ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

FACULTY OF SCIENCE

DEPARTMENT OF BIOLOGY

EVALUATION OF YEAST BIOMASS PRODUCTION USING MOLASSES AND SUPPLEMENTS

BY
TAMENE MILKESSA JIRU

A Thesis Submitted to the School of Graduate Studies of Addis Ababa University in Partial Fulfillment of Degree of Master of Science in Biology (Applied Microbiology).

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Addis Ababa

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LIST OF ABBREVIATIONS

ADY	active dry yeast
BA	commercial baker`s yeast
$CaCl_2.2H_2O$	calcium chloride hydrated
DNS	dintro salicylic acid
E.C.	Ethiopian calendar
ETB	Ethiopian birr
gm	gram
HCl	hydrochloric acid
H_3PO4	phosphoric acid
H_2SO_4	sulphuric acid
hrs	hours
IDY	instant dry yeast
KH_2PO_4	potassium ortho phosphate
$MgSO_4.7H_2O$	magnesium sulphate hydrated
YMPGA	yeast extract, malt extract, peptone, glucose and agar
YMPG	yeast extract, malt extract, peptone and glucose
$(NH_4)_2SO_4$	ammonium sulphate
NaOH	sodium hydroxide
NH_3	ammonia
Na_2CO_3	sodium carbonate
$NaHCO_3$	sodium bicarbonate
OD	optical density
RPM	revolution per minute
SCP	single cell protein
TE	local yeast strain isolated from *teff* dough
TL	local yeast strain isolated from *tella*

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ABSTRACRT

Three yeast strains of *saccharomyces cerevisiae*, namely commercial baker's yeast (BA), an isolate from *teff* dough (TE) and an isolate from *tella*(TL) were cultivated in the laboratory by submerged method to determine biomass yield. The biomass of these yeast strains was compared with respect to molasses concentrations(3% w/v,5% w/v,8% w/v and 10% w/v), pH(3.5,4.0,4.5,5.0 and 5.5),growth temperatures (25^0C,30^0C and 37^0C), duration of incubation(24,48,72 and 96 hrs) and the effect of addition of supplements as treatments; T1-$(NH_4)_2SO_4$ (0.5 % w/v),T2-$(NH_4)_2SO_4$ (0.5 % w/v) and KH_2PO_4 (0.3 % w/v),T3-$(NH_4)_2SO_4$ (0.5 % w/v), KH_2PO_4 (0.3 % w/v) and peptone (2% w/v),T4-$(NH_4)_2SO_4$ (0.5 % w/v), KH_2PO_4 (0.3 % w/v),yeast extract(1%w/v) ,$MgSO_4.7H_2O$ (0.05 % w/v) and $CaCL_2.2H_2O$ (0. 004 % w/v),T5-$(NH_4)_2SO_4$ (0.5 % w/v), KH_2PO_4 (0.3 % w/v), peptone (2%w/v),yeast extract(1%w/v),$MgSO_4.7H_2O$ (0.05%w/v) and $CaCL_2.2H_2O$ (0.004% w/v), biotin(0.005%w/v) and calcium panthetonate (0.0001% w/v). The contents of molasses were analyzed before the cultivation process and it was found that the molasses used for this study contains 43.1 % sugar, 0.25% total nitrogen, 1.56 % crude protein, 17.9 % moisture content, 82.1% dry weight and 11.7 % total ash. With respect to molasses concentration, BA isolate showed maximum biomass yield at 5%, 8% and10% concentrations, whereas TE isolates showed the same trend at 5% and 8% concentrations. TL isolate was found to accumulate the maximum yield at 8% molasses concentrations. In all cases, the isolates showed similarity in high biomass accumulation when they were grown at 8% w/v molasses concentration. Concerning the effect of pH on the growth of yeasts, isolate BA was found to be effective at all pH values except pH 5.5; whereas TE isolate was effective at pH 4.0, pH 4.5 and 4.5. Furthermore, isolate BA and isolate TE were also effective at pH 4.5. At pH 3.5 and 5.5, there was a steady decrease in biomass yield by all the isolates. With respect to incubation temperatures, the different isolates displayed biomass yield ranging from 1.27g/l to 3.25g/l. All isolates showed slow growth at 25^0C, and 37^0C with subsequent slow increase as the incubation temperatures increased. The highest biomass was observed at 30^0C by isolates BA (2.98-3.2g/l in 24-72 hrs), TE (2.91-3.1g/l); whereas isolate TLshowed biomass increase of 2.81g/l .Supplementing molasses media with $(NH_4)_2SO_4$ (0.5% w/v) (Treatment1) increased the biomass of TL (5.6-5.9g/l), TE (5.6-6.2g/l), and BA (6.1-6.4g/l within 24 and 72 hrs. In all cases the maximum biomass was achieved within 48 hrs. When this compared with biomass accumulation on molasses alone, the inclusion of the supplemental nitrogen source showed 1.5-2 fold increase in yeast dry weight by all isolates. Comparing the growth of the isolates on molasses and ammonium sulphate as control the isolates did not show significant difference in biomass with further treatments (T2-T5). The incorporation of all the necessary supplements resulted in maximum biomass production by BA (8.0 g/l), followed by TE (7.5 g/l) and TL (6.5 g/l). In all the biomass propagation processes, the commercial baker's yeast strain, BA was superior in giving high biomass yield. Further more the leavening action of the two yeast strains, i.e., an isolate from *teff* dough (TE) and commercial baker's yeast (BA) was compared at room temperature and 30^0C. BA was found to be higher than TE both at room temperature and 30^0C.

Key words/phrases/: Baker's yeast (*Saccharomyces cerevisiae*)**,** Biomass, Leavening action, Molasses, Supplements

DECLARATION

I the undersigned person declare that this thesis is my original work and that all sources of material used for the thesis have been genuinely acknowledged.

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Signiture: ----------

Date: ----------

This thesis has been submitted for the examination with my approval as

Adivisor`s Name: Dawit Abate (Ph.D)

Signiture: ----------

Date: ----------

1. INTRODUCTION

Yeasts are unicellular, eukaryotic and polyphyletic organisms classified in the kingdom fungi. They are ubiquitous, and commonly found on fruits, vegetables and other plant materials. Some yeasts are found in association with soil and insects (Sláviková and Vadkertiová, 2003; Suh *et al.*, 2005). Approximately 100 genera comprising more than 700 species of yeast have been described (Mueller *et al*., 2004).

They are facultative anaerobes and can respire and survive under both aerobic and anaerobic conditions. In the absence of oxygen, they can ferment sugar into alcohol (ethanol) and carbon dioxide and low biomass. In well-aerated conditions, the cells could be able to get enough energy and convert sugar into high biomass (Rose and Harrison, 1969). The useful physiological properties of yeasts have led to their use in the field of industrial microbiology. Fermentation of sugars by yeast is the oldest application in the making of bread, beer and wine.

Apart from the production of bread and beverages, ethanol production *per ce* is vital for different applications. It is the most common organic solvent, an intermediate in the production of liquid detergents, used in the making of perfumes, paints, explosives plastics, plasticizers, cosmetics, antifreeze etc. (Hook, 1988; Akpan *et al.*, 2005). These days there are lots of interests to use bioethanol as an alternative/supplementary energy source Gunasekaran and Chandra, 1999).

Yeasts are also involved in single cell protein production. The main elements present in baker's yeast are carbon, hydrogen, oxygen and nitrogen, which normally account for as much as 94% of the dry matter (White, 1954). These four elements are present on the yeast in the form of carbohydrates (glycogen, cellulose, and yeast mannan), protein and lipids (true fats, lecithin, and sterols). Yeasts also contain high amount of vitamin B complex, nucleic acids and organic bases (pyramidine and purine bases).

The impressive advantages of microorganisms for single cell (SCP) production compared with conventional sources of protein (soybeans or meets) are well known. Microorganisms have high protein content and short growth time, leading to rapid biomass production. Moreover, they contain lower amount of nucleic acids than bacteria making them more acceptable as food supplement (Bhattacharjee, 1970). The large-scale production of yeast for nutritional uses was experienced in Germany during both world wars. Eventually, about 16,000 tons of *Candida utilis* was incorporated in to human food per year during the Second World War by Germans (Bhattacharjee, 1970).Single cell protein from yeasts is also used as a protein supplement of fodder mixtures in the nutrition of domestic animals (Bekatorou *et al*., 2006).

The yeasts can be propagated using cheap raw materials and easily harvested due to their bigger cell sizes and flocculation abilities. The raw materials used as substrates for industrial yeast biomass production are usually agricultural, forestry and food waste by-products. These are conventional materials like starch, molasses, distiller's wash, whey, fruit and vegetable wastes, wood, straw, etc., and unconventional ones like petroleum by-products, natural gas, ethanol and methanol (Jay, 1996). After fermentation, the yeast biomass is harvested and may be subjected to downstream processing steps, like washing to remove the impurities, cell disruption, protein extraction and purification to extract enzymes, molecules like RNA,DNA,B vitamins etc., (Bekatorou *et al.*, 2006).

The significance of yeasts in food technology in a world of low agricultural production and rapidly increasing population makes the production of food grade yeasts extremely important (Bekatorou *et al.,* 2006). In view of the fact that a large part of the earth's population is malnourished, due to poverty and inadequate of food, scientists are concerned whether the food supply can keep up with the world population increase (Zheng, *et al.*, 2005).

The natural energy resources such as fossil fuel, petroleum and coal are being utilized at a rapid rate and these resources have been estimated to last over a few years.

With the increasing shift towards a bio-based economy, there is rising demand for developing efficient cell factories that can produce fuels, chemicals, pharmaceuticals and food ingredients using yeasts. The yeast *Saccharomyces cerevisiae* is extremely well suited for the above purposes (Nowak, 2001; Soares *et al.*, 2002; Oliveira, 2006). Intense research has been carried out for obtaining efficient fermentative organisms, low cost fermentation substrates, and optimum environmental conditions for fermentation to occur. *Saccharomyces cerevisiae* can produce ethanol to give concentrations up to 18 % through strain improvement program (Lin and Tanaka, 2006).

Statement of the problem

Ethiopia is a developing country with a high demand of baker's yeast from a number of bakeries. As a result the use of commercial baker's yeast is increasing day to day. Moreover, a number of alcohol and beverage industries (beer and wine) are built and these industries need tremendous amount of yeast. There is no baker's yeast producing plant in the country. Baker's yeast for the country's demand is imported from many partner countries.

As reported by the Ethiopian Statistical Agency (ESA, 2008) the country imported a total of 2,131,571 and 2,213,404 kilograms of active and inactive dry yeast in the year 2006 and 2007, respectively. This was equivalent to 31,107,845 ETB (3,110,785 US $) and 33,089,615 ETB (3, 308, 962 US $). This necessitates the need for alternative import substitution of baker's yeasts for the national development. Furthermore, it is high time to mobilize resources to improve human food and animal feed with SCP using different types of microorganisms and yeasts.

In Ethiopia there are several fermented foods such as *tella*, *tej*, *kocho*, *teff*, etc. A lot of research was undertaken on the microbial profile of these commodities. In most cases, strains of *Saccharomyces cervisiae* were found to dominate the fermentation process.

Although the pattern and diversity of microbial fermentation of these foods and beverages were well documented (Samuel Sahle and Birhanu Abegaz Gashe, 1991; Mogessie Ashenafi, 2002), the selection and optimization of these organisms as potential inputs for small-scale and large-scale production of bioethanol and biomass is very limited. Against this background, the following objectives were set to evaluate the local yeast isolates in comparison with the imported Baker's yeast.

General Objectives

- To isolate yeast from local fermented beverages and food and optimize the conditions for high growth and biomass yield using molasses and a variety of supplements.

Specific Objectives

- To analyze the contents of locally available molasses (Wonji/Shoa Sugar Factory)
- To isolate, identify and compare yeasts strains from *teff* dough and *tella* (local beverage) with commercial baker's yeast
- To determine the effect of addition of supplements to yeast biomass yield
- To compare the leavening effectiveness of local yeast strains from *teff* dough and commercial baker's yeast

2. LITERATURE REVIEW

2.1 The Yeasts

Yeasts belong to the group of living things called fungi. Yeast cells are usually spherical, oval or cylindrical in shape (Brock and Madigan, 1991).Yeast size can vary greatly depending on the species, typically measuring 3–4 µm in diameter, although some yeasts can reach over 40 µm (Walker *et al*., 2002).

Yeasts are able to reproduce both sexually and asexually. Asexual reproduction is usually by budding (Prescott and Dunn, 1959; Pelczar *et al.*, 1986; Brock and Madigan, 1991). Sexual reproduction is through conjugation and production of ascospores. Ascosporogenous yeasts, which belong to the tribe Saccharomycetaceae, undergo the formation of ascospores inside asci by the process involving meiotic division referred to as sporulation (Rose and Harrison, 1969).

