*Rhamnus prinoides*), which is different from hop (*Humulus lupulus*) is widely cultivated in Ethiopia and is available dried in the local market. Although *gesho* may have antibacterial effect against some groups of bacteria, its main purpose in the process is to impart the typical bitter taste to *tella*. The fermentable grains for *tella* preparation are usually prepared in two forms. Flours of millet, barely or teff (dark

variety) are toasted, milled, mixed in water and baked on a wide metal pan into *kita* (unleavened bread). The *kita* is broken into small pieces. Barley flour is separately toasted on a metal pan sprinkling water on it during toasting until it turns dark brown. This is called *enkuro*. The color of *tella*, which may vary from light yellow to dark brown, is determined by the extent of baking the *kita* or toasting the *enkuro*.

Samuel Sahle and Berhanu Abegaz Gashe (1991) described the processes and microbiology of *tella* fermentation (Table 15). The fermentation is divided into four phases. During the first phase, powdered leaves of *gesho* are mixed with water in a small earthen pot and allowed to ferment for four 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*, pieces of *kita* and water. This is left to ferment for two more days. During the third stage, chopped pounded stems of *gesho*, *bikil*, *enkuro* 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 then 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. The clear liquid is *tella*. In general, about 1 kg of *gesho* (leaves and pounded stems), 0.5 kg of *bikil*, 15 kg of grains, in the form of *kita* (5 kg) and *enkuro* (10 kg) are mixed with 30 liters of water to prepare *tella*. Good quality *tella* has a final ethanol content of 2-8% (v/v) and the pH is 4-5 (Samuel Sahle and Berhanu Abegaz Gashe, 1991). When the clear *tella* is completely decanted from the sediment, fresh water is added to the sediment and mixed well. This is left to ferment. The resulting beverage is known as *kirari* and is weaker than the regular *tella*. It is most often used for family consumption, and sometimes is given to children. The better quality is often kept for guests.

Sometimes, at the end of the third stage, a smaller volume of water is mixed with the *difdif* and a more concentrated *tella* is obtained by filtering the *difdif* through a cotton cloth and keeping it in a closed container. Such *tella* is known as filtered *tella*.

Samuel Sahle and Berhanu Abegaz Gashe (1991) reported that the first phase was important to extract the components of *gesho*. The liquid at this stage was very dark in color with a strong bitter taste. The microbial count increased markedly towards the end of the phase and reduction in content of

total carbohydrate and reducing sugar occurred. The microbial flora consisted of molds, *Lactobacillus* spp. and other bacteria. Molds disappeared, however, towards the end of the phase. Ingredients added in the subsequent phases served as sources of fermenting microorganisms and increased amounts of carbohydrates and reducing sugars. Active fermentation resulted in vigorous foaming and bubbling.

Table 15 Changes in total aerobic count, moisture content and pH occurring during the fermentation of *tella*.

Phase	Fermentation time (days)	pH	Moisture (%)	Total aerobic count CFU*/ml
I (0-4 days)	0	5.2	95.6	$2x10^3$
	1	5.2	95.6	$4x10^3$
	2	5.1	95.6	$1x10^4$
	3	5.0	94.8	$2x10^6$
II (4-6 days)	4	4.7	94.5	$3x10^6$
	5	4.6	83.1	$3x10^7$
III (6-8 days)	6	4.8	84.8	$7x10^7$
IV (8-12 days)	8	4.6	74.0	$1x10^8$
	10	4.5	93.4	$1x10^8$
	12	3.9	96.6	$9x10^7$

*CFU = Colony forming units
Source: Samuel Sahle and Berhanu Abegaz Gashe (1991)

The fermenting organisms were composed of *Saccharomyces* spp., (mostly *S. cerevisieae*) and *Lactobacillus* spp. (mostly *Lactobacillus pastorianumi*). The yeasts dominated the fermenting flora after the end of the first stage till the completion of fermentation (Table 16). 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 sour 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%.

Table 16 The microbial flora of fermenting *tella* (CFU/ml).

