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School of Graduate Studies

Faculty of Technology

Department of Chemical Engineering

STUDIES ON THE DEVELOPMENT OF BAKER'S YEAST USING CANE MOLASSES

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By: ***Wondimagegen Damtew***

Advisors: ***Dr. Eng. Shimelis Admassu***

Dr. R. Amutha

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Food Engineering Program

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By
Wondimagegen Damtew

Approved by the Examining Board:

_____________________ _____________________
Chairman, Department's Graduate Committee

_____________________ _____________________
Advisor

_____________________ _____________________
External Examiner

_____________________ _____________________
Internal Examiner

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L IST OF SYMBOLS AND ABBREVATIONS

A	Area of heat exchanger
ADY	Active dry yeast
ATP	Adenosine tri phosphate
Conc.	Concentration
DNS	Dintro salicylic acid
EDTA	Ethylene diamine tetra acetic acid
IDY	Instant dry yeast
KNO_3	Potassium nitrate
Mola	Molasses
MYPG	Maltose, yeast extract, peptone and glucose
N	Normality
N_C	Number of runs
NH_4NO_3	Ammonium nitrate
$(NH_4)_2SO_4$	Ammonium sulphate
Ppm	Parts per million
Q_S	Rate of steam supply
Q_W	Rate of cooling water
SCP	Single cell protein
S_O	Mass of fermentable sugar
t_c	Cycle time
V	Volume of broth
W	Weight
WLN	Wallerstien Laboratories Nutrient agar
W/V	Weight per volume
W/W	Weight per weight
YPD	Yeast extract, peptone and dextrose

ABSTRACT

Three yeast strains were used for biomass production of baker's yeast using wine, brewer and baker's yeast strains. The effect of growing yeast *saccharomayces cerevisiae* on cane molasses with respect to baker's yeast production was assayed**.** All yeast strains were cultured in YPD microbiological media and cane molasses media; their growth properties and biomass yield were examined using different substrate concentration of cane molasses and different nitrogen source chemical compounds. The studies on the feasibility of different types of yeast growth on cane molasses has been analyzed here. As a result of the kinetics study of growth of three yeast strains shows that baker's yeast strain gives the best results to know a generation time reduced high growth rate and a high quantity of biomass. The maximum biomass yield and protein concentration was obtained with the 5 %(w/v) cane molasses of bakers` yeast; the effect of different concentration of cane molasses (5%, 10% and 15% sugar concentration) on biomass yield and protein concentration of bakers, wine and brewery yeast strains were studied. Biomass and protein formation was affected by the type of nitrogen present in the medium; high yield of protein being achieved in fermentation medium containing 2 % (w/v) $(NH_4)_2SO_4$. Conversely medium, containing potassium nitrate had suppressive effect of protein production. Baker's yeast production was industrialized using baker's yeast strain in 5%(w/v) cane molasses with addition of ammonium sulphate.The results obtained on industrial scale feasibility study shows that the unit costs for the baker's yeast production are $2.4 per kilogram, while the local market price for the baker's yeast is $7.00/kg. It has good market demand and profitability analysis also provides positive results.

Keywords: Baker's yeast (*saccharomayces cerevisiae)*, Biomass, molasses and Single protein.

1. INTRODUCTION

1.1 Background

Yeasts can be used in many industrial processes, such as the production of alcoholic beverages, biomass (baker's and fodder) and various metabolic products. Among these categories, biomass in general and baker's yeast specifically has developed from sugarcane molasses by using different strains and substrate concentration *(Hensirisak,* 1997*).* Humans have known yeasts before thousands of years ago; they have been used in fermentation processes like the production of alcoholic beverages and bread leavening. Fermentation is the process of using microorganisms to valuable products such as food, antibiotics, industrial enzymes and chemicals. Baker's yeast (*Saccharomyces cerevisiae*) is one of the oldest products of industrial fermentation that was used traditionally. It is still one of the most important in industries based on its use for bread-making, a stable food for large section of world's population. Baker's yeast production is aerobic fermentation process whose efficiency is strongly dependent on the transfer of oxygen and nutrients to the microorganisms (*Bekatorou et al.,* 2006).

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*).* Yeasts are included in starter cultures, for the production of specific types of fermented foods like bread, sourdoughs, fermented meat and vegetable products, *etc*. 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 single cell protein (SCP) production compared with conventional sources of protein (soybeans or meat) are well known. Microorganisms have high protein content and short

growth times, leading to rapid biomass production, which can be continuous and is independent from the environmental conditions(*Lee et al.,*1996).The most widespread and commonly used substrates for SCP production have been those where the carbon and energy source is derived from carbohydrates, molasses, whey, methanol and cellulose. This is because their building blocks (mono and disaccharides) are natural microbial substrates, and that the raw materials are a renewable resource, which is widely, distributed.

The principal raw materials used in developing baker's yeast are the pure yeast culture and cane molasses. Cane and beet molasses are used as the principal carbon sources to promote yeast growth and supplies all the sugar that yeast needs for growth and energy along with part of the needed nitrogen (*John,* 1954*)*. The yeast strains most used widely are baker's yeast (*Saccharomyces cerevisiae),* wine and brewery yeast *(saccharomyces carlsbergensis)*. Metabolically, yeasts are mostly facultative aerobes, capable of growing either in the absence of air (fermentative) or in its presence (oxidative). Therefore, in the absence of aeration, yeast has the ability to instantaneously change its respiratory metabolism from oxidative to fermentative *(Meyerhof,* 1926*)*. The rational study of this work was investigating the effect of biomass and protein concentration changes associated with the active and rapid growth of baker's yeast (*Saccharomyces cerevisice*), wine and brewery yeast (*Saccharomyces carlsbergensis*) under cane molasses and produced in industry level. Such a growth is essentially aerobic, and necessitates nitrogen source compounds were added in the experimental conditions which fulfill this requirement, and accordingly differ from the conditions suitable for the study of biomass development. The fast growth of these organisms is the strain of *Saccharomyces cerevisiae* used in the brewing and baking industries. *S. cerevisiae* is the organism commonly referred to this strain of yeast has been extensively studied and applied widely both in the laboratory and in industry. Baker's yeast is manufactured by large-scale aerobic fermentation of selected strain, *S. cerevisiae* and 5 % (w/v) substrate concentration with adding ammonium sulphate. Baker's yeast is studied in laboratory with plotting of time growth curve on substrate concentration and proteins concentration curve. Using laboratory experiments results it has scaled up industry level production of yeast crops in large with selecting appropriate technological set up and assessing the economic viability of the production.

1.2. The scope of the study

Raw material cane molasses was analyzed and prepared in different substrate concentration. Yeast growth was characterized on three different yeast strains that is baker's, wine and brewery yeast strains to give better biomass and protein yield. Identification of the beast strain with suitable substrate concentration was studied and using experiment results, it was scaled-up to industry level production.

1.3. Objectives

General objectives

The aim of this study was developing baker's yeast growth from cane molasses with analyzing the effect of different substrate concentration and addition of nitrogen source chemical compounds on yield capacity of biomass and protein on baker's, wine and brewery yeast strains as well as their effect, on the growth of all the strains.

Specific objectives

- ❖ Identifying, comparing the basic biochemical composition of raw material cane molasses for bakers yeast development.
- ❖ Identifying the suitable strain for single cell protein production.
- ❖ Optimizing and characterization of yeast growth
- ❖ Study the growth of yeast on different sugar concentration.
- ❖ Comparison of different types of yeast strain and substrate concentration for suitable biomass yield and protein concentration on cane molasses.
- ❖ Improving the protein content by adding different nitrogen source.
- ❖ Scale-up to industry level using experiment and other data.
- ❖ Study industrial process of baker's yeast production using cane molasses and evaluating economic viability of the process.

2. LITERATURE REVIEW

2.1 Raw materials

Cane or beet molasses is the primary raw material for bakery yeast production. It supplies all the sugar that yeast needs for growth and energy along with part of the needed nitrogen. Molasses is a by-product of the sugar manufacturing process. The concentrated sugar solution obtained from the milling of sugar cane or sugar beet is cooled allowing the sugar to crystallize. 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*)*. 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. Besides its high sugar content, molasses contains minerals, organic compounds and vitamins, which are valuable nutrients in fermentation processes. According to the Association of American feed control officials describes the following types of molasses.

Cane Molasses**: -** is a by-product of the manufacture or refining of sucrose from sugar cane. It must not contain less than 46% total sugars expressed as invert. If its moisture content exceeds 27%, its density determined by double dilution must not be less than 79.5^0 Brix.

Beet Molasses**: -** is a by-product of the manufacture of sucrose from sugar beets. It must contain not less than 48% total sugars expressed as invert and its density determined by double dilution must not be less than 79.5^0 Brix.

Citrus Molasses**: -** is the partially dehydrated juices obtained from the manufacture of dried citrus pulp. It must contain not less than 45% total sugars expressed as invert and its density determined by double dilution must not be less than 71.0^0 Brix.

Hemi cellulose Extract**: -** is a by-product of the manufacture of pressed wood. It is the concentrated soluble material obtained from the treatment of wood at elevated temperature and pressure without use of acids, alkalis, or salts. It contains pentose and hexose sugars, and has a total carbohydrate content of not less than 55%.

Starch Molasses:-is a by-product of dextrose manufacture from starch derived from corn or grain sorghums where the starch is hydrolyzed by enzymes and/or acid. It must contain not less than 43% reducing sugars expressed as dextrose and not less than 50% total sugars expressed as dextrose. It shall contain not less than 73% total solids *(AAFO,* 1982) Ethiopia has three large sugar factories, which can produce molasses as by-product that is methehara, Fincha, and Wonji/Shoa sugar factories. (*Ethiopian Stastisc Authority,* 2006) reported average annual molasses production is 40,000-50,000 tons per year among the sugar factories in Ethiopian. Methehara sugar factory is the largest molasses producer. The capacity of molasses production in methehara sugar factory for the last five years are shown in the table 3.1

Table 2.1 Annual molasses production in methehara sugar factory

Years (G.C)	2001/2002	2002/2003	2003/2004	2004/2005	2005/2006
Cane molasses (tons)	36,655	39,500	35,749	37,727	41,343

2.2 Yeast Metabolism

Yeasts are facultative anaerobes and can grow with or without oxygen. In the presence of oxygen, they convert sugars to CO_2, energy and biomass. In anaerobic conditions, as in alcoholic fermentation, yeasts do not grow efficiently, and sugars are converted to intermediate by-products such as ethanol, glycerol and CO_2 (*Balls et al.,* 2007*)*. Therefore, in yeast propagation, the supply of air is necessary for optimum biomass production. The main carbon and energy source for most yeast is glucose supplied from molasses, which is converted to the glycoltic pathway to pyruvate and by the Krebs cycle to anabolites and energy in the form of ATP. Yeasts are classified according to their modes of further energy production from pyruvate to 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 cell alive. This process is called

fermentation, in which the sugars are not completely oxidized to CO_2 and ethanol *(Bekatorou et al.,* 2006 *and Scragg,* 1991*).*

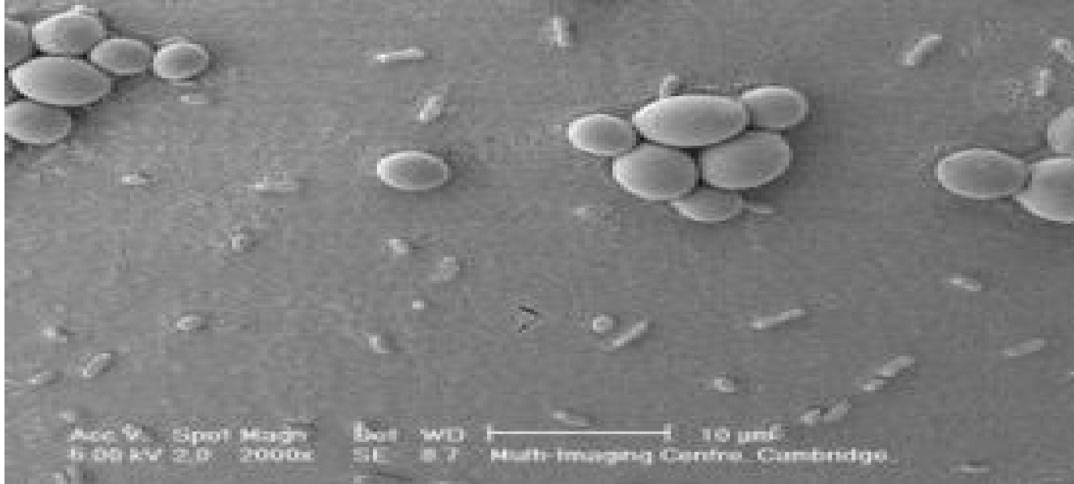


Fig 2.1 **Y**east *Saccharomyces cerevisiae* (large cells) surrounded by the bacteria *E. coli* (smaller cells)

Yeasts can metabolize various carbon substrates but mainly utilize sugars such as glucose, sucrose and maltose. Sucrose is metabolized after hydrolysis into glucose and fructose by the extra cellular enzyme invertase. Maltose is transferred in the cell by maltose permease, and metabolized after hydrolysis into two molecules of glucose by maltase. Some yeast can utilize a number of unconventional carbon sources, such as biopolymers, pentoses, alcohols, hydrocarbons, fatty acids and organic acids (*Bailey et al.,* 1977*)*. Elements like N, P, S, Fe, Cu and Zn are essential to all yeasts and are usually added to the growth media. Most yeast is capable of assimilating directly ammonium ions and urea, while very few species have the ability to utilize nitrates as nitrogen source. Phosphorus and sulphur are usually assimilated in the form of inorganic phosphates and sulphate, respectively.

