THE MICROBIOLOGY OF TELLA FERMENTATION

A THESIS PRESENTED TO

SCHOOL OF GRADUATE STUDIES

A D D I S A B A B A U N I V E R S I T Y

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR

THE DEGREE OF MASTER OF SCIENCE IN BIOLOGY

By:

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JUNE, 1990.

A C K N O W L E D G E M E N T S

I would like to express my sincere appreciation and thanks to my advisor, Dr.Berhanu A. Gashe, for his close supervision, guidance and encouragement throughout this work. I am also grateful to Dr. Getachew Tefera for allowing me to use FFLP Mufle furnace.

I am also indebted to Ato Admasu, Ato Assefa and Ato Haile who cooperated in providing me the apparatus necessary for this work. I am thankful to my friends Amare, Mulugeta, Adane, Meshesha, Yemane and Eyoualem. problems in the laboratory.

The Equipment, Media and Chemicals of the Biotechnology project, Biology Department, AAU is also gratefully acknowledged.

I am specially grateful to W/ro Hiwot, W/ro Asnakech and W/o Welansa who brewed tella in the laboratory during the study period. I am so much thankful to Ato Befekadu Regassa who helped me in typing this thesis.

Finally I am thankful to the Swedish Agency for Research Cooperation with Developing Countries (SAREC) for financial assistance towards the costs incurred in the research.

TABLE OF CONTENT

TABLE OF CONTENT PAGE

LIST OF TABLES AND FIGURES PAGE

A B S T R A C T

Four major phases in Tella fermentation were identified. In each phase, different microorganisms were isolated and enumerated.

Six genera of bacteria were identified. They were: Arthrobacter, Acetobacter, Lactobacillus, Bacillus, Proteus and Achromobacter. In addition two genera of yeasts, Saccharomyces and Rhodotorula were identified, out of these Saccharomyces was found to be responsible for tella fermentation.

In addition to microbiological studies, chemical and physical characteristics of the four phases were studies. In phase I (0-96 hr), the p^H droped from 5.2 to 4.73 at the end of this phase. There is a slight difference between temperature of fermenting material and the room temperature. The total solid content, amount of reducing sugar and total carbohydrates ranging from 4.4% to 5.49%, 4.04 to 12.0 mg/ml and 18.5-33.4 mg/ml respectively.

In phase II (96-144 hr), the p^H decreased from 4.73 to 4.71. Likewise the temperature of the fermenting material increased from 21°C to 25°C at the end of this phase. The total solid content, amount of reducing sugar,

total carbohydrates and ethanol content ranged from 15.22 to 21.1%, 8.75 to 28.37 mg/ml, 43.16 to 98.08 mg/ml and 0 to 3.17% respectively.

In phase III (144 - 192), the p^H fell from 4.75 to 4.67 at the end to this phase. The temperature increased from 24°C to 27°C at time 192 hr. The amount of total solids, reducing sugar, total carbohydrates and ethanol content ranged from 26.0 to 28.76, 5.2 to 14.83 mg/ml, 98.57 to 118 mg/ml and 2.77 to 5.13% respectively.

In phase IV, the p^H decreased from 4.58 to 3.34 at the end of this phase. The temperature also decreased from 24.6°C to 21.67°C. The total solid, reducing sugar, total carbohydrate and ethanol contents ranged from 4.3 to 11.73%, 2.35 to 5.2 mg/ml, 34.6 to 78.57 mg.ml and 4.3 to 5.13% respectively at the end of period I (192 - 264 hr) of phase IV.

In addition a comparison was made between the laboratory brewed and locally collected tella samples with regard to microbial populations, chemicals and physical characteristics. The microorganisms responsible for its fermentation and spoilage were found to be similar.

I N T R O D U C T I O N

Fermentation is one of the most economical methods of producing and preserving foods and beverages which are acceptable to man. The Asians, centuries ago, taught the world how to produce meat-like flavours from vegetable proteins. The Indonesians discovered a means of introducing texture into vegetable products, which serves as meat substitute in their diets. Much of the developing world depend upon various fermented foods, for daily consumption.

Fermentation yielding alcohol and/or acid generally offer low-cost ways of preserving food in a world where the majority of the people cannot afford canned, frozen or dehydrated foods (except those that are sun-dried), (Steinkrous, 1983).

Production of ethyl alcohol seems to have been associated with man through recorded history. Primitive man without a real understanding of the process, learned its preparation through observation and experience. The production of fermented foods and alcoholic beverages from a wide variety of fruits and grains, the curing of hay and silage, and the production of lactic and acetic acids were among the early domestic application of

fermentation techniques (Harrar, 1964).

According to historical accounts, the ability of yeasts to make alcohol in the form of beer was known to the Sumerians and Babylonians before 6,000 B.C. (Maqueen, 1987). Evidences from archeological excavation have shown that brewing operations were in existence in Ur and ancient Chinese and Japanese practiced the art skillfully (Robb, 1978).

It is true that man has been using mocro-organisms with scientific intelligence for only 75 years (Ordeal, 1987). The presence of yeast cells in fermenting beer was first recorded in 1980 by Anton Van Leeuwen Hook (Phaff, 1981). Yeasts were regarded as undesirable scum. A study of the disease of beer (Beer sickness) led Pasteur to discover that yeasts bring about the desired fermentation to alcohol, and that the sickness in beer was caused by bacteria that produced lactic acid, acetic acid or other unwanted by-products. This scientific approach to brewing also helped to open up the field of microbiology and biochemistry. Later on, the Danish botanist Emil Christon Hansen, working at the Carlsberg Institute isolated a pure culture of yeast for bottom fermenting beer (Ross, 1981).

agronomy, physics and related subjects. There is also a very promising advancement in a modern brewing process. It is not just only the pots and pans that work well, the biochemistry of fermentation is also finely tuned (Macqueen, 1987). Man is constantly finding new kinds of yeasts, molds and bacteria from among the 100,000 or more known and described species, by doing improvements through mutation and selection (Ordeal, 1987; Stackman, 1964).

Fermented Beverages.

Fermented beverages of the world represent independent discoveries. Therefore, they vary considerably in kind or type (Pederson, 1979). It is known that before the advancement of distillation techniques as introduced into Europe by the Arabs, the oldest alcoholic drinks were fermented beverages of relatively low alcoholic content (Gray, 1959; Desta, 1977). These include groups of beer and wine.

Nutritionally, alcoholic drinks have an appreciable caloric content. In some parts of the tropics consumption of local beer per head is thought to average a quart a day. This can provide 10% of the caloric requirement of the individual. Amongst some population which are near the margin of vitamin deficiency, such drinks (i.e. fermented beverages) can also serve as sources of

vitamins (Nicholsen et al., 1969; Okafor, 1977a; Sanchez-Marroquin, 1977; Herrera et. al., 1977; Escobar, 1977; Steinkrous, 1983).

In nearly all areas of the world, some types of alcoholic beverages native to the region is prepared and consumed (Pederson, 1975). Obviously fermented beverages are in a rather different class being prepared chiefly for their alcoholic content. Apart from moslems and a few other groups, who abstain from drinking alcohol, most people of the world devote, a part of their farm acreage for fermentation (Nicholson et al., 1969). There are a number of traditional fermented beverages, throughout the world. These beverages are grouped into two main catagories, depending on the types of substrates used for preparation and production of ethyl alcohol.

The first groups of fermented alcoholic beverages are those in which sugars are the principal fermentable carbohydrates. These types of beverages include: Honey wines (i.e., Mead, Methglin), (Morse and Steinkrous, 1975); Ethiopian Tej (Vogel and Gobezie, 1977); Sugar cane wines, which includes Phillippine basi (Sanchez, 1977; Tanimura et al., 1978); Palm wines (Toddys) (Okafor, 1977a; Odeymi, 1977; Fupurusi, 1977; Theirendirarajah et al., 1977a, b; Samarajeewa, 1977; Nyako, 1977; Merican, 1977; Shuaib and Azemey,

1977; Wong and Jackson, 1977; Ekonon and Nadodawithana, 1977); Mexican pulgue (Shanchez-Marroquin, 1977; Herrera et al.; 1977; Goncalves de Lima); Indian jack fruit wine (Dahiya and Prabhu, 1977); Kenyan urawaya (Harkishor, 1977); commercially produced grape juice wine and related groups.

The second group of fermented alcoholic beverages are those in which starch is used as sole source of carbohydrate to produce alcohol. Such kind of fermented beverages are again classified into three main groups, depending on the ways by which starch itself is hydrolyzed to simple sugars. One of these which employ a unique starch hydrolysis step is, the one where saliva serves as the source for the amylolytic agent. A typical example of such type of beverage is that of the American maize drink Chicha (Escobar, 1977; Escobar et al.; 1977). Chicha can also be fermented from malt. The second type of fermented beverage in which starch hydrolysis is accomplished by malting (germination). Malting or germination of cereals is a major means by which starch is converted to simple sugars.

Beverages produced from malt are the most commonly distributed groups of alcoholic beverages. These are the commercially produced beers (Lewis, 1987; Macqueen, 1987; Rubb, 1976); African Kaffir beer or Bantu beer (Novellie, 1968); Mexican tesgino (Taboda et al., 1977); Tephuno (Navital and Pennington, 1969);

Egyptian Bouza (Marcos, 1977); Nigerian Pito (Ekundayo, 1977); Kenyan Busaa (Hakishor, 1977); Zambian Opaque Maize beer (Lovelace, 1977); and the Ethiopian Tella (Vogel and Gobezie, 1977; Pederson, 1979).

In the third type of alcoholic beverages starch hydrolysis during fermentation is accomplished by amylolytic molds and yeasts. The alcoholic beverages of the Far-East souch as Japanese Sake (Yoshizawa, 1977) and the Korean Yakuju and Takju (Park et al., 1977) are good examples of this kind of beverages.