Fermentation time (days)	*Arthrobacter* spp.	*Bacillus* spp.	*Acetobacter* spp.*	*Lactobacillus* spp.**	*Saccharomyces* spp.***	Molds
0	$1x10^2$	$1 x10^2$	$1 x10^2$	$1 x10^2$	-	$2 x10^2$
1	$1 x10^3$	$3 x10^2$	$2 x10^2$	$4 x10^2$	-	$2 x10^2$
2	$1 x10^3$	$3 x10^2$	$5 x10^2$	$1 x10^3$	-	$2 x10^3$
3	$2 x10^5$	$3 x10^3$	$6 x10^2$	$4 x10^3$	$2 x10^3$	-
4	$2 x10^6$	$4 x10^5$	$8 x10^3$	$2 x10^4$	$6 x10^5$	-
5	$2 x10^5$	$8 x10^5$	$2 x10^4$	$1 x10^5$	$2 x10^7$	-
6	$2 x10^5$	$1 x10^6$	$6 x10^4$	$7 x10^5$	$6 x10^7$	-
8	$2 x10^5$	$8 x10^4$	$5 x10^5$	$3 x10^6$	$9 x10^7$	-
10	$5 x10^5$	$4 x10^2$	$5 x10^6$	$2 x10^7$	$9 x10^7$	-
12	$3 x10^5$	-	$8 x10^7$	$7 x10^6$	$3 x10^6$	-

**Acetobacter xylinum* was the most predominant species
***Lactobacillus pastorianum* was the most abundant species
****Saccharomyces cerevisieae* was the most abundant species
Source: Samuel Sahle and Berhanu Abegaz Gashe (1991)

Tej fermentation

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 substrate. 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 glowing splinters of *Olea africana* and smoldering *gesho* (*Rhamnus prinoides*) and put back to the fermenting must. Honey, which may contain various impurities including wax, is mixed with water and placed in the smoked pot. The pot is covered with cloth and allowed to ferment in warm place for 2-3 days. At this stage, the wax and top scum is removed. A portion of the fermenting honey is boiled with pieces of washed *gesho* bark and stems and it is put back to the fermenting must. The pot is covered and fermented continuously for five more days, in warmer weather, or for 15-20 days, in cooler environments. The mixture is stirred daily and finally filtered through cloth to remove sediment and *gesho*.

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*. (2006) studied microbial variability in *tej* samples collected from various production units at different production times (Table 17). They reported that mean counts of aerobic mesophilic bacteria and aerobic spores for the different production units were $<10^3$ cfu/ml. 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 for the different production units. A total of ten different species made up the yeast microflora in the *tej* samples. Major yeast species in *tej* were *Saccharomyces cerevicieae*, *Kluyveromyces bulgaricus*, *Debaromyces phaffi* and *Kluyveromyces veronae*. There was no production unit in this study with samples containing one yeast species only. Samples from five production units contained yeast isolates belonging to two or three genera, whereas the other five had samples containing four to six genera. Yeast counts, too, showed significant variations within samples of a production unit.

Lactic acid bacteria also had counts $>10^6$ cfu/ml 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 homofermentative lactobacilli constituted part of the dominant lactic flora followed by *Leuconostoc* spp. and heterofermentative lactobacilli. In most of the samples, the lactic flora was dominated by only two or three groups of lactic acid bacteria. Domination of the lactic flora by only one group was rare.

Thus depending on number and type of yeast species and the dominating flora of lactic acid bacteria, and depending also on their metabolites, the chemical composition and organoleptic property of the products would vary markedly.

Table 17 Mean counts (log cfu/ml) of microorganisms in various *tej* samples from different production units.

Unit	pH	AMB	Yeasts	*Lactobacillus*		*Leuco nostoc*	*Strepto coccus*	*Pedio coccus*
				Homo fermentative	Hetero fermentative			
A	3.47	2.02	6.71	6.41	6.09	6.29	6.33	6.29
B	3.95	2.08	6.95	6.36	6.40	6.25	6.14	6.46
C	3.98	1.91	6.66	6.33	6.12	6.13	6.20	6.37
D	3.82	2.39	6.79	6.27	6.63	6.31	6.23	6.27
E	3.63	1.67	6.55	5.78	6.07	5.82	6.13	6.06
F	3.68	2.27	6.80	6.28	6.43	6.32	6.20	6.15
G	3.58	1.88	6.74	6.34	6.42	6.41	6.19	6.75
H	3.80	2.55	6.85	6.14	6.43	6.18	5.78	6.38
I	3.82	1.82	6.65	6.29	6.38	6.33	6.08	5.83
J	3.87	2.17	6.54	6.11	6.28	6.41	6.26	5.97

AMB, aerobic mesophilic bacteria (adapted from Bekele Bahiru *et al*., 2006)