2.3 Baker's yeast

Industrial microbiology deals with microorganisms that have an economic value. The economic criterion implies that microorganisms have some specific type of growth or metabolic activity to produce one or more products. These considerations make it apparent that industrial microbiology is a very broad area for study. Industrial microbiology also deals with the isolation and description of microorganisms from natural environments, such as soil or water, and cultural conditions required for obtaining specific products from these organisms in the laboratory and in large-scale culture vessels commonly known as fermenters (*Parakulsuksatid,* 2000*).*Thus, the ability of microorganisms to convert inexpensive raw materials (substrates) to economically valuable organic compounds obviously is of considerable interest. The valuable organic compounds (fermentation products) may be components of the microbial cells, intracellular or extra cellular enzymes, or chemical produced or converted by the microorganisms. Some commercially important products of microbial activity of different micro organism are antibiotics, organic solvents, gases, beverage, foods, flavoring agents, glycerol, amino acids so on (*Casida,* 1968). Yeasts are the most important and the most extensively used microorganisms in industry. They are cultured for cell mass, cell components, and products that they produced during the fermentation. Industrial production of yeast cells for their biomass and production of alcohol by yeast are two quite different processes. The first process is aerobic process, requires oxygen for maximum production of cell, whereas the alcoholic fermentation process is anaerobic and takes place only in the absence of oxygen. The yeast widely used for both industrial and domestic processes is *Saccharomyces cerevisiae*.

2.4 General characteristics of fermentation process

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)

The basic components of a fermentation process are given in Figure 2.2 This figure shows the flow of steps in a development of product which is designed to gradually improve the overall efficiency of the fermentation. Before a fermentation process is established, a producer organism has to be isolated, and perhaps modified, such that it produces the desired product. Then, the proper environmental conditions have to be verified. Scale-up and plant design are the next step to achieve the product needed in commercial quantities.

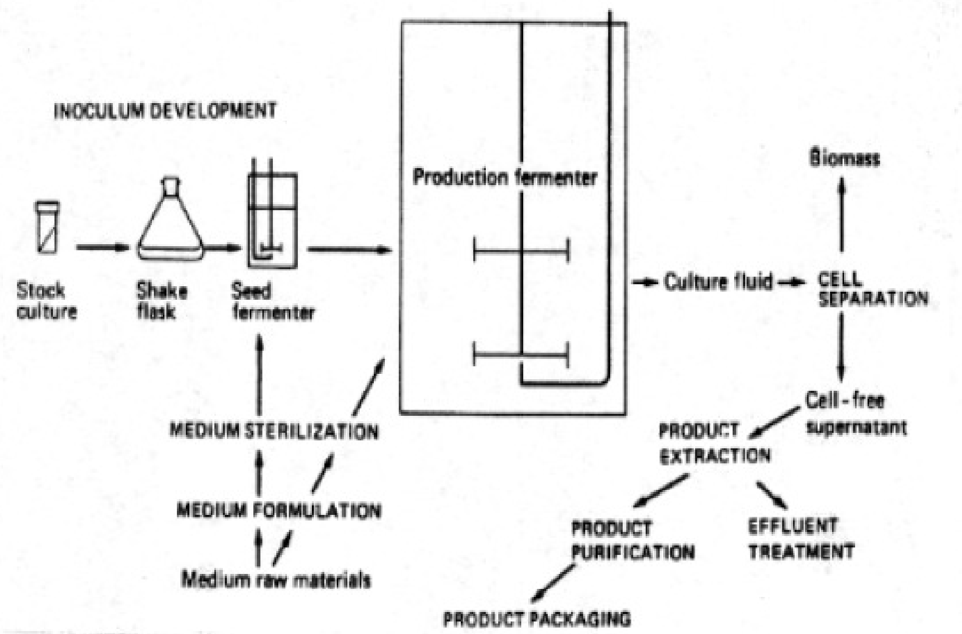


Fig 2.2 A generalized schematic representation of a typical fermentation process

2.4.1 Aeration and agitation in aerobic fermentation

Aeration supplies the necessary oxygen to the microorganisms, and agitation maintains uniform conditions within the fermenter. Altogether, the aeration and agitation are important in promoting effective mass transfer to liquid medium in the fermenter. The main function of a properly designed bioreactor is to provide a controlled environment in order to achieve the optimal growth and/or product formation in the particular cell system employed. In laboratory shake flasks, aeration and agitation are accomplished by the rotary or reciprocating action of the shaker apparatus. In pilot-scale and production-scale fermenters, oxygen is generally supplied by compressed air, and mechanical devices are used to agitate the liquid broth. In aerobic fermentation processes, oxygen is a key substrate, and because of its low solubility in aqueous solutions, a number of studies to enhance the efficiency of oxygen mass transfer have been conducted. The concentration of dissolved oxygen in a suspension of respiring microorganisms generally depends on the rate of oxygen transfer from the gas phase to the liquid, on the rate at which oxygen is transported to the site of utilization, and on the rate of its consumption by the microorganism. In the conventional water soluble carbohydrate substrate processes, it has frequently been found that the rate of oxygen transfer from dispersed air bubbles can become the rate limiting factor by the rate of supply of oxygen. *Humphrey (*1967) has reviewed the engineering problems associated with hydrocarbon fermentations. One such problem is that of supplying sufficient oxygen to meet the relatively-high microbial respiration rate demands in these fermentations. *Darlington (*1964) has shown that hydrocarbon substrate growth requires about 2.7 times the rate of oxygen transfer from air as does microbial growth on glucose.

2.4.2 Characteristics of baker's yeast fermentation

A selected strain of baker's yeast, *S. 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 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). Under aerobic conditions, *S. cerevisiae* uses sugars such as glucose to grow cell mass rather than produce alcohol, studied a general model for aerobic yeast growth in batch culture. This model was developed by comparing the rate of sugar transport into the cell and the rate of transport of respiratory intermediates (pyruvate) into the mitochondrion. If the rate of sugar uptake is higher than the transport rate of respiratory intermediates into the mitochondrion, the metabolism favored ethanol production and limited the specific oxygen uptake rate. If the respiratory intermediates transport rate into the mitochondrion was equal, the transport of sugar into the cell, carbon dioxide (CO_2) was the major metabolite with little or no ethanol produced and a much higher specific oxygen uptake rate occurred (*Gelinas et al.,* 1998). In aerobic batch fermentation, *S. cerevisiae* can only produce a limited amount of respiration enzymes. If the glucose concentration is more than 5% in the medium, the respiratory intermediate enzymes are suppressed and the ethanol mechanisms dominate. A concentration of glucose at 0.1 g l^{-1} is the maximum growth condition for cell growth in continuous fermentation; the dilution rate is equal to the growth rate when steady state is achieved. At steady state, the glucose concentration in the fermenter will be near zero. The low glucose concentration in the fermenter causes the yeast to completely oxidize all the glucose it consumes. A high glucose concentration in the feed stream decreases cell growth. In the presence of excess glucose, the yeast converts some glucose to ethanol, thereby reducing the cell mass yield. To get the high conversion of glucose to cell mass, the glucose concentration in the fermentation had to remain near to zero and new glucose has to be added continuously. In fed batch fermentation, the nutrients (glucose) are fed intermittently during the production phase (log phase). This technique has been found to be particularly effective for processes in which substrate inhibition, catabolic repression, and product inhibitions are important. The glucose is fed to the yeast at a rate almost equal to the glucose consumption rate (*Tadeusz et al.,* 2005). There is no inhibition effect due to high glucose concentration in fed-batch fermentation. It is not easy to equilibrate the glucose feed rate with the consumption rate by the yeast. Since the yeast cell number is growing, the glucose feed rate should increase as the yeast cell number increase, but in a practical fermentation there is only one feed rate.

2.5 Food Grade Yeasts

Various microorganisms are used for human consumption worldwide as SCP or as components of traditional food starters, including algae (*Spirulina, Chlorella, Laminaria, Rhodymenia, etc.*), fungi (*Aspergillus*, *Penicillium, etc.*) and yeasts (*Saccharomyces, Candida, Kluyveromyces, Pichia* and *Torulopsis*). Among the yeast species, *Saccharomyces cerevisiae* and *Candida utilis* are fully accepted for human consumption, but very few species of yeast are commercially available. The most common food grade yeast is *Saccharomyces cerevisiae,* also known as baker's yeast, which is used worldwide for the production of bread and baking products. *S. cerevisiae* is the most widely used yeast species, whose selected strains are used in breweries, wineries and distilleries for the production of beer, wine, distillates and ethanol. Baker's yeast is produced utilizing molasses from sugar industry by-products as a raw material. Commercial *S. cerevisiae, S.carlsbergenesis* and other yeast products available to cover the needs of the baking and single cell protein fermentation industries or for use as nutritional supplements for humans (*Bekatorou, et al.,* 2006 *and Haider, et al.,* 2003*).*

2.6 Market & Growth Drivers

From the *industrial extension bureau of Mott macdonald,2007* report, the growth of baker's 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 of bakery items, form the total bakery production, the bread production alone was estimated a growth rate of 7.5%. Baker's yeast market in Ethiopia is demanding because of its population and emerging new food processing industries in the country. In Ethiopia, food habits have started to change from traditional system.

Table 2.2 Manufacturing data of baker product in Ethiopia

Years (E.C)	1993	1994	1995	1996	1997
Manufacture of bakers products	104	124	127	141	197

Source *Ethiopia statistic Authority,* 2006

Increasing consumption of bread in city and semi urban areas as a staple food rather than just a breakfast item and industries are registering increasingly which indicates good prospects for baker's yeast demand in the domestic market. Demand for bakery products is increasing as they are an essential content of many fast food items and people now increasingly prefer convenience bakery products to traditional food items.

3. MATERIALS AND METHOD

3.1 Materials

3.1.1 Sugar cane molasses

The raw material cane molasses collected from wonji /shoa sugar factory and analyzed its necessary elements and nutrients before its use to fermentation. Weighing out desired amount of cane molasses in gram and diluted with distilled water.

3.1.2 Chemicals and Reagents

It has used different analytical grade chemicals and reagents for raw materials and product analysis.

3.1.3 Biological material

Biological materials used are constituted of three different yeast strains. A brewery yeast strain (saccharomyces calesbergensis) is collected form Meta ambo brewery factory. Baker's yeast (saccharomyces cerevisiae) has available on commercial market and wine yeast form the laboratory. All strains are kept in the refrigerator to avoid of contamination.

3.2 Biochemical compositions analysis of cane molasses

3.2.1 Determination of total solids

Weight out of 10 gm cane molasses of well mixed and diluted with 50gm of distilled water to bring to 60gm of total weight and stirred with glass rod until completely dissolved. Filter through a fluted what man No 41, filter paper covered on the funnel with a watch glass to minimize evaporation. It has ejected the first 20ml of filtrate collected sufficient filtrate in a 150ml beaker for determination of refractometric brix at 20 ^{0}C. It has taken reading from refractometer after bring temperature of the molasses solution to 20 ^{0}C using water bath. The refractive index of the solution at 20 ^{0}C converted to degree Brix at 20 ^{0}C from the instrument guide table *(Ethiopian standard,* 2004*).*

3.2.2 Determination of sulphated ash

Weigh out 20gm cane molasses sample and add 20gm water, with a 1:1 w/w solution. After the sample has been mixed well, it has been prepared by taking minimum value and Added equal quantity of water that was 10gm of distilled water and mixed after a further gentle reheating. It added 2 ml of sulphuric acid, then heated with crucible and Bunsen burner in a fume cupboard until the sample was completely carbonized. Sample crucible has been placed in a muffle furnace at 550 ^{0}C for 2 hours. It has cooled and added 2ml H_2SO_4 acid solution and evaporated on the Bunsen burner in a fume cupboard. It incinerated at 650 ^{0}C for 30 minutes and cooled in the desiccators. It was taken final weight of the samples and calculated sulphated ash of the cane molasses in percent *(Ethiopian standard,* 2004*).*

3.2.3 Determination of total reducing sugars

This method relies upon reducing sugar to reduce Fehling`s solution under standard conditions. 50 g/l hydrolyzed cane molasses solution has prepared and 25ml of the cane molasses solution transferred in to a 250ml volumetric flask. Add 5 ml of the 6.34 N Hcl acids from a 25ml burette mix gently. The flask has been immersed in the water bath at 60 ^{0}C and swirled gently for 3 minutes to raise the temp of the sample. It should be stayed in the water bath for further 12 minutes. The flask cooled and diluted the solution to approximately 125ml with water and added a few drops of phenolphthalein solution. Then 2N NaoH was added to impart a red dish color to the solution. During the addition of alkaline, the solution should be gently agitated. The red color of the solution has been discharged by adding a few drops of 0.5N Hcl, 4.0ml of the EDTA solution mixed well and make up to the volume with water at 20 ^{o}C. 20 ml Fehling's s solution was pipette in the boiling flask which had 20 ml of the hydrolyzed cane molasses solution with 2 – 4 drops paraffin. The flask which had Fehling`s solution and standard invert solution on has been Placed on Bunsen burner to reach boiling point and added 4 drops of the methylene blue solution. Titration was preceded by initially adding increments of 2ml molasses solution and progressively reducing the additions down to 0.2ml and attempting to obtain the end point in about 2 minutes from the time the solution commencing boiling. The end point is denoted by the disappearance of the blue color.

It has carried out a further testing on the next sample by adding 20ml of fehling`s solutions to the boiling flask, adding sufficient water to give volume 75 ml. After 2 minutes boiled and added four drops of methylene blue solution**.** It has been taken titration result by adding the hydrolyzed molasses solution drops wise and calculated total reducing sugars concentration according to *Ethiopian standard, 2004 and John,* 1954.

Percent total reducing sugar (as inverted sugar) $=100/C*T_i$

C= concentration of molasses test (gm/100ml)

T_i = volume of molasses solution used in ml

3.2.4 Determination of nitrogen

Using Kjeldahl method all nitrogen was converted to ammonia by digestion with a mixture of conc. sulphuric acid and conc.orthophosphoric acid containing potassium sulphate as boiling point raising agent and selenium as catalyst. The ammonia released after alkalinization with sodium hydroxide was steam distilled into boric acid and titrated with Sulphuric acid. Weighing out molasses sample transferred into a tecator tube, Place it in the tecator rack. Adding 5 ml of NH_4cl solution in to each tecator tubes and 6 ml of the acid mixture that was mixed of 5 parts of conc. Orthophosphoric acid with 100 parts of conc. Sulphuric acid. It has mixed the molasses sample and acid carefully. 3.5 ml of hydrogen peroxide was added step-by-step. There was a violent reaction. By adding 3gm of the catalyst mixture for 30 min before digestion. Digestion was going on for more than 3 hour at 370 ^{0}c. Distillation has been placed on a 250 ml conical flask contain 25 ml of the boric acid – indicator solution under the condenser of the distiller with its tip immersed into the solution. Transfer the digested and diluted solution into the sample compartment and added 25 ml of the 40 % sodium hydroxide solution into the compartment, it has been rinsed down with a small amount of water, and switch on the steam. It has been distilled until 100 ml then continued until a total volume of a few ml of water before the receiver was removed. Then it was titrated with 0.1N sulphuric acid to a reddish color using the radio meter P^H stat.

$$\text{Total percent of nitrogen in a sample} = \frac{(T-B)*N*14*100}{W}$$

T: Volume in ml of the standard sulphuric acid solution used in the titration for the test

B: Volume in ml of the standard sulphuric acid solution used in the titration for the blank determination.