Although some of these beverages mentioned are produced industrially, the majority are prepared traditionally in small scale or in small quantities in house holds. There are a number of problems, associated with traditional modes of fermentations. Some of these are, the variability in proportion of ingredients allocation, duration of fermentation, alcoholic content, problems of sanitation and decreases in shelf-life.

Mode of Fermentation.

The traditional mode of fermentation of alcoholic beverages varies from one group of beverage to another. It even varies within the group itself.

Honey wines (Dwojniack and Tronjniack) are prepared by mixing water and honey based on weight

The rate of fermentation of all honey wines can be increased by addition of growth factors that stimulate the growth of yeasts (Steinkraus and Morse, 1966). In some types of wines hops are added for enhancement of flavour and aroma. In addition the tannins from hops help in precipitating out proteins (Prescott and Dunn, 1959).

The Phillippines basi and Kenyan muratina are grouped under the sugar-cane wines, and use sucrose as substrate for ethyl alcohol production.

The Phillippine basi is made by fermenting boiled freshly extracted sugarcane juice with a moxture of yeasts, bacteria, and molds, or with organisms found on samac (Macharange tanarius or M. gradifolin Linn.) leaves, bark, or fruit. (Sanchez, 1977; Tanimura et al., 1978b).

In Muratina fermentation, the dried muratina (sausage tree) fruit is fastened to the bottom of a barrel with the twigs, the sugarcane juice is added, and incubation continued for 1 or 2 days in a warm place. Frequently sugarcane juice is added, probably, to increase the ethyl alcohol yield. The barrel is filled to the top with juice and this, with a blanket of CO_2 gas which is produced during fermentation, keeps the juice anaerobic. Completion of fermentation is determined by taste.

The fermentation of palm wine is as follows: Palm sap is collected from different palm trees. The sap collected is mized with sugar and water, and allowed to ferment for a few hours. This is filtered and stored in a container for consumption (Merica, 1977).

The Mexican pulque fermentation starts by collecting the agave juic and pouring it into wooden, leather or fiber glass tanks. Fermentation is the result of natural inoculum from a previously used pulque fermentation to the tank. The fermentation lasts for 8 to 30 days (Sanchez-Maraquin, 1977).

Indian jack fruit wine is prepared by extracting the juice of Artocarpus metrophyllus with a bamboo basket seive. A small amount of fermented juice is added as inoculum to start the fermentation. The liquid portion is removed by decanting the collected extract in earthen-ware pots and mixed with banana leaves. It is allowed to ferment at temperatures of 18 to 30°C. for about a week. This is decanted and served (Dahiya and Prabhu, 1977).

In Kenyan Urwaga, dry banana leaves are burnt in the pits, and the ash formed is covered with green banana leaves. Banana are placed on the ash, covered with leaves and then left for five to seven days. It is then peeled and filtered. Flour from roasted

sorghum, millet or maize, is mixed with the filtered banana juice in a pot. Then it is incubated for 12 to 24 hours. Urwaga is served thereafter (Harkishor, 1977).

The South American beverage chicha from maize is produced by moisting the maize flour with water or saliva and rolling it into a ball of appropriate size. This is placed into the mouth and thoroughly mixed with saliva. The salivated rolled maize flour (gobs) is known as muko. It is then allowed to sun-dry. After drying it is ground and then mixed with water. This is boiled for 3 to 5 hours. Thereafter, it is cooled and filtered. The strained liquid is poured into 'pots' for fermentation. After inoculation with the desirable organisms, the fermentation is continued for an extra day. Chicha is then said to be ready for drinking (Escobar, 1977).

There are several kinds of malt beverages such as those mentioned earlier. The African beer (Bantu beer), production starts when the sorghum malt is mashed with water at 50°C. Cereal adjuncts after cooking and mixing with soured mash is strained and pumped to fermentation tank and allowed for 1 day at 22° to 30°C. Thereafter it is packed or containerized for bulk sales (Novellie and Schaepd-rijver, 1979).

The Mexican Tesguino preparation begins by soaking maize kernels placed in baskets. This is allowed to

germinate. The germinated kernels are ground, mixed with water and then boiled. After addition of catalysts fermentation is continued for 2 to 3 days. The Tesguino which is produced is then ready for serving (Taboda et al., 1977).

Egyptian Bauza is produced from wheat grains. The wheat is moistened with water and allowed to germinate for 3 to 5 days. It is then sun-dried and coarsely ground. The malted ground, wheat is mixed with lumps of bread, and water in a wooden barrel. Bauza from a previous fermentation is added, and fermentation is continued for 24 hours at room temperature. The mash is then passed through a seive to remove large particles. The fluid is then ready for consumption (Marcos, 1977).

The Nigerian Pito preparations begin with germination of maize or sorghum. The germinated (malt) maize ground is moxed with water and boiled for 6 to 12 hours. After cooling and filtering, it is allowed to ferment for 12 hours. It is again boiled for another 12 hours and allowed to cool. Thereafter starter culture from a previous brew is added and incubated overnight. Then pito is ready for serving (Ekundayo, 1977).

The Kenyan Busaa production begins by mixing maize flour with water to form dough. The dough is toasted. Malt finger millet is ground to flour, mixed with water

and allowed to form a slurry. This is incubated for 2 to 3 days. After fermentation it is filtered and consumed as beer or distilled to changue (Harkishor, 1977).

The Zambian opaque maize beer is produced in a way that malt and ground maize are mixed with maize porridge. This is allowed to sour, the mixture again boils and cools. After addition of more malt the mash is allowed to ferment for 3 days, and is ready for serving (Lovelace, 1977).

In the Zambian munkoyo preparation, the roots of munkoyo plant (Rhynchosia venolose) are pounded and soaked in water. Cooked and cooled maize porridge is then mixed with the munkoyo extract. After one day of fermentation it becomes ready for consumption (Lovelace, 1977).

The most popular fermented beverage where starch hydrolysis is accomplished by amylolytic mold and yeast is the Japanese sake. Its preparation begins as follows, washed, steeped and steamed rice is mixed with spores of Aspergillus oryzae for Koji production. Then Koji and steamed rice are mixed with the starter (muto). It is then allowed to ferment for about 3 weeks. This is filtered to provide fresh Sake (Kodama and Yashizawa, (1977).

The Korean Yakju and Takji preparation begins by mixing the starter, nuruk, (produced by inoculation of molds, bacteria and yeasts on wheat flour) with steamed and cooled rice. This is allowed to ferment for a few days. Thereafter the fermented mash is filtered under pressure. The liquid is then aged and bottled (Park et al., 1977).

ORGANISMS RESPONSIBLE FOR FERMENTATION

There are a number of organisms responsible for the fermentation of different alcoholic beverages. The fermentation of honey wines is generally carried out by osmophilic yeasts and lactic acid bacteria (Sanchez, 1977; Tanimura et al., 1978b).

The fermentation of Muritiana is dominated by yeasts. The organisms encountered in palm wine fermentation vary widely. Lactic acid bacteria, yeasts and acetic acid bacteria as well as Zymomonas and Micrococcus spp. are involved in the fermentation (Okafor, 1972 a,b). Leuconostoc and Lactobacillus spp. are initial inhabitants of the palm sap, but it is Saccharomyces cerevisiae, that is usually responsible for the production of ethyl alcohol (Faparusi, 1977). Saccharomyces sp., Geotricum candium, Candida spp., Lactobacillus plantarum and Micrococcus spp. were isolated from Ghanian wine (Nyoko, 1977).

The primary ethanol producing yeast in Mexican pulque is S. cerevisiae. In addition homo and hetro fermentative Lactobacillus spp. were encountered.

The acidity is mainly due to the production of lactic and other acids in pulque (Sanchez-Macrofuin and Hope, 1953). Zymomonas mobilis is reported to be responsible for pulque fermentation (Swenys and Deley, 1977).

The Indian jack fruit wine fermentation is assumed to be carried out by yeasts which resemble Endomycopsis (Dahiya and Prabhy, 1977).

The Kenyan Urwga fermentation is thought to have both yeasts and lactic acid bacteria. However, in depth studies have not been made on the process and the microorganisms (Harkishove, 1977).

Soriamo (1938) reported the involvement of S.cerevisiae and bacteria of the genus Lactobacillus in the fermentation of chicha, Gomez (1949) also found various yeasts, bacteria and molds in Colombian chicha.

Souring which is a desirable characteristic in Kaffir beer fermentation is caused by lactic acid bacteria, but production of ethyl alcohol is the result of S. cerevisiae (Vander-Walt, 1956).

The yeast S.cerevisiae is involved in the alcoholic fermentation of tesguino (Herrera et al., 1973). Bacillus megaterium has been isolated from agave tesguino (Ulea et al., 1974).

There is lack of information on the microbial flora of Egyptian Bouza. But there is an assumption that the high acid production is due to lactic acid bacteria. Ethyl alcohol production is suspected to be the result of yeast fermentation (Marcos, 1977). Leuconostoc spp., Lactobacillus sp., Saccharomyces sp. and Geotricum candidum were found to participate in the fermentation of the Nigerian pito (Dkundaya, 1977). The micro-organisms responsible for lactic acid and ethanol production in Busaa fermentation are L. brevies, L. salivarus. L. plantarum, L.casei, L. buchoneri, Candida Krusei and S. cerevisiae, Microbiological studies have not been reported on the Zambian Opaque maize beer and Zambian Munkoyo (Lovelace, 1977; Mbugna, 1977).

Complex microbial interraction take place in the Japanese Sake fermentation. Koji is produced as a result of the activities of A. Oryzae. Along with the Koji S. Sake is inoculated into the main mash. In the traditional fermentation process the first organisms to grow actively in the mash are Pseudomonas, Achromobacter, Flavobacterium and/or Micrococcus spp. (Murakami, 1972).