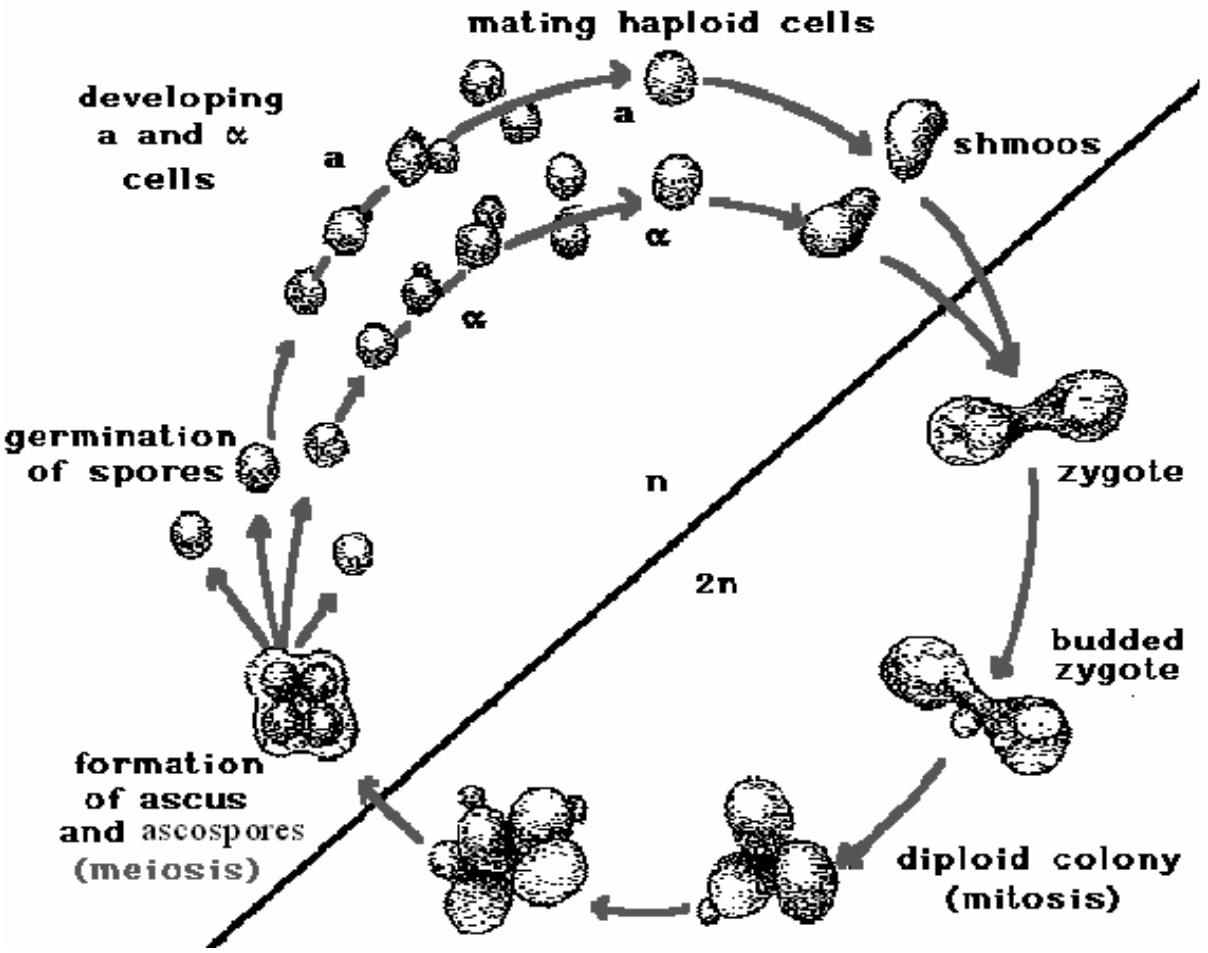

Fig.1 Yeast life cycle **(**http: //www.phys.ksu.edu/gene/al.).

Yeasts are facultative anaerobes. They can respire and survive under both aerobic and anaerobic conditions. In the absence of oxygen, they can metabolize sugar into alcohol (ethanol) and carbon dioxide and low biomass. In well aerated conditions, the cells could be able to get enough energy and convert sugar into carbon dioxide, water and biomass (Rose and Harrison, 1969; Bekatorou *et al.*, 2006).

In terms of energy, the yeast cells under aerobic conditions can get higher amount from the glucose as shown below

$$C_6H_{12}O6\ (\text{glucose}) + O_2 \rightleftharpoons 6CO_2 + 6H_2O + \text{Energy}$$

$$\Delta G = -686\ \text{Kcal / mol}$$

In anaerobic conditions the cells of yeast get very little amount of energy as shown in the reaction below.

$C_6H_{12}O_6$ (glucose) $\rightleftharpoons$ $2C_2H_5OH + 2CO_2$

$\Delta G = -54$Kcal/ mol

Therefore, yeasts increase in number and yield higher biomass under aerobic conditions and ethanol under anaerobic conditions (Rose and Harrison, 1969; Bekatorou *et al.*, 2006).

2.2 *Saccharomyces cerevisiae*

Scientific classification

Kingdom: Fungi
Phylum: Ascomycota
Subphylum: Ascomycotina
Class: Saccharomycetes
Order: Saccharomycetales
Family: Saccharomycetaceae
Genus: *Saccharomyces*
Species: *S. cerevisiae*

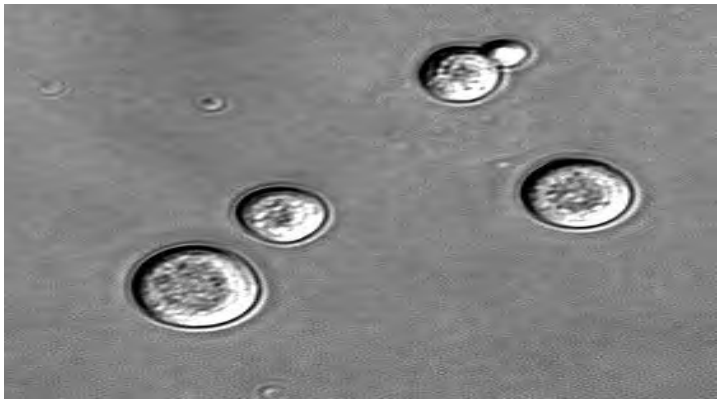

Fig.2 Saccharomyces *cerevisiae* (http://en.wikipedia.org/wiki/Yeast)

Saccharomyces cerevisiae is a species of budding yeast. It is perhaps the most useful yeast owing to its use since ancient times in baking and brewing. It is believed that it was originally isolated from the skins of grapes. It is one of the most intensively studied eukaryotic model organisms in molecular and cell biology, much like *Escherichia coli* as the model prokaryote. It is the microorganism behind the most common type of fermentation. *Saccharomyces cerevisiae* cells are round to ovoid, 5–10 micrometers in diameter. It reproduces by a division process known as budding **(**Ostergaard *et al*., 2000). "*Saccharomyces*" derives from Greek, and means "sugar mold". "*cerevisiae*" comes from Latin, and means "of beer" Balasubramanian and Glotzer (2004) .

Other names for the organism are:

- Brewer's yeast
- Ale yeast
- Top-fermenting yeast
- Baker'**s** yeast
- Budding yeast

2.3 Criteria Used in Yeast Identification and Classification

Criteria used in yeast identification and classification are described by (Cook, 1958; Lodder, 1971) as follows.

Morphological characteristics:-These include characteristics of vegetative cells, form, size and shape of cells and method of asexual reproduction. Asexual or vegetative reproduction occurs in yeasts by fission, by budding or by combination of the two.

Sexual reproductive characteristics: - These include ascus and ascospore types and interfertility in ascomycetus yeasts are used as taxonomic criteria.

Cultural characteristics:-Cultural characteristics include ability to grow in liquid or in solid media. The shape, size and color of colonies in solid media are also criteria used in yeast identification.

Physiological characteristics:- physiological characteristics include utilization carbon compounds, fermentation sugars, oxidation of carbon compounds, splitting of arbutin, utilization of nitrogen compounds**,** growth in vitamin free medium, acid production, growth on media of high osmotic pressure, growth at elevated temperature, hydrolysis of urea, fat splitting, pigment formation and ester production.

2.4 Substrates of Yeast

For their nutrition, yeasts require a source of carbon for growth and energy, a nitrogen source for synthesis of protein and other nitrogenous materials, inorganic nutrients for the build up of the normal functioning and structure of the cell, as well as vitamins (White, 1954). The raw materials used as substrates for industrial yeast biomass production are usually agricultural, forestry and food waste by-products.

There are two types of raw materials depending on the grown microorganism: conventional materials like starch, molasses, distiller's wash, whey, fruit and vegetable wastes, wood, straw, etc., and unconventional ones like petroleum by-products, natural gas, ethanol and methanol (Jay, 1996).

2.4.1 Molasses

Molasses is a by product of the sugar industry. It is residue after the crystallization of the main fraction. When no more sugar can be crystallized out of solution, the resulting liquid (molasses), containing about 50% sucrose is eliminated. For every 100 Kg of plant, some 3.5 to 4.5 Kg of molasses may be obtained (Curtin, 1983).

Based on its origin, it can be called cane molasses or beet molasses (White, 1954; Cook, 1958; Crueger and Crueger, 1990) and is the cheapest source of carbohydrate (Goksungur and Zorlu, 2001). It contains 45-55% fermentable sugars including sucrose, glucose, fructose, raffinose, melibiose and galactose (Atiyeh and Duvnjak, 2003; Bekatorou *et al.*, 2006). The fact that molasses may be extracted from at least two sources of plant adapted to tropical and temperate climates permits the obtainment of molasses in a wide range of geographical locations. The use of molasses for the production of yeast biomass has simplified the manufacturing process in many ways. Its cost is reduced as compared with the use of grain and other raw materials (Roman, 1957; Crueger and Crueger, 1990).

The molasses is used as a source of carbon, energy and other essential nutrients. Molasses could not supply all the essential nutrients for yeast growth. Therefore, the addition of supplements such as $(NH_4)_2SO_4$, urea, yeast extract or Peptone as nitrogen source, KH_2PO_4, H_3PO_4 as phosphorus source, other macro elements such as calcium in form of calcium salts, magnesium in the form of magnesium salts, microelements such as iron, zinc, copper, manganese are necessary for maximizing biomass yield of *Saccharomyces cerevisiae* or any other types of yeasts. Vitamins are also required for yeast growth (biotin, inositol, panthotenic acid and thiamine) (White, 1954; Cook, 1958; Rose and Harrison, 1971).

The composition molasses may vary quite widely depending on the location, soil type, the climatic conditions and the production process of each individual sugar factory (Curtin, 1983; Crueger and Crueger, 1990). Table1 shows the percentage composition of both beet and cane molasses.

Table1. Percentage composition of cane and beet molasses (White, 1954).

Constituent	Molasses type	
	Beet (%)	Cane (%)
Total sugar as invert	48-58	50-58
Nitrogen	0.2 -2.8	0.08 -0.5
Total solid	78- 85	78-85
P_2Os	0.02 -0.07	0.009 -0.07
MgO	0.01- 0.1	0.25 -0.5
K_2O	2.2 -4-5	0.8 -2.2
Carbon	28- -34	28 -33
SiO_2	0.1 -0.5	0.05 -0.3
Al_2O_3	0.0005 -0.06	0.01 -0.04
Fe_2O_3	0.001 -0.02	0.001 -0.01
Total ash	4.0 - 8.0	3.5 -7.5

Moasses replaced malted grain for yeast production during the First World War. It is then became common practice to develop yeast on beet and/or cane molasses and ammonium salts (such as sulphate, phosphate or chloride), with aqua ammonia as an additional source of nitrogen and as an aid in controlling the pH of the medium (White, 1954).

Clarification of molasses

For industrial propagation of yeasts molasses can be clarified either mechanically or chemically.

1. Mechanical clarification: - Molasses is usually diluted with hot water. Dilution of the molasses is used to reduce its viscosity (Reyed and E1- Diwany, 2008). The hot solution is then passed through a battery of centrifugal clarifiers arranged in parallel. After centrifugation the pellet is discarded and the supernatant is used for yeast propagation (Cook, 1958).

2. Chemical clarification- This method frees the molasses coloring matter and impurities that it contains by the number of treatments. The most widely used is the entrainment of impurities with a precipitate of tricalcium phosphate and calcium sulphate. In this process the molasses is diluted to a specific gravity of 20^0 Balling (approximately to a 20% sugar solution) acidified with sulfuric acid, heated to 65^0C with steam and calcium super phosphate is added.

The mixture is boiled, allowed to stand for an hour and then neutralized with calcium oxide. After a further 8 hours standing, the sediment of calcium salts and organic matter sinks to the bottom and the clarified solution is decanted for use (Cook, 1958).

Currently in Ethiopia there are three factories engaged in the processing of sugar cane to sugar and other products. Ethiopian Statistical Agency, ESA (2008) reported total annual production of about 141,363 tons of molasses per year by the three sugar factories. Total annual production of cane molasses in Ethiopia in the three sugar factories in 2000 E.C. is presented in table 2.

Table 2. Total annual production of cane molasses in Ethiopia in 2000 E.C. in three sugar factories (ESA, 2008).

Sugar factory	Molasses production (in tons)
Metahara	41,363
Wonji/Shoa	20,000
Fincha	80,000
Total	141, 363

2.4.2 Whey

Whey is the main waste of the dairy industry. It is produced worldwide in large amounts and its disposal causes serious environmental problems due to its high organic load, which makes its full treatment impossible (Bekatorou *et al*., 2006). On the other hand, whey has a significant nutritional value since it contains respectable amounts of proteins, lactose, organic acids, fat, vitamins and minerals. Therefore, its conversion to products of added value is a major concern for science and industry. The composition (high salt concentrations) and temperature of whey at the moment of its production in the factory do not allow easy microbial utilization. Lactose, the main sugar constituent in whey, can be metabolized only by a few species of the *Kluyveromyces* and *Candida* yeasts. The yeast *Saccharomyces cerevisiae* cannot utilize lactose because it lacks the enzyme β-galactosidase and lactose permease. *Kluyveromyces marxianus* is the only strain used for biomass production from whey on a commercial scale (White, 1954;Bekatorou *et al*.,2006).

2.4.3 Starch

Saccharomyces cerevisiae can utilize starch, only after its conversion to fermentable sugars, glucose and maltose. Hydrolysis of starch to glucose can be done either by treatment with acid or non-yeast enzymes. Enzymatic treatment includes three different processes: gelatinization by heating, liquefaction by thermostable α-amylases, and saccharification by mixed enzyme activities (Nigam and Singh, 1995). Nevertheless, processes like these imply considerable costs, which is the main limiting factor in industrial utilization of starch for yeast biomass production. Starch can be utilized by mixed cultures of yeasts and amylolytic fungi like *Aspergillus* species for SCP or ethanol production (Nigam and Singh, 1995; Jay, 1996). According to Jay (1996) and Nigam and Singh (1995) using starch for yeast biomass yield has disadvantages. These are;

1. It has competing value as food
2. Production costs are high due to the high energy consumption in the cooking process
3. The addition of large amounts of amylolytic enzymes.

2.4.4 Residues of forestry and agriculture

Wastes of agriculture and forestry are rich in cellulose, hemicellulose and lignin. Their enzymatic conversion to fermentable sugars requires chemical pretreatment that leads to various polymer fragments. *Saccharomyces cerevisiae* does not have the variety of enzymes required to hydrolyze these polymers. As a result, yeast biomass production on lignocellulosic wastes implies a high economic cost. A solution to this problem could be the use of mixed cultures of *Saccharomyces cerevisiae* and cellulolytic microorganisms, but this process is today applied for ethanol production in pilot plants only (Cuzens and Miller, 1997).

2.5 Factors Affecting Yeast Growth

Yeast growth is affected by a number of factors. These include composition of medium commonly sugar source, aeration (oxygen), agitation of the medium, pH, temperature and period of propagation. Some of the factors affecting yeast growth are discussed below.

2.5.1 Sugar feed (media composition)

The main carbon and energy source for most yeast is glucose which is converted via the glycolytic pathway to pyruvate and by the Krebs cycle to anabolytes and energy in the form of ATP.Yeasts are further classified according to their modes of further energy production from pyruvate: respiration and fermentation. These processes are regulated by environmental factors, mainly glucose and oxygen concentrations.

In respiration, pyruvate is decarboxylated in the mitochondrion to acetyl- CoA which is completely oxidized in the citric acid cycle to CO_2, energy and intermediates to promote yeast growth.