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 (Table 18). The range of titratable acidity was 0.1 g/100 ml to 1.03 g/100 ml and mean values for production units ranged between 0.34 and 0.6 g/100 ml. About 65% of the *tej* samples had titratable acidity values of ≥4 g/100 ml. 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%. In general, the range for alcohol content of *tej* is very wide and values lower than 5% would be obtained in cases where the fermentation is far from complete. The higher titratable acidity combined with significant amount of alcohol would result in sweet-sour alcoholic flavor which would make *tej* preferable by consumers. This combination would also give *tej* the required microbiological stability, which would permit certain degree of preservation without the use for highly specific techniques as observed in wine. Producers do not determine the end-point of the fermentation and *tej* is consumed while in a state of active fermentation. Aerobic conditions would result in the formation of acetic acid from alcohol making the product more and more sour, a condition termed as 'dryness' by consumers. Fusel oil content of samples ranged between 0.1 g/100 L and 88 g/100 L. Mean values for production units was 13.6-27.4 g/100 L. About 50% of the samples had fusel oil contents of >20 g/100 L. Fusel oils may contribute to the flavor and odor of *tej*. At higher levels, however, fusel oils can be toxic and, thus, hazardous to health. Producers of traditional alcoholic beverages do not have control on products of natural fermentation processes. It is, thus, likely that home-brewed products can have higher fusel oil contents than commercially brewed ones. 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). The large number of fermentative microorganisms in *tej* could be responsible for the reduction of total sugars in *tej*. On the other hand, however, the dead and living microbial cells in *tej* could contribute to increased protein content of the final product. Variations in the various chemical and nutritional parameters were significant within samples of the same production unit.

Table 18 Mean values of chemical and nutritional parameters in various *tej* samples from different production units.

Unit	pH	%TA	Alcohol (%)	Fusel oil (g/100 L)	Carbohydrate (mg/ml)	Lipid (mg/ml)	Protein (mg/ml)	Reducing sugars (mg/ml)
A	3.47	0.57	10.85	ND	1.49	1.34	0.39	0.58
B	3.95	0.40	10.17	22.91	3.08	0.31	0.43	1.18
C	3.98	0.52	8.33	13.90	2.81	0.56	0.33	2.09
D	3.82	0.34	9.80	13.58	3.21	0.98	0.43	1.69
E	3.63	0.60	9.58	23.47	2.99	0.64	1.47	1.36
F	3.68	0.44	8.25	19.80	1.81	0.44	3.32	0.46
G	3.58	0.43	8.17	15.49	2.10	0.36	4.66	0.99
H	3.80	0.42	10.05	27.38	3.73	0.71	1.90	1.72
I	3.82	0.43	6.98	25.72	3.52	0.87	1.62	1.71
J	3.87	0.40	8.12	23.12	2.89	0.73	1.40	0.92

TA, titratable acidity (adapted from Bekele Bahiru *et al*., 2001)

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, reported that the ethanol content of *tej* ranged from 13-13.3%.

Tej is a widespread home industry of considerable social and economic importance. The skills for its preparation have passed from generation to generation informally and orally. The basic raw materials and preparation skills may not be markedly different among *tej* producers. However, as honey price goes up, producers have started to partly use cane sugar as fermentable substrate. In addition, some producers add roots, barks, leaves and stems of herbal plants to increase the "strength" of *tej*. This would result in differences in physico-chemical properties of the final product. Although the fermentation is spontaneous and depends on the microflora naturally present in the substrates, on utensils and equipment used, the different metabolic products of the randomized microflora at different stages, the physical and chemical environments, duration of fermentation and concoction practices have influence on the succession of microorganisms during the fermentation and would, thus, result in microbial

and physico-chemical variations in the product. Further work on determination of appropriate substrates, selection of desirable fermenting cultures and optimization of process parameters would help to produce *tej* with better keeping quality and acceptable levels of alcohol, fusel oils and other hazardous metabolites.