In Korean Yakju and Takju, the nuruk is the result of the activities of A. oryzae, A. niger, aerobic bacteria and yeasts (Kim, 1968). Saccharomyces, S. cerevisiae, Hansenula anomala, H. Subpelliculosa, Pichia polymorpha, Torulopsis sake, T. inconspieva, Bacillus subtilis, B. megateirium, B. pumilus, Lactobacillus plantarum and Leuconostoc mesnteriodes are involved in the fermentation of Yakju and takju (Shin and Cho, 1970; Kim, 1970; Kim and Lee, 1970; Lee and Rhee, 1970). Saccharomyces cerevisiae is generally considered the most important only amongst the group production of ethanol. Hansenula spp. is considered important for flavor development (Kim and Lee, 1970).

SPOILAGE ASPECTS IN FERMENTED BEVERAGES

Microbial spoilage is common in many of the fermented alsoholic beverages. It becomes particularly acute for those beverages with no effective sterilization technique. Heqn,. spoilage is the most prominent problem in traditional fermented beverages. This is also a problem in commercially produced alcoholic beverages. Spoilage occurs if and only if undersirable change occurs on the already produced food and beverages (Banwart, 1979).

Spoilage of fermented beverages is the results of the activities of five different families of bacteria.

They belong to the families Pseudomonadaceae, Bacillaceae, Achromobacteriaceae, Micrococcaceae and Lactobacillaceae (Prescott and Dunn, 1959). The presence of some of the bacterial groups mentioned might be desirable, in one group of fermented beverage while it could be considered undesirable in another.

ETHIOPIAN BEVERAGES

There are many types of traditionally fermented beverages in Ethiopia, the most common and widely known alcoholic beverages are grouped into three major kinds. These are Tej, Araki (spirit) and Tella.

Tej:- It is grouped under the honey wine in which sugar (honey) is a substrate for ethyl alcohol production. It has yellow to olive-green colour. In the past, Tej used to be breved only on special occasions and in the home of the rich. A less expensive Tej is made by replacing part or all of the honey with sugar. A natural yellow food colour is added to it. Such type of Tej is at present available in most cities and is normally very cheap. (Desta, 1977; Vogel and Gobezie, 1977).

Araki (Katikala):- It is a distilled form of a fermented product. Araki is made from a variety of grains. The ingredients used are Gesho (Rhamnus perinoides), Bikil (malt), kitta in which flour of teff (Eragrostis tef), Dagussa (Elusine coracana) or other cereals are mixed with water to form a dough. This is baked into cakes on metal plate. Thereafter, it is broken into pieces used as adjuncts. (Evaldson, 1969; Desta, 1977; Berhanu, 1989).

Tella:- Tella is the most commonly consumed alcoholic beverage in Ethiopia. The word "Tella" has a debatable

origin. The clergy argue that, the word has its root in "Geez" meaning "life". "Tallih" in Arabic means "life" giver, therefore it is synonymous to the clerical definition. But in Amharic "Tellanih" means "we hated" (Tegegne, 1956).

The origin of Tella is hidden in the most of history. Some say that it has existed in Ethiopia for centuries, but others deny this. Nevertheless, Tella remains typically Ethiopian. It is served during wedding ceremonies, religious and national holidays and festivals occasions. It is sold in most localities.

According to an Addis Ababa Census published in 1988 by Central Statistical Office (1988), about 11,000 individuals are engaged in the brewing and sale of traditional alcohol beverages. The majority of them are however involved in the browing and sale of Tella.

There are several recipes for making Tella. Every house wife (Tella brewer) has her own recipe for brewing Tella. It may have light-yellowish to dark-brown coloration and may be turbid. The colour of the Tella is determined by the housewife preparing it.

Unlike the industrially brewed beverages, the mode of fermenting Tella is variable. The amount of adjuncts,

duration in fermentation, colour, alcohol content, viscosity, etc. varies greatly. The biochemical changes taking place, the micro-organisms involved in the fermentation and those which bring about undesirable changes are not known (Vogel and Gobezie, 1977; Desta, 1977).

OBJECTIVES

The study of this work was conducted with the following specific objectives in mind.

1. To isolate the different groups of micro-organisms involved in Tella fermentation.

2. To identify the micro-organisms responsible for the desirable fermentative changes.

3. To identify those micro-organisms which are involved in its spoilage.

4. To record the major biochemical changes taking place during fermentation.

5. To generate base line data which will be essential for large-scale production of Tella.

Materials and Methods.

Materials Used in Tella Fermentation;

The raw materials used for brewing Tella were the following:

Barley (*Hordeum volugare*): serves as source of Bikil (malt), and "Enkuro" (roasted, ground and steamed barley).

"*Gesho*" (*Rhamunus prinoides*): is a cultivated indigenous shrub. It is also known to occur as far as Cameroon to the west, and as far south as South Africa. It may serve the same purpose as hops in commercial beer.

"*Tef*" (*Eragrostis tef*): serves as source of kitta(sour unleavened bread).

"*Girawa*" (*Vernonia amygdoliana*): is a woody plant whose leaves are used for cleaning, and washing the containers used for brewing.

"*Woyra*" (*Olea* sp.): a wood plant used for smoking the containers used for Tella fermentation.

The barley, "Tef", "Gesho", and "Woyra" used in this study were purchased from local market in Addis Ababa.

Treatment of the Raw Materials.

"*Bikil*" (malt): Barley was first cleaned to remove dirt and extraneous materials and then steeped in clean

water for about a day. Then next day, excess water was strained off and the grain spread on the ground, covered with leaves, and allowed to germinate for five days. After germination the leaves were removed, and the malt was sun-dried and then milled to fine powder.

"Gesho" (R. prinoides): The leaves and stems of R. prinoides were chopped into pieces using a small axe and then allowed to sun-dry. It was then pounded into finner powder using a wooden mortar ("Muketcha") and pestle ("Zenezena").

Adjuncts: The adjuncts used in brewing "Tella" were "Kitta" and "Enkuro".

"Kitta" Preparation: "Tef" (E. tef) flour was mixed with water to form thick dough, and left to ferment for about 24 hours. The sour dough was then baked on "Mitad" (circular earthen gridle). The bread was broken into small pieces.

"Enkuro" Preparation: Barley was roasted on metal gridle to make "Asharo" (roasted grain). The amount of heat treatment was controlled by the housewife. When light-yellow or light-brown tella is required, it is heated gently, but when dark-brown or black is desired, it is roasted to dark brown or blackish colour. In this study it was roasted to brown coloration. The roasted grain ("Asharo") was then milled to powder (granulates), water was sprinkled on the powder and then

placed on hot metal plate ("Mitad") and steamed while mixing. The "Enkuro" thus prepared is used as an adjunct in tella fermentation.

Equipment Used for Tella Preparation.

"Ensra" (an earthen jar whose capacity was about 30 lit) and "Gan" (earthen jar whose capacity was nearly 60 lit.) served for brewing tella.

The Ensra was used at the initial stage of fermentation, but the Gan was used furing the addition of adjuncts. Both containers were washed and scrubbed with fresh leaves of "Girawa" (V.amygdoliana), and then smoked with splinters of "Woyra" (Olea sp.) before use. Washing and scrobing was intended for cleaning the jar; but the reasons for smoking process though mandetary are not known.

Tella was prepared on three occasions in the laboratory by three housewives following the traditional way of brewing method during the study period.

Steps in Tella Fermentation.

A. Tinsis:

This is the initial step in the fermentation of tella. Ground leaves of Gesho and water in the ratio of 1kgm: 8L were mixed in the "Ensra" and allowed to ferment for 72 to 96 hours. The fermentation takes longer hours when the ambient temperature is low.

B. Addition of Kitta and Bikil to Tinsis:

After 72 - 96 hours of fermentation of the "Tinsis", pieces of kitta, ground Bikil, Gesho (pounded stem) and water were added in the ratio of 9.5 kg: 0.8 Kg:0.18 kg: 9.3L respectively into the "Tinsis". After mixing it was transferred into a "Gan". As a result of this the volume of the fermenting material increased to 37L. The fermentation was then allowed to proceed for another 48 hours.

C. Addition of "Enkuro" (Difdif preparation):

After the completion of the fermentation in step B, chopped splintered stems of "Gesho" (size less than 3 cm. in length and less than 0.5 cm. in width), Bikil, Enkuro, and water were added in the proportion of 0.85 kg:0.6 kg: 19.21 kg.:6.61L respectively. The whole fermenting mass is known as "Difdif". This was then left to ferment for another 48 hours.

D. Final Stage of Fermentation:

To the "Difdif" which was kept fermenting for about 48 hours, 34.35 liters of water was added and the whole content was mixed thoroughly. This was the full capacity of the "Gan". After filling the "Gan" to the rim, it was sealed with cheese cloth and mud placed on top of it to create anaerobic condition. Fermentation was continued for 72 hours. At the end of this stage it was ready for consumption.

The taste and other qualities of the tella were assessed by 10 to 21 individuals.

Sampling.

Samples from the Gesho, malt, barely, kitta, Enkuro, water used for brewing and rinse solutions from the container were taken for bacteriological and chemical analysis. In addition, enrichment for yeasts using malt extract originated from Gesho, malt (Bikil) and the container was made. Samples were also removed aseptically from the fermenting mash (from Ensra nad Gan) every 24 hours for 22 days for analysis. The volume of sample removed for analysis was 300 ml.

Isolation and Enumeration of Micro-organisms.

For microbiological analysis, 50 ml of the sample, was diluted into 450 ml of 0.1% sterile peptone water. Further dilutions were prepared as desired using peptone water (Banwart, 1979).