In anaerobic conditions, glucose is slowly utilized to produce the energy required just to keep the yeast cells alive. This process is called fermentation, in which the sugars are not completely oxidized yielding CO_2 and ethanol (Scragg, 1991; Bekatorou *et al.*, 2006) as final product.

When the yeast cell is exposed to high glucose concentration, catabolite repression occurs, during which gene expression and synthesis of respiratory enzymes are repressed, and fermentation prevails over respiration(Rincon *et al.*,2001: Bekatorou *et al.*,2006).

In industrial practice, catabolite repression (repression of gluconeogenesis, the glyoxylate cycle, respiration and the uptake of less preferred carbohydrates) by glucose and sucrose, also known as Crabtree effect, may lead to several problems, such as incomplete fermentation, development of off flavors and undesirable by products as well as decreased biomass and yeast vitality (Verstrepen *et al*., 2004; Bekatorou *et al*., 2006). Industrial production of *Saccharomyces cerevisiae* is therefore, performed in aerobic, sugar limited fed-batch cultures (Van Hoek *et al.,* 1998; Mickiewicz and Borowiak, 2005).

2.5.2 Aeration

Highly aerobic culture conditions are used in the production of yeast specifically baker's yeast to maximize cell growth (Campelo and Belo, 2004). The modern technique of baker's yeast production is based on applying the principle of the Pasteur reaction at the limit value of its aeration. Pasteur defined fermentation as life without air. Its biochemistry involves the breakdown of carbohydrates only to the stage of ethanol.

Under aerobic conditions, however, maximum growth occurs and the efficiency of utilization of carbohydrate increases as respiration and the breakdown of the carbohydrate to carbon dioxide and water becomes complete (Cook, 1958).

Generally oxygen has the following basic functions (Prescott and Dunn, 1959).

1. Inhibits fermentation
2. Increases respiration
3. Agitation of the medium
4. Removal of toxic end products
5. Stimulation of vegetative growth

Oxygen is used in the synthesis of unsaturated fatty acids and sterols which form the cell membrane. These molecules are important for both growth and fermentation and serve as a means for storing oxygen within the cell. They are also necessary for increasing cell mass (growth) involving the over all uptake of nutrients and determining alcohol tolerance. Oxygen stimulates the synthesis of molecules necessary for yeast to metabolize and take up maltose and other sugars

2.5.3 Temperature

The temperature most favorable to the growth of baker's yeast varies from strain to strain. Optimum temperature is usually between 25^0C-35^0C. The maximum survival temperature is 37^0C (Cook, 1958). Propagation at low temperature, the rate of growth is slower and gives a decreased yield of yeast. Yeast grown in low temperature is less stable when stored and transported as a pressed cake and the dry matter of a yeast cake of standard consistency becomes progressively less at low temperature, i.e., it affects the relationship between intracellular and extra cellular water content.

2.5.4 pH

Yeasts grow well at acidic pH (acidophilic organisms). For industrial propagation low pH is helpful in restricting the development of many bacterial contaminations; however, the color of the yeast may be affected at low pH. The pH of the media is commonly adjusted by the addition of H_2SO_4, NH_3, Na_2CO_3 or $NaHCO_3$ (Prescott and Dunn, 1959) to the substrate.

2.6 Yeast Dry Matter

The main elements present in baker's yeast are carbon, hydrogen, oxygen and nitrogen which normally account for as much as 94% of the dry matter (White, 1954). Table 3 shows elementary analysis of yeast dry matter.

Table3. Elementary analysis of yeast dry matter (White, 1954).

Constituent	Percent of yeast dry matter
carbon (C)	45.0-49.0
Hydrogen (H)	5.0- 7.0
Oxygen (O)	30.0 -35.0
Nitrogen (N)	7.1 -10.8
Total ash	4.7- 10.5
Phosphate (P_2O_5)	1.9- 5.5
Potash (K_2O)	1.4- 4.3
Calcium (CaO)	0.005 – 0.2
Magnesium (MgO)	0.1 – 0.7
Aluminum (as Al_2O_3)	0.002 – 0.02
Sulphate (as SO_4)	0.01- 0.05

These four elements are present on the yeast in the form of carbohydrates (glycogen, cellulose, and yeast mannan), protein and lipids (true fats, lecithin, and sterols). Yeasts also contain high amount of vitamin B complex, nucleic acids and organic bases (pyramidine and purine bases). The inorganic substances may constitute up 6 to 8 % of the yeast dry matter (White,1954;Roman,1957) Table 4 gives the quantities of some substances present in yeast dry matter (White, 1954).

Table 4. Composition of yeast dry matter (White, 1954).

Constituent	Percent of yeast dry matter
Ash	Normally 6 -8
Glycogen	1 -30
Fat soluble fraction(true fats, sterols. lipids	1 -2. 2
Yeast gum (mannan)	Up to 4
Cellulose (yeast)	Up to 5
Proteins and organic nitrogenous bases	44 – 47

2.7 Production of Baker's Yeast (*Saccharomyces cerevisiae*)

2.7.1 History of production

The first written record of the actual existence of bread dates to around 2600 B.C. in Babylonia. This discovery of leaven bread was generally attributed to ancient Egyptians. In ancient Jerusalem for example, public bakeries were established which "produced small beads similar to the present day bread rolls (Haider *et al.*, 2003). The practice of using beer yeast in sourdough fermentation continued up to the 19th century and commercial bakers obtained their yeast supplies from local brewers.

Due to its bitter test and variable fermentation activity, brewers yeast was gradually replaced by baker's yeast. The earliest production of pressed baker's yeast probably occurred around 1781 in Holland with the so called Dutch process, the yield of pressed yeast was equivalent to only 4-6 % of the weight of the raw material used (Cook,1958).

In 1846, Maunter developed the Vienna process. In this process, yeast was recovered from the entire batch by continuously collecting the foam produced during fermentation. The yield of compressed yeast was increased to about 14% plus a concurrent yield of 30% ethanol (Cook, 1958; Prescott and Dunn, 1959). Marquardt introduced aeration of the grain mash in 1879, resulting in the yield of yeast biomass to 50% -60% and decreased ethanol to 20%. In 1919, a process was invented by Sac in Denmark and Hayduck in Germany in which sugar solution was fed to an aerated suspension of yeast instead of adding yeast to a diluted sugar solution. This process was known as "Zulaufverfahren". An incremental feeding or fed batch process was thus introduced for the first time. At about the same time, molasses, because of the food shortage during World War I replaced the traditional grain mash. These refinements gradually raised the yield of compressed yeast to the theoretical maximum of 50% by weight of raw material used, with no concomitant ethanol formation. Such accomplishments eventually led to the development of a baker's yeast industry independent of alcoholic beverage production (Cook, 1958).

Today, the scientific knowledge and technology allow the isolation, construction and industrial production of yeast strains with specific properties to satisfy the demands of the baking and fermentation industry (beer, wine, baking) (Phaff,1990*).* A selected strain of baker's yeast, *Saccharomyces cerevisiae*, is used for industrial-scale production. These strains are selected for stable physiological characteristics, vigorous sugar fermentation in dough, and cellular dispersion in water, no autolysis during the fermentation, rapid growth and high cell yields, and easy maintenance during storage.

The industry required that, the fermentation of baker's yeast has to produce a product with minimum variation in yeast performance, maximum yield on raw material and minimum production of undesirable side products (Haider *et al.,* 2003).

2.7.2 Commercial practice of baker's yeast production

2.7.2.1 Propagation processes

Yeasts were used to raise bread in Egypt from 4000 BC, and fermented dairy products Such as cheese and yogurt were developed early in history (Haider *et al.,* 2003*).* The ability to cultivate large quantities of organisms is achieved by using a vessel known as a fermenter or bioreactor. A fermenter is a vessel in which an organism is cultivated in a controlled manner to produce the organism cell mass itself, or a product produced by the cell. In some specialized cases, fermentation is used to carry out specific reactions. The use of such vessels has allowed laboratory results to be scaled up as large as 260,500 liter and become a commercial process. Currently, there are approximately 200 commercial fermentation products. These products can be classified in three general groups

(1) Those that produce microbial cells.

(2) Those where the product is produced by the cells.

(3) Those that modify a compound, which is added to the fermentation, a process known as biotransformation *(*Acourene *et al.,* 2007).

Industrial propagation of yeast is done on abundantly available and cheap agricultural and industrial wastes, mainly molasses, by successive submerged fermentation. After fermentation, the yeast biomass is harvested and may be subjected to downstream processing steps, like washing to remove the impurities, cell disruption, protein extraction and purification to extract enzymes, molecules like RNA,DNA,B vitamins etc., (Bekatorou *et al.*, 2006).

Industrial yeast production generally involves the following stages: propagation involving a number of fermentation processes, harvesting, concentration and / or drying, packaging and shipment. Fig.3 presents a commercial baker's yeast propagation scheme.

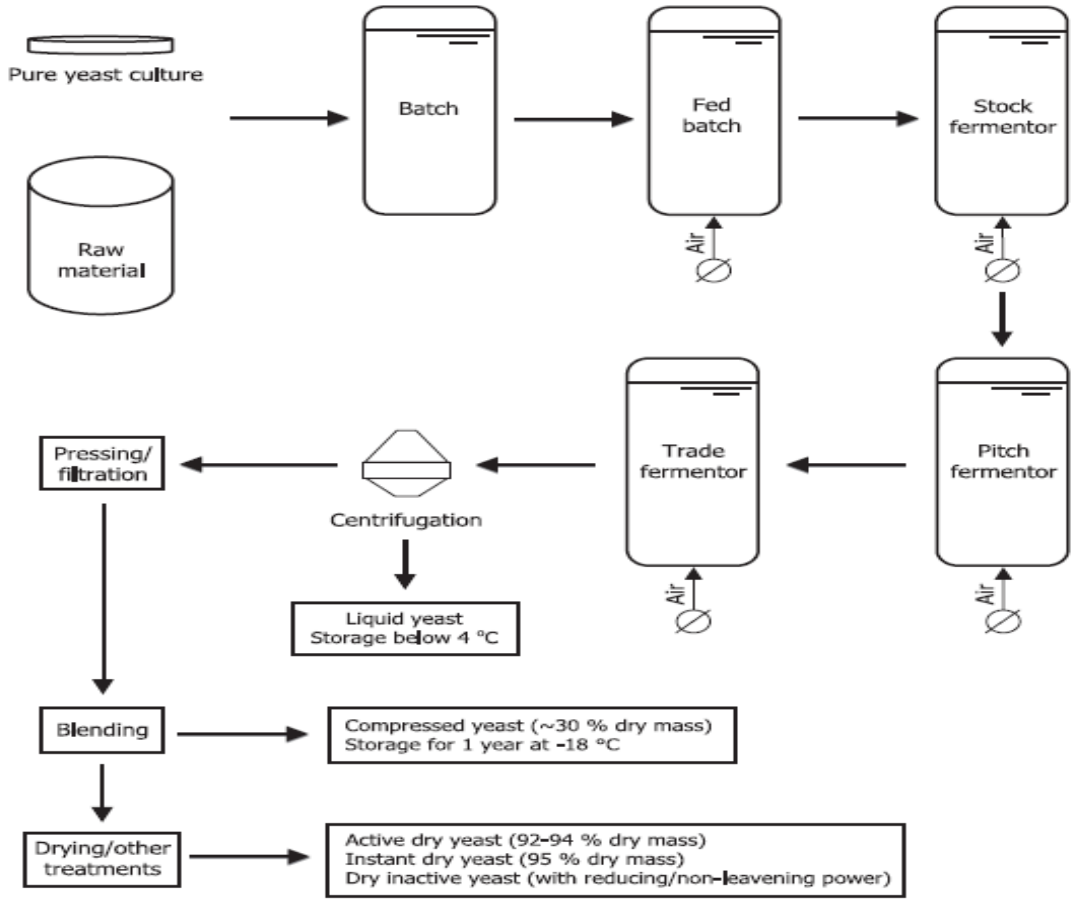

Fig.3 Propagation scheme for the production of baker's yeast (Randez-Gil *et al*., 1999).

Yeast cells are grown in a series of fermentation bioreactors, which are operated under aerobic conditions to promote yeast growth. Initially, cells from a pure yeast culture are grown on a suitably adjusted mixture of molasses in the laboratory and the produced biomass is transferred aseptically into one or more bioreactors which operate in batch mode with out air supply.

The next bioreactor usually operates in fed batch mode with air supply, and the produced biomass is used to pitch the stock bioreactor. The biomass produced in this bioreactor is harvested by centrifugation and used in the next stage, the pitch fermentation. Both of these stages operated in the fed batch mode with vigorous aeration and incremental addition of nutrients. The biomass produced in the pitch bioreactor is used to pitch the final production fermentation. At the end of the process, the content in the production bioreactors is aerated for an additional time period and this is the maturation stage. The amount of yeast biomass produced increases from stage to stage to time and the sequence and the number of fermentation stages vary among manufacturers.

2.7.2.2 Treatment and packaging

The yeast in the final production bioreactor is concentrated by centrifugation and finally harvested by a filter press or a rotary vacuum filter. The yeast cake is blended with suitable amounts of water and emulsifiers and cutting oils (soybean or cotton seed oil) to obtain its extrudable form.

The yeast is then packaged and shipped as compressed fresh baker's yeast or thermolyzed and dried to form various types of dry yeast. The dried yeast is packed under vacuum or nitrogen atmosphere.

2.7.2.3 Formulations of baker's yeast

Baker's yeast as a commercial product has several formulations that can be grouped into two main types: compressed yeast, called fresh yeast, has moisture content 70-75 % (Cook, 1958) and the dried yeast (Daramola and Zampraka, 2007).

Compressed yeast is the traditional formulation of baker's yeast, and is ready for immediate use. Dried (dehydrated) yeast is available in two forms: Active dry yeast (ADY) and instant Dry yeast (IDY). Active Dry yeast (ADY) is normally sold in air tight packages, vacuum seal or filled with an inert gas such as nitrogen. It is not a problem to maintain quality, but it should be rehydrated before use. Unlike ADY, instant day yeast (IDY) does not have the cell damage during rehydration (Daramola and Zampraka,2007).

Inactive dry yeast: - is a product without leavening properties, used for the conditioning of dough properties in baking or the development of characteristic flavor (Bekatorou *et al.*, 2006).