N: Normality of standard sulphuric acid

W: Weigh in grams of the test material.

3.2.5 Determination of calcium

Wet ashing method is used to test calcium content of the cane molasses using 60ml tri-acid solutions to 4gm molasses sample in beaker or conical flask cover with a watch glass and allow standing for 8hours. Initially, it has digested at low heat on a hot plate, then increased temperature to 200 ^{0}C, and digested until oxidation is completed. It was added 2 ml to 4 ml concentrated HNO_3 and reheated. Increase temperature until more fumes was evolved. Cooled and added 20ml 2N HCL and boiled for 2-4 hrs. It has transferred to 100ml volumetric flask filtered through what man no 541. It was prepared 1ppm, 2ppm and 3ppm standard solution for calibration and reference reading of sample testing in the atomic absorption. It has taken the atomic absorption reading and calculates its calcium content of the sample cane molasses.

3.3 Experimental procedure

3.3.1 Preparation of culture media and sugar cane molasses

Dark and thick jelly cane molasses was collected from wonji/shoa sugar factory. It can store for long time isolated from biological materials with out significant quality change. The amount of sugar was estimated in the substrate cane molasses by DNS method, and then diluted suitably according to the need using distilled water. 99 ml distilled water was added to 1ml cane molasses to dilute and black solution was prepared with out any sample.0.1mLdiluted cane molasses was taken and add 2.9ml distilled water and followed by 1ml DNS reagent. Samples and blank were boiled for 10minuts at 90 ^{0}C. After cooling, reading was taken at 540nm wave length using spectrophotometer. Absorbance reading was converted corresponding sugar amount from standard curve. Standard graph was prepared using different sugar concentration by DNS method (*Miller et al.,* 1959*)*.Based on this result culture media of sugar cane substrate has been prepared in 5 % (w/v), 10 % (w/v) and 15 % (w/v) sugar concentration. It was adjusted the pH 5-5.6 and sterilized at 120 °C for 20 minutes in the autoclave.

3.3.2. Production of bakers yeast (*saccharomycess*)

Preparation of biological solid medium:

Yeast is generally grown in different solid media, Common media used for the cultivation of yeasts include; YPDA (yeast extract, peptone and dextrose agar), MYPG (maltose, yeast extract and peptone) and yeasts mould agar or broth. Accordingly, the best media, which is appropriate for yeast growth is MYPG (maltose, yeast extract and peptone) and YPD media (yeast extract, peptone and dextrose) (*Gunasekaran,* 1995).

YPD broth and agar medium was prepared by taking 10g/l yeast extract, 20g/l peptone, 20 g/l dextrose, 20 ml/l acid and with and with out 20 g/l agar into 250 ml flask. The medium was sterilized in the autoclave at 21lbs pressure for 15 minutes with 121 ^{o}c and let to cool. YPD media was poured in Petri plate with the technique is shown in Fig 3.1 (*Dasilva et al.,* 2005)

Preparation of the inoculums:

Inoculating 5gm of all the three strains of baker's, wine and brewer yeast separately into 100ml YPD broth and aerated for more than 12 hrs with 120 rpm. Pure yeast culture strains were inoculated into Petri dish of YPD agar media with streak plate techniques. It was incubated the inoculated YPD agar plate in 30 ^{o}c incubators for 12hrs.It was maintained on agar media at 4 ^{o}c. Transferring of single colonies of baker's , wind and brewery yeast into 5 ml YPD media and grow under aeration by rotating over night at 120 rpm.(*Acourene et al.,* 2007*, Haider et al.,* 2003 *and Gunasekaran,* 1995)

Preliminary yeast growth experiment

The experiment was carried out by taking 5 gm baker's, wine and brewery yeast strains into YPD media and 5 gm molasses with 1.8 % $NaNO_3$ in 100 ml distilled water. Yeast growth was measured every two hrs for 14 hrs period at 540 nm wave length and plot the curve of microbial growth condition of each strains.

3.3.3 Biomass yield of the three strains in YPD and different substrate concentration

100 ml YPD medium was prepared and sterilized at 121 ^{o}c for 15 minutes. It was inoculated 5 gm pure strain of baker's, wine and brewery yeast and shaking with 120rpm for more than 12 hrs. It was transferred 10 ml of cultured yeast in test tubs and centrifuged with 4500 rpm for 20 minutes. supernatant was removed and the pellet was collected and inoculated into 5 % (w/v), 10 % (w/v) and 15 % (w/v) cane molasses samples which has enriched in nutritious elements of 2 % (w/v) $NaNo_3$,$(NH_4)_2SO_4$ and $KNO_{3.}$

3.4 Analytical method used for measuring and analyzing yeast growth

3.4.1 Kinetics of growth

It has taken off 5ml of medium sample every 2 or 12 hrs time interval until 36 hrs. It has measured optical density of the samples at 540 nm weave length in the spectrophotometer and blank solution was used for reference reading.

Recorded the result and plot a curve to see the yeast cell growth of each strain in different sugar concentration of cane molasses and YPD medium (*Acourene et al.,* 2007).

3.4.2 Analysis of baker's yeast

Biomass Estimation:

At the end of the yeast growth, the culture was harvested using centrifugation at 4500 rpm at 20 minutes. During centrifuging the supernatant and pellet was separated. The liquid part of the culture that is supernatant was utilized for the determination of the residual sugar estimation. The remaining pellet was diluted with 5 ml distilled water and measured optical density of the biomass on different substrate concentration at 540 nm. Record the result and plot a curve to see biomass yeast cell growth condition of each strain (*Acourene et al.,* 2007 *and Bill et al.,* 1996).

Reducing sugar estimation by dinitrosalicylic acid (DNS) method

The glucose concentration in the different substrate concentration was determined by the 3, 5-dinitrosalicylic acid (DNS) method (*Miller,* 1959).In this method, DNS reagent was prepared from 3, 5-dinitrosalicylic acid ,10.6g , Rochelle salt (Na-K) ,306g, phenol, 8.132 g, sodium metabisulfite, 8.3 g, Phenolphthalein, 3 ml, NaOH, 19.8 g, and 0.1 N HCl were mixed in 1416 ml distilled water. Standard glucose solutions were prepared in concentrations of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0 g l^{-1}. 1 ml of standard glucose solution was pipetted into a test tube and 3 ml of DNS reagent was added to it. The mixture was placed in boiling water for 5 minutes to develop color. After 5 minutes, the sample was cooled to room temperature. About 2 ml of the sample was placed in a cuvette and the absorbance at 540 nm was measured. Blank was prepared with all reagent but without sample instead of sample, water was used. The absorbance of the standard glucose solutions were all measured and Plotted against the concentration of glucose. The plot was linear with slope of 1.31 and the correlation coefficient (r) 0.993. The glucose content of the supernatant solution was measured by the DNS method using the glucose calibration curve determined above.

Total protein concentration estimation

The capacity of protein concentration estimation in baker's, wine & brewery yeast in different substrate concentration of sample was determined by the (Lowry *et al.,* 1951 *and Palanivelu,* 1997 *method).*

Lowry et al method is used the following reagents in analytical form

SOLUTION-A: - 50 ml of 2 % sodium carbonate mixed with 50 ml of 0.1N NaOH Solution, (0.4 gm in 100 ml distilled water.)

SOLUTION-B: - 10 ml of 1.56 % copper sulphate solution mixed with 10 ml of 2.37 % Sodium potassium tartarate solution.

SOLUTION-C: - was prepared by mixing 1 ml of SOLUTION-B with 99 ml of SOLUTION-A

SOLUTION-D: - was prepared by diluting Folin - Ciocalteau reagent solution commercial reagent (2N) with an equal volume of water

Sample to be analyzed has been recentrifuged with 4500rpm for 20 minutes after tested the yeast cell growth. Supernatant was removed; the remaining pellet was diluted with 5 ml distilled water and added 1 ml of 0.85 % (w/v) Nacl solution. 0.1 ml pellet solution was dissolved with 2.9 ml in different test tubes and followed by 1ml alkaline copper sulphate reagent that is solution (c). It was mixed well and incubated at room temperature for 10 minutes. Then added 0.5 ml solution (d) to each tube, vortex it well and incubated for 30 min in dark area. Blank has been used without sample. Regarding to the intensity of the color, spectrophotometer reading were observed as optical density at 720 nm. Protein concentration (g/l) was calibrated using appropriate standard graph BSA.

4. BAKER'S YEAST PRODUCTION IN INDUSTRY LEVEL

The combined effect of yeast growth, sugar assimilation rate and protein yield has taken from the laboratory results. Baker's yeast, s.cervisiae is used for industrial-scale production. This strain is selected for stable physiological characterization, vigorous fermentation and cellular dispersion in water. During yeast growth, there will be rapid growth, high cell yields and easy maintenance during storage (*Parakulsuksatid,* 2000). The fermentation of baker's yeast has produced a product with minimum variation in yeast performance and maximum yield on 5-7 %(w/v) substrate concentration (*Bouleuard,* 1992*)*.Industrial production of baker's yeast is used to utilize more than 15,000 tons of molasses per year. It can satisfy more than 192 baker item manufacturing industries and saving foreign currency which has allocated for importing baker's yeast. Considering some amount of molasses has tried to use in alcohol, K-50 and other activities in the country. It has assumed to use one –third of average annually production of cane molasses that is 15,000 tons/year, assuming 300 working days for industrializing the process and evaluating the economic viability of the project.

4.1 Preparation of molasses

The raw material cane molasses containing 45-55% sugar as inverted sugar and 50 tons/day is used as the sugar source for yeast productions. Concentrated molasses should be prepared as solution, 7 % (w/v) of sugar concentration with adding 150 tons/day hot water using a mechanical stirrer. The solution is then brought up to the necessary sterilization temperature with live steam preferably in a stainless steel boiling tank. After sterilizing the molasses must next be clarified 10 % of the solution of sludge which is normally contains in the molasses. The sludge is mainly inorganic, containing much calcium sulphate, silica and earthy material, but it also contains small amount of precipitated proteins and fatty and vegetable cellular materials. It has selected mechanical clarification of molasses by means of the modern centrifugal clarifier (*Dallas,* 1994). The necessary equipments and its specification for molasses treatment plant were listed in (Table 4.1).

Table 4 .1 Equipment specification of molasses treatment plant

Equipments	Quantity	Type	Capacity
Storage tanks	**2**	**Vertical cylinder**	**300 m^3**
Mixers	**1**	**Vertical cylinder**	**253 m^3**
Clarifier	**1**	**Centrifuge**	**8 m^3/hr**
Sterilizer	**1**	**Plate heat sterilizer**	**7 m^3/hr**
Mash tanks	**1**	**Vertical cylinder**	**214 m^3**

4.2 Fermentation process at industry scale

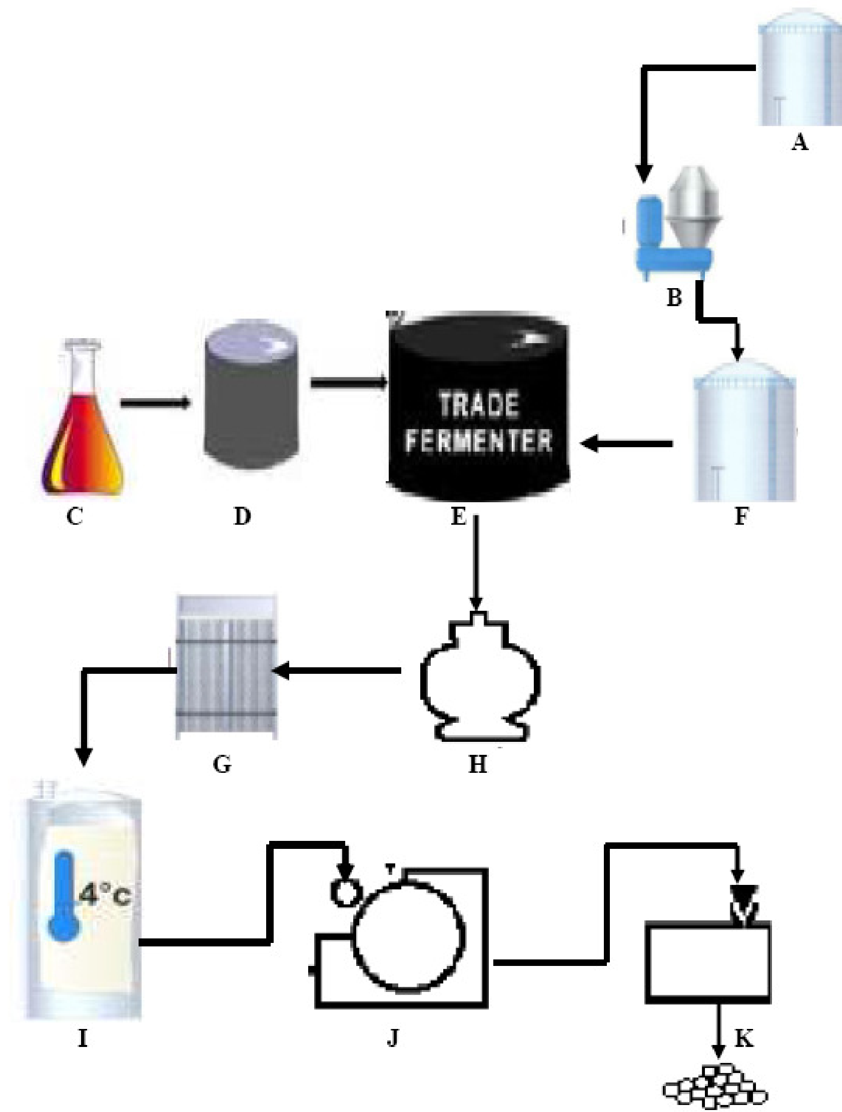
Yeast cells are grown in a series of fermentation vessels. A typical fermentation process is shown in Figure 4-1. In general, the process consists of placing a laboratory-grown pure yeast culture, along with the other feed materials, into the first fermenter and allowing the yeast to grow. Yeast is propagated when the mixture is placed in consecutive fermenters that are equipped for batch or Incremental feeding of the molasses solution. Yeast fermentation vessels are operated under aerobic conditions (free oxygen or excess air present) because under anaerobic conditions (limited or no oxygen), the fermentable sugars are consumed in the formation of ethanol and carbon dioxide, which results in low yeast yields. To maximize yeast yields, it is important to supply enough oxygen to keep the dissolved oxygen content in the liquid surrounding the yeast cells at an optimal level *Stone, (*1998*)*. In practice, however, oxygen transfer rates are often inadequate. Under such conditions, some ethanol is formed. In addition, it is also important to control the amount of fermentable sugars present, so that the yeast assimilates the sugar as fast as it is added. The following subsections describe each stage in the fermentation sequence.