Appropriate aliquots of samples were transferred into sterile petri dishes. Sterile tryptone soya agar (TSA) (Oxoid) supplemented with 0.5% dextrose and 0.5% yeast extract for aerobic cell count, Rogosa agar for Lactobacilli, Malt extract agar (oxoid) for yeasts and molds, Dextrose trypton agar, Macconkey agar (oxoid) for califorms, and Azide blood agar (oxoid) for streptococci were used for culturing. About 20 ml of agar was poured into each petri dish. The plates were incubated at 30°C. in an inverted position.

Bacterial colonies were counted after 24-48 hours of incubation on TSA. Yeast and mould colonies were counted after 72-96 hours growth on the malt extract agar (MEA). Lactic acid bacteria were counted from Rogosa plates.

All the colonies were examined and the colony morphology, pigmentation, shape, size, edge and optical character were recorded using magnifying lense.

The different colonies which appeared on the various plates were transferred onto slants whose composition were similar to the original isolation media and then incubated at 30°C. To check for purity of the isolates repeated streaking on nutrient agar or on appropriate media were carried out. After growth of the isolates the purity of the colonies were rechecked. Later the pure colonies were transferred into the slants containing nutrient agar and incubated at 30°C. and then used for biochemical analysis.

Identification of Microorganisms.

Bacteria:- The morphological (such as shape, presence and absence of spores, etc) cultural (such as turbidity, pelicle formation, nature of pelicle etc) physiological and biochemical characteristics of each of the isolates were recorded. Based on these information standard manuals such as Bergey's Manual of Determinative Bacteriology (1974) were consulted for the proper naming of the

bacteria to the genus or the species level.

Yeast:- In order to identify and characterize each yeast isolate, several morphological, cultural, sexual and physiological information were gathered, based on criteria and methods of classification set by Vander-Walt (1971). Then manuals entitled "the Yeasts" (Lodder, 1971) and the biological and chemical characters of yeasts (Cook, 1958) were consulted for the proper naming of the yeasts to the genus or species level.

PHYSICAL PARAMETERS STUDIED.

Changes in Fermentation Temperature.

Variation in temperature between the fermentation vat and the environmental temperature at every sampling period were recorded. Temperature recording were carried out using a Philip-Harris model thermometer (caliberation -10+100°C.).

Determination of Moisture Content.

Moisture content of each sample was determined after drying the specimens in an oven at 80°C. for 24 hours.

Ash Content.

The ash content was determined after burning the sample at 550°C. for 4 hours in a muffle furnace.

Change in Fermentation p^H:-

The change in fermentation p^H during sampling was recorded using a Corning 140 Model p^H meter (Corning, UK).

CHEMICAL ANALYSIS.

Determination of Total Carbohydrate Content.

The amount of total carbohydrate during each of the sampling periods was determined using the phenolsulphuric acid method (Dubois et al,1956). The reagent employed contains the following:

Reagent A: 5.5 ml liquid phenol (90%) was added to 94.5 ml of water to get 5% final concentration.

Reagent B: Concentrated Su;phuric acid.

One ml of the sample and one ml of Reagent A were mixed with a Vortex-mixer (Gallenkamp). Then 5 ml of Reagent B was added into it mixed thoroughly and left for 10 minutes. Thereafter it was transferred into a water bath (Gallenkamp) whose temperature was adjusted to 25°C for 15 minutes at room temperature. The colour developed was read on a spectronic 2'UVD spectrophotometer (Bausch and Lomb) at 488 nm using a 1 cm light path cuvette. The blank contained all the ingredients except the sample.

Reducing Sugar Content.

The amount of reducing sugars present at each sampling period was estimated using the method of Nelson (1944) as modified by Clark and Switzer (1977). The reagents contained the following:

Reagent A:- 12.5 g sodim carbonate, 12.5 potassium-sodium tartarate, 10 g sodium bicarbonate, 100 g sodium sulphate plus enough distilled water to make a final volume of 500 ml.

Reagent B:- 7 g of hydrated copper sulphate ($CuSO_4.5H_2O$), one drop of concentrated sulphuric was dissolved in 50 ml of distilled water.

Reagent C:- Arseno Molybdate Reagent:- 25 g of ammonium molybdate was dissolved in 450 ml of distilled water, and then 21 ml of concentrated sulphuric acid was added to it. Separately prepared 3.0 g of sodium arsenate in 25 ml of distilled water then included into the solution.

The working reagent was composed of 12.5 ml of reagent A and 0.5 ml of reagent B.

One ml each the sample and working reagent were mixed with a mixer, and then immersed in boiling water for exactly 20 minutes. At the end of 20 minutes the samples were immersed in running water to cool them

down immediately. Thereafter, 1 ml of Reagent C was added into the samples and mixed. This was allowed to stand for 5 minutes at room temperature. The final volume of the sample was then adjusted to 10 ml with distilled water. The colour produced was read on a spectronic-2'UVD spectrophotometer (Bausch and Lomb) at 540 nm, using a 1 cm light path cuvette against a blank which contained all the ingredients except the sample.
A sample containing a known concentration of glucose was run during the analyses to check for reproducibility of the results.

Protein Content Determination.

The protein content of the sample was determined using the method of Lowry (Lowry et al., 1951). The reagents contained the following:

Reagent 1:- 2 g sodium hydroxide (NaOH) and 10g of sodium carbonate (Na_2CO_3) dissolved in 500 ml of distilled water (2% Na_2CO_3).

Reagent 2:- 1g of sodium tartarate dissolved in 50 ml of distilled water (2%).

Reagent 3:- 1 g of hydrated copper sulphate ($CuSo_4.5H_2O$) dissolved in 100 ml of distilled water (1%).

One part of Folin's phenol reagent (2N) mixed with one part of water freshly prepared.

The working reagent contained combinations of reagents 1,2 and 3 in the ratio of 98: 1:1: respectively.

One ml of the sample and 5 ml of the working reagent were mixed and left for 10 minutes to react. Thereafter 0.5 ml of Folin phenol reagent was added and mox thoroughly. After 30 minutes, the colour produced was read on spedtronic-2 UVD spectrophotometer (Bausch and Limb) at 750 nm using 1 cm light path cuvette against a blank which contained all the ingredients except the sample.

Alcohol Content.

The amount of ethyl alcohol produced during fermentation was estimated by distillation, using a distillation apparatus, by comparing it with known' amount of ethonal content.

RESULT

Major Events in the Fermentation of Tella

Four major events in the fermentation of Tella were identified. Each phase had its own unique chemical, physical and biological properties.

The major activities recorded are shown in the flow chart (Fig. 1). Phase I could extend for up to 96 hr. This is followed by events in Phase II which take about 48 hr i.e. from 96-144 hr of fermentation. The third and fourth phases required 48 hr and 312 hr. respectively. The fourth Phase however could be subdivided into two stages. Stage 1, required 72 hr. This completes the fermentation time; stage 2 was truely the aging period (264 hr.) and also the time required at which the fermented Tella has to be consumed. Prolongation of the aging process beyond 264 hr. resulted in the acidification of Tella and in the overall quality deterioration.

Major Characteristics of the different Phases

Phase I of Tella Fermentation

Water and "Gesho" (R. prenoids) in the proportion of 8L to 1.0 kg respectively were mixed in 'Ensra' (Earthen jar) which has been previously washed and smoked. This mixture is known as 'Tinsis'. This was mixed

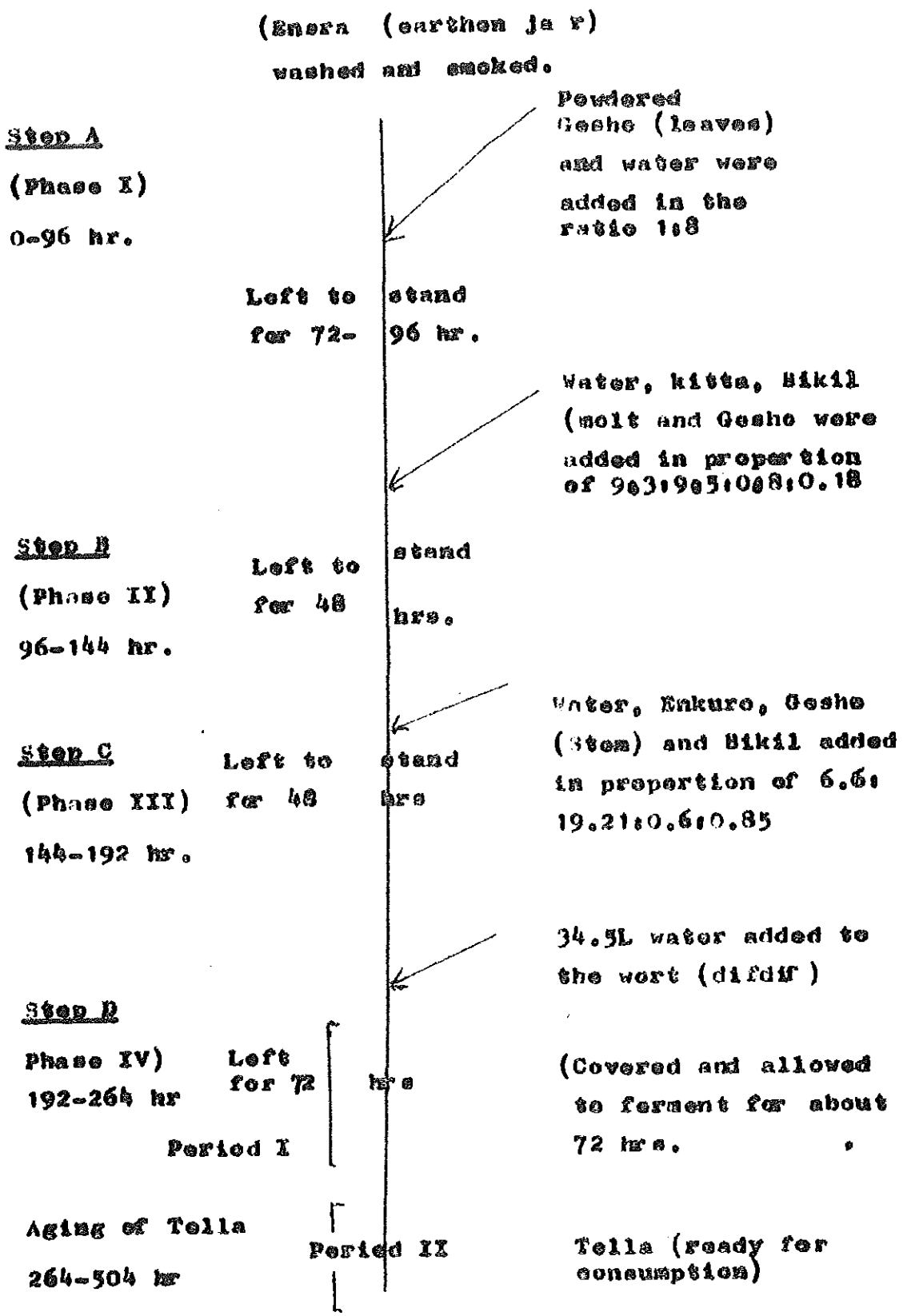

Fig. 1. Flow sheet describing major events in the fermentation of Tella.