2.8 Industrial and Related Uses of Yeasts

The useful physiological properties of yeasts have led to their use in the field of biotechnology. Fermentation of sugars by yeast is the oldest and largest application of this technology. Many types of yeasts are used in making many types of foods and beverages. Baker's yeast in bread making, brewers yeast in beer fermentation and wine yeast in wine fermentation. Yeasts also used in single cell protein production. Industrial biotechnology is a rapidly growing field. The genus *Saccharomyces* contains yeasts used in the field of biotechnology. The most commercially used yeast is baker's yeast. Baker's yeast is composed of almost exclusively of cells of one or more selected strains of *Saccharomyces cerevisiae* (Soares *et al.*, 2002; Oliveira, 2006).

With the increasing shift towards a bio-based economy, there is rising demand for developing efficient cell factories that can produce fuels, chemicals, pharmaceuticals and food ingredients. The yeast *Saccharomyces cerevisiae* is extremely well suited for the above purposes.

As one of the most intensely studied eukaryotic model organisms, a rich density of knowledge detailing its genetics, biochemistry, physiology, and large-scale fermentation performance can be capitalized upon to enable a substantial increase in the industrial application of this yeast.

Developments in genomics and high-throughput systems biology tools are enhancing one's ability to rapidly characterize cellular behavior, which is valuable in the field of metabolic engineering where strain characterization is often the bottleneck in strain development programmes (Nielsen and Jewet, 2007).

2.8.1 Production of Yeast cells

(a) Baker's yeast:-the yeast, *Saccharomyces cerevisiae*, is used in baking as leavening agent where it converts the fermentable sugar present in the dough into carbon dioxide. This causes the dough to expand or rise as the carbon dioxide forms pockets or bubbles. When the dough is baked it sets and the pockets remain, giving the baked product a soft and spongy texture (Nishida et *al*, 2004). According to the Industrial Extension Bureau of Mott MacDonald India (2007) report, European and Asian regions produced 51 million tons of baker`s yeast in the year 2004-2005.

(b) Single cell protein: - The significance of yeasts in food technology as well as in human nutrition, as an alternative source of protein to cover the demands in a world of low agricultural production and rapidly increasing population makes the production of food grade yeasts extremely important (Bekatorou *et al.*, 2006). A large part of the earth's population is malnourished, due to poverty and inadequate of food. Scientists are concerned whether the food supply can keep up with the world population increase, with the increasing demands for energy, the ratio of land area required for global food supply or production of bioenergy, the availability of raw materials, as well as the maintenance of wild biodiversity (Zheng, *et al.*, 2005).

Therefore, the production of microbial biomass for food consumption is a main concern for the industry and the scientific community. The impressive advantages of microorganisms for SCP production compared with conventional sources of protein (soybeans or meets) are well known. Microorganisms have high protein content and short growth time, leading to rapid biomass production, which can be continuous and is independent from the environmental conditions.

The yeasts can be propagated using cheap raw materials and easily harvested due to their bigger cell sizes and flocculation abilities. Moreover, they contain lower amount of nucleic acids than bacteria (Bhattacharjee, 1970) making them more acceptable as food supplement. The large scale production of yeast for nutritional uses was experienced in Germany during both world wars. Eventually, about 16,000 tons of *Candida utilis* was incorporated in to human food per year during the Second World War by Germans (Bhattacharjee, 1970).

Single cell protein from yeasts is also used as a protein supplement of fodder mixtures in the nutrition of domestic animals (Bekatorou *et al*., 2006).

Yeast is recommended as a food supplement because of the following characteristics (Bhattacharjee, 1970; Pederson, 1979).

(a) Ability to utilize cheap raw materials
(b) Rapid growth rate
(c) Palatability
(d) Lack of pathogenicity
(e) High content of valuable proteins
(f) Genetically stable
(g) Easily digested giving high nutritional value.

A young chicken or pig may double its weight in a month, but a yeast cell does this in less than two hours. A cell factory with 10 large sized fermenting tanks can provide 10 tons of yeast per day on a continuous basis. The equivalent of these yeasts would require killing of 80 pigs a day or 30,000 pigs in a year (Bhattacharjee, 1970).

Human and animal protein such as rennin, growth hormone and hepatitis B vaccine are produced from yeast (*Saccharomyces cerevisiae*) cells (Brock and Madigan, 1991; Torrado *et al.*, 2005; Bechem *et al.*, 2007; Sidorenko *et al*., 2008).

2.8.2 Yeasts for Bioethanol Production

The natural energy resources such as fossil fuel, petroleum and coal are being utilized at a rapid rate and these resources have been estimated to last over a few years. Bioethanol is considered as alternative energy resource for the above energy resources (Gunasekaran and Chandra, 1999). Ethanol is a renewable energy resource produced by yeast (Humphrey and Caritas, 2007).

As described by Hook (1988) and Akpan *et al.*, (2005), in addition to energy resource, ethanol has the following applications. It is the most common organic solvent, it is as an intermediate in the production of liquid detergents, used in the making of perfumes, Paints, explosives plastics, plasticizers, cosmetics, antifreeze, reduces global warming, helps diversification of agricultural industries, increases employment, lignin utilization in electricity generation(future project),increase research and technology and related scientific fields.

Brazil is the pioneer in large-scale motor fuel ethanol production through the fermentation of sugar cane molasses by yeast, producing in the year 2004 about 14.2 billion liters of bioethanol (Marcos *et al*.,2006), most of which is fermented using hexose sugars present in cane syrup (Monte *et al.*, 2003).

Intense research has been carried out for obtaining efficient fermentative organisms, low cost fermentation substrates, and optimum environmental conditions for fermentation to occur. For this purpose Saccharomyces *cerevisiae* is found to have high potential for ethanol production (Nowak, 2001).Efforts are underway to improve the industrial performance of yeasts to ferment sugars to ethanol. The efforts are not only restricted to yeasts but also to bacteria such as *Zymomonas mobilis.* Efforts include improving the efficiently by which sugars are converted to ethanol (i.e., yield of 90% of theoretical yields needed), increasing the rate of productivity, development of strains tolerant high ethanol levels and resistant microorganisms to fermentation inhibiting compounds (Ladisch and Dyck, 1979; Dien , 2003).

2.8.3 Alcoholic beverages from yeast

Industrial strains of *Saccharomyces cerevisiae* are used by much food companies as starters for fermentative processes (Torrado *et al.,* 2005). Fermentation products from yeast include ethanol (industrial alcohol), glycerol, and beverage alcohol such as beer and wine, distilled beverages such as whisky, brandy, vodka and rum (Brock and Madigan, 1991; Torrado *et al.*, 2005).

2.8.4 Other yeast products

Yeast extract- Yeast extract is the product of enzymatic digestion of the yeast cell constituents by endogenous and exogenous yeast enzymes. It is rich in peptides, amino acids, nucleotides and vitamins; therefore it is good for use as supplement in culture media. It is also used in pharmaceuticals, as well as flavor and taste enhancer (replacing glutamate and nucleotides) in many canned foods (Bekatorou *et al.,* 2006).

Other yeast products include B vitamins (Peterson and Elvehjem, 1939; Brock and Madigan, 1991), vitaminD, enzymes for industry (invertase, galactosidase), Biochemicals; for research, ATP, NAD, RNA.Biochemicals for research are used as model in genetic studies of eukaryotes (Brock and Madigan, 1991).

2.8.5 Flavor enhancing property

Yeasts contribute to aroma and flavor during the making of bread dough (Brock and Madigan, 1991; Van Hoek *et al.*, 1998; Nishida et *al*, 2004). Baker's yeast produces the CO_2 that results in dough leavening and contributes to the flavor and crumb structure of bread (Randez-Gil *et al.*, 1999).

2.9 Market and Growth Drivers

The principal use of baker's yeast is as an essential bakery ingredient for causing fermentation in the dough used in making bakery items. This process helps making soft and fluffy bakery items like variety of breads, bread rolls, pizza base, cracker biscuits, sweet breads and burger buns etc.

From Industrial Extension Bureau Mott MacDonald India (2007) report , the growth of bakers yeast market is directly linked to the increasing trend of processed and fast food consumption, especially bakery items. The European and Asian regions produced 51 million tons of bakery items, valued at US $ 107 billion, in the year 2004-2005. As per the emerging global trend China is presently one of the most promising markets for baker's yeast, as its demand is continuously increasing with the rise in population and changing demand of bakery products. Baker's yeast market in developing countries is touching new highs with increasing demand for processed foods and a consistent growth in bakery items production, compensating for the slow growth averaging 1% to 2% in developed countries, where the market is saturated.

India's bakery production in the year 2004-2005 registered a growth rate of around 20 % producing approximately bakery items. From the total bakery production, the bread production alone was estimated a growth rate of 7.5%.

Growth Drivers

- Increasing consumption of bread as a staple food rather than just a breakfast
- Increasing number of people in urban and semi urban areas and changing their food consumption habits and the pattern of people will drive the growth of bakery industry and in turn the growth of baker's yeast demand.
- Demand for bakery products is increasing as they are aṇ essential content of many fast food items and people now increasingly prefer convenience products over traditional food items

2.10 Current Status of Baker's Yeast Demand in Ethiopia

Ethiopia is a developing country and there is a high of baker's yeast demand from a number of bakeries. The urban people are somewhat changing their food habit. This is because of the fast food culture of the western world. One of the basic ingredients of these fast foods is bread. As a result the use of commercial baker's yeast is increasing day to day. Moreover, a number of alcohol and beverage industries (beer and wine) are built and these industries need tremendous amount of yeast. There no baker's yeast producing plant in the country. Baker's yeast for the country's demand is imported from many partner countries.

Table 5 shows total amount of yeast (active and inactive) imported to Ethiopia, the cost and partner countries in the years 2006 and 2007 (ESA, 2008).

Table 5. Amount of yeast (active dry and inactive dry) in kilograms, year imported, partner countries and the cost in ETB (ESA, 2008).

Year	2006				2007			
Type of yeast	Active dry yeasts		Inactive dry yeasts		Active dry yeasts		Inactive dry yeasts	
Source	Net wt	Birr	Net wt	Birr	Net wt	Birr	Net wt	Birr
Belgium	59,560	1,084,408	-	-	16,720	380,606	1	72
China	-	-	1	71	66,164	1,173,064	78	20,058
Croatia	-	-	-	-	78	59,982	-	-
Denmark	2	4,298	-	-	3	9,735	-	-
Djibouti	637,502	12,382,702	-	-	-	-	-	-
Egypt	-	-	-	-	26,650	409,788	-	-
France	42,617	1,176,276	65	47,809	631,674	12,371,443	3	529
Greece	-	-	-	-	550	8,314	-	-
India	-	-	86	13,490	-	-	1	207
Iran	220,540	1,226,953	-	-	386,824	2,271,311	-	-
Italy	31,192	746,108	43	5,660	-	-	-	-
Malaysia	-	-	-	-	71	1,152	-	-
Malta	-	-	-	-	1,584	28,784	-	-
New Zeeland	-	-	-	-	1	137	-	-
Qatar	-	-	-	-	73	68,362	-	-
Tokelau	296,830	757,688	-	-	-	-	-	-
Turkey	839,651	13,636,353	5	452	1,017,629	15,675,662	32,040	521,344
Turtles and Caicos Islands	-	-	-	-	33,230	88,710	-	-
UAE	3,676	25,477	-	-	27	267	-	-
United Kingdom	1	100	-	-	-	-	-	-
USA	-	-	-	-	-	-	3	88
Total	2,131,571	31,040,363	200	67,482	2,181,278	32,547,317	32,126	542,298

As reported by the Ethiopian Statistical Agency (ESA, 2008) the country has imported a total of 2,131,571 kilograms active and inactive dry yeasts in the year 2006 and 2,213,404 kilograms of active and inactive dry yeast in the year 2007. The amount of yeast imported to the country in the year 2007 has increased by 81,831 kilograms from the 2006 import. A considerable amount of foreign currency is spent to meet the annual demand of our bakery ingredients and alcohol industries. This is because baker's yeast is purchased with dollars and there is extra cost for transportation.

For instance the national expenditure of money in Ethiopia upon importing active and inactive dry yeasts together in the years 2006 and 2007 as shown in table 5 was 31,107,845 ETB(3110785 US $) and 33,089,615 ETB (3308962 US $) respectively. The country in 2007 has spent cost 1,981,770 ETB (198,177 US $) from the 2006 cost. Baker's yeast producing plant is needed to be established in our country to fulfill the demand and to save foreign currency.

Wild yeasts play the major role in the fermentation of local fermented foods and beverages such as *injera*, *kocho*, *tella* and *tej* (Pederson, 1979; Samuel Sahle and Birhanu Abegaz Gashe, 1991; Mogessie Ashenafi, 2002).

3. MATERIALS AND METHODS

3.1 Yeast Strains

3.1.1 Isolation of yeast strains from *teff* dough and *tella*

Teff dough and *tella* samples were obtained from two house holds in Addis Ababa, Yeka Sub City, and Kebele 09/10. One ml of each of the samples were transferred to 9 ml of sterile distilled water and mixed thoroughly. Serial dilutions (10^{-1} -10^{-6}) were done. At each successive step of the series, the dough or the *tella* samples were maintained in increasing dilute aqueous suspensions. Aliquots of 0.1ml from appropriate dilutions were spread plated on YMPGA medium.

The medium contains gm/l: yeast extract 3, malt extract 3, peptone 5, glucose 10 and agar 20. Chloramphenicol (0.1 gm/l) was added to each media to inhibit bacterial growth. The samples were incubated at a temperature of 25^0C for 48 hours. Colonics were enumerated, characterized and recorded. The colonics were transferred to slantYMPGA cultures and preserved at 4^0C for further study.

3.1.2 Isolation of active dry baker's yeast into pure culture

The active dry baker's yeast, *Saccharomyces cerevisiae* by the name saf- levure, France was bought from a supper market. 0.5 gms of this yeast were used for this experiment and suspended in sterile distilled water aseptically.

Serial dilutions (10^{-1} -10^{-6}) were made to reduce the number of yeast cells as described above. Aliquots of 0.1 ml of the suspensions were spread plated on to YMPGA media. The cultures on the agar plate were incubated at 25^0C for 48 hours.

3.1.3 Characterization of yeasts

A single colony was picked and transferred to YMPGA slant under aseptic conditions and preserved at 4^0C for future work. The yeast cells were observed under the microscope (400×) (Bosch and Lomb) to determine shape of cells and budding formation.

Induction of ascospore formation and observation

For production of ascospores from *tella* and *teff* dough yeasts, the methods of Lodder (1971) and Kirsop and Kurtzman (1988) were followed. Accordingly, two types of sporulation media were prepared. These were Gorodkowa agar and Macclary acetate agar medium.

Gorodkowa agar medium

Ten grams of peptone, 1.0 gram glucose, 5.0 grams of NaCl, and 10 grams of meat extract and 20 grams of agar were dissolved in a liter of distilled water. The contents were boiled in a water bath. Then, they were dispensed into tubes and were sterilized by autoclaving. They were allowed to lean to make slants.