Laboratory Stage: - The initial fermentation stage takes place in the laboratory.
A portion of the pure yeast culture is mixed with the molasses solution in a sterilized flask. The total contents of the flask are typically less than 5 liters (l) and the yeast is allowed to grow in the flask for 2 to 4 days. The entire flask contents are then used to inoculate the second fermentation stage.

Pure Culture Stages:-Typically, the next stage consists of pure culture fermentations. The capacities of the fermentation vessels used in this stage range from 1,140 l to 26,500 l. The pure culture fermentations are batch fermentations in which the yeast is allowed to grow for 13 to 24 hours (hr). The contents of the fermenter from the first pure culture stage are added to the next fermentation vessel, which already contains the nutrient-rich molasses solution. These fermentations are a continuation of the flask fermentation, except that they have provisions for sterile aeration and aseptic transfer to the next stage. The yeast yield in the pure culture fermentations is approximately 27 kilograms (kg) in the first fermenter and 600 kg in the second fermenter *(Bouleuard,* 1992). The critical factor in the pure culture operation is sterility. Rigorous sterilization of the fermentation medium prior to inoculation is conducted by heating the medium at 165 ^{0}C under pressure or by boiling it at atmospheric pressure for extended periods. If a sterile environment is not provided, contaminating microorganisms can easily outgrow the yeast. The need for process control in the pure culture medium is limited. However, microbiological testing of the medium before, during, and after each fermentation is essential. The molasses concentration of the fermenter is often standardized to between 5 and 7.5 percent sugar. Once the pure culture fermentation is started, the only controllable parameters are the temperature and the degree of aeration *(Dallas,* 1994*).*

Main Fermentation Stages: - The majority of the yeast yield grows in the final fermentation stages. The main fermentation steps may take place in a two-stage. The fermenters used in the final stages are usually constructed of stainless steel and vary considerably in size, ranging from 37,900 l to over 283,900 l. These vessels have diameters in excess of 7.0 meters (m) and heights up to 14 m. The larger vessels are associated with the final fermentation stages. The fermentation vessels are typically operated at 30^{0}C (86^{0}F). Fermenters are usually equipped with an incremental feed

system. This incremental feed system may be a pipe or a series of pipes that distributes the molasses over the entire surface of the fermenter liquid. The rate at which the molasses is fed is critical and may be controlled by a speed controller connected to a pump or by a valve on a Rota meter, which delivers a certain volume of molasses at regulated time intervals. Nutrient solutions are kept in small, separate tanks and are added through rotameters into the fermenter, but the rate of feed is not as critical as with molasses. Fermenters must also be equipped with heat exchangers to remove the heat produced from the production process and to cool the fermenter. The type of heat exchanger system used depends on the size of the fermentation vessel. Because large volumes of air are supplied to the fermentation vessels during this stage of production, the fermenter size influences the type of aeration system selected. The different types of aeration systems include horizontal, perforated pipes; compressed air and mechanical agitation; and a self-priming aerator. In the horizontal, perforated pipe system, air is blown through a large number of horizontal pipes that are placed near the bottom of the fermenter. With this aeration system, the only agitation of the fermenter liquid is carried out by the action of the air bubbles as they rise to the surface. Typically, this type of aeration system requires from 25 to 30 cubic meters (m^3) 0.45 kilograms (kg). The efficiency of aeration with a given volume of air is greatly increased by mechanical agitation. In a compressed air/mechanical agitation aeration system, air under pressure is supplied to a circular diffuser pipe. Directly above the air outlets, a horizontal turbine disk provides mechanical agitation, which distributes the air bubbles uniformly. Agitation systems have baffles to keep the fermenter liquid from rotating in the direction of the motion of the disk. This uniform distribution of air bubbles reduces the volume of air needed to grow the yeast. In an agitated system, only 10 to 15 m^3 of air are required to produce 0.45 kg of yeast. The final trade fermentation stage has the highest degree of aeration, and molasses and other nutrients are fed incrementally. Large air supplies are required during the final trade fermentations, so these vessels are often started in a staggered fashion to reduce the size of the air compressors required. The duration of each of the final fermentation stages ranges from 11 to 15 hr. In each of the above stages, the liquid is aerated for an additional 0.5 to 1.5 hr after all of the required molasses has been fed into the fermentor. This extended aeration period permits further maturing of the yeast and more stability in refrigerated storage. (*Bouleuard,* 1992 *and Dallas,* 1994*)*



A (cane molasses).,B(clarifier),C (seed yeast in flask),D(Seed propagation),E(trade fermenter),F(mash tank),G(cooler),H((centrifuge),I(cream yeast tanker),J(vacuum drum filter),k(granulator)

Fig 4.1 Process flow diagram of the production of baker's yeast

4.2.1 Material and Energy Balance

The major operation of baker's yeast production is fermentation. Fermentation is the heart of the baker's yeast process so that material and energy balance has conducted in the fermenter to determine the yield of the fermenter and sizing of post harvesting product treatment equipments. After treating 200 tons/day cane molasses solution, it has clarified 10 % of it. Then 180 tons/day molasses solution and ammonium sulphate in the ratio of one to three was processed.

Molasses solution = 180 tons/day

$(NH_4)_2SO_4$ = 14 tons/day

V = Volume of materials in the fermentor

$$V = \frac{\text{mass of molasses solution with ammonium sulphate}}{\text{Density of the solution}}$$

$$V = \frac{(180+14)\ \text{tons/day}}{1052\ \text{kg/m}^3}$$

$$V = 184\ m^3$$

Assuming three fermenters and 70 % of the vessel volume was filled with substrate solution, with gas space occupying the top portion of the fermenter. To facilitate the reaction with the combined action of aeration and agitation of the liquid need space in the vessel. The volume of the each fermentor is 88 m^3 and calculating the amount of fermentable sugar in the molasses using data collected from sugar factory, molasses for (10-17) percentage reducing sugar has 30-37% pole. Based on this 7% sugar concentration molasses has 13 % pole.

$$\text{Mass of fermentable sugar} = \left(\frac{\text{Mass of molasses}}{\text{Day}} - \frac{\text{Mass of } (NH_4)_2SO_4}{\text{Day}}\right)*0.13 \text{---------} (4.1)$$

$$So = (M_{mola} - M_{(NH4)2\ SO4})*0.13$$

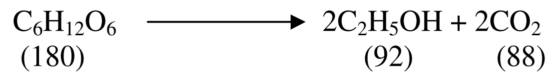
$$So = (180{,}000 - 14000)\ kg * (0.13)$$

$$So = 21{,}580\ kg/day$$

$$So = 7193 \text{ kg/d*f}$$

Mass of fermentable sugar (S_o), mass of molasses (M_{mola}) and mass of ammonium sulphate ($M_{(NH4)2SO4}$).

From theoretical equation of the alcohol to yeast substance transformation theory as shown in the equation



$$\underset{(180)}{C_6H_{12}O_6} \longrightarrow \underset{(92)}{2C_2H_5OH} + \underset{(88)}{2CO_2}$$

The carbon from 180 parts of hexose will yield 92 parts of alcohol, which will be transformed into yeast substance. Four carbon atoms from the six in the original hexose will go to from yeast substance carbon – that is 180 parts of sugar yield 48 parts of yeast carbon. The average carbon content of yeast dry matter is about 47%, so that 48 parts of carbon from 180 grams of hexose sugar will form (48*100)/47 = 102.1 part of yeast dry matter. Maximum yield obtainable from 180 part of hexose sugar be 102.1 part of yeast dry matter, and 100 parts of hexose sugar would yield 57 parts of yeast dry matte. Theoretical annual bakers yeast production has been calculated 57% * Amount of hexose sugar used from *John (*1954*)*. Theoretical Annual baker yeast is 0.57 *21,580 kg/d = 12301kg/d or 513 kg/hr. 171 kg/hr bakers yeast will be produced from each fermenter. From cell kinetic in exponential growth phase the progressive doubling of cell number results in a continually increasing rate of growth in the population. The rate of the cell population increase (Rx) at any particular time is proportional to the number of density (x) of yeast cell present at that time will be calculate by *(Zang,* 2003).

$$R_x = \frac{dx}{dt} = \mu x \text{ -- (4.2)}$$

Where the constant μ is known as the specific growth rate (hr^{-1})

$$x = x_o \exp(\mu t) \text{ --- (4.3)}$$

Where, x is cell mass concentration (g/l)

t is time (h) and μ is known as the specific growth rate (hr^{-1})

Batch fermentation has been selected for this process and 24 hrs used as one batch including sterilization time of the fermentor. Active dry yeast is one of the common types of baker yeast. So that it was considered the product on dry cell mass that is 4104 kg/d .f From experimental data, it has found that only 80% of the yeast biomass is viable the rest 20 % are dead and taking critical specific growth rate μ is 25 (hr^{-1}) *(Hoek, et al.,* 1998*)*.
It has calculated seed cells which is used for each batch is 12 kg.

The physical characteristics of a typical commercial fermentation vessel are shown in Fig (4.2). There is design assumption for fermentation processes. Fermentation vessels are usually constructed from stainless steel to minimize corrosion problems and contamination of the fermentation broth by unwanted metallic ions. It needs care in the overall process to avoid dead spaces and cervices where solids resistant to sterilization can accumulate and where microbiology films can grow. Typically 70 to 80 % percent of the vessel volume is filled with liquid to have gas space. Agitation and aeration will promote foaming in the liquid surface. Foams can interfere with the effectiveness of the fermentation by impending gas mass transfer from broth to the head space. It is necessary to use antifoams agent to avoid this problem *(Baily,* 1977). To determine the shape and basic internal dimension of the fermenter. It should analyze the total pressure exerted to the fermenter from the combined effect of static head of the solution, the CO_2 evolved during the process and atmospheric pressure.

Static head pressure = $P_s = \rho_s * g * h$

Pressure caused by CO_2 = $P_{CO2} = \frac{n*R*T_f}{V_C}$ assuming ideal gas law

and atmospheric pressure (P_A)

$$P_T = P_A + P_S + P_{CO2} \qquad (4.4)$$

P_T = 2.7 bar

Where, P_T = Total pressure in the fermenter, ρs = density of solution was taking water density 1000 kgm^{-3}, h = height of solution in fermenter was assumed 7m, g = gravitational acceleration (9.8ms^{-1}), R = universal gas constant (8.314$JK^{-1}mol^{-1}$), V_C = volume of CO_2 (1954 m^{-3}) and T_f = temperature of fermentor (30 ^{O}C).

According to the total pressure exerted on the fermenter, torispherical head is beast. It operates less than 15 bar pressure, easier and cheaper to fabricate (*Sinnott,* 1993*).*Internal geometrical dimension of the fermenter is shown in the Fig 4.2 and the beast geometrical correlation is reported from (*Fryer et al.,*1997 *and Bally et al.,*1977).

Total volume of the fermenter vessel is the sum of cylinder volume and torispherical volume.

V = volume of cylinder + 2 * volume of torispher-------------------- (4.5)

$V = \prod * r^2 * H_S + 2*0.07\ Dt$

$V = 1.18\ Dt^3 + 0.14\ Dt^3$

Diameter of the fermenter was calculated 4 m and from geometric relation diameter of the impeller was 1.3 m.

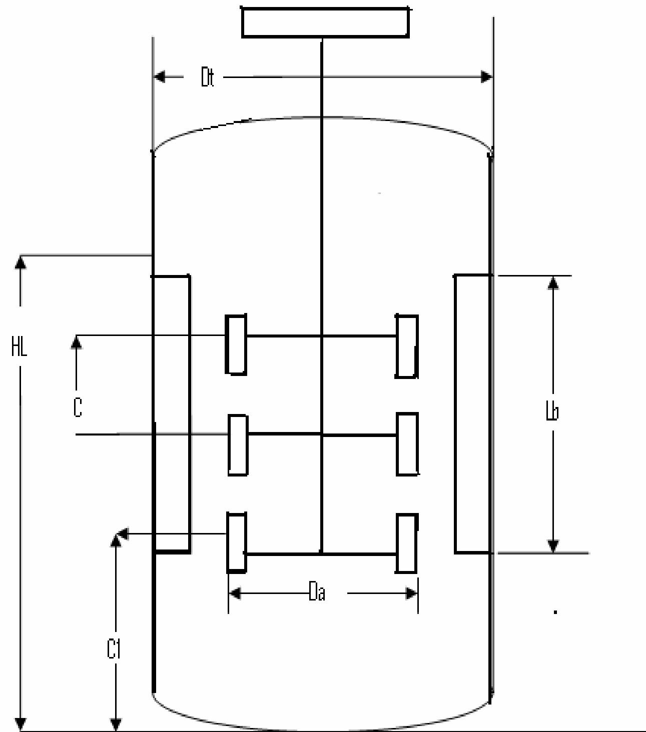


Fig 4.2 Sectional diagram of fermenter used for baker's yeast production

Da = diameter of the impeller; Dt= diameter of the fermenter, L_b = baffle length, H_L = height of the solution, H_S = height of cylinder, C = the gape between the impeller disk and C_1 the impeller height form the bottom

Geometrical correlation of the fermenter internal parts is reported (*Fryer et al.,* 1997 *and Bally et al.,* 1977)

Geometric ratio	Typical rang value	Standard geometry
Da/D_t	1/4—2/3	1/3
C_1/D_t	1/4—1/2	1/3
H_L/D_t	1—3	1.75
C/Da	1.5—3	1.5
C_1/Da	~1	1
H_S/Dt	3/2	3/2
L_b/Dt	1/12—1/10	1/10

4.2.2 Estimated impeller power consumption of fermenter

Estimation of power consumption of each fermenter from the impeller was calculated from *coulson and Richardsons, (*1996*), Sinnott, (*1993*) and Perry, (*1984*).*the shaft power of an agitator can be estimated using the following dimensionless equation. Calculating *Reynolds* number and correlate the power number from the graph.

$$N_{Re} = \frac{Da^2 * N * \rho}{\mu} \quad (4.6)$$

$$N_P = \frac{P}{N^3 * D_a^5 * \rho} \quad (4.7)$$

P = 356 k watt

Using impeller diameter of (Da) is 1.3 m, taking water density (ρ) and viscosity (μ) respectively with taking optimum revolution of impeller that is 2 revolutions per seconds. Shaft power (P) is 365 kwatt for single agitator.