thoroughly and allowed to ferment. The fermentation was characterized by vigorous bubbling and foaming. Depending upon the ambient temperature, the fermentation in this phase could reach its peak within 96hr. In triplicate experiments it took 72, 96 and 96 hrs.

Eventhough the temperature of both 'tinsis' and the room were 20°C at time 0, the temperature of the fermenting mash increased to 22°C at the end of this phase. However, that of the room temperature was 20-21°C.

The p^H at the initial stage of the tinsis preparation was 5.2, however, this declined to 4.73 of p^H at the end of the fermentation in phase I. The total solid content initially was 4.4%, but this was increased to 5.5% at the end of Phase I.

The total carbohydrate and the reducing sugar contents at the initial stage of this phase were 37.4 mg/ml and 12.5 mg/ml, respectively. These decreased to 18.5 mg/ml (total carbohydrates) and 4.04 mg/ml (reducing sugars). However, these concentrations in total carbohydrates and reducing sugars amount did not lead to the production of ethnol during this Phade I (Table I).

The contribution of 'Gesho' to the microflora of the 'tinsis' are shown on Table II. The water used for

brewong did not contribute organisms significantly. The ensra and Gesho served as source of the yeasts and the bacteria during the fermentation.

The total aerobic count at time 0 was $1x10^4$CFU/ml. The population increased gradually and reached $5x10^7$ CFU/ml at 96 hrs.

The yeast count was very low initially (48 hr) but appeared in great numbers after 48 hr ($7x10^3$CFU/ml). The proportion of yeasts to bacteria, between 48-96 hr in about the same (Table 1). This yeasts were present initially in Gesho and container used. The yeasts were primarily of Saccharomyces spp.

The most predominant bacterial species which appeared early during the fermentation process were Arthrobacter piscens, A. oxydans, A. simplex, unidentified Arthrobacter spp., Acetobacter xylenum, unidentified Acetobacter spp., Bacillus maceranus, unidentified Bacillus spp., Proteus retigeri and Lactobacillus spp.

Molds were present at the initial stage of Phase I but, they disappear from the fermenting mash after 72 hr of fermentation.

Phase II (96-144 hr)

At the end of Phase I, the thinsis was transferred into a "Gan" and 9.5 kg of 'kitta', 0.8 kg of Sikil, 0.18 kg of Gesho and 9.32 l of water were added to it

and sixed thoroughly (Fig. 1).

The p^H fell slightly by a unit of 0.03 (from 4.73 to 4.71) as a result of the introduction of ingredients during the beginning of this phase. It dropped further from 4.71 to 4.5 at the end of the Phase II. Likewise, the temperature of ' tinsis' dropped by 1°C as a result of the addition of ingredients. But the temperature of the fermenting material again increased from about 21°C to 25°C at the end of the phase (Table V). This temperature was higher by 4°C than that of the room in which the fermentation was being carried out.

The reducing sugars and total carbohydrate concentrations initially (Phase II) were 28.37 mg/ml and 98.08 mg/ml respectively. These decreased to 8.75 mg/ml (reducing sugars) and 43.16 mg/ml (carbohydrates) respectively. On the other hand protein and total solids were between 14.67-21.7 mg/ml and 15.22%-21.1% respectively.

(4) As can be noted in Table V, the concentrations of various chemicals analyzed dropped. The early increament noted was that of ethanol. The alcohol content at this phase was 3.17%. The increment in ethanol content was directly related to increases in population of yeasts and decreases in reducing sugars and total carbohydrates, in the fermenting mash (Table V).

TABLE I.

CHEMICAL AND PHYSICAL CHANGES DURING PHASE I OF TELLA FERMENTATION

Time of Isolation (hrs)	Temperature°C		pH	Total Solid %	Ethanol %	Reducing Sugar %	Total Carbohydrates mg/ml	Protein Concentration mg/ml
	Ferm. Temp.	Room Temp.						
0	20.67	20.33	5.2	4.4	0	12.65	37.4	18.4
24	21.33	20.67	5.17	4.58	0	10.33	28.67	17.0
48	21.67	20.33	5.13	4.56	0	9.58	24.33	23.33
72	21.67	20.67	4.95	5.24	0	5.94	20.67	23.73
96	22	20.67	4.73	5.49	0	4.04	18.5	15.46

TABLE IX.

SOME PHYSICAL, CHEMICAL AND MICROBIAL ACCOUNTS OF INGREDIENTS USED FOR BREWING

Ingredients	Total Viable Cell Counts	Bacterial Population CUF/gm	Mold CUF/gm	PH	Moisture Content %	Ash	Reducing Sugar mg/gm	Total Carbo-hydrates mg/gm	Protein mg/gm
Gesho (R. prinoides) leaves stem	$1x10^4$	$1x10^4$	$2x10^3$	6.59	7.68 7.8	7.14 3.7	25	186	42
Bikil (Malt)	$9x10^4$	$9x10^4$	$1x10^4$	5.68	10.0	4.4	264	298	3.0
Kitta (Unleaven bread)	----	----	----	6.1-5.8	39.57-36.3	11.11	44	271	5
Enkuro (roasted millet & steamed barley	----	----	----	4.8-4.69	33.35-31.2	5.3	40	191.8	62

Table III.

Microorganisms Isolated from Ingredients Used for Brewing Tella.

Ingredients	Arthrobacter Parsens	A. flavus	Arthrobacter spp.	Proteus vulgaris	A. aerogenes	Proteus spp.	Bacillus Mycoides	Bacillus spp.	Aerobacter spp.	Pseudomonas spp.	Molds.
"Gesho"	$2x10^3$	$1x10^3$	----	$5x10^3$	----	$3x10^3$	$1x10^3$	$5x10^2$	----	$6x10^2$	$2x10^2$
Nikil	----	----	$4x10^3$	$3x10^4$	$6x10^3$	$1x10^3$	----	$1x10^3$	$4x10^3$	---	$1x10^2$

Table IV.

MICROBIAL POPULATION DYNAMICS IN PHASE I OF TELLA FERMENTATION

96	72	48	24	0	Time of Ibolation (Mr.)
$5x10^7$	$2x10^7$	$4x10^4$	$4x10^3$	$1x10^4$	Total viable cell counts CPU/ml
$2x10^5$	$1x10^3$	$3x10^2$	-	-	Seccharomyces cerevisiae
$6x10^6$	$2x10^5$	$7x10^3$	-	-	Saccharomyces spp.
-	-	$2x10^3$	$2x10^2$	$2x10^2$	Molds
$5x10^6$	-	-	$6x10^2$	$5x10^2$	Arthrobacter Pascens
-	-	-	$3x10^2$	$1x10^3$	Arthrobacter oxydans
$1x10^7$	$4x10^6$	$7x10^2$	$4x10^2$	$2x10^2$	A. simplex
$3x10^6$	$2x10^6$	$5x10^2$	$5x10^2$	$4x10^2$	Arthrobacter spp.
$1x10^6$	$1x10^6$	$2x10^3$	$1x10^3$	$7x10^2$	Acetobacter xylenium
$2x10^7$	$9x10^6$	$4x10^3$	$1x10^2$	$4x10^2$	Acetobacter spp.
$3x10^5$	$3x10^3$	$3x10^2$	$3x10^2$	$3x10^3$	Bacillus maceranus
$1x10^5$	$4x10^2$	-	-	-	Bacillus spp
-	-	-	-	$2x10^3$	Proteus rettuger
$1x10^6$	$2x10^3$	$6x10^2$	$2x10^2$	$1x10^2$	Home fermentative Lactobacillus sp.
$1x10^6$	$2x10^3$	$5x10^2$	$2x10^2$	$1x10^2$	L. Pastorianus

The total aerobic count was $4x10^7$CFU/ml at the beginning of this phase and it increased by 75% at the end of the phase ($7x10^7$CFU/ml).

Additional micro-organisms were again introduced into the fermenting material from the 'Bikil'. These were mainly acetic acid bacteria and Arthrobacter spp. (Table VI).

The population of Saccharomyces spp., S.cerevisiae and that of Lactobacillus pastorianus increased through time during this phase (Table VI). However, those of Arthrobacter spp. and some of the acetic acid bacteria disappeared from the fermenting mash when the ethanol content increased from 0.77% to 3.17% (at the end of phase II). Nevertheless, it was the yeasts (genus Saccharomyces) which were dominant than the bacterial spp. after the 96^{th} hr of fermentation.

Phase II was also characterized by vigorous fermentation foaming and bubbling.