Macclary acetate agar medium

Two point five grams of yeast extract, 1.0 gram of glucose, 8.2 grams of sodium acetate trihydrate, 1.8 grams of potassium chloride and 15 grams of agar were dissolved in a liter of distilled water. The constituents were heated and dispensed into tubes. They were autoclaved and slanted. In both media, loopful of yeast samples (24hours culture) were inoculated and incubated at 25^0C for 3 weeks.

Observation of ascopores

Yeast samples were wet- mounted on a glass slide to observe types of ascospores. They were also heat fixed and spore stained according to Lodder (1971). Accordingly the heat fixed samples were flooded with 5% aqueous malachite green for 30-60 seconds. They were heated to steaming 3 to 4 times. The excess stain was run off under running tap water for half a minute. The preparations were then counterstained with 0.5% safranin red for about 30 seconds. The excess stain was gently washed with running tap water for half a minute. The preparations were observed both under high power (400×) and oil immersion objectives (1000×).

3.2 Preparation of Molasses

Cane molasses was obtained from Wonji/Shoa sugar factory at low cost. For each experiment the molasses was diluted1:3 using distilled water. The diluted molasses was then boiled and allowed to cool for the sedimentation of insoluble materials.

The substrate was repeatedly centrifuged with a high speed at 5000×g for 10 minutes for retrieving the soluble materials in the supernatant.

The supernatant soluble was adjusted to appropriate pH using HCl and NaOH, autoclaved at 121^0C for 15 minutes and preserved at 4^0C to be used as substrates for further experiments.

Fig.4 Molasses prepared for the propagation of commercial baker`s yeast, *teff* dough and *tella* yeasts.

3.3 Physical and Biochemical Characterization of Molasses

3.3.1 Determination of dry weight molasses

Dry weight of the molasses sample was determined by following Krishna and Ranjhan (1980). About 5 grams of the molasses sample was weighed in pre weighed empty crucible. The crucible with the molasses sample was kept in an oven at 105^0C for 2hrs.The crucible with the molasses sample was cooled in a desiccator and weighed.

The process of heating, cooling and weighing was repeated until constant dry weight was obtained. The percentage dry weight of the molasses sample was determined as follows.

Moisture, percent $= \frac{(W_1 - W_2)}{(W_1 - W)} \times 100$

by weight

Where: W_1-Weight in grams of the crucible with the molasses sample before drying

W_2-weight in grams of the crucible with the dried sample

W- Weight in grams of the empty crucible.

Dry weight, percent =100-moisture percent

3.3.2 Determination of total ash

Total ash in the molasses sample was determined following the methods described by AOAC (2000) and Krishna and Ranjhan (1980). Crucible was dried in an oven at 100^0C for six hours. It was removed from an oven and kept in a desiccator and after its constant weight was taken. Five grams of the molasses sample (wet weight) was taken and placed in weighed crucible. Then the crucible with molasses sample was kept in a muffle furnace and the temperature was set at 550^0C for 2 hours. Then it was removed from the muffle furnace and allowed to cool in a desiccator and weighed. At last the following simple formula was applied to estimate total ash in percentage.

$$\text{Total ash \%} = \frac{(W_2 - W)}{(W_1 - W)} \times 100$$

Where: W- weight of empty crucible

W_1 – weight of the crucible plus the dried molasses

W_2 - weight of the crucible plus ash

3.3.3 Estimation of total sugar

Total sugar of the molasses sample was determined by DNS method (Miller, 1959). The DNS solution was prepared by dissolving sodium sulfite 0.5 gm, phenol 2.0gm, sodium hydroxide 10.0 gm, potassium sodium tartarate 200 gm and 3-5dinitrosalicylic acid 10.0 gm in a liter of distilled water. For hydrolysis, 5.0 ml of distilled water and 1.0 ml of concentrated HCl were added to 5.0 ml molasses. The contents were boiled for one minute, followed by cooling, neutralizing with 4.4 ml of 2.5 N NaOH and diluted appropriately according to Sheoran *et al.*, (1998).

A standard glucose curve was made by using the standard proportion of glucose according to Miller(1959).The standard curve for glucose was prepared by adjusting the final concentration to 0.2 mg/ml as 0,0.02,0.04,0.06,0.08,0.1,0.12,0.14,0.16,0.18,0.2 where the actual proportion of glucose solution to water was 0:1000 20:980, 40:960, 60:940, 80:920, 100:900, 120:880, 140:860, 160:840,180:820, and 200:800.

Two ml of DNS solution was added into each dilution. This was followed by boiling the mixture for five minutes. Absorbance of the mixture was read in a spectrophotometer at 540 nm after having cooled at room temperature. The standard formula Y= aX +b was developed based on the result of the standard curve.

Where X- the amount of glucose equivalent

Y- Absorbance value

3.3.4 Determination of total nitrogen and crude protein

Total nitrogen (nitrogen of protein and other compounds) was determined using Kjeldahl method according to Krishna and Ranjhan (1980). A digestion flask containing about l gram molasses sample , 6 ml of acid mixture (concentrated sulfuric acid and concentrated orthophosphoric acid) and about 3 gram of catalyst mixture (K_2SO_4 selenium, and$CuSO_4$) were boiled to about 370^0C in order to allow digestion. Then distillation took place by adding 25ml of 40 % NaOH and using 25 ml of 2% boric acid and 10 drops of indicator solution. Finally, the distillate was titrated with standardized 0.1N sulfuric acid to a reddish color. The total nitrogen percent and crude protein content was determined using the following formulae.

$$\text{Total nitrogen (percent) by weight} = \frac{(V_2 - V_1) \times N \times 0.014}{W} \times 100$$

Where: V_2 - volume in ml of the standard sulfuric acid solution used in the titration to the test material (molasses sample).

V_2 - volume in ml of the standard sulfuric acid used in the titration for the blank determination

N- Normality of standard sulfuric acid solution

W -Weight in grams of the test material in dry matter basis.

Crude protein content, percent per weight = total nitrogen X 6.25(AOAC, 2000)

3.4 Inoculum Preparation

The yeast strains were grown in a broth culture medium containing gm/l: yeast extract, 3.0;peptone,5.0;glucose,2.0;sucrose,15.0;$Na_2HPO_4.12H_2O$,2.4;$MgSO_4.7H_2O$,0.075 and $(NH4)_2SO_4$,5.1 (O-Neto *et a*l., 1997).The medium was sterilized at 121^0C for 15-minutes inoculated with a loopful of yeast samples from the slant. The broth culture was allowed to grow for 24hours. 0.9% V/V inoculum size was used in each case.

3.5 Effect of Molasses Concentration on Yeast Growth

To evaluate the effect of molasses concentration on yeast biomass yield the following experiment was carried out. 0.9 % v/v of inoculum from each yeast strain was dispensed into 250ml flasks containing 3 % w/v, 5 % w/v, 8 % w/v and 10 % w/v of molasses. The pH was adjusted at 4.5 before sterilization of the molasses media using HCl and NaOH. Flasks were incubated at temperature of 30^0C. Yeast biomass yield was recorded after 24, 48, 72 and 96 hrs growth. The optimum concentration (8% w/v) of molasses was used for further work (investigation).

3.6 Effect of pH on Yeast Growth

The effect of pH on yeast biomass yield was tested as follows. Molasses having a concentration of 8% w/v and pH values of 3.5, 4.0, 4.5, 5.0 and 5.5 were prepared and inoculated with 0.9 % v/v of yeast inoculum and incubated at 30^0Cfor 24, 48, 72 and 96 hrs.

3.7 Effect of Temperature on Yeast Growth

The effect of temperature on yeast biomass yield of the three yeast strains was tested. The process of biomass propagation was undertaken at temperatures of 25^0C, 30^0C and 37^0C using 8% w/v molasses. The pH of the medium was maintained at 4.5. Yeast biomass yield was recorded after 24, 48, 72 and 96 hrs growth.

3.8 Optimization of Yeast Biomass

To increase yeast biomass yield different amounts of nutrients were added to the molasses. The effectiveness of each supplement was evaluated at pH 4.5 and temperature of 30^0C in terms biomass after 24, 48, 72 and 96 hrs incubation. Each supplement was added according Van Hoek *et al.*, (1998).The different treatments were:

Treatment1 (T1) -8 w/v % molasses + 0.5 % w/v $(NH_4)_2\ SO_4$

Treatment2 (T2) -8 w/v% molasses + 0.5 % w/v $(NH_4)_2\ SO_4$ + 0.3 % w/v $KH_2\ PO_4$

Treatment3 (T3) -8% w/v molasses +0.5% w/v $(NH_4)_2SO_4$ +0.3% w/vKH_2PO_4 +2% w/v peptone

Treatment4(T4) - 8 % w/v molasses + 0.5 % w/v $(NH_4)_2SO_4$ + 0.3% w/v KH_2PO_4 +1 % w/v yeast extract +0.05 % w/v Mg $SO_4.7\ H_2O$+0.004% w/v $CaCl_2.2H_2O$.

Treatment5(T5) - 8 % w/v molasses + 0.5 w/v $(NH_4)_2\ SO_4$ + 0.3% w/v $KH_2\ PO_4$ + 1% w/v yeast extract, 2%w/v peptone + 0.05% w/v $MgSO_4.7H_2O$ +0.004 % w/v $CaCl_2.2H_2O$ + 0.005 % w/v biotin + 0.0001% w/v calcium panthetonate.

3.9 Cell Harvest and Biomass Determination

In series of the propagation processes cell harvest and biomass determination was done according to Campelo and Belo (2004). The Erlenmeyer flasks containing the inoculated molasses alone and molasses with various supplements were kept at different P^H values (3.5, 4.0, 4.5, 5.0 and 5.5), different temperatures (25^0C,30^0C and 37^0C) and different incubation periods (24, 48, 72 and 96hrs). After incubation periods, the suspensions in each flask were centrifuged at 5000 ×g for 10 minutes several times with intermittent washing with cold distilled water. Each yeast biomass (pellet) was dried in an oven at 60^0C to a constant weight. Dry weight of yeast was measured using Methler balance (Scaltec).

3.10 Leavening Action

The leavening action of the yeast isolates the method of from *teff* dough and commercial baker's yeast was compared following Azmuda *et al.*, (2006). Bread making dough was prepared by mixing wheat flour (250gm), yeast cells (2gm), table salt (1.5gm), table sugar (10gm) and distilled water (200ml) in 1000 ml measuring cylinders each.

The dough in each container was incubated four 2-4 hours at room temperature and 30^0C for fermentation. The height of the dough was measured from the graduated surface of the cylinder before and after fermentation and the net increased volume was calculated.

4. RESULTS

4.1 Characterization of Yeasts

Commercial baker`s yeast and local yeast isolates from *teff* dough and *tella* used in this study were compared using colony characteristics, budding features and ascospore formation (sexual characteristics). Similar colonies were obtained with creamy color and circular appearance (Fig .5). They also showed colony diameters ranging from 1-2 mm upon incubation for 3-5 days. The shape of some yeast cells was ovoid whereas others displayed spheroidal shape. Although all isolates showed 4 ascospores in one ascus, the diameters of cells of the commercial isolate was found to be greater in size compared to the local *teff* and *tella* isolates (Fig. 6) .Like wise, the isolates displayed similar pattern of multipolar budding.

Fig.5 Colonies of commercial baker`s yeast, *teff* dough yeast and *tella* yeasts.

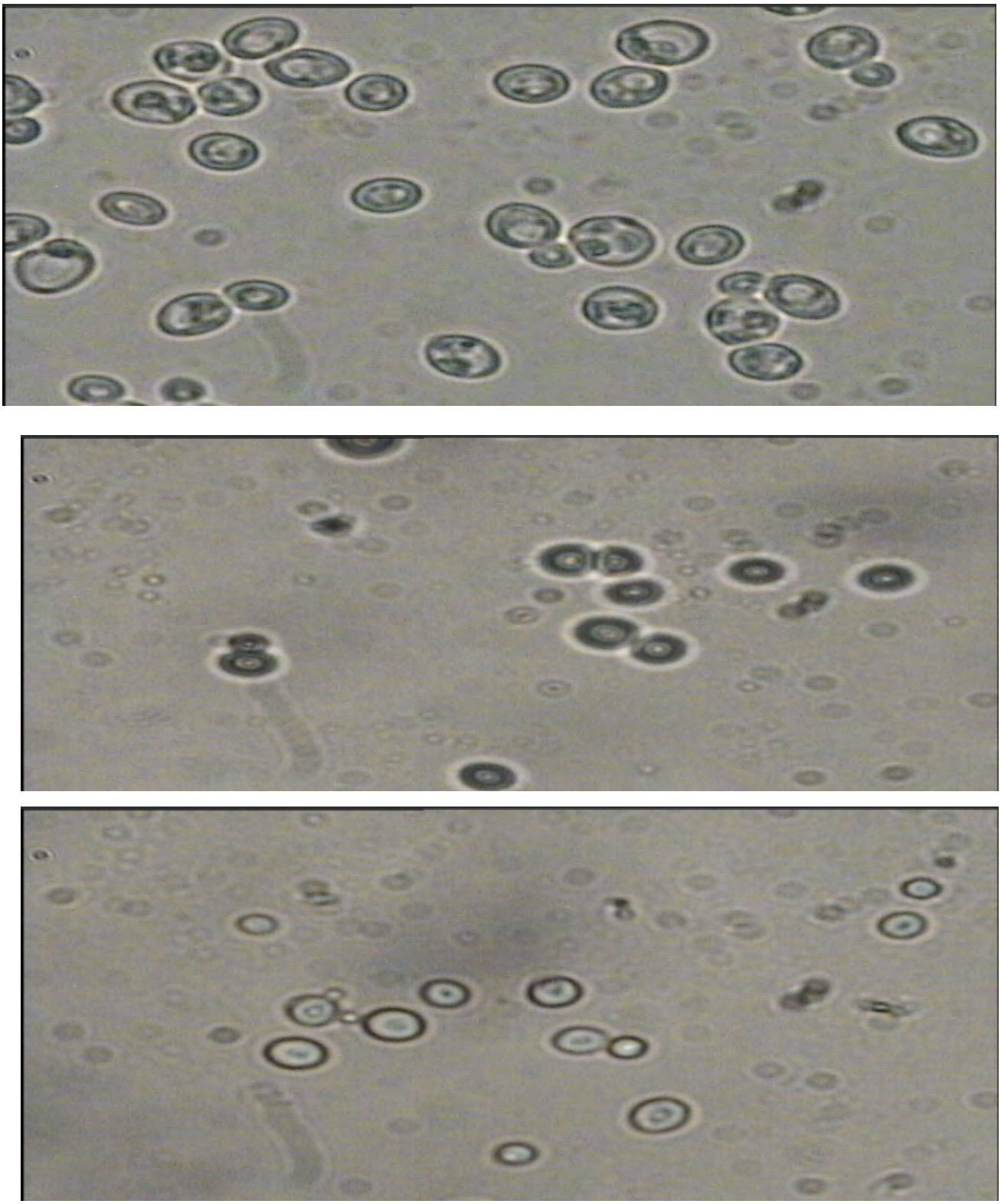

Fig.6 Microphotographs of budding in commercial baker`s yeast, *teff* dough yeast and in *tella* yeast (from top to bottom).