Time –cycle of operation

The time- cycle of the various operations, from the cleaning of the fermenter to the discharge of the broth in Fig (5.1) is needed in order to define the annual numbers of runs. The cycle time, tc for each batch run is given by *Ghose, (*1977*).*

$$tc = to_1 + to_2 + to_3 + t_F + to_5 \quad \text{------} \quad (4.8)$$

Data and assumptions was collected from *Ghose, (*1977*)* in advances in biochemical engineering and from fermentation process *Bouleuard, (*1992*) and Dallas,(* 1994*)* was listed below t_f (time for reaction =52hr), to_1(time for cleaning the reaction vessel=1hr), (to_2 time required for sterilization of the vessel=3hr), (to_3= time for continuous Sterilization of the culture medium),(to_4= time need for inoculation=1hrs) and (to_5=time for discharge of broth=1hrs).

Taking the following parameters to calculate the annual number of runs, Nc, in annual production from *Parakulsuksatid, (*2000*) and Ghose, (*1977*)* in equation (4.9).

PA = annual amount of specific product [126,720kg], t_A = annual working period [300 day or 7200hrs], N_f = number of vessels [N_V =3], V = Volume of broth in vessel [$61m^3$] and ρ = cell mass concentration [8 kg/m^3]

$$Nc = \frac{P_A}{V*\rho*N_F} = \frac{t_A}{tc} \quad \text{------} \quad (4.9)$$

$$N_C = 87$$

The time required for each batch run is (tc) = 83 hrs and for continuous sterilization of culture medium using the above calculated date is to_3 = 8.5 hr

Amount of steam supplied continuously in the culture medium for sterilization is reported by *Ghose, (*1977*)* in equation (4.10)

$$Q_s = \frac{\frac{C\rho V (T-T_O)}{to_3}}{\lambda + (T- T_O) C} \quad \text{------} \quad (4.10)$$

Q_s = the rate of steam supply ton/h, C = Specific heat of culture medium assume equal to that of Water (10^3 K Cal ton $^{-1}$ $^0c^{-1}$), V = volume of material in the fermentor T = Sterilization temp (120 ^{0}c), λ = latent heat of vaporization of water = $494*10^3$ Kcal ton $^{-1}$, Ts = steam temp (165^0c) at $6kgcm^2$,

ρ = density of culture medium assuming water (1 ton m^{-3}), T_o = room temp (20^oc)

Amount of steam required for sterilization of the medium is 4 ton /hr.After sterilization of the culture medium to be free from the microorganism and cooling to optimum temperature with heat exchanger. The heat transfer area of the heat exchanger is explained by *Ghose, (*1977) in equation (4.11)

Data and assumption is listed below

A = heat transfer area of heat exchanger (m^2), T_C = is temperature of cooling water (^OC), T_F= medium temperature at exit of heat exchanger (^OC), T_X = temperature of cooling water at exit (^OC) and U = over all heat transfer coefficient (Kcal $m^{-1}h^{-1O}C^{-1}$)

$$A = \frac{\frac{C \rho \underline{V} (T-T_F) \ln \frac{T- T_F}{T_F-T_c}}{to_3}}{U \{(T-T_F) - (T_F- T_C)} \quad \text{-- (4.11)}$$

The heat transfer area of heat exchanger is 17 m^2 for each heat exchanger.

Fermenter also should be cooled to keep the desired temperature for yeast growth. The rate of cooling water supply to maintain the desire temperature for fermentation is 122 tons /hr given by *Ghose, (*1977) equitation 4.12

$$Q_w = \frac{\frac{C \rho \underline{V} (T-T_F)}{to_3}}{(T_x-T_c)\ c} \quad \text{-- (4.12)}$$

Table 4.2 Specification of fermentation process Equipment

Equipment	quantity	type	capacity
Fermenter (pure culture)	1	Vertical Cylinder	$8m^3$
Fermenter (2nd stage)	1	Vertical Cylinder	$40\ m^3$
Fermenter (3rd stage)	3	Vertical Cylinder	$88\ m^3$
Heat exchanger	3	Plate type	$17\ m^2$

4.3 Product treatment

Once an optimum quantity of yeast has been grown, the yeast cells are recovered from the final trade fermenter by centrifugal yeast separators. The separators used in this process are continuous dewatering centrifuges. After pass through the separators, yeast solids content of 8 to 10 percent can be acquired from fermenter liquor containing 3.5 to 4.5 percent solids. The yeast is washed with water and passed through the separators a second time. The second pass usually produces concentrations of 18 to 21 percent solids. If the concentration of yeast solids recovered from the second pass is below 18 percent, then another washing and a third pass through the separators is normally required. The yeast cream resulting from this process can be stored for several weeks at a temperature slightly above 0°C (32°F). The centrifuged yeast solids are further concentrated by pressing or filtration. Two types of filtering systems are used: filter presses and rotary vacuum filters. In the filter press, the filter cloth consists of cotton duck or a combination of cotton duck and synthetic fibers so tightly woven that no filter aid is necessary. Filter presses having frames of 58 to 115 centimeters (cm) are commonly used, and pressures between 860 to 1,030 kilo Pascal are applied. Yeast yields between 27 and 32 percent solids may be obtained by pressing. Rotary vacuum filters are used for continuous feed of yeast cream. Generally, the filter drum is coated with yeast by rotating the drum in a trough of yeast cream or by spraying the yeast cream directly onto the drum. The filter surface is coated with potato starch containing some added salt to aid in drying the yeast product. The filter drum rotates at a rate of 15 to 22 revolutions per minute (rpm). As the drum rotates, blades at the bottom of the drum remove the yeast. After a filter cake of yeast is formed and while the drum continues to rotate, excess salt is removed by spraying a small amount of water onto the filter cake. From this process, filter cakes containing approximately 33 percent solids are formed. (*Bouleuard,* 1992 *and Dallas,* 1994*)*

Table 4.3 Equipments specification on product treatment

Equipments	quantity	Type	Capacity
Separator	3	centrifuge	14 m^3
Cream storage tank	2	Vertical cylinder	63 m^3
Filter	3	Drum vacuum	10m^2
Granulator	3	Conventional	469 kg/hr
Dryer	2	Fluidized bed	230 kg/hr

5. RESULTS AND DISCUSSION

5.1 Biochemical composition of cane molasses

Yeast growth has influenced by different factor like sugar, vitamins, biotin and minerals content of the molasses. Biochemical composition analysis was focused only certain of it. The composition of molasses shows wide variation. Its composition is influenced by factor such as soil type, ambient temperature, moisture, season of production, variety and technology of sugar mills can control the amount of sucrose extracted. Because of this the sugar content of molasses produced in different countries will vary according to the production technology employed. According to *Curtin (*1983*)*, changes in the design of centrifuges used to separate sugar and syrup constitute one of the major advancements in the cane sugar industry. Continuous centrifugation now results in more sugar extracted with a corresponding decrease in the amount of sugar left in molasses. The obtained result shows that cane molasses composition presented in table 4.1, reflect, there was similar result from analysis presented in the following publications (*Curtin, John, and Ethiopian standards).* However, trace element composition of molasses was not analyzed, it improves biomass yield of the yeast.

Table 5.1 Summary of laboratory results and comparing with other publication

Ser/no	characteristics	Ethiopian standards (w/v)	John (w/v)	Curtin (w/v)	Laboratory results (w/v)	remarks
1	Total solids	85%	79%	79.5%	80%	✓
2	Total reducing sugar	50%	45-55%	46%	47.7%	✓
3	Reducing sugar	14%	10-15%	-----	11.16%	✓
4	Nitrogen	---	0.15-.8%	------	0.88%	✓
5	calcium	---	0.08-.5%	0.8%	0.42%	✓
6	Ash	14%	8.1%	8.1%	8.3%	✓

Ethiopian standards, 2004, Curtin, 1983 and John, 1954

✓ = Acceptable or within the standard

5.2 Preliminary study of the kinetic growth of the three strains in molasses

From preliminary experiments, all strains of yeast cells were grown in cane molasses and normal microbial growth curve in YPD, molasses and molasses with 1.8 % NH_3NO_3 in all the three strains which is shown in Fig 5.1, Fig 5.2 and Fig 5.3. Molasses with ammonium nitrate did not have significance growth difference in all strains but it supported the growth in lag phase.

Different yeast cultures have able to reduce sugar in the cane molasses regardless of the initial total sugar content. Table 5.2 is percentage sugar utilization of baker's, wine and brewery yeast strain in YPD and molasses medium. Baker's yeast had maximum sugar utilization capacity for all media comparing to the other two yeast strain. In the other hand, brewery had minimum. The other effect which was observed from preliminary test was addition of 1.8 % (w/v) ammonium nitrate did not have any significant effect on brewery yeast strain.

Table 5.2 Percentage of sugar assimilation efficiency on baker's, wine and brewery yeast strain in YPD, molasses & molasses with 1.8 % NH_4NO_3

Types	Baker's yeast			Wine yeast			Brewery yeast		
Media	YPD	mola	Mola + NH_4NO_3	YPD	mola	Mola + NH_4NO_3	YPD	mola	Mola + NH_4NO_3
Sugar assimilation In percent	92.7	88.6	79	93	71	30.8	24	49	5.5

YPD=Yeast extract, peptone and dextrose medium

Mola= cane molasses

NH_4NO_3 = ammonium nitrate

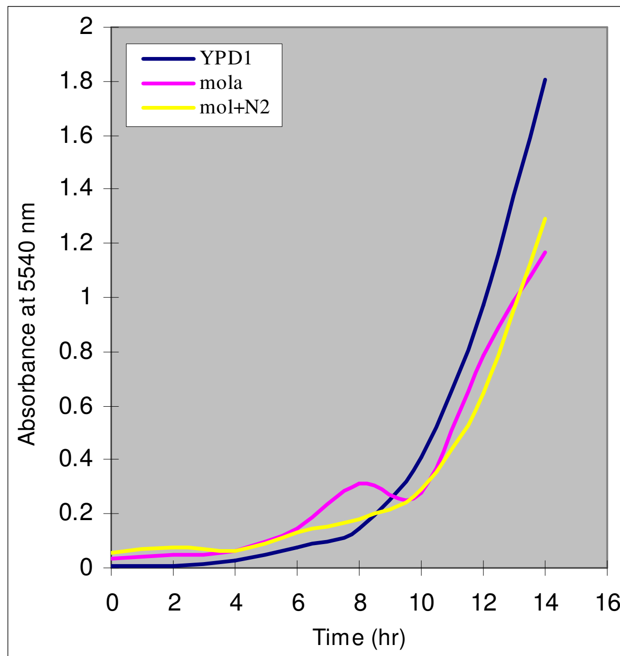


Fig 5.1 Baker's yeast growth on YPD, molasses and molasses with NH_4NO_3

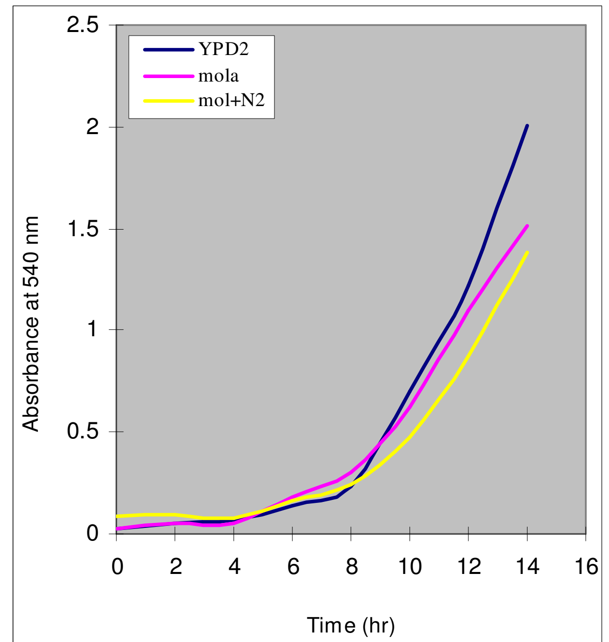


Fig 5.2 Wine yeast growth on YPD,molasses and molasses with NH_4NO_3

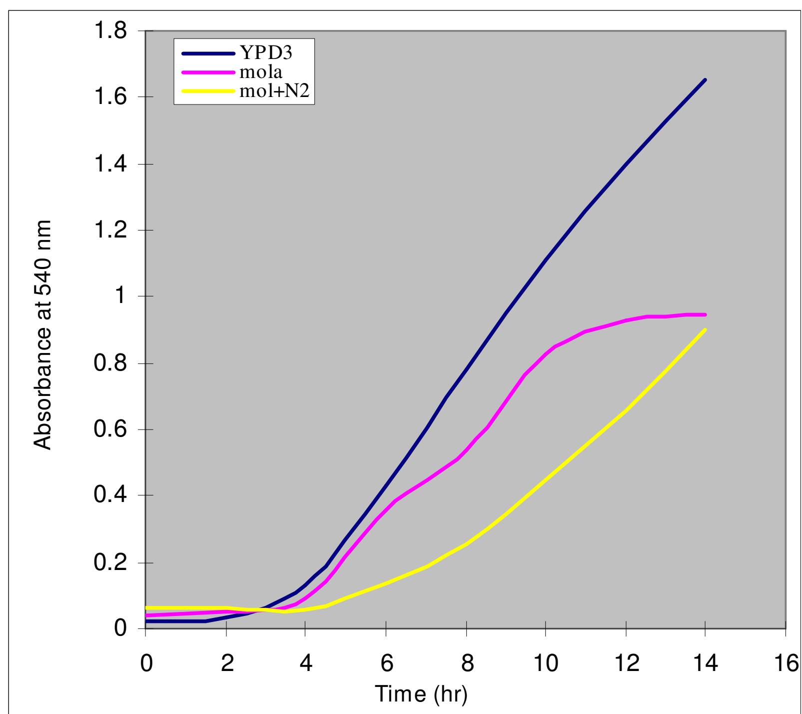


Fig 5.3 Brewery yeast growth on YPD, molasses and molasses with NH_4NO_3

5.3 Study on the kinetic and biomass yield of the three strains on different substrate concentration.

Sugar concentration decreased as the cell grows on molasses due to substrate utilization for metabolism. In order to identify the optimum sugar concentration of different types of yeast strains, experiments were carried out in the following ways. First attempt, It has been investigated by carrying out yeast growth test and sugar assimilation rate experiments with different substrate concentration in the range of 5 %-15 % (w/v) of substrate concentration *(Ryohei,* 2003). Second attempt to confirm the best substrate concentration, all the yeast strain were allowed to produce protein in 5 % (w/v), 10 % (w/v), 15 % (w/v) substrate concentration.