Phase II (144-192 hr)

The termination of phase II was identified by taste and smell of the fermenting mash, by the brewer. The smell of the alcohol from the mash and the relatively high temperature within the mash served as the main

characteristics for the commencement of this phase. The content of phase II was transfered into larger vat called "Gan".

Phase II, was characterized by the addition of 19.2kg of Enkuro, 0.85 kg of Bikil, 0.6 kg of pluverized "Gesho" (Stem) and 6.6L. of water into the mash. This was mixed thoroughly. The fermentation was then allowed to continue for 48 ht (i.e., from 114^{th} up to 192 hr). Foaming evolution of gas and bubbling are again characteristics of this phase (Fig. 1).

The p^H which was 4.53 at the end of phase II, increased to 4.75 as a result of the addition of the adjuncts mentioned above. As the fermentation proceeded, the p^H fell slightly, and reached 4.67 at the end of the third phase. (Table VII).

The temperature in the room in which the experiments were carried, varied by only $0.3^{o}C$., within the 48 hr (Table VII). On the other hand, the fermenting mash increased from $24^{o}C$ at time 144 hr to $27^{o}C$ at time 192 hr. This indicated that, vigorous fermentation was taking place in the fermentation vat.

The amount of reduced sugars and total carbohydrates at the initial stage of this phase was 14.83 mg/ml and 118.5 mg/ml, respectively. This decreased to 5.2mg/ml

Table V.

CHEMICAL AND PHYSICAL CHANGES OCCURING IN PHASE II.

Time of Isolation (hr)	Temperature (°C)		pH	Total Solids %	Ethanol Content %	Reducing sugar mg/ml	Total Carbo-hydrates mg/ml	Protein Concentration mg/ml
	Ferm. Material	Room Temp.						
96	21.33	20.67	4.71	21.1	0	28.37	98.08	21.7
120	24	21.33	4.62	16.94	9.77	15.53	80.13	14.67
144	25	21.0	4.5	15.22	3.17	8.75	43.16	17.7

Table VI.

MICRODIAL POPULATION DYNAMICS IN PHASE II OF TELLA FERMENTATION

144	120	96	Time of Isolation (hrs)
$7x10^7$	$5x10^7$	$4x10^2$	Total viable cell count CFU/ml
$8x10^6$	$4x10^6$	$1x10^5$	*Saccharomyces cerevisiae*
$6x10^7$	$2x10^7$	$4x10^6$	*Saccharomyces* spp.
-	-	$4x10^6$	*Arthrobacter Pascens*
-	$1x10^6$	$9x10^6$	*A. simplex*
-	$1x10^7$	$2x10^6$	*Arthrobacter* spp.
-	$2x10^7$	$2x10^7$	*Acetobacter xylenium*
-	$1x10^7$	$1x10^7$	*Acetobacter* spp.
-	-	$3x10^5$	*Bacillue maceranus*
$4x10^6$	-	$1x10^5$	*Bacillus* spp.
$3x10^6$	$4x10^6$	-	*Proteus rettigeri*
$1x10^6$	-	$1x10^6$	Home formentative *Lactobacillus* spp.
$4x10^6$	$2x10^6$	$1x10^6$	*L.Pastorianus*

(reducing sugars) and 78.57 mg.ml (total carbohydrates) Table VII). These decrease in reducing sugars and total carbohydrates were directly related to the production of ethanol and total increases in population of the micro-organisms involved in the fermentation. Eventhough the ethanol content stood at 2.77% at the 144^{th} hr, it reached 5.13% after 48 hr, when this phase came to completion.

The total viable cell count at the initial step of this phase was $7x10^7$ CFU/ml, but it increased to $1x10^8$ CFU/ml at the end of the phase (Table VIII).

The bacterial groups, Arthrobacter spp., Acetobacter spp. and Bacillus maceranus, that were not seen during the early step, appeared at the end of the phase (168-192 hr). On the other hand Proteus rettgeri disappeared at the later steps of phase III. Again the Rhodotorula spp. which were not seen up to 144 hr of fermentation, appeared after 144-168 hr of this phase. Regardless of these, Phase III was dominated by Saccharomyces spp. Lactobacillus pastorianus and other homofermentative Lactobacillus spp.

Phase IV (192-504 hr).

This phase can be sub-divided into two periods. Period I took 72 hr. and could be considered as the time required for maximum production of ethanol. Period

Table VII. Phase III.

CHEMICAL & PHYSICAL CHANGES OCCURRED DURING FERMENTATION

Time of Isolation. (hr).	Temperature (°C)		pH	Total Solid %	Ethanol %	Reducing Sugar mg/ml	Total Carbohydrate mg/ml	Protein Concentration mg/ml
	Fermenting Material	Room. Temp.						
144	24	21	4.73	28.76	2.77	14.83	113.37	12.67
168	27	21	4.69	24.83	3.33	10.73	93.0	12.3
192	27	21.3	4.67	26.0	3.13	5.2	74.57	10.67

Table VIII. MICROBIAL POPULATION DYNAMICS IN PHASE III OF TELLA FERMENTATION

192	168	144	Time of Isolation (hrs)
$4x10^6$	$1x10^7$	$7x10^6$	Saccharomyces cerevisiae
$3x10^7$	$5x10^7$	$5x10^7$	Saccharomyces spp.
$6x10^6$	$1x10^6$	-	Rhodotorula spp.
$2x10^7$	$4x10^7$	-	Arthrobacter Pascens
-	$3x10^5$	-	A. simplex
$3x10^7$	-	-	Arthrobacter xyleniun
$4x10^7$	$1x10^6$	-	Acetobacter spp.
$1x10^7$	$4x10^5$	-	Bacillus maceranus
$1x10^6$	$1x10^6$	$3x10^6$	Bacillus spp.
-	-	$3x10^6$	Proteus rettigori
$1x10^6$	$6x10^5$	$1x10^6$	Home fermentative Lactobacillus spp.
$2x10^6$	$7x10^5$	$3x10^6$	L. Pastorianus
$1x10^6$	$2x10^5$	-	Arthrobacter spp.

II (264 -504 hr) could be classified as period of maturation (aging), and it was also at this period that tella could be served.

During the initial stage Period I of phase IV, 34.5L of water was added into the fermentation vat("Gan". This was enough to fill the vat to the rim. This was sealed using cheese cloth and mud to create anaerobic conditions. Then it was allowed to ferment for 72 hr. i.e., from 192th upto 264 hr (Fig. 1).

The total solid content which was 26% of at the end of phase III, decreased to 11.73% as a result of the addition of water. However, as fermentation proceeded, the total solid content dropped and reached 3.4% at the end of period I of Phase IV. This reduction may be due to settling of spent stillage ("Attela"). Tella could be served, 24hr after the beginning of period I (Table X).

The p^H before the addition of water at the end of phase IV was 4.67. But as a result of addition of water it fell by 0.09 unit of p^H, at the initial step of Phase IV (i.e., 192 hr). Thereafter the p^H continued to decrease from 4.58 [illegible] from 4.09 to 3.34 in period II (264-504 hr).

The temperature of the mash decreased by $3^{o}C$ in period I, and by $1^{o}C$ in period II (i.e., 264-312 hr). This forms no change with room temperature throughout phase IV (i.e., 21.3-20.67^{o}C).

The reducing sugars and total carbohydrates content initially (phase IV) was 5.2 mg/ml and 78.57 mg/ml, respectively. Thereafter it decreased to 2.35 mg/ml and 34.6 mg/ml in period I (Table IX). In period II (264-360 hr), the amount of reducing sugars and total carbohydrates decreased again to 1.96 mg/ml and 26 mg/ml, respectively. The ethanol content initially increased (196-216). This was related to decrease in reducing sugars and total carbohydrates. But the ethanol content decreased continously from 5.33% to 4.3% in period I, and from 4.3% to 2.33% in period II (264-360 hr).

The protein content which was 10.67 mg/ml decreased to 3.16 mg/ml in period I. There was further decrease from 3.16 mg/ml to 2.0 mg/ml in period II of phase IV.

The total aerobic cell count at the initial step of this phase (period I) was $1x10^{8}$ CFU/ml. This figure increased to $3x10^{8}$ CFU/ml at the completion of period I (i.e. 264 hr) and at the beginning of period II of phase IV. This population decreased to $6x10^{7}$ CFU/ml at the end of phase IV. This was an 80% decrease in population (Table IX).

The Saccharomyces spp., which were $4x10^7$ CFU/ml reached a population of $6x10^7$ CFU/ml at the end of period I. But in the period II the population decreased from $6x10^7$ CFU/ml to $2x10^6$ CFU/ml.

Achromobacter spp. appeared at the end of period I and at the early stage of period II. Bacillus spp disappeared, but Lactobacillus casei appeared at the end of Phase IV.

In phase IV the dominant bacterial groups were Arthrobacter spp., acetic acid bacteria and both homo and hetro fermentative Lactobacillus spp. However, the reduction in p^H and ethanol content in phase IV was the result of the activities of the dominant groups of bacteria. These are the groups of acetic acid bacteria and lactic acid bacteria (Table X).

TABLE XI. PHASE IV.