Borde fermentation

Borde is a traditional fermented beverage made from maize, barley or wheat and their malts. Its production is based on natural fermentation of the ingredients. It is an opaque, effervescent light brown beverage consumed while at an active stage of fermentation. It is a very popular meal replacement consumed by both children and adults in southern Ethiopia and some other parts of the country. Maize is the most common ingredient for the preparation of *borde*. The malt is usually made of a mixture of cereals. Kebede Abegaz *et al*. (2002) described in detail the processes of *borde* preparation as practiced in southern Ethiopia. Cereal for malting is carefully cleaned, rinsed in water several times and soaked in clean water until malting. The malt is then sun-dried and a portion is milled into flour for immediate use. Equipment used for processing, such as clay pots, grinding stones, straw sieves, gourd bottles, etc, are locally available. Production of *borde* has four major phases. In phase I, maize grits are immersed in water in a clay pot and left to ferment for 44 to 72 hours. The contents are apportioned in three parts at different periods (44h, 66h and 72h). In the second phase, the portion obtained at 44h of phase I fermentation is cooked on a hot metal pan at 90^{o}C for 30-45 minutes, into a well roasted granular mass (*enkuro*). The *enkuro* is allowed to cool down and fresh malt flour is added to it and blended in water in a clay pot. The clay pot is beforehand washed with water and fresh leaves of *Vernonia amygdalina* and smoked with glowing splinters of *Olea africana*. This mixture is known as *tinsis* and is allowed to ferment for about 24 hours. At this stage, three quarters of the malt component and a quarter of the unmalted ingredient is utilized. In phase III, a 66h fermented mass from phase I is slightly roasted, cooled, thoroughly kneaded with more flour and water and molded into dough balls. This is steam-baked in a clay pot for 1-1.5 hours and results in cooked dough with pleasant aroma of fresh bread. This is known as *gafuma*. The *gafuma* is cooled and blended with *tinsis* and water into a thick brown mash called *difdif*. This is allowed to ferment for 18 hours. At phase IV, porridge is made from flour and mixed with fermenting mass obtained from 72h fermentation of phase I. The thick porridge is blended with fermented *difdif* along with some additional malt and water. This is followed by repeated

wet-milling, each followed by slurrying with water and sieving. The filtrate is collected and diluted to the right consistency and allowed to ferment for 4 to 6 hours to yield *borde* (Fig. 6).

Borde is usually consumed by low-income groups and, on the average, a laborer consumes two to three liters of *borde* per day. This amount will sustain the consumer for a good part of the day. It is consumed even in large quantities at cultural festivals, on market days and at collective work gatherings (Kebede Abegaz *et al*., 2002). 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 when added to grains such as maize, wheat, etc. According to Kebede Abegaz *et al*. (2002), *borde* is also traditionally used for medical and ritual purposes. Mothers are encouraged to consume *borde* after giving birth to enhance lactation. It is believed to alleviate problems related to malaria, diarrhea, constipation and abscesses. Children are fed with *gafuma* and blended *borde* as meal replacement. According to consumers and brewers, the most important sensory properties of good quality *borde* are active effervescence, refreshing aroma, uniform turbidity, thick consistency, sweet sour taste and fairly smooth texture (Kebede Abegaz *et al*., 2002). *Borde* has a short shelf life as it turns too sour to consume after about 4 hours after completion of phase IV of the fermentation. It is, nevertheless, one of the important nutritious and low alcohol beverages in Ethiopia.