From the first test which has indicated on table 5.3, Baker's and wine yeast had maximum sugar assimilation in 5 % (w/v) substrate concentration. Good sugar utilization was obtained in 5 % substrate concentration in baker's and wine yeast at 36 hr yeast growth time. Good sugar utilization is obtained with initial sugar concentration up to 10 % (w/v) but the utilization decreases significantly when its concentration increased from 15 % to 20 %. The decreased sugar conversion with the highest concentration is probably due to osmotic effects of yeast cell wall (*Vincent,* 1998). The maximum amount of protein concentration was obtained in 5 % (w/v) wine yeast and 10 % (w/v) substrate concentration of baker's and brewery yeast strain. Reduction in protein concentration formation was observed when the sugar concentration of molasses was increased beyond 10 % (w/v). It may be due to the high sugar concentrations and high specific growth rates trigger alcoholic fermentation, even under fully aerobic conditions (*De deken,* 1996). Alcoholic fermentation during the industrial production of baker's yeast is highly undesirable, it reduces the biomass yield on the yeast production. The biomass yields and protein concentration are given in Fig 5.4, Fig 5.5 and Fig 5.6. It was observed that protein concentration and biomass productivity increase with increasing sugar concentration up to 15 % (w/v) substrate concentration. Generally, 5 % (w/v) and 10 % (w/v) sugar concentration had better biomass yield and protein concentration in all yeast stains throughout the yeast growth time, especially, the highest value of protein concentration and biomass productivity are obtained at baker's yeast. Based on the above results 5 %(w/v) and 10 %(w/v) of sugar concentration was selected for further experiments.

Table 5.3 Residual sugar concentration of 5%, 10% &15% sugar conc. of the three strains

	Baker's yeast			**Wine yeast**			**Brewery yeast**		
Time	**5 % (w/v)**	10 % (w/v)	15 % (w/v)	**5 % (w/v)**	10 % (w/v)	15 % (w/v)	5 % (w/v)	**10 % (w/v)**	15 % (w/v)
0 hr	63.5	103	161.5	71.5	145	162	82.5	102	165
12 hr	43	89	92	31	29.5	130.5	78	72	62
24 hr	40	69.5	79	24.5	60	64.5	73	72.6	40.3
36 hr	**40**	54.2	86	**24**	58	61	64.5	**24.5**	36.5

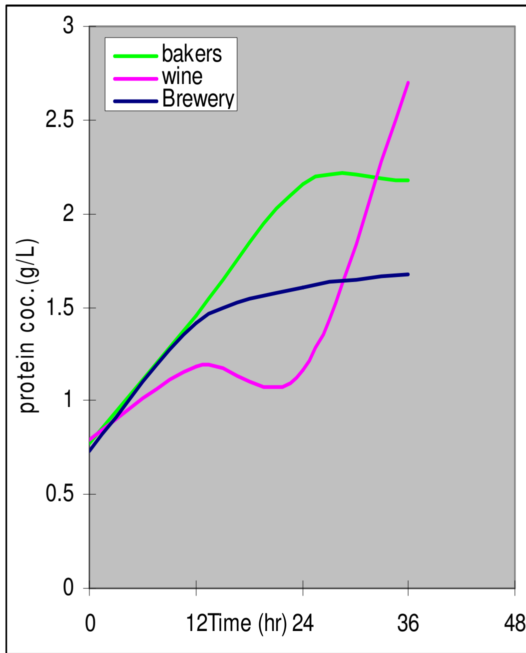


Fig 5.4 Protein conc. on baker's, wine and brewery yeast on 5% sugar conc.

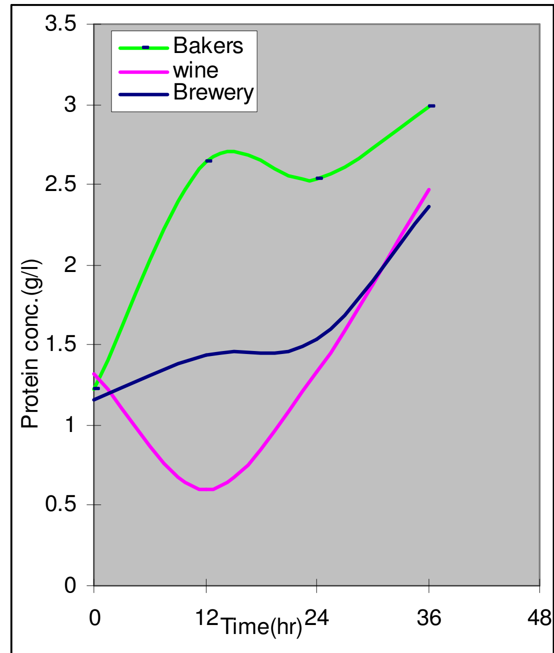


Fig 5.5 Protein conc. on baker's, wine and and brewery yeast strains on 10 % sugar conc.

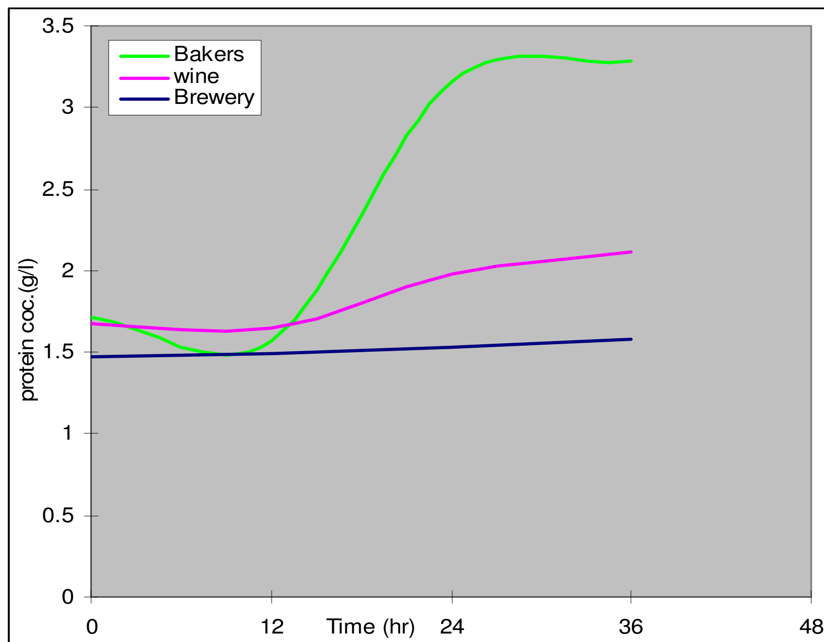


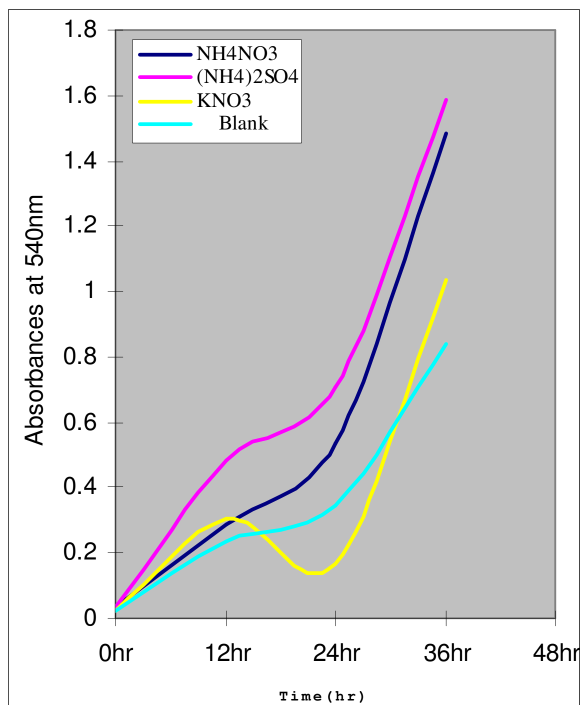
Fig 5.6 Protein conc. of baker's, wine and brewery yeast strain on 15 % sugar conc.

5.4 Study on the effect of NH_4NO_3, $(NH_4)_2SO_4$ and KNO_3 on the kinetic and biomass yield of the three yeast strains in 10 % molasses

*Curtin,(*1983*)* explained cane and beet molasses are shortage of basic elements for yeast growth that is nitrogen ,phosphors ,calcium and magnesium, the most one to enhance the yeast cell growth is nitrogen should be supplied as aqueous ammonia, ammonium sorts, salts, soluble proteins. For this experiment, It was supplied 2 % (w/v) NH_4NO_3, $(NH_4)_2SO_4$ and KNO_3 salts to for this experiment to analyze the effect of yeast growth, sugar assimilation rate and protein concentration in different yeast strains.

Generally, there was yeast growth in all strains in all nitrogen source chemicals. Yields of biomass varied considerably and were found depend on the strain and nitrogen source compound. From growth curve indicated in Fig 5.7, Fig 5.8 and Fig 5.9, substrate which had 2 % (w/v) $(NH_4)_2SO_4$ had maximum growth. Where as KNO_3 substrate had lower effect. This indicates that KNO_3 had inhibitory effect considerably. A significant difference in maximum growth rate on 10 % substrate concentration between the three ammonium salts could be seen. However, the maximum growth rate on baker's and wine yeast strains did not differ between the two yeasts. In order to identify the best strain and nitrogen source chemical, it was investigated sugar assimilation and protein concentration rate in 10 % substrate concentration of all strains are shown in Table 5.4 and Table 5.5. When they were compared it, baker's yeast utilized maximum sugar in all ammonium salts, similarly, there was high protein yields produced. Specifically, 2 % (w/v) $(NH_4)_2SO_4$ in baker's yeast was the highest one because more than 98 % sugar converted to 84% protein concentration. *(Preson,*1975) explained that, If sugars are fermented in the presence of adequate amount of nitrogen ,less alcohol is formed due to the environment is more favorable to growth of the yeas by reducing pH of the medium. And additions of ammonia on the molasses will hydrolysis some of the sucrose to reducing sugar. In case of brewery strain no sugar utilization at all so that no proteins yield in 36 hr fermentation time. Followed by low protein concentration. Addition of nitrogenous source compounds in brewery yeast did not have any protein enhancement effect. *Gellissen,* 2000 said that very few species have ability to utilize nitrate as nitrogen source.

The comparative study of the different source of nitrogen shows that the ammonium sulfate gives more protein than the other source of nitrogen (Table 5.5).Protein concentration yield in 10 % and 5% substrate concentration with 2 % (w/v) $(NH_4)_2SO_4$ of baker's yeast is greater than the other (Table 5.5). From these result, it was noted that ammonium sulphate act as a good nitrogen source and baker's yeast strain is the best protein producer on cane molasses.