4.2 Physical and Biochemical Characterization of molasses

The molasses substrate that was used to grow the different yeast species was evaluated in terms of its sugar and nitrogen contents, water holding capacity, dry ash concentration (Table 6). Accordingly, the molasses sample was found to contain higher sugar content of 43.1%, and lower nitrogen and protein contents of 0.25% and 1.56% respectively. It also showed moisture content of 17.9% (Table 6).

Table 6. Relative composition of ingredients in molasses from different sources.

No	**Moisture content**	**Dry weight**	**Sugar content**	**Nitrogen content**	**Protein content**	**Dry ash content**	**Reference**
1	17.9%	82.1%	43.1%	0.25%	1.56%	11.7%	This work
2	-	85%	50%	-	-	14%	Ethiopian standard (2004)
3	-	77-84%	54.6%	0.5-1.5%	-	7-11%	Rhodes and Fletcher (1966) Imre (1969)
4	-	79-85%	50-58%	0.08-0.5%	-	-	White (1954)

4.3 Effect of Molasses Concentration on Yeast Growth

To determine the effect of molasses concentration on biomass yield of yeasts, the yeast isolates were grown at 3% w/v, 5% w/v, 8% w/v and 10 % w/v) (Table 7). In general, the three isolates displayed biomass accumulation in the range of 2.14-2.91g/l for TE isolate, 2.10-2.78g/l for TL isolate and 1.97-2.98g/l for BA isolate within the incubation period of 24-96 hrs.

This shows that the TL, TE, and BA isolates showed differences between the lowest and highest values in biomass to the tune of 24.5%, 26.4%, and 34%, respectively at different incubation periods and different substrate concentrations.

The pattern of substrate utilization by the isolates on the different concentrations of molasses showed variations among one another. BA isolate showed maximum biomass yield at 5%, 8% and 10% concentrations within 24-48 hrs; whereas TE isolates showed the same pattern at 5% and 8% concentrations after 48 hrs growth. Unlike the other isolates, TL isolate was found to accumulate the maximum yield at 8% molasses concentrations after 48hrs (Table7). This shows that similarities and differences in biomass accumulation occur as a function of time and substrate concentrations. Although the isolates showed a steady increase at first, they showed reduced pattern in accumulation of biomass when they were further grown for 72-96 hrs. In all cases, the isolates showed similarity in high biomass accumulation when they were grown at 8% w/v molasses concentration. Consequently, this concentration was taken as optimal for further characterizations.

Table 7. Effect of molasses concentration on the growth of the different yeast isolates at pH 4.5 and temperature of 30^0C.

Incubation Hours		**24hrs**			**48hrs**			**72hrs**			**96hrs**		
Yeast biomass yield (g/l)		TE	TL	BA	TE	TL	BA	TE	TL	BA	TE	TL	BA
Molasses Concent-ration	3%w/v	2.52b	2.50b	2.22d	2.69b	2.56b	2.67b	2.14d	2.10d	2.52b	2.15b	2.30c	1.97d
	5%w/v	2.69b	2.69b	2.80a	2.79a	2.55b	2.55c	2.20d	2.64b	2.73b	2.25c	2.41c	2.35c
	8%w/v	2.69b	2.74b	2.86a	2.91a	2.78a	2.98a	2.64b	2.64b	2.76a	2.25d	2.47c	2.27c
	10% w/v	2.52b	2.73b	2.73b	2.63b	2.59b	2.80a	2.54b	2.42c	2.68b	2.58b	2.47c	2.40c

Values followed by different letters are significantly different at<0.05 level of significance.

4.4 Effect of pH on the Growth of Yeasts

In order to evaluate the effect of pH on biomass yield of yeasts 8% w/v molasses at different pH values were prepared. The temperature was kept at 30^0C. The growth of yeasts was recorded after 24, 48, 72 and 96 hrs. The minimum and maximum record along the tested pH values was 1.81-2.86g/l for TE isolate, 1.71g/l-2.74g/l for TL isolate, and 2.05-2.97g/l for isolate BA. This gives differences in biomass of each isolate to the tune of 37%, 37%, and 31%, respectively. With regard to inter-isolate difference in biomass accumulation BA isolate was found to accumulate 11% and 23% more biomass than the maximum yield recorded by TE and TL isolates. Generally, isolate BA was found to be effective at all pH values except pH 5.5; whereas TE isolate was effective at pH 4.0 and pH 4.5 at 48 hours, and at pH 4.5 at incubation time of 72 hrs(Table 7). Furthermore, isolate BA and isolate TE were also effective at pH 4.5 within 24 hrs incubation. At pH 3.5 and 5.5, there was a steady decrease in biomass yield by all the isolates. Table 7 shows the effect of pH on the growth and biomass yield of yeasts.

Table 8. Effect of pH on the growth of yeasts at 8% substrate concentrations and 96 hrs of incubation.

Incubation hours		**24hrs**			**48hrs**			**72hrs**			**96hrs**		
Yeast biomass yield (g/l)		TE	TL	BA	TE	TL	BA	TE	TL	BA	TE	TL	BA
pH	3.5	2.4c	2.31c	2.37c	2.65b	2.46c	2.76a	2.36c	2.24c	2.65b	2.37c	2.34c	2.15d
	4.0	2.45c	2.32c	2.46c	2.86a	2.54b	2.84a	2.67b	2.46c	2.73b	2.39c	2.35c	2.18d
	4.5	2.55b	2.56b	2.87a	2.83a	2.74b	2.94a	2.76a	2.63b	2.71b	1.81e	2.13d	2.05d
	5.0	2.54b	2.30c	2.97a	2.64b	2.45c	2.89a	2.71b	2.70b	2.56b	2.41c	1.73e	2.52b
	5.5	2.13d	2.23c	2.51b	2.39c	2.17d	2.53b	2.47c	2.16d	2.54b	2.32c	1.71e	2.18d

Values followed by different letters are significantly different at<0.05 level of significance

4.5 Effect of Temperature on the Growth of Yeasts

To know the optimum temperature for maximum yeast biomass yield, the isolates were inoculated into molasses medium 8% w/v adjusted to 4.5 and incubated at 25^0C, 30^0C and 37^0C. The data showed that the different isolates displayed biomass yield ranging from 1.27g/l to 3.25g/l within the different temperatures. All isolates showed slow growth at 25^0C, and 37^0C with subsequent slow increase as the incubation temperatures increased (Table 9). The highest biomass was displayed at 30^0C by isolates BA (2.98-3.2g/l in 24-72 hrs), TE (2.91-3.1g/l in 24-72 hrs); whereas isolate TL showed biomass increase of 2.81g/l in 48 hrs. There was a rapid decrease in cell number of all the isolates at 96 hrs of incubation.

Table 9. Effect of temperature on the growth of yeasts in 8% w/v molasses.

Incubation hours		**24 hrs**			**48 hrs**			**72 hrs**			**96 hrs**		
Yeast biomass yield (g/l)		TE	TL	BA	TE	TL	BA	TE	TL	BA	TE	TL	BA
Temperature	25^0C	2.19c	2.12c	2.70b	2.54b	2.81a	2.94a	2.85a	2.62b	2.71b	2.61b	2.51b	2.60b
	30^0C	2.91a	2.71b	2.96a	3.1a	2.94a	3.2a	3.07a	2.88a	2.98a	2.47b	2.60b	2.69b
	37^0C	1.49d	2.27c	2.50b	2.15c	2.25c	2.51b	2.32c	2.51b	2.48b	1.44d	2.31c	1.27d

Values followed by different letters are significantly different at<0.05 level of significance

4.6 Optimization of Biomass with different supplements

After investigating the effect of different concentrations of molasses and pH and different temperatures on the biomass production of yeasts under study at RPM of 140 and different incubation periods (hours), optimization of biomass yield was investigated through the addition of supplements. The biomass production of yeasts through the addition of supplements was characterized at pH 4.5, temperature of 30^0C, RPM of 140 and at different incubation periods (24, 48, and 72 and96 hrs).

4.6.1 Effect of addition of supplemental $(NH_4)_2SO_4$ (0.5% w/v) on biomass production of the different isolates (Treatment 1, T1)

In order to show the efficiency of molasses to be used a substrate alone or not, different experiments were undertaken by supplementing molasses with different ingredients. Treatment 1(T1) was done by the addition of $(NH_4)_2SO_4$ (0.5% w/v) to the 8% w/v molasses media. The temperature and pH were kept at 30^0C and 4.5, respectively. Fig.7 shows the effect of addition of $(NH_4)_2SO_4$ (0.5% w/v) on the biomass yield of yeasts.

The data showed that the inclusion of ammonium sulphate increased the biomass of TL (5.6-5.9g/l), TE (5.6-6.2g/l), and BA (6.1-6.4g/l) within 24 and 72 hrs (Fig.7). In all cases the maximum biomass was achieved within 48 hrs, except BA isolate that continued to steadily increase up to 72 hrs of incubation. When this compared with biomass accumulation on molasses alone, the inclusion of the supplemental nitrogen source (ammonium sulphate) showed 1.5-2 fold increase in yeast dry weight by all isolates.

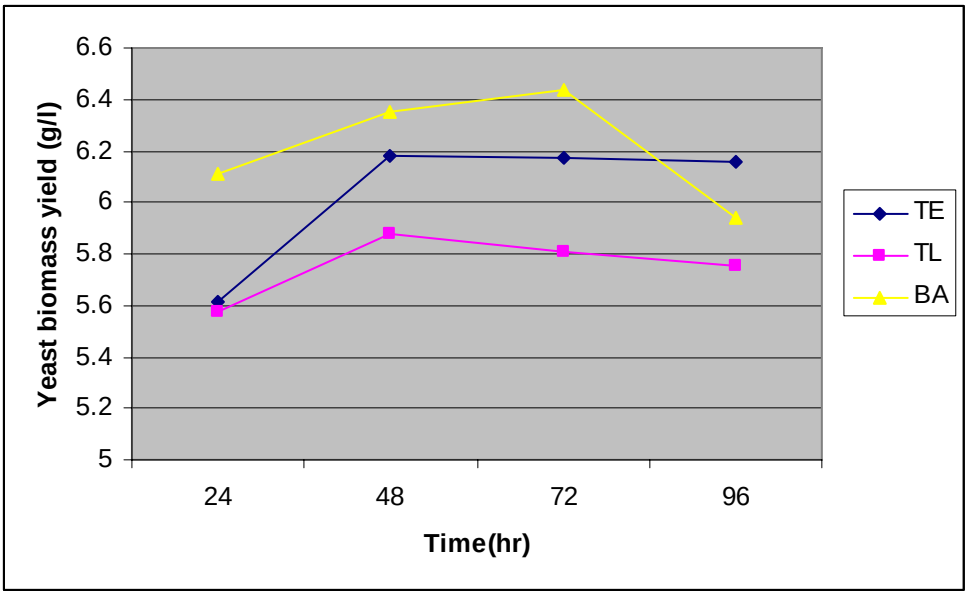

Fig.7 The effect of addition of $(NH_4)_2SO_4$ (0.5% w/v) on the biomass yield of yeasts.

4.6.2 Effect of addition of $(NH_4)_2SO_4$ (0.5% w/v) and KH_2PO_4 (0.3% w/v) (Treatment 2, T2)

The effect of addition of $(NH_4)_2SO_4$ (0.5% w/v) and potassium orthophosphate (KH_2PO_4)(0.3% w/v) to the 8% w/v molasses media on yeast biomass yield was evaluated. The temperature and pH were kept at 30^0C and 4.5 respectively. Each yeast strain was allowed to grow for 24 hrs, 48 hrs, 72 hrs and 96 hrs. As shown in Fig.8, the isolates showed increase in biomass starting from 24 hrs with their highest growth at 48 hrs. The pattern of increase in growth was recorded ranging 5.5-5.9g/l for TL and 5.8-6.3g/l for TE, and 6.4-6.5g/l for isolate BA, respectively (Fig.8). All isolates showed decrease in growth after 72 hrs. The data showed that isolate BA showed increases of 3% and 9% in biomass compared to TE and TL, respectively.

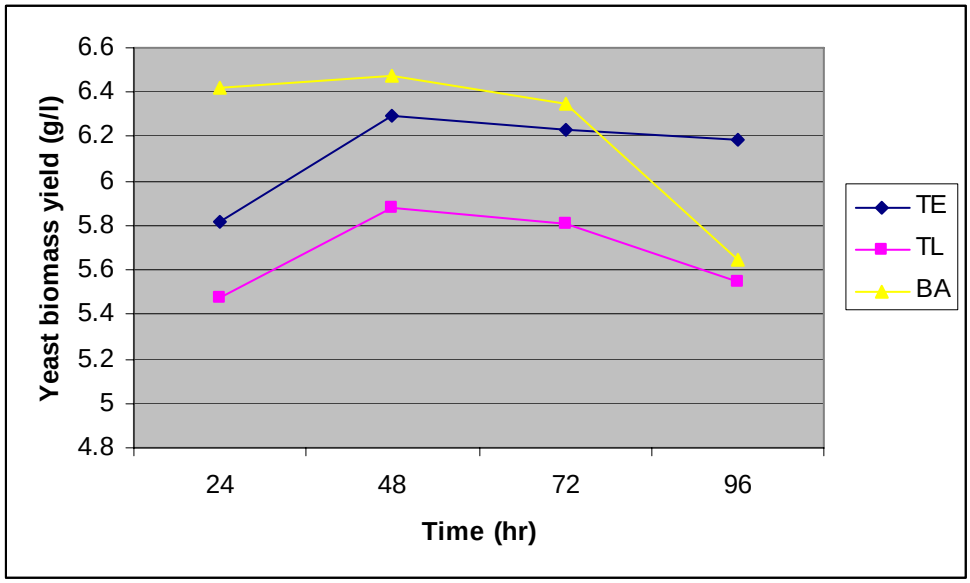

Fig.8 Effect of addition of $(NH_4)_2SO_4$ (0.5% w/v) and KH_2PO_4 (0.3% w/v) on the growth of yeasts at pH 4.5 and temperature of 30^0C.