CHEMICAL AND PHYSICAL CHANGES OCCURED DURING FERMENTATION

Time of Isolation (hrs)	Temperature(°C)		pH	Total Solid %	Ethanol %	Reducing Sugar mg/ml	Total Carbo-hydrates mg/ml	Protein Concentration mg/ml
	Ferm. Temp.	Room. Temp.						
192	24.67	21.3	4.58	11.73	5.13	5.2	78.57	10.67
216	23.83	20.67	4.59	6.57	5.33	4.06	64.96	8.2
240	22.67	20.67	4.46	5.23	4.67	3.17	53.43	4.13
264	21.67	21	4.08	3.4	4.3	2.35	34.6	3.16
288	20.67	20.67	3.93	3.05	3.6	1.98	31.67	2.35
312	20.67	21.3	3.78	3.04	3.13	1.96	26.5	2.0
360			3.57		2.33			
432			3.43					
504			3.34					

Table X. MICROBIAL POPULATION DYNAMICS IN PHASE IV OF TELLA FERMENTATION

isolation(hrs)	Total viable cell count. CFU/ml	Saccharomyces cerevesiae	Saccharomyces spp.	Rhodotorula spp.	Arthrobacter Pascens	Arthrobacter spp.	Acetobacter xylenium	Acetobacter spp.	Bacillus Macernus	Bacillus spp.	Achromobacter delicatus	Lactobacillus casi	Home fermentative Lactobacillus spp.	L. Pastorianus
92	$1x10^8$	$4x10^6$	$3x10^7$	$6x10^6$	$2x10^7$	$1x10^6$	$3x10^7$	$4x10^7$	$1x10^6$	$2x10^6$	-	-	$1x10^6$	$2x10^6$
16	$1x10^8$	$1x10^7$	$6x10^7$	-	$1x10^6$	$2x10^6$	$2x10^7$	$4x10^7$	$8x10^5$	$2x10^6$	-	-	$4x10^6$	$7x10^6$
40	$1x10^8$	$2x10^6$	$5x10^7$	-	$2x10^6$	$3x10^6$	$1x10^7$	$2x10^7$	$3x10^6$	$5x10^6$	$5x10^6$	-	$1x10^7$	$1x10^7$
64	$3x10^8$	-	$6x10^7$	$2x10^6$	$1x10^6$	$2x10^6$	$3x10^7$	$4x10^7$	$4x10^5$	-	$5x10^6$	-	$3x10^7$	$5x10^7$
88	$1x10^8$	$1x10^6$	$3x10^7$	-	$1x10^6$	$2x10^6$	$3x10^7$	$4x10^7$	$1x10^6$	-	$3x10^6$	-	$2x10^7$	$2x10^7$
12	$8x10^7$	-	$4x10^7$	-	$1x10^6$	$1x10^6$	$3x10^7$	$2x10^7$	$1x10^6$	-	-	-	$4x10^6$	$1x10^7$
60	$7x10^7$	-	$3x10^7$	$1x10^6$	$1x10^7$	$3x10^6$	$1x10^7$	$2x10^7$	-	-	-	$2x10^6$	$4x10^6$	$6x10^6$
32	$4x10^7$	-	$2x10^6$	-	$1x10^7$	$2x10^6$	$1x10^7$	$4x10^6$	-	-	-	$1x10^6$	$4x10^6$	$4x10^6$
04	$5x10^7$	-	$2x10^6$	-	$2x10^7$	$3x10^6$	$2x10^7$	$2x10^7$	-	-	-	-	$5x10^6$	$7x10^6$

PERIOD II PERIOD I.

Some Chemical, Physical and Microbiological Surveys on Tella Collected from Households in Addis Ababa Region.

Some chemical and physical characteristics of collected tella samples, collected from Addis Ababa are shown in Table XI.

The p^H, total solid, ethyl alcohol, total carbohydrates, reducing sugars, protein and ash content varied from 3.5 to 4.38, 2.0% to 4.4%, 4.5% to 6%, 32 mg/ml to 40 mg/ml, 2.0 mg/ml to 4.4 mg/ml, 3.0 mg/ml to 3.4 mg/ml and 0.1% to 0.13%. One of the collected samples, had higher ethyl alcohol, total carbohydrates and reducing sugars content. This sample was traditionally known as 'Filter tella'. This type of tella is filtered from the mash at the end of phase III after slight addition of some water (Table XII).

The total aerobic count of the locally collected samples are shown in Table XII. Its range was from $5x10^7$ to $3x10^8$ CFU/ml. Acetic acid and lactic acid bacteria and in addition to *Arthrobacter* spp., and *Bacillus* spp. were isolated. Their range of proportion varies from $2x10^6$ CFU/ml to $8x10^7$ CFU/ml.

Yeasts belonging to genus *Saccharomyces* were isolated from all of the samples. Their count range from $1x10^7$CFU/ml to $6x10^7$ CFU/ml. They were the most predominant species of the isolates obtained from the sample.

Table X. SOME CHEMICAL AND PHYSICAL CHARACTER OF LOCALLY COLLECTED TELLA SAMPLES

Sample Collected from Kefitegna	House Code	pH	Total Solids %	Alcohol Content %	Reducing Sugar mg/ml	Total Carbo-hydrates mg/ml	Protein mg/ml	Ash %	Remark
16	1	4.55	8.13	9.2	26	78	6.1	0.29	"Filter Tella"
13	2	3.5	3.8	[illegible]	[illegible]7	32	3.2	0.12	
14	3	3.5	4.4	5.0	4.0	40	3.1	0.13	
15	4	4.26	2.36	4.5	2.5	36	3.4	0.12	
5	5	4.38	3.6	5.5	2.7	34	3.0	0.11	
9	6	4.05	2.0	6	2.35	40	3.1	0.1	
10	7	4.0	3.02	6	2.37	36	3.2	0.12	
8	8	3.78	2.59	5.2	2.2	32	3.22	0.112	
11	9	4.05	3.02	5.35	4.4	38	3.13	0.11	
12	10	4.2	3.5	5.5	4.4	40	3.2	0.14	
Lab brewed		4.13	3.46	5.05	2.0	35.4	3.16	0.13	

TABLE XII.. MICROFLORA OF LOCALLY COLLECTED TELLA SAMPLES.

ample ollected efitegna	House Code	Total Viable Cell Count CFU/ml	Sacch-aromyces spp. CFU/ml	Acetic[1] Acid Bact.	Lact.[2] Acid Bact.	Other[3] Bacterial Groups
16	1	$6x10^6$	$2x10^6$	$1x10^6$	$2x10^6$	$1x10^6$
13	2	$5x10^7$	$2x10^7$	$7x10^7$	$8x10^6$	$4x10^6$
14	3	$3x10^8$	$2x10^7$	$2x10^8$	$8x10^7$	$5x10^6$
15	4	$6x10^7$	$2x10^7$	$2x10^7$	$2x10^7$	$8x10^6$
5	5	$5x10^7$	$1x10^7$	$2x10^7$	$8x10^6$	$4x10^6$
9	6	$7x10^7$	$5x10^7$	$2x10^7$	$2x10^6$	$3x10^6$
10	7	$6x10^7$	$4x10^7$	$2x10^7$	$1x10^6$	$2x10^6$
8	8	$6x10^7$	$2x10^7$	$3x10^7$	$1x10^7$	$4x10^6$
11	9	$6x10^7$	$3x10^7$	$2x10^7$	$4x10^6$	$2x10^6$
12	10	$5x10^7$	$3x10^7$	$1x10^7$	$4x10^6$	$3x10^6$
Lab.	Brewed	$2x10^8$	$6x10^7$	$7x10^7$	$8x10^7$	$3x10^6$

Note:

[1]Acetic Acid Bacteria includes *Acetobacter* spp. and *Acetobacter xylenium*.

[2]Lactic acid Bacteria includes Both Homo and Hetro Fermantative *Lactobacillus* spp. such as *Lactobacillus pastorianus*.

[3]*Bacillus* spp. and *Arthrobacter* spp. such as *Bacillus Maceranus*, *Arthrobacter* papcensch as Bacillus

DISCUSSION

The process involved in the fermentation of tella was found to be more complex than malt beverages such as kaffir beer (Nolvellie and Schaepdrijver, 1979); Bouza (Marcos, 1977) and Pito (Ekundayo, 1977). Its fermentation has some resemblance to that of comercial beer. However, unlike commercial beer which has starter cultures, it relies on micro-organisms present on the ingredients and in the containers used for brewing.

The ingredients are added singly or in combinations into the fermentation vats (Fig. 1).

During the fermentation of tella continuous changes in p^H, temperature, reducing sugars, total carbohydrates, soluble proteins, alcohol content, ash and total solids occured. There was also variability in the number and kinds of micro-organisms involved in it during the different phases or stages of fermentation (Table I,V, VII and X).

The p^H feel from the near neutral to the acidic side. This kind of decline in p^H is considered as a desirable characteristic in commercial beer production (Prescott and Dunn, 1959). Unlike other fermented malt beverages

which do not contain clear cut stages (Kaffir beer, Egyptian Bauza, etc), the rate of change in p^H was found to be variable during the different phases of tella fermentation. In phase I, p^H decrease was not associated with increase in ethanol production (Table III). The decrease in p^H during phase II, III and IV (ie., the 192^{th} - 216^{th} hr), however, influenced the tempo fermentations. Vigerous fermentation and increments in ethanol production were apparent after this decline in p^H of the mash. It is known that p^H plays an important role in the mash. The p^H increases the quantity of extracts and activity of enzymes, modifies the nature of the mash, serves in selection of the micro-organisms involved, during fermentation and serves in the inhibition of undesirable micro-organisms (Novellie and Scheapdrijver, 1979; prescot and Dunn, 1959). This selective nature of p^H in the type of fermenting micro-organisms and/or numbers noticed in other beverages also apparent in tella fermentation (Table V,VI, VIII and IX).

In addition to the factors mentioned above, the decrease in p^H was also directly related to the ambient temperature in which fermentation was being carried out. Unlike beer fermentation the fermentation takes place at room temperatures (Prescot and Dunn, 1959). Ambient temperatures vary from 10^oC up to 30^oC in most regions

of Ethiopia where tolla is traditionally brewed. Hence, ambient temperature affects the duration of fermentation. On the average, it takes 10 -15 days.