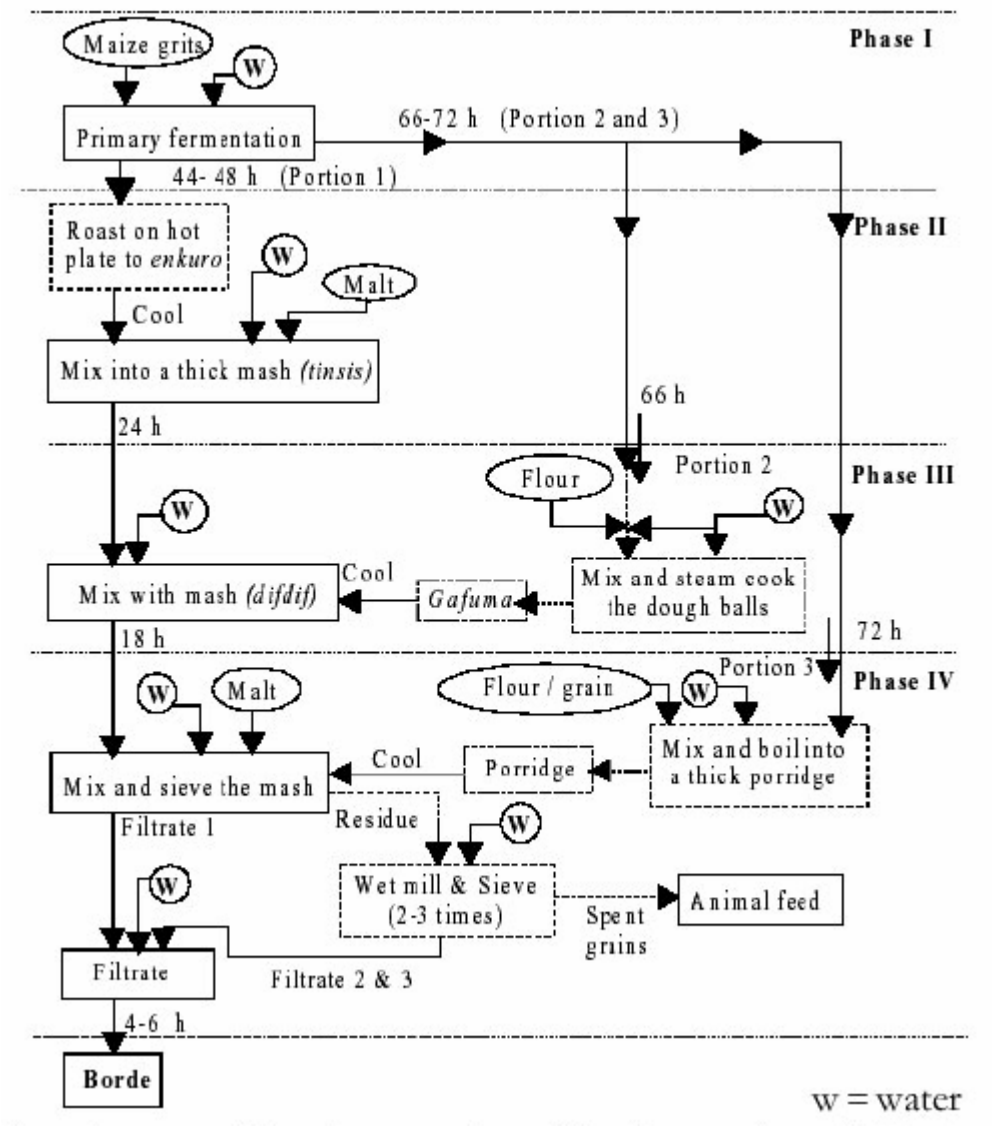

Fig. 6. Flow charts: traditional preparation of *borde* in southern Ethiopia (From Kebede Abegaz *et al*., 2002).

It appears that the ingredients and processing of *borde* fermentation vary among *borde* producing communities. Maize was reported to be the major ingredient in southern Ethiopia (Mogessie Ashenafi and Tetemke Mehari, 1995; Kebede Abegaz *et al*., 2002) whereas wheat was the preferred ingredient in Addis Ababa (Ketema Bacha *et al*., 1998). *Borde* preparation in Addis Ababa is a one-phase process and fermentation is initiated through

backslopping. Soaked and deeply roasted wheat flour is mixed with malt and water and allowed to ferment for 24 hours to give *borde*. 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. Counts of Enterobacteriaceae was around 10^6 cfu/ml, and yeast count ranged between 10^7 and 10^8 cfu/ml. 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 increased protein, fat and ash contents of the finished product.

Ketema Bacha *et al*. (1998) studied the microbial dynamics of *borde* fermentation as practiced in Addis Ababa 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 and reached counts as high as 10^9 cfu/ml 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 (Table 19).

Table 19 Changes in counts (cfu/ml) of major microorganisms during *borde* fermentation.

Fermentation time (h)	pH	Lactobacilli		Streptococci	Entero-bacteriaceae	Yeasts	Molds
		Homo-ferementative	Hetero-fermentative				
0	5.0	$<10^4$	$2.5x10^5$	$3.4 x10^4$	$1.0 x10^5$	$3.1 x10^4$	$2.5 x10^4$
4	4.28	$<10^4$	$1.1 x10^6$	$3.3 x10^6$	$4.7 x10^5$	$4.6 x10^4$	$1.5 x10^4$
8	3.96	$<10^4$	$7.7 x10^7$	$9.5 x10^6$	$2.0 x10^6$	$1.8 x10^5$	$7.1 x10^3$
12	3.80	$<10^4$	$3.7 x10^8$	$1.2 x10^7$	$4.7 x10^6$	$1.9 x10^5$	$1.4 x10^3$
16	3.76	$<10^4$	$5.1 x10^8$	$1.4 x10^6$	$5.6 x10^6$	$9.2 x10^5$	<10
24	3.60	$<10^4$	$9.8 x10^8$	$7.0 x10^5$	$2.1 x10^6$	$3.6 x10^6$	<10

Source: Ketema Bacha *et al*., (1998)

Borde is one of the various nutritious and low alcoholic traditional fermented beverages in Ethiopia. The scaling up of such products, although important, may have to be undertaken with great care so as not to lose the nutritive value as well as the public acceptance of the beverages.