4.6.3 Effect of addition of $(NH_4)_2SO_4$, KH_2PO_4 and peptone (2% w/v) (Treatment 3, T3)

The incorporation of ammonium sulphate, potassium orthophosphate and peptone on the molasses medium showed differential response by the yeast isolates (Fig.9). Fig.9 demonstrates that the isolates displayed a steady increase in biomass after 24 hrs where isolates BA and TE accumulated 7.5g/l and 6.5g/l dry weight in 48 hrs, respectively. However, isolate TL reached the maximum of 6.2 g/l after 72 hrs of incubation time. This shows that isolate BA responded better to the supplements by increasing biomass by 13 and 17% compared to TE and TL isolates, respectively.

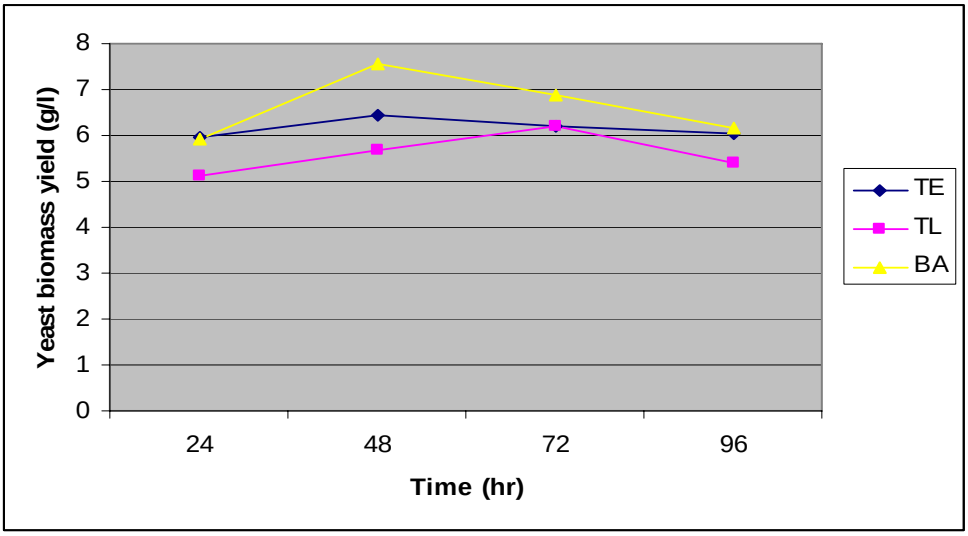

Fig.9 Effect of addition of $(NH_4)_2SO_4$ (0.5% w/v), KH_2PO_4 (0.3%w/v) and peptone (2% w/v) on the growth of yeasts at pH 4.5 and temperature of 30^0C.

4.6.4 Effect of addition of $(NH_4)_2SO_4$,KH_2PO_4 , yeast extract, $MgSO_4.7H_2O$ $CaCl_2.2H_2O$ (Treatment 4, T4)

The effect of addition of different minerals and yeast extract on the molasses medium (T4) to grow the different isolates is depicted in Fig.10. Accordingly, the isolates showed the same pattern of high growth at 48 hrs, and the medium enabled the isolates to accumulate biomass 8.04g/l (BA), 6.5g/l for both TE and TL isolates.

Although BA showed 19% more growth than both isolates in this medium, TL isolate was stimulated to grow better than on other media in the previous experiments.

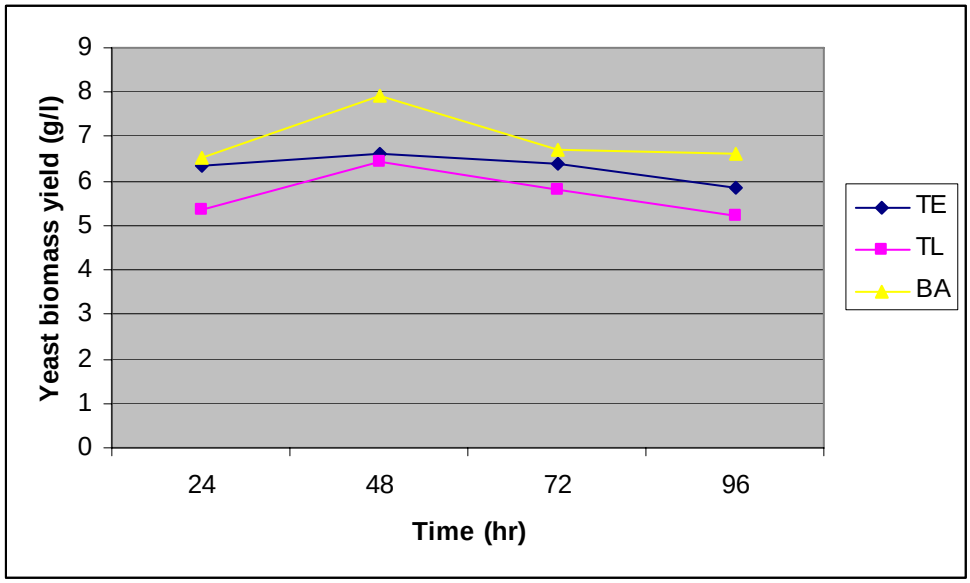

Fig.10 Effect of addition of $(NH_4)_2SO_4$ (0.5%w/v),KH_2PO_4 (0.3%w/v), yeast extract (1%w/v),$MgSO_4.7H_2O$(0.05% w/v),$CaCl_2.2H_2O$(0.004% w/v) at pH 4.5 and temperature of 30^0C.

4.6.5 Effect of addition of $(NH_4)_2SO_4$, KH_2PO_4, Yeast extract, Peptone, $MgSO_4.7H_2O$, $CaCl_2.2H_2O$, Biotin, and Calcium panthetonate (Treatment 5, T5)

In this experiment all the hitherto ingredients and two vitamins (Biotin, calcium panthetonate) were incorporated on the molasses medium to evaluate the growth and biomass yield of the yeast isolates. Fig.11 shows that medium increased the biomass of BA, TE, and TL to 8.0g/l, 7.5g/l, and 6.5g/l at 48 hrs of incubation time. Although the medium did not bring significant difference between BA and TE isolates, it enhanced more growth (13%) for TE and (19%) for BA isolate compared to the TL isolate that was found to be equally effective growth as TE on the previous medium (T4).

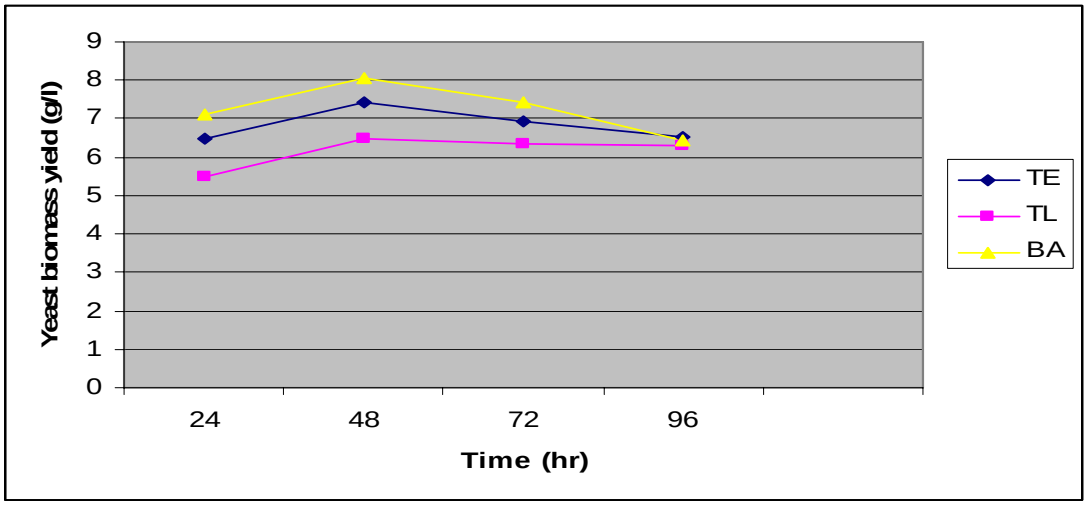

Fig.11 Effect of addition of $(NH_4)_2SO_4$ (0.5% w/v), KH_2PO_4(0.3%w/v), yeast extract(1 % w/v),peptone(2 % w/v),$MgSO_4.7H_2O$(0.05 % w/v),$CaCl_2.2H_2O$ (0.004 % w/v), Biotin (0.005 % w/v) and Calcium panthetonate (0.0001 % w/v)

Table 10. Summary of optimization of growth and biomass yield of the yeast isolates on the different media after 48 hrs (g/l).

No	**Control (molasses alone)**	**T1**	**T2**	**T3**	**T4**	**T5**
TL	2.78	5.9 (2.1)*	5.9 (2.1)	6.2 (2.1)	6.5 (2.3)	6.5 (2.3)
TE	2.91	6.2 (2.1)	6.5 (2.2)	6.5 (2.2)	6.5 (2.2)	7.5 (2.6)
BA	2.98	6.4 (2.1)	7.5 (2.5)	7.5 (2.5)	8.0 (2.7)	8.0 (2.7)

* The parenthesis shows the rate of biomass accumulation compared with control

Table10 showed the overall performance of the three isolates in biomass production on the molasses medium supplemented with additional ingredients. The highest biomass yield of 8.0g/l was recorded by isolate BA with treatments T4 and T5, followed by 7.5g/ l by isolate TE with T5. Isolate TL did not respond well to various treatments compared to the T1. In general, there is no significant difference in biomass of the yeast isolates grown on the different treatments (T2-T5) with one another compared with the difference between T2 and the control.

4.7 Leavening Action

The leavening actions or raising power of both commercial baker's yeast and local yeast isolate from *teff* dough were compared by mixing 250 grams of wheat flour ,10 grams of table sugar,1.5 grams of table salt and 2 grams of yeast with 200 ml of distilled water in 1000ml measuring cylinders each . The raising power of bath yeast strains was evaluated both at room temperature and 30^0C after 2 hrs, 3 hrs and 4 hrs. The results are presented in Table 11.

Table 11. Comparison of leavening action of yeast isolate from *teff* dough and commercial baker's yeast.

Yeast type	Incubation temperature	Leavening hour	Dough volume change(in ml)	Dough volume change (in %)
TE	Room temperature	2hr	20ml	4%
		3hr	30ml	6%
		4hr	60ml	13%
	30^0C	2hr	50ml	11%
		3hr	160ml	35%
		4hr	200ml	44%
BA	Room temperature	2hr	180ml	40%
		3hr	340ml	76%
		4hr	350ml	78%
	30^0C	2hr	230ml	51%
		3hr	430ml	95%
		4hr	460ml	102%

As shown in Table 11, dough volume increase by the *teff* dough isolate (TE) after 2, 3 and 4 hrs at room temperature in order 20ml (4%), 30ml (6%) and 60ml (13%). This yeast strain caused dough volume increase of 50ml (11%), 160ml (35%) and 200ml (44%) at 30^0C after 2, 3 and 4hrs incubations respectively.

The dough volume increases by the commercial yeast strain (BA) after 2, 3 and 4 hrs fermentation at room temperature was 180ml (40%), 340ml (76%) and 350ml (78%) respectively. At 30^0C and 2 hrs fermentation, BA caused the dough volume increase by 230ml (51%). After 3 and 4 hrs fermentation the dough volume increase by BA at 30^0C was 430ml (95%) and 460ml (102%) respectively.

5. DISCUSSION

In this study, two yeast isolates from local fermented food and beverage (*teff*, *tella*) were isolated and compared with the commercial baker's yeasts. Based on their colony characteristics (white and creamy texture), ovoid microscopic shape, the presence of 4-ascospore in ascus, and budding pattern (multipolar), all isolates were found to belong to *Saccharomyces* type unicellular ascomycete according to Lodder (1971) and Kirsop and Kurtzman (1988). These results were consistent with the previous finding that corroborates yeasts from *teff* dough and *tella* are *Saccharomyces* types (Birhanu Abegaz Gashe *et al.*, 1982; Samuel Sahle and Bihanu Abegaz Gashe, 1991).

The different isolates showed variations in dry mass accumulations depending upon the substrate concentrations. The pattern of substrate utilization by the isolates on the different concentrations of molasses showed variations among one another. BA isolate showed maximum biomass yield at 5%, 8% and 10% substrate concentrations within 24-48 hrs; whereas TE isolates showed the same trend at 5% and 8% concentrations after 48 hrs. Generally, all isolates showed high yield in biomass at a concentration of 8 % and 48 hrs incubation times. The inter-isolate difference between the highest biomass yield of BA isolate and the TL and TE isolates was 3-7%. This insignificant difference shows that the local isolates are equally effective in biomass accumulation (Table 7). However, the rapid growth and biomass production by BA isolate in 24 hrs compared to other is a good attribute in selection. According to Benitez *et al.*, (1996), one of the most important requisites in the commercial production of baker's yeast is rapid growth and high biomass yield.

The fact that BA isolate grew effectively on 5% molasses concentration support the finding of Al-Mudhaffar (1978) as opposed to isolates TE and TL. This is particularly important in industrial production of *Saccharomyces cerevisiae* since the system perform better in aerobic; sugar limited fed-batch cultures (Van Hoek *et al.,* 1998; Mickiewicz and Borowiak, 2005).

Bekatorou *et al*., (2006) and Verstrepen *et al*., (2004) showed that high substrate concentration would lead to catabolite repression by glucose and sucrose, which is known as Crabtree effect, may lead to several problems, such as incomplete fermentation, development of off flavors and undesirable by products as well as decreased biomass and yeast vitality.

When one compares the over-all pattern of growth of the three isolates along the substrate concentrations and incubation periods, they showed differences in dry mass accumulation of 24.5%-34% suggesting that optimization is very important to obtain the maximum yield. In general, the biomass accumulation of the different isolates was found to be between 1.97g/l-2.98g/l indicating that molasses support considerable growth for yeasts. This was corroborated by the contents of the molasses analyzed (Table6).Although the different components analyzed were within and outside the values of the previous findings (Table 6) (White, 1954; Rhodes and Fletcher, 1966; Imrie, 1969; Ethiopian standards, 2004), the high sugar contents and the relatively low nitrogen content and other unmeasured ingredients supported the growth of the yeasts. Mormeneo and Sentandreu (1982) successfully grew yeasts by providing molasses as sole nutrient source.