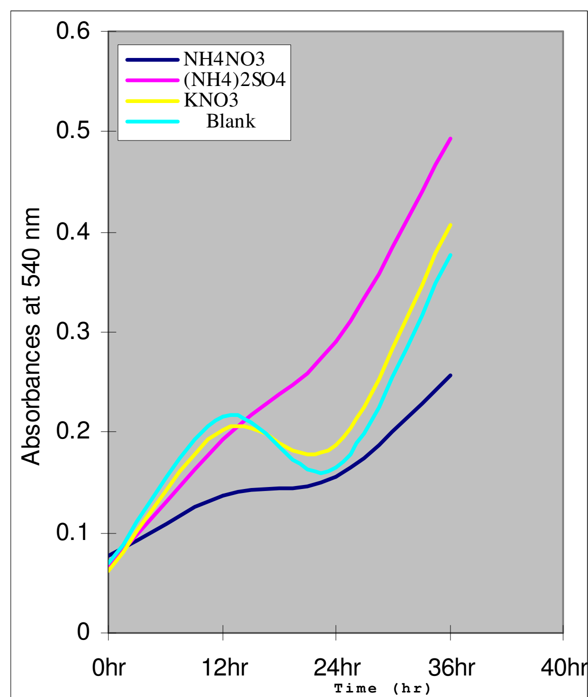


Fig 5.7 Growth of 10 % sugar conc. on baker's Yeast with NH_4NO_3, $(NH_4)_2SO_4$ and KNO_3

Fig 5.8 Growth of 10 % sugar conc.on wine yeast with NH_4NO_3, $(NH_4)_2SO_4$ and KNO_3

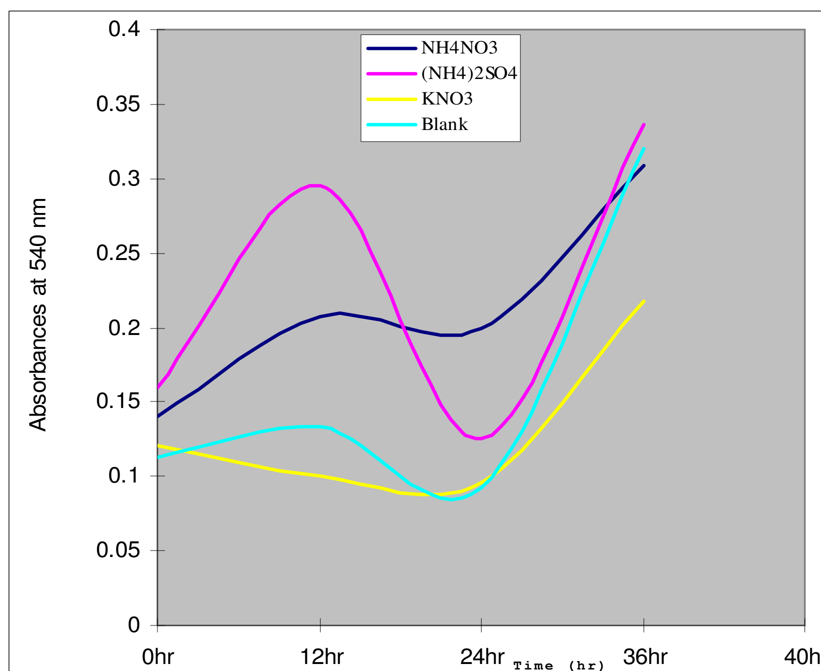


Fig 5.9 Growth of 10 % sugar conc.on brewery yeast with NH_4NO_3, $(NH_4)_2SO_4$ and KNO_3

Table 5.4 Residual sugar concentration of 10 % (w/v) substrate concentration in all the three strains at every 12 hr in (g/L)

	Bakers yeast				Wine yeast				Brewer yeast			
T(hr)	**+1a**	**+1b**	**+1c**	**+1d**	**+2a**	**+2b**	**+2c**	**+2d**	**+3a**	**+3b**	**+3c**	**+3d**
0	135	82	111	116	66	60	70.3	59.5	105	89	125	140
24	33	42	85	91	60	53.5	62.5	60	87	109	93.5	94.5
36	18.5	**1.45**	46.5	44	59	48	55	**44.7**	113	**96**	110	137

Table 5.5 Protein concentration of 10 % (w/v) substrate concentration in all three strains at every 12 hrs in (g/L)

	Bakers yeast strain				Wine yeast strain				Brewery yeast strain			
T(hr)	**+1a**	**+1b**	**+1c**	**+1d**	**+2a**	**+2b**	**+2c**	**+2d**	**+3a**	**+3b**	**+3c**	**+3d**
0	1.13	0.82	1.18	1.46	0.88	1.26	0.98	1.08	1.13	1.00	0.76	1.17
12	1.40	1.87	1.37	2.00	1.50	2.29	1.08	1.25	0.47	0.85	1.07	1.09
24	2.17	3.53	1.65	3.3	1.07	1.73	1.23	1.27	1.14	0.98	1.06	0.94
36	4.52	**5.07**	2.66	3.11	1.53	1.15	1.32	**2.07**	1.01	0.97	0.90	**1.05**

+a = NH_4NO_3 1= bakers yeast

+b = $(NH4)_2SO_4$ 2 = wine yeast

+c = KNO_3 3 = brewery yeast

+d = blank with out addition of any sample solution

T (hr) = time in hour

5.5 Study on the kinetic and biomass yield of baker's yeast with 2% $(NH_4)_2SO_4$ on different sugar concentration of cane molasses

From the previous experiment results, it was selected bakers yeast strain and ammonium sulphate was the best for biomass and protein yield in short fermentation time. So that the experiment was carried on further to select suitable substrate concentration on bakers yeast strain with 2 % (w/v) ammonium sulphate.

Although there was different growth pattern in baker's yeast strain in different substrate concentration with and with out 2 % ammonium sulphate. It had similar lag phase for the first 12hr yeast growth time in all substrate concentration. According to Fig 5.10, the sugar amount became depleted within 24 hr fermentation time in 5 % substrate concentration. When substrate concentration was increased from 10 % -15 %, yeast growth time was longer and longer like Fig 5.11 and Fig 5.12. In aerobic batch fermentation, S.cervisiae can produce a limited amount of respiration enzymes. If the glucose concentration is more than 5 % in the medium, the respiration intermediate enzymes are suppressed and ethanol mechanisms dominate (*Hoek, et al.,* 1998). The result comes out for the experiments has indicted in Fig 5.13, Protein concentration in bakers yeast with 2 % (w/v) ammonium sulphate in 5% (w/v), 10 % (w/v) and 15 % (w/v) sugar concentration of cane molasses were analyzed. Under low concentration of molasses, the microorganism has completed fermentation almost at 24hr and maximum protein was obtained. In the case of 10 % (w/v) and 15 % (w/v) molasses the fermentation process was not completed at 36hr and with minimum protein.

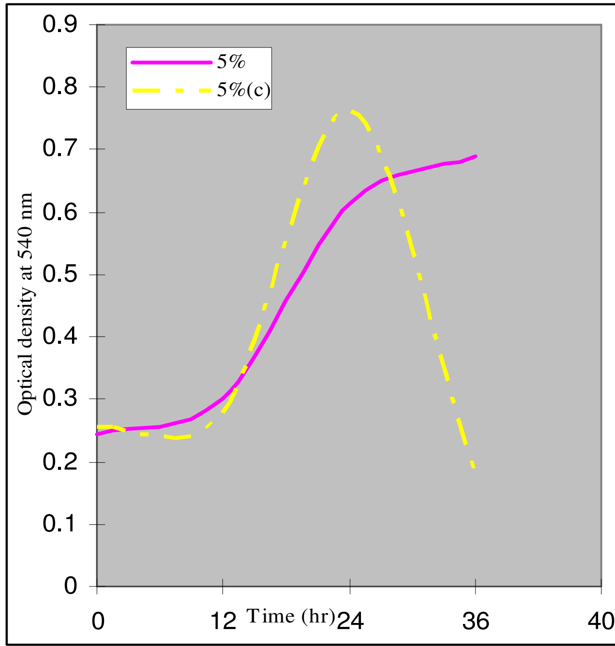


Fig 5.10 Growth curve of 5% molasses in baker's yeast with 2% $(NH_4)_2SO_4$

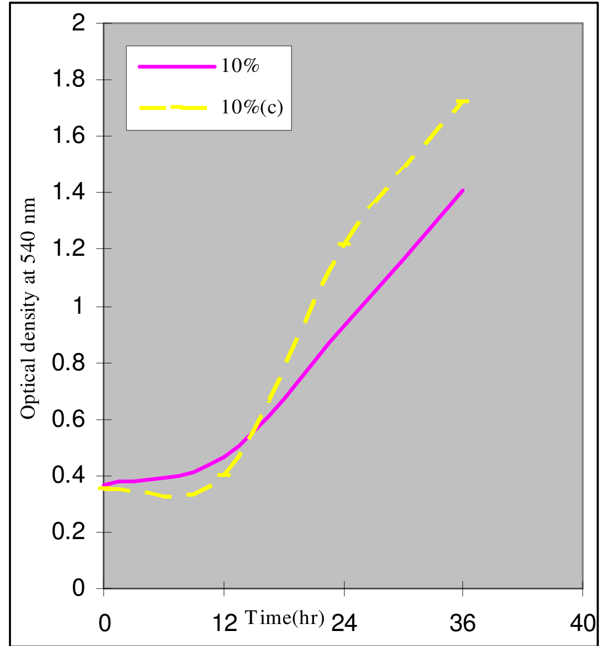


Fig 5.11 Growth curve of 10% molasses in bakers yeast with 2% $(NH_4)_2SO_4$

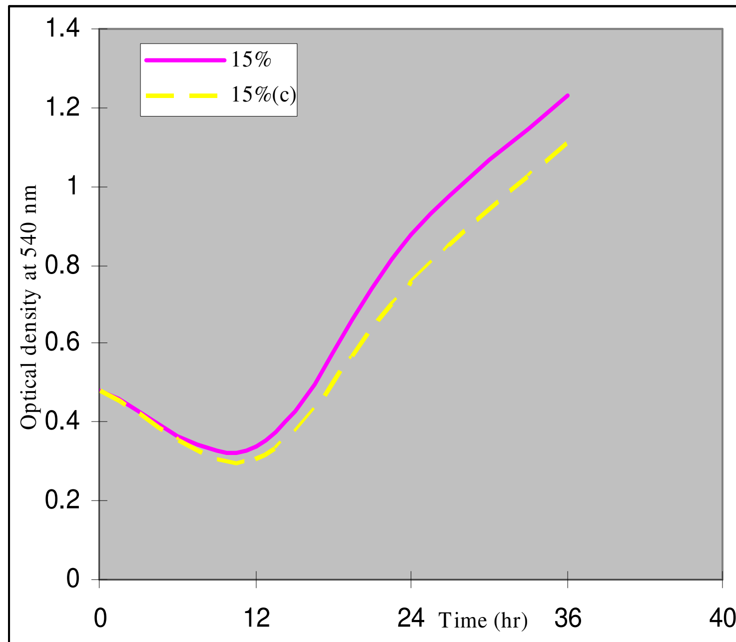


Fig 5.12 Growth curve of 15% molasses in baker's yeast with 2% $(NH_4)_2SO_4$

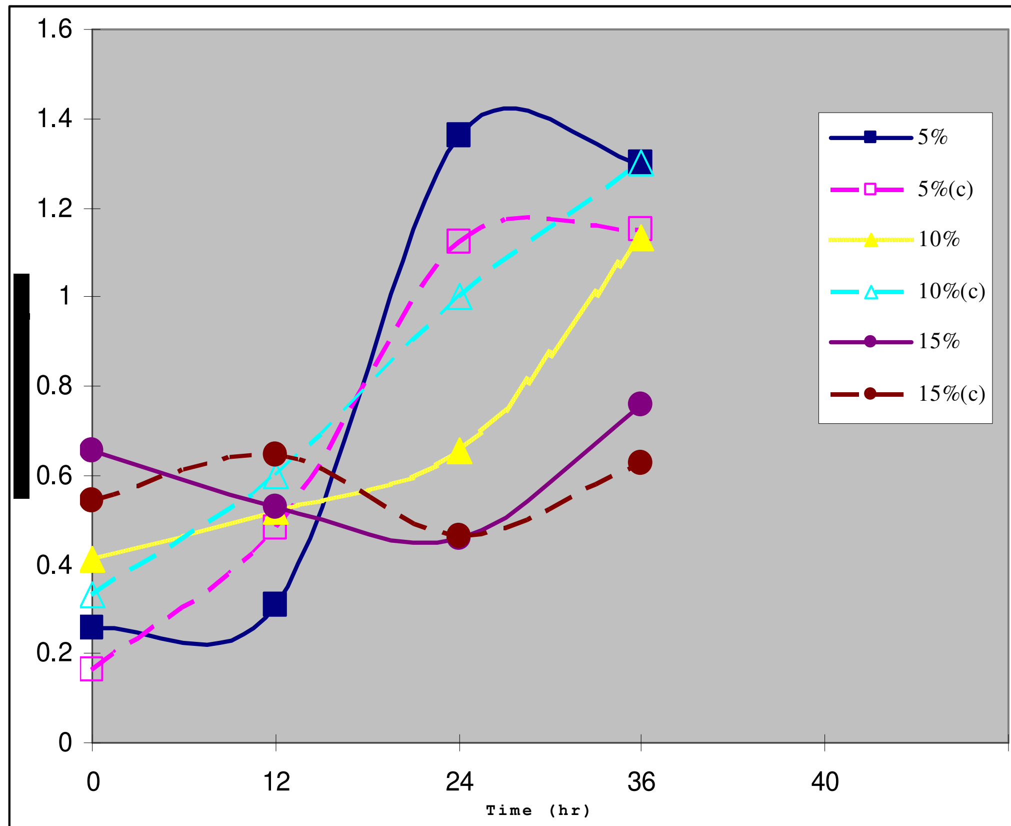


Fig 5.13 Protein concentration curve of 5%, 10% and 15 % molasses in baker's yeast with 2% $(NH_4)_2SO_4$

5 % = 5 %(w/v) sugar concentration with $(NH_4)_2SO_4$

5 %(C) = 5 %(w/v) with out $(NH_4)_2SO_4$

10 % = 10 %(w/v) sugar concentration with $(NH_4)_2SO_4$

10 %(C) = 10% (w/v) with out $(NH_4)_2SO_4$

15 % = 15 %(w/v) sugar concentration with $(NH_4)_2SO_4$

15 %(c) = 15 %(w/v) with out $(NH_4)_2SO_4$

6. ECONOMIC EVALUATION OF THE PROJECT

The proposed project in large scale setting up of a baker's yeast manufacturing unit for dry yeast with the assumption of the project for 12 years and 300 operating days to cater to the domestic market of bakery industry. The capacity of the proposed project is processing one- third of renewable source cane molasses of the country that is around 15,000 tons per annum and evaluate the economic viability.