Identification of the strains important for fermentation and optimization of the process parameters should be done in detail to design mechanisms for production of industrial-based products. Kebede Abegaz *et al*. (2004), for example, studied effect of technological modification on fermentation of *borde* and suggested simpler and shorter process that can yield acceptable *borde*, but the microbial safety of the product was questionable. Girum Tadesse *et al*. (2005b) studied survival of *E. coli* O157:H7, *Staphylococcus aureus*, *Shigella flexneri* and *Salmonella* spp. in fermenting and ready to consume *borde*. The fermentation markedly reduced the number of the pathogens but most were detected at low levels at 24 h. The various genera of lactic acid bacteria isolated from *borde* inhibited the test pathogens at different rates (Girum Tadesse *et al.*, 2005a), and the same test pathogens could survive in ready-to-consume fresh *borde* for 12 to 24 hours (Girum Tadesse *et al*., 2005b).

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. These are mixed with water, usually in the evenings, and the product is ready for consumption the next day (Fig. 7). 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.

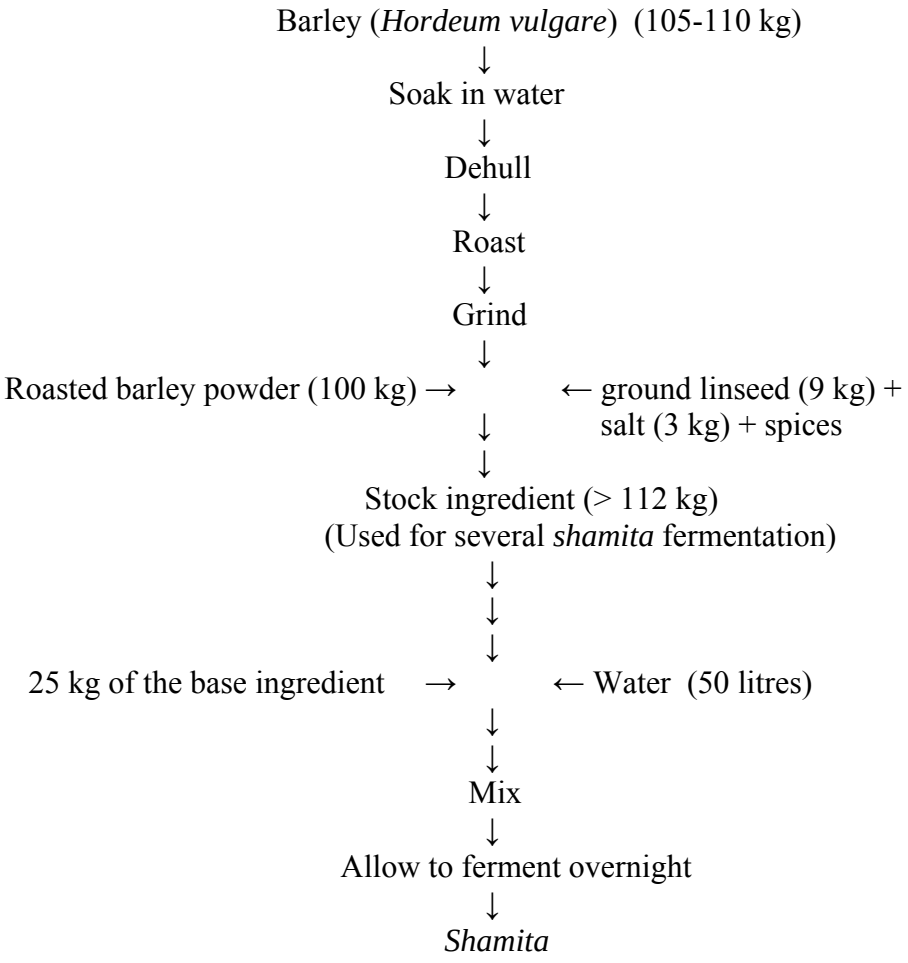

Fig. 7. Flow chart of laboratory *shamita* fermentation process (From Ketema Bacha *et al*., 1999).

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) 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) 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, respectively.

Table 20 Changes in counts (cfu/ml) of major microorganisms during *shamita* fermentation.