The optimization of growth and biomass yield was also evaluated on the basis of different pH (pH 3.5-pH 5.5) at 8% substrate concentration and 30^0C. The isolates showed the same pattern of accumulating biomass of 2.05-2.97g/l for isolate BA, 1.81-2.86g/l for TE isolate, and 1.71g/l-2.74g/l for TL isolate. This shows that BA isolate was found to accumulate 11% and 23% more biomass than the maximum yield recorded by TE and TL isolates. Generally, isolate BA was found to be effective at all pH values except pH 5.5; whereas TE isolate was effective at pH 4.0 and pH 4.5 at 48 hours, and at pH 4.5 at incubation time of 72 hrs(Table 8). This shows that isolates BA and TE were more tolerant to pH than the TL isolate. In a similar study, yeasts, specifically *Saccharomyces cerevisiae* (baker`s yeast) were found to grow at pH levels ranging from 3.6 to 6.0 (Azmuda *et al.,* 2006). The ability of the yeasts to perform well below pH 4.0 is said to offer an advantage of using yeast in less than aseptic equipment to minimize loss due to bacterial contaminants (Hettenhaus, 1998).

The effect of temperature on growth and maximum biomass yield showed that the highest biomass was displayed at 30^0C by isolates BA (2.98-3.2g/l in 24-72hrs), TE (2.91-3.1g/l in 24-72hrs); whereas isolate TL showed biomass increase of 2.81g/l in 48hrs. There was a rapid decrease in cell number of all the isolates at 96 hrs of incubation at all tested temperatures (Table9**).** Even if all isolates were found to decrease 22-29% in biomass when they were grown at 37^oC, tolerance to high temperature *per ce*, they may have the potential to be used under high temperatures without investment for cooling equipment in order to reduce the capital cost (Hettenhaus, 1998).

After having identified the optimization substrate concentration, pH, and temperature, additional nutrient supplements were also tested to further increase of yeast biomass. Consequently, the supplementation of additional nitrogen source (ammonium sulphate) in the medium alone (T1) was found to increase the cell mass of the isolates by more than 2-fold (Table10; Fig.8). This showed that the nitrogen content of the molasses (0.25% (Table 6) was very small to limit the growth and biomass yield of the isolates.

It was interesting to note that the addition of other ingredients other than the nitrogen source (T1) did not stimulate significant growth of the isolates (Table10). Table10 showed the overall performance of the three isolates in biomass production on the molasses medium supplemented with additional ingredients. Comparing the growth of the isolates on molasses and ammonium sulphate as control the isolates did not show significant difference in biomass with further treatments (T2-T5). However, the incorporation of the necessary supplements showed the same pattern in terms of biomass yield i.e., BA produced the maximum biomass (8.0432 g/l), followed by TE (7.432 g/l) and TL (6.489 g/l). After subsequent incubation period there was no significant increase in cell number or biomass yield probably due to the shortage of nutrients, accumulation of growth inhibitory by products or deviation of the pH of the medium (Azmuda *et al.*, 2006). Although the isolates showed a steady increase when other ingredients were incorporated (T2-T5), it was isolate BA that responded better with (2.5-2.7 fold increase in biomass compared to the other isolates. However, isolate TE isolate was also found to perform better in a medium supplemented with minerals, nitrogen and vitamin additives (2.6-fold) (T5).

The data generally showed that TE and TL isolates could not show significant difference in biomass production when the number of ingredients increased (T2-T4); whereas BA isolate responded better in the accumulation of 15-20% more than the treatment T1 (molasses+ammonium sulphate). Given that the incorporation of many ingredients requires more input into the production of biomass, the incorporation of ammonium sulphate is considered as cost-effective.

In general, the isolate from *teff* was found to perform close to the commercial BA yeast with respect to pH and temperature tolerance, different concentrations of the substrate molasses with or without additional nutrient supplements. In most cases it was found as fast growing with high biomass as the commercial isolate. It is established that rapid growth and high biomass yield are the most important requisites in the commercial production of baker's yeast (Benitez *et al*., 1996; Randez-Gil *et al.,* 1999; Zamani *et al.,* 2008).

The effect of leavening or dough raising power of TE and BA was compared at room temperature and 30^0C (Table 11). The commercial strain was higher in dough volume increase both at room temperature and 30^0C incubation. After 2hrs incubation TE gives 4% dough volume increase at room temperature and 11% dough volume increase at 30^0C,whereas BA gives 40% dough volume increase at room temperature and 51% dough volume increase at 30^0C.After 4 hrs incubation, TE caused dough raising power of 13% (room temperature) and 44% (30^0C),whereas BA gives78% (room temperature) and 102% (30^0C). From this one can understand that BA is time effective and economical.

It is generally recognized that, in baker's yeast, a good dough-leavening activity correlates with a high potential glycolytic activity, the ability to adapt rapidly to changing substrates, a high potential maltose-fermentation rate, strong hypertonic adaptation which is relevant to sweetened dough and the ability to grow and synthesize enzymes and coenzymes under anaerobic conditions (Higgins *et al*.,2001; Randez-Gil *et al.*, 1999). The commercial baker`s yeast probably fulfills the above mentioned features.

6. CONCLUSION

The molasses sample was found to contain higher sugar content (43.1%) but lower nitrogen content (0.25%). Even though, the molasses used for this study contains lower contents of nitrogen, it still supported the growth of all yeast strains.

Similar colonies of the three yeast strains were obtained with creamy color and circular appearance. They also showed colony diameters ranging from 1-2mm, 4 ascospores in one ascus, the diameters of cells of the commercial isolate was found to be greater in size compared to the local *teff* and *tella* isolates. They also showed multipolar budding.

The pattern of substrate utilization by the isolates on the different concentrations of molasses showed variations among one another. All isolates showed high yield in biomass at a concentration of 8 % and 48 hrs incubation times.

Isolate BA was found to be effective at all pH values except at pH 5.5, whereas TE isolate was effective at pH 4.0 and pH 4.5 at 48 hours, and at pH 4.5 at incubation time of 72 hrs. This shows that isolates BA and TE were more tolerant to pH than the TL isolate.

The effect of temperature on growth and maximum biomass yield showed that the highest biomass was displayed at 30^0C by isolates.

The supplementation of additional nitrogen source (ammonium sulphate) to the molasses medium alone (T1) was found to increase the cell mass of the isolates by more than 2-fold.

In all cases yeasts yielded maximum biomass after 48 hrs incubation period. After subsequent incubation period, especially after 96 hrs incubation period there was a decrease in cell number or biomass yield

The leavening actions of TE and BA were compared. BA was higher than TE in the dough volume increase both at room temperature and 30^0C. BA was time effective with respect to leavening activity.

7. RECOMMENDATION

Ethiopia produces about 141,363 tons of molasses from three sugar producing factories. Much of the molasses is disposed as waste. Some of it as already stated is currently used in the production of ethanol. The country has no baker`s yeast producing plant. The demand of the country is fulfilled by import and a lot of foreign currency is lost. Therefore, it is economical and advisable to establish activities involved in the industrial manufacturing of yeasts in Ethiopia, since there is sufficient amount of molasses for producing baker`s yeast. If baker`s yeast is produced in the country, it serves multi purposes. These include for baking activities, for beverage and alcohol producing plants and to solve the problem of protein deficiencies since yeasts serve as single cell protein.

On the other hand, from the present study in general it can be concluded that BA meets the desired characteristics with respect to both biomass propagation and leavening action. Indeed, the local yeast isolate from *teff* dough (TE) was also good with respect to biomass yield and leavening action. A further study on the physicochemical properties and molecular biology of the local yeast isolate (TE) is needed to be carried out in order to improve the performance in biomass propagation and the leavening action of TE.

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9. APPENDIX

Fig.a Inoculums of commercial baker`s yeast,*teff* dough and *tella* yeasts

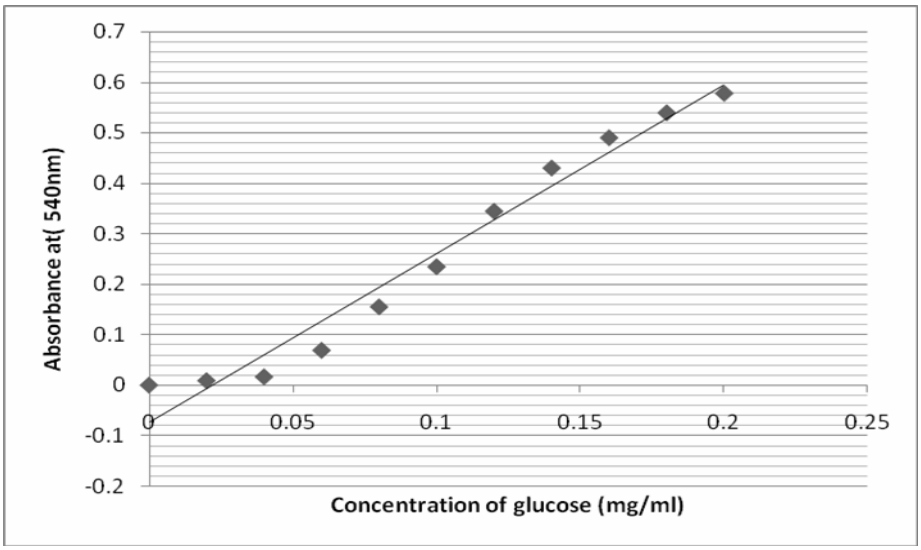

Fig.b The standard curve is used to estimate total sugar.
The standard formula obtained was Y= OD ×3.338-0.73 and the total sugar in molasses sample was estimated based on the formula.

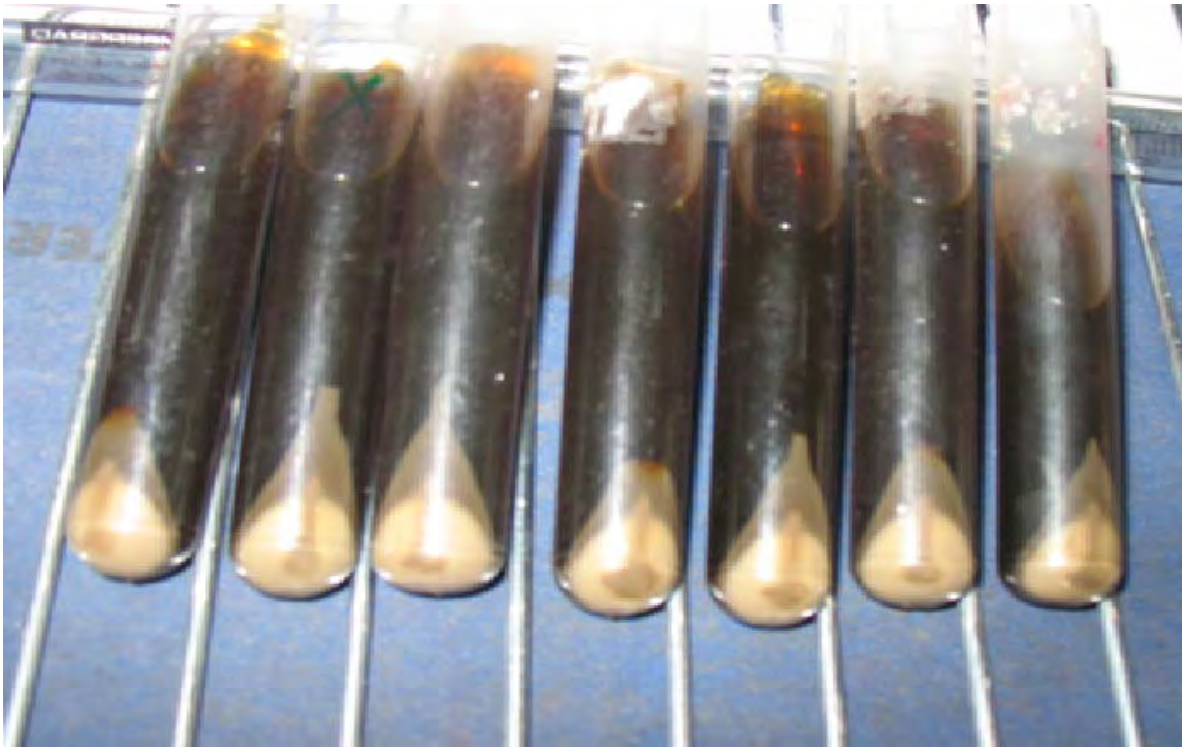

Fig.c Yeast biomass after centrifugation: Pellet at the bottom of the centrifuge tubes

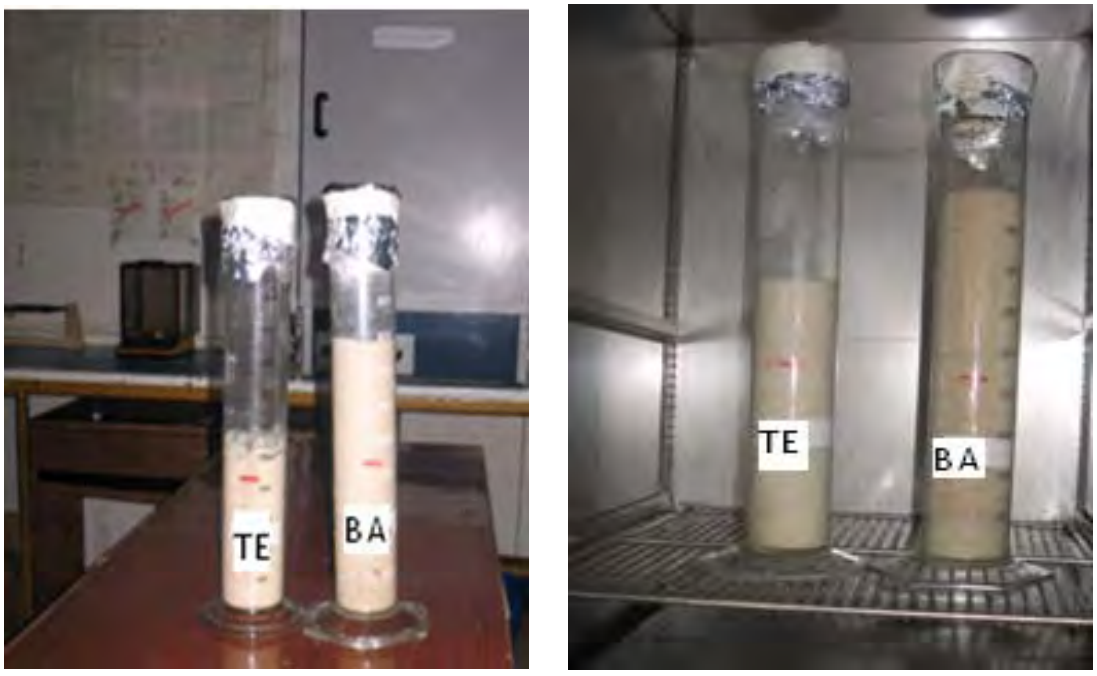

Fig.d Comparisons of leavening actions TE and BA at room temperature (left) and 30^0C(right) with BA higher dough volume increase.