The fermenting mash had higher temperature than that of the room where fermentation was carried out. This of course implies that microbil activity was higher between 120th (5 days) to 216th hr (9 days) when the temperature of the fermenting mash was high (Table IV and VI). The decline in p^{H} after the 216 hr (9th day) in fermenting tella (Phase IV) was brought about as a result of the increments in number of acetic acid bacteria increased. Acetic acid bacteria belonging to the genera of Acetobacter and Gluconobacter use ethanol as carbon/energy source to primarily produce acetic acid (Banwart, 1979).

The temperature of the mash started delcining when increments in microbial population started declining and at the same time the alcohol content also decreased from 5.33% to 2.33%. The decrease in total pupulation of micro-organisms was from 1x10^{8} CFU/ml to 6x10^{7} CFU/ml was clearly visible (Table X). The rise in temperature of the fermenting mash to some extent can increase the activity of both and amaylases within the system (Prescot and Dunn, 1959; Novellie and Schaepdrijver, 1979) thereby increasing total reducing sugars content

which could be available to the micro-organisms.

The fluctuation in temperature and the decrease in p^H were associated with the disappearance of simple sugars or decrease in total carbohydrates in the fermenting material. The sample taken during phase I, contained lower concentration in both total carbohydrates and reducing sugars as compared to the other phases (ie., phases II, III, and Iv (period I). This is because more ingredients were added in subsequent phases.

Initially during "tinsis" preparation (Phase I), Gesho (powdered leaves) was the only ingredient used. Gesho leaves have low quantities of total carbohydrates and raducing sugars than "Bikil", kitta and "Enkuro" which are mainly made up to starch (Table III). High reducing sugar and total carbohydrate content were characteristic of phase II and III.

Both the simple sugars and total carbohydrates decrease significantly during phase II and III, when microbial activity was high. As the sugars decreased in concentration, that of the ethanol increased concomitantly. This relation has been observed previously by others (Lewis, 1987).

Ethanol production is one of the major events which takes place during tella production (Desta, 1977). The

ethanol content and the rate of its production at the different phases was found to be variable (Tables I, V, VII and IV). In phase I, ethanol production was not observed. The production of ethanol in the fermenting material was only observed in the initial stage of phase II, after bikil and kitta have been added to tinsis. Maximum production of ethanol was observed during phase III and at the initial stage of phase IV (i.e., between 192^{th} and 216^{th} hr). After the initial stage of phase IV, the content of ethanol decreased.

The production of ethanol has an impact on the spectrum of micro-organisms (Haas, 1960). This was shown during 96^{th} to 144^{th} hr of fermentation (Table V), when there was decrease in number as well as types of micro-organisms involved in tella fermentation. The decrease in ethanol content during phase IV gave way to reduction in number of yeasts from tella. This was the result of the settling of the yeasts to the bottom and relatively increment of the bacterial population on the surface of tella.

Tella can be consumed 24 hr after difdif preparation. This is known as "Gush" (unaged). However, it is considered aged and of proper quality after the 264^{th} hr (11 days after fermentation).

It is consumed by the brewer and the resident of the house to check for the quality and progress of the fermentation.

At the end of period I of phase IV tella is said to be ready for drinking. However, according to this study this period also showed reduction in ethanol content and acidification of tella. Further storage of tella after this period lead to increment in sourness and decrease in ethanol content. Similar condition is known to happen in Kenyan Busaa and other indigenous fermented beverages. (Harkishor, 1977).

There was change in total solid content of the fermenting material during fermentation. The trend in each phase showed devreases initially followed by increases at later stages (except phase IV). This was the result of absorption, solubilization of the ingredients and evaporation. Higher amounts of total solid content was attained during phase II and III. This was due to the addition of 'Bikil' and adjuncts such as (Kitta and Enkuro) to the mash. But in phase IV (period I) it showed sharp decrease in total solid contents. This was as a result of the addition of water (Fig. 1) to extract the contents of the fermenting materials and to allow the settling of spent stillage ("Attela") (Solomon, 1984).

The number and kinds of micro-organisms in the fermenting material showed variability (Table IV, VI, VIII and X). This variability happened between and within the groups of micro-organisms involved in the fermentation and the variations in number and kinds was evident from the initial phase of the fermentation.

The number of micro-organisms was initially relatively smaller ($1x10^3$-$1x10^4$ CFU/ml). But it has reached the highest population at the beginning of phase III and period I of phase IV. This has declined after period I of phase IV, when tella was ready for consumption. This occured at the same time when the particulate material was settling.

The variation in kinds of organisms during fermentation continued until the end of phase IV. All the organisms found in the 'Gesho', 'Bikil' and the fermenting vat did not grow during the fermentation. Most of the initial isolates from these sources where inhibited and only a few of them found the environment conductive to growth and attain high population within the system. One of these members were the yeasts belonging to the genus (Saccharomyces). These yeasts, initially were only found in very : mass numbers in 'Gesho' and 'Bikil'.

The increment in number of organisms was directly related to the availability of nutrients in the fermenting

material. After the initial stage of phase II there was general decrease in bacterial population and a concomitant increment in yeasts population. This is most probably the result of the ethanol production, some among them isolates were unable to metabolize starch or occasionally glucose. In phase IV, it is the bacterial pupulation that was predominant in the fermenting mash. Thos was because of the settling of the yeasts members to the bottom of the vat in phase IV. The rise in the number of bacterial population in phase IV resulted in the decline of p^H and ethanol content.

Initially bacteria, molds and yeasts appeared in the fermenting materials. Their dynamics of growth and change within the group varied tremendously.

During tinsis preparation 0 hr) coliforms, molds, Arthrobacter spp., Bacillus spp., acetic acid bacteria and lactic acid bacteria were isolated both from gesho and/or the container used for the fermentation. Coliforms were present in gesho. However, they disappeared after 24 hr of fermentation. Again they reappeared when the p^H was at 4.74 during phase II (This was associated with the introduction of ingredients at 120 hr), they disappeared totally from the dermenting material after the initial stage of phase III (144 hr) (Table IV).

Coliforms are known to account for a wide range of metabolic products which impart off flavour odours to the wort, that may cause sweet and fruity tasts or resemble the smell of cooked cabbage in commercial beer. The presence of coliforms in malt beverage is known to be common (Klegn and Hough, 1971).

Molds were isolated from Gesho used; but, they disappeared after 72 hr of fermentation. The disappearance of molds after 72 hr appears to show, the unability of molds to compete with bacterial and yeast population. According to Novellie and Schaepdrijver (1979), when yeasts are active, any dissolved oxygen is quickly and continuously taken by them, thus generating unfavourable conditions for fungal growth.

Bacillus spp., were isolated from tinsis at 0 hr. These spore-forming members were present in the fermenting material upto the later stage of phase IV (321^{th} hr), when the p^H was 3.78 (Table X). The Bacillus spp., isolated were found to produce acid during biochemical characterization. Hence, they might have contributed to the acidity of the mash. This is at least true in beer and tesquino (Vlea, et al., 1974; Haas, 1940). But they disappeared after 312 hr of fermentation.

The acetic acid bacteria were isolated from tinsis. They increased in number gradually during the

fermentation periods. These groups of bacteria have the capacity to oxidize ethanol to acctic acid. They also account for the frequent cause or acidity, off-flavours and turbidity. They cause yeast cell to die and form dextraneous "rope" in substantial quantities. These conditions are common in most of the fermented beverages such as beer (Banwart, 1979; Haas, 1960). This was no difference in the case of tella.

Both groups of lactic acid (homo and hetro fermentative) bacteria were found throughout the fermentation period. These groups of bacteria are the most frequent contaminats of beer. By far the most common of the group in beer is Lactobacillus pastorianus (Haas, 1960). In tella, L.pastorianus was also the predominant lactic acid bacterial isolates. This bacterium produces lactic, acetic and formic acid. It also produces alcohol and CO_2. It is responsible for the undesirable effects, such as sourness, silky turbidity and off-flavour at least in beer. These bacteria may also reinfect the yeasts (Kleyn and Hough; 1971; Prescot and Dunn, 1959).

The other genus which isolated initially and existed throughout the fermentation period was, Arthrobacter spp., The source of Arthrobacter spp. was the Gesho used in the preparation of tella (Table II). This group is known to cause spoilage in

brewing and food fermentations. However, these are aerobic, and detected to produce methyl mercapton from sulfur containing amino acid (Banwart, 1979).

Yeasts appeared after 48 hr of fermentation. The sources of yeasts were the fermentation vat, gesho and bikil. However, initially the populationswas much lower than the bacterial isolates.

Yeasts belonging to genus Saccharomyces were the most predominant members found in fermenting tella. This group of yeasts were responsible for the production of alcohol (ethanol). This is true for most fermented beverages such as palm wine (Faparusi, 1977) beer (Banwart, 1979) maize chicha (Soriano, 1983) and others.

Rhodotorula spp. appeared in the fermenting material at the initial period of phase IV, but it disappeared during the latter stage of phase IV. The number of Rhodotorula spp. were smaller than that of Saccharomyces spp. They do not play any positive role in the fermentation (Lodder, 1971), but they are considered to spoil the final products of beer (Kleyn and Hough, 1971).

Achromobacter spp. appeared in 240th hr and disappeared at 288th hr of fermentation. This period was recognized as the period when tella is ready for consumption. The organism is a common beer spoilage organism and is

responsible for causing turbidity, off-odours and off-flavours (Haas, 1960).

Similar microbial, chemical and physical characteristics were shown from tella samples collected around Addis Ababa (Table XI and XII). This proves the relationship of tella brewed in the laboratory with the household brewed ones.

The fermentation of tella has close resemblance to the production of commercial beer. Heance, large scale commercial production is likely possible. As it is indicated in a Central Statistical Office (1988) report the rate of production of traditional beverages including tella has increased significantly in recent years. The price of tella when compared to the commercially produced alcoholic beverages is very low.

The development of starter culture will contribute to the development of large scale production of tella in the not-too-far future.

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