Time (h)	pH	Lactobacilli		Streptococci	Micrococci	Staphylococci	*Bacillus* spp.
		Homo fermentative	Hetero fermentative				
0	5.80	$6.44x10^5$	$3.0 x10^4$	$< x10^2$	$1.5 x10^5$	$2.1 x10^5$	$2.4 x10^4$
4	5.52	$6.9 x10^6$	$3.1 x10^6$	$< x10^2$	$7.2 x10^5$	$2.5 x10^5$	$3.6 x10^4$
8	4.77	$7.6 x10^6$	$1.1 x10^8$	$< x10^2$	$1.1 x10^6$	$5.4 x10^4$	$2.6 x10^4$
12	4.43	$3.2 x10^7$	$1.3 x10^8$	$< x10^2$	$1.6 x10^6$	$1.9 x10^4$	$2.2 x10^4$
16	4.26	$4.4 x10^7$	$1.8 x10^8$	$< x10^2$	$2.9 x10^6$	$1.8 x10^4$	$2.1 x10^4$
24	4.03	$1.1 x10^9$	$2.2 x10^8$	$< x10^2$	$5.0 x10^4$	$1.4 x10^4$	$4.6 x10^3$

Source: Ketema Bacha *et al*. (1999)

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 (Table 20). 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).

Conclusion

Food preparation is predominantly a household phenomenon in Ethiopia. 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 a suitable environment for the microorganisms to result in a desirable product is based on women's indigenous knowledge, which has improved through generations.

The majority 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. People living in other regions of Ethiopia either have their own distinct fermented products or have a different version of a product consumed by those living in other regions. Microbiological 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 application.

Most of the fermentation studies hietherto attempted to describe the microbiological successions and the accompanying chemical changes during the fermentation process. As can be observed in previous studies, 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.

Attempts should 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 flavor 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, in addition to improving the keeping quality of the products, has the advantage of reducing wastage during processing, which is significant at household level.

Quite a substantial amount of food is lost at household level due to two major reasons. The first is direct loss due to microbial spoilage. One can imagine how much *enjerra* is lost to molding at the household 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 most households cannot afford cooling devices to extend the keeping quality of the food. This undue consumption of foods in shorter time merely because longer keeping would result in spoilage and, thus wastage, is uneconomical and may be considered as “loss”. 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 household

level. The dictum is "think globally, act locally".

Food safety studies should also be done extensively to determine the fate of the important food-borne pathogens during fermentation of the product and to determine the correct temperature-time combination to guarantee complete elimination of the pathogens.

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. 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 pathogens 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. Studies are, thus, needed to control the spoilage process of fermented products.

REFERENCES

Abraham Besrat, Admasu, A. and Ogbai, M. (1980a). Critical study of the iron content of tef (*Eragrostis tef*). *Ethiop. Med. J.* **18**: 45-52.

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.

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.

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.

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

Almaz Gonfa, Foster, H.A. and Holzapfel, W.H. (2001). Field survey and literature review on traditional fermented milk products of Ethiopia. *Int. J. of Food Microbiol*. **68**: 173-186.

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.

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

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.

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.

Bekele Bahiru, Tetemke Mehari and Mogessie Ashenafi (2006). Yeast and lactic acid flora of 'tej', an indigenous Ethiopian honey wine: variations within and between production units. *Food Microbiol*. **23**: 277-282.

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. Africa*. **6**: 104-108.

Belachew Desta. (1977). A survey of the alcoholic content of traditional beverages. *Ethiop. Med. J.* **15**: 65-68.

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.

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.

Berhanu Abegaz Gashe. (1987a). Kocho fermentation. *J. Appl. Bacteriol.* **62**: 473-477.

Berhanu Abegaz Gashe. (1987b). Spoilage organisms of kocho. *MIRCEN J. Appl. Microbiol. Biotechnol.* **3**: 67-74.

Blandino, A., Al-Aseeria, M.E., Pandiellaa, S.S., Canterob, D. Webba, C. (2003). Review: Cereal-based fermented foods and beverages. *Food Res. Int.* **36**: 527–543

Campbell-Platt, G. (1994). Fermented foods: a world perspective. *Food Res. Int.* **27**: 253.

Chaltu Gifawesen and Abraham Besrat. (1982). Yeast flora of fermenting tef (*Eragrostis tef*) dough. *SINET: Ethiop. J. Sci.* **5**: 21-25.

Coppock, D.L., Holden, S.J., O'Connor, C.B. (1991). Milk processing and peri-urban dairy marketing in semi-arid Ethiopia and prospects for development. ILCA, Addis Ababa, Ethiopia, 40 pp.

CSA. (1995). Report on livestock, poultry and beehives population (private peasant holdings). Central Statistical Authority Statistical Bulletin, Addis Ababa, Ethiopia, 243 pp.

Demeke, T. (1986). Is Ethiopia's *Ensete ventricosum* crop her greatest potential food? *Agric. Int.* **12**: 362-365.

Eden Ephraim and Mogessie Ashenafi (2005). Fate of *Salmonella typhimurium* DT 104 during the fermentation of 'Siljo', a traditional Ethiopian fermented legume condiment, and during product storage at ambient and refrigeration temperatures. *World J. Microbiol. Biotechnol.* **21**: 1259–1265.

EHNRI (1997). Food composition table for use in Ethiopia. Part III. Ethiopian Health and Nutrition Research Institute. Addis Ababa. pp. 34.

Esser, P., Lund, C., and Clemensen, J. (1983). Antileukemic effects in mice from fermentation products of *Lactobacillus bulgaricus*. *Milchwissensch* **38**: 257–260.

Fekadu Beyene and Abrahamsen, R.K. (1997). Farm-made fermented milk and cottage cheese in southern Ethiopia. *Trop. Sci.* **37**: 75-79.

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.

Girum Tadesse, Eden Ephraim and Mogessie Ashenafi. (2005a). Assessment of the antimicrobial activity of lactic acid bacteria isolated from Borde and Shamita, traditional Ethiopian fermented beverages, on some food-borne pathogens and

effect of growth medium on the inhibitory activity. *Internet J. Food Safety* **5**: 13-20.

Girum Tadesse, Mogessie Ashenafi and Eden Ephraim. (2005b). Survival of *E. coli* O157:H7, *Staphylococcus aureus, Shigella flexneri* and *Salmonella* spp. in fermenting 'Borde', a traditional Ethiopian beverage. *Food Control*. **16**: 189-196.

Gulilat 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.

Gulilat 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.

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

Jay, J. (1996). **Modern Food Microbiology**. Aspen Publishers, Maryland, USA.

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

Kebede Abegaz, Fekadu Beyene, Langsrud, T. and Narvhus, J.A. (2002). Indigenous processing methods and raw materials of borde, an Ethiopian traditional fermented beverage. *J. Food Technol. Afr.* **7**: 59-64

Kebede Abegaz, Langsrud, T., Fekadu Beyene and Narvhus, J.A. (2004). The effects of technological modifications on the fermentation of Borde, an Ethiopian traditional fermented cereal beverage. *J. Food Technol. Afr.* **9**: 3-12.

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.

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.

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.

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.

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.

Mattila-Sandholm, T. (1998). VTT on lactic acid bacteria. *VTT Symposium*, 156, pp. 1–10.

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.

Mehtzun Tedla and Yewelsew Abebe. (1994). Study of "Enset" Processing and Development of "Enset" Processing Tools in the Southern Region of Ethiopia (Monograph). ACA/NORAGRIC Research Project. Awassa College of Agriculture, Addis Ababa Unversity, Ethiopia.

Mekonnen Tsegaye and Mogessie Ashenafi. (2005). Fate of *Escherichia coli* O157:H7 during the processing of Ergo and Ayib, traditional Ethiopian dairy products and effect of product storage temperatures on its survival. *Int. J. Food Microbiol.* **103:** 11–21.

Mekonnen Tsegaye, Eden Ephraim and Mogessie Ashenafi. (2004). Behaviour of *Escherichia coli* O157:H7 during the fermentation of Datta and Awaze, traditional Ethiopian fermented condiments, and during product storage at ambient and refrigeration temperatures. *Food Microbiol.* **21**: 743-751.

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

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.

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.

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.

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

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

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.

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.

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

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

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.

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

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

Mogessie Ashenafi. (1996). 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.

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.

O'Connor, C.B., Mezgebu, S. and Zewde, Z. (1993). Improving the efficiency of butter making in Ethiopia. *FAO Wrld. Anim. Rev*. 50-53.

O'Mahoney, F. and Bekele, E. (1985). Traditional butter making in Ethiopia and possible improvements. ILCA Bulletin. 22, pp. 9-14.

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

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.

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.

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

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

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

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

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

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.

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

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

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.