6.1 Estimation of equipment costs

Assuming new equipment is similar to a base item where cost data is available, Equation 6.1 explains the equipment cost estimation process, where EPC is Equipment Purchased Cost, Q and Q_O are the new and base equipment capacities, respectively, and a is the exponent of the power law function given by the user. By default, this exponent is set as 0.6 for estimating new equipment cost (*Whiteside,* 2007 *and Harriso,* 2003*).*The cost of major equipment was evaluated based on cost data on 1997, 2000 and 2004 and bring back to equivalent cost to 2006 applying cost index techniques. Equipment cost was in U.S. dollars

$$EPC = C_O\,(Q/Q_O)^{a} \quad \text{--} \quad (6.1)$$

Table 6.1 Specification and costs of the major equipment required for baker's yeast production (Year 2006 prices)

Name	Size	Size Reference	Unit cost ($)	Unit	Total cost ($)	Baseline Equipment Data			
						source	size	Price $	year
Storage Tanks	300	m^3	122,400	2	224,800	A&D	300 m^3	102,000	2000
Mixer Tanks	253	m^3	278,175	1	278,175	A	80 m^3	11,600	2000
Clarifier	8	m^3/hr	72,000	1	72,000	A	8 m^3/hr	27,000	1997
Heat Sterilizers	7	m^3/hr	240,354	1	240,354	A&D	18m^3/h	353000	2000
Main Fermenter	88	m^3	316,950	3	1,639,200	A&D	260m^3	950,000	2000
Heat Exchanger	17	m^2	4,927	3	14,781		24 m^2	4,800	2000
Separators	2.6	m^3/hr	78,000	3	234,000	E	0.5m	30,000	1997
Cream Storage Tank	63	m^3	42,354	2	84,708	A&E	300m^3	102,000	2000
Air Filter	7,667	m^3/hr	12,100	2	24,200	B&E	0.68m^3/s	8,000	2000
Filter	10	m^2	52,000	3	156,000	A&D	25.5m^2	102,000	2000
Dryer	230	kg/hr	117,850	2	235,698	A&E	5,654,275	147,000	2004
All listed Equipment					3,203,916				
Unlisted equipment (0.20*All listed Equipment)					640,783				
Total					**$ 3,844,700**				

A. Harrison, G, et al., 2003
B. Zhuang, J., 2004
C. Material and energy balance of the process.
D. Whitesides, W., 2007
E. Sinnott, R.K 1993.
F: Cost is set at zero because it is not yet available
Equipments data colleted for Table 5.1, Table 5.2 and Table 5.3
Cost data is obtained from similar equipment, but not exactly the same equipment better data sources are recommended for the future
*Size Reference: L = Liter; m = meter; m^2 = square meter; m^3 = cubic meter; kg = kilogram; h = hour

6.2 Direct fixed capital cost calculation

The direct fixed capital costs calculation is the basis for further economic analyses such as the operating cost analysis used to determine the unit costs for this thesis and cash flow analysis used to determine the profitability. Based on the specification of major equipment costs shown in Tables 6.1 direct fixed capital (DFC) costs are estimated in Table 6.2. Equation 6.2 is used to calculate the DFC costs, which consist of direct costs (DC), indirect costs (IC) and other costs (OC).

$$DFC = DC + IC + OC \qquad (6.2)$$

Direct Costs: - direct costs include purchase costs, installation costs, piping, instrumentation, insulation, electrical facilities, buildings costs, yard improvements and auxiliary facilities. Purchase costs are the sum of the equipment costs, including unlisted equipment used for baker's yeast production. Unlisted equipment costs are estimated as 20 % of equipment costs by default. Installation costs are the sum of costs related to installation of all listed equipment. The factor method was used in the estimation of the costs for piping, instrumentation, insulation, electrical facilities, buildings, yard improvements and auxiliary facilities based on purchase costs (PC). Similar factors are used in the estimation of operating costs, etc. All factors used are listed in Table 6.3.

Indirect Costs and Other Costs: - Indirect costs consist of engineering costs and construction costs. The factors 0.25 and 0.35 are used by default to estimate these two costs respectively, based on direct costs (see Table 6.3). Other costs consist of contractor's fees and contingency costs. Factor 0.05 and 0.10 are used by default to estimate these two costs respectively, based on the sum of direct costs and indirect costs (see Table 6.3).

Table 6.2 Direct fixed capital costs estimates for baker's yeast production.

Items	Cost ($)
Total Direct Cost (TPDC):	
Equipment purchase costs (PC)	3,844,700
Installation	1,537,880
Processing piping	1,345,645
Instrumentation	1,537,880
Insulation	115,341
Electrical	384,470
Buildings	1,730,115
Yard Improvement	576,705
Auxiliary Facility	1,537,880
Total Direct Costs (TPDC)	12,610,616
Total Indirect Costs (TPIC):	
Engineering	3,152,654
Construction	4,413,716
Total Indirect costs(TPIC)	7,566,370
Other Costs (OC)	
Contractor's Fee	1,008,849
Contingency	2,017,700
Total other costs (OC)	3,026,549
Total Direct Fixed Capital (DFC) Costs	**$23,203,535**

Source: Data from Table 6.1

Economic parameter from baker's yeast production processing data and materials and energy balance

Table 6.3 Factors in estimation of new items based on old items

Estimated items	Factors	Base Items
Piping	= 0.35 *	Purchase costs (PC)
Installation	= 0.40 *	Purchase costs (PC)
Instrumentation	= 0.40 *	Purchase costs (PC)
Insulation	= 0.03 *	Purchase costs (PC)
Electrical	= 0.10 *	Purchase costs (PC)
Building	= 0.45 *	Purchase costs (PC)
Yard Improvement	= 0.15 *	Purchase costs (PC)
Auxiliary Facilities	= 0.40 *	Purchase costs (PC)
Unlisted purchased Costs (UPC)	= 0.25 *	Purchase costs (PC)
Maintenance (MAI)	= 0.06 *	Direct fixed capital (DFC) cost
Insurance (INS)	= 0.01 *	Direct fixed capital (DFC) cost
Local Taxes(LT)	= 0.02 *	Direct fixed capital (DFC) cost
Factory Expenses(FE)	= 0.05 *	Direct fixed capital (DFC) cost
Laboratory /Q_C/Q_A/	= 0.15 *	Total Labor costs(TLC)

Source: design data from Harrison (2003), Sinnott (1993) and Zhung (2004)

QC= Quality control, QA= Quality Analysis

6.3 Operating cost calculation

Operating costs are calculated and equal the sum of the following items as specified within the processes: (1) Raw materials; (2) Labor-Dependent; (3) Facility-Dependent; (4) Laboratory/QC/QA (QC=Quality Control; QA=Quality Analysis); (5) Utilities; and (6) Miscellaneous. Once all six parts are calculated, then operating costs can be derived

Raw materials: three different raw materials are examined in the baker's yeast production process in this thesis for economic purposes: cane molasses, nitrogen source ammonium salts and water. Itemized raw material costs are summarized and listed in Table 6.4

Utilities: three different utilities are examined in the baker's yeast production process in this thesis for economic purposes: electricity, heating steam and cooling water. Itemized utility costs are summarized and listed in Table 6.5.

Labor-Dependent: Estimated total manpower requirements for the process is 48 persons that will include 5 people at managerial grade, 30 people as staff in the manufacturing section , 3 persons in quality control and 10 other staff members for other departments including purchase and marketing assistants. Assuming 3 shift systems with 40 hrs per week and $0.36per hr.

{(3*30*48*43*0.45) + (5*43*40*2.5) + (13*48*43*0.85)}=$127,900

Table 6.4 Raw materials for baker's yeast production

Raw materials	unit cost ($/kg)	Annual amount(kg)	Annual cost ($)
Molasses	0.066	15,000,000	990,000
Ammonium sulphate	0.15	4,200,000	630,000
Water	$4.3 * 10^{-3}$	45,000,000	198,000
Total			**$ 1,818,000**

Source: fermentation process material balance in fermentor section 5.2.1

Table 6.5 Utility costs for baker's yeast production

Utility	unit cost ($/unit)	Annual amount	Annual cost($)
Electricity (Kwh)	0.067	7,700,132	515,842
Steam (kg)	0.0012	28,800,000	345,600
Cooling water (kg)	0.0001	878,400,000	87,840
Total			**$ 949,282**

Sources: fermentation process of materials and energy calculation section 5.2.1

Facility-Dependent: the facility-dependent costs (FDC) accounts for depreciation (DEP) of direct fixed capital (DFC) costs, equipment maintenance (MAI), insurance (INS), local taxes (LT), and the possibly other overhead-type of factory expenses (FE). Equation 6.3 is used to calculate the FDC.

$$FDC = DEP + MAI + INS + LT + FE \quad (6.3)$$

The depreciation (DEP) item is calculated using a straight-line depreciation method, considering a salvage value fraction (f) of the direct fixed capital (DFC), which is assumed 10 % in this analysis by default. The depreciation period (n) is set to ten years by default. Equation 6.4 is used to calculate depreciation:

$$DEP = \frac{DFC * (1- f)}{n} \quad (6.4)$$

The factor method is used in the estimation of the equipment maintenance (MAI), insurance (INS), local taxes (LT), and other overhead-type of factory expenses (FE) respectively, based on the direct fixed capital (DFC). See the Table6.3 for a detailed list of factors (the factors are all default values in the production process).

Laboratory/QC/QA: this accounts for the costs of off-line analysis and quality control costs. In this thesis, it is estimated by default as 15% of total labor costs. See the Table 6.3 for a detailed list of factors. Miscellaneous operating costs include research and development costs, process validation expenses and others, which are estimated as all zero.

Table 6.6 Annual operating costs (year 2006 prices) for baker's yeast production

Cost Items	U.S. dollar($)	%
Raw materials	1,818,000	22
Labor dependent	127,900	1.52
Facility dependent	5,108,008	65.4
Laboratory	19,185	0.22
Utility	949,282	11.3
Miscellaneous	0	0
Total	**$ 8,022,375**	**100**

Unit Cost Analysis

The unit costs for baker's yeast production are calculated as the quotient of the annual operating cost divided by the annual production rate.

$$\text{Unit Cost (\$/Kg)} = \frac{\text{Operating Cost (\$)}}{\text{Annual production (kg)}} \text{ ------------------------------------- (6.5)}$$

The unit cost for baker's yeast production is equals $ 2.4 per kilogram From Table 6.6 operating costs for each component (raw materials, labor-dependent, facility-dependent, laboratory/QC/QA, utilities and miscellaneous), From equation 6.5, the operating costs are in the numerator, so that the share of the annual operating costs (for each component) can be used to calculate the share (for each component) of the overall unit costs.

Baker's yeast selling Price

In order to conduct the profitability analysis, the selling price for baker's yeast must be first identified. From local market of the current baker's yeast price is $7per kilogram and international selling price is $ 2 per kilogram of baker's yeast *(mott MacDonald,* 2003). So the unit baker's yeast price per kilogram is calculated and equals $2.4/kg. This price level is lower than locale price where as slightly higher than the unit costs for international price.

Profitability Indicators

There are two profitability indicators discussed in this section: (1) payback period and (2) net present value; In order to calculate these indicators, annual net cash flows must be identified. Table 6.7 presents the detailed cash flow information for baker's yeast production. Profitability indicators, defined below, are also provided in the Table 6.7, given by the "economic evaluation report" and "cash flow analysis report" from the whole baker's yeasts processing.

Payback Period: - the payback period is a simple indicator measuring how long it takes to recover the initial investment in the baker's yeast production plants. When choosing among a few mutually exclusive projects, the project with the quickest payback is preferred. The payback period is calculated as the quotient of the total capital investment divided by the net profit as shown in equation 6.6:

$$\text{Payback period} = \frac{\text{Total capital investment}}{\text{Net profit}} \text{ ------------------------------ (6.6)}$$

The payback period is calculated and equals 3 years, which implies that it takes less than four years to recover the initial investment for the baker's yeast production plant.

Net Present Value: - the net present value (NPV) indicates the expected impact of the project on the value of the production plant. Projects with positive NPV are expected to increase the value of the baker's yeast production plant. When choosing among mutually exclusive projects, the project with the largest positive NPV should be selected. The NPV is calculated as the present value of the project's net cash flow (NCF), the annual benefits minus costs. Equation 6.7 is used to calculate the NPV.

$$NPV = \sum_{t=0}^{T} \frac{NCF_t}{(1+d)^t} = NCF_0 + + \frac{NCF_1}{(1+d)^1} + \frac{NCF_2}{(1+d)^2} + \text{--------} \frac{NCF_T}{(1+d)^T} \text{----------} \quad (6.7)$$

T= the project life (12 years in this analysis); NCF_O = the net cash flow for the year t (t=1…T.) d=the discount rate.

Table 6.7 shows the net present values are calculated and equal $25,114,000 at the discount rate 7%.

Table 6.7 Cash flow and profitability indicators for baker's yeast production ($1000)

Years	capital Investment	sales revenues	operating cost	Gross profit	Depreciation	Taxable income	Taxes	Net profit	Net cash flow
1	-8,500	0	0	0	0	0	0	0	-8,500
2	-8,500	0	0	0	0	0	0	0	-8,500
3	-7,363	0	0	0	0	0	0	0	-7,363
4	0	19,872	8,022	11,850	2,088	9,762	3,417	6,345	8,433
5	0	19,872	8,022	11,850	2,088	9,762	3,417	6,345	8,433
6	0	19,872	8,022	11,850	2,088	9,762	3,417	6,345	8,433
7	0	19,872	8,022	11,850	2,088	9,762	3,417	6,345	8,433
8	0	19,872	8,022	11,850	2,088	9,762	3,417	6,345	8,433
9	0	19,872	8,022	11,850	2,088	9,762	3,417	6,345	8,433
10	0	19,872	8,022	11,850	2,088	9,762	3,417	6,345	8,433
11	0	19,872	8,022	11,850	2,088	9,762	3,417	6,345	8,433
12	0	19,872	8,022	11,850	2,088	9,762	3,417	6,345	8,433
Payback period =3 years						**Discount rate = 7%** **NPV = 25,114** **DFC Salvage Fraction =0.1**			

Source: "Economic evaluation report" and "Cash flow analysis report" from *Harriso,* 2003 *and* Sinnott, 1993 and baker's yeast production data.

Note: NPV=net present value

DFC=direct fixed capital.

7. Conclusions and recommendation

7.1 Conclusions

This study was investigated primarily aimed to investigate baker's yeast production in the laboratory and industry level using cane molasses which is produced from three large sugar factories. Additionally, it has planned to satisfy local market. This would help to analyses raw material cane molasses for yeast growth by identifying the basic biochemical composition. Kinetic yeast growth was characterized in different substrate concentration of baker's, wine, and brewery yeast strains. All the yeast strains are able to convert cane molasses into biomass and protein concentration. The result obtained shows that baker's yeast strain can produce maximum protein yield in low sugar concentration. Addition of nitrogen source chemical on baker's yeast in 5 % (w/v) and 10 % (w/v) sugar concentration has an effect on protein yield. Economic analysis of industrial scale baker's yeast production was conducted by assuming 15,000 tons molasses per years and using the first four chapter experimental results data of baker's yeast strain on 5 % (w/v) substrate concentration with ammonium sulfate. Production scales, as well as other technical and economic parameters specified in the thesis. Profitability analysis is conducted to asses the profit making ability of baker's yeast production plant. The results is that pay back period is three years which implies that investment can be recovered in less than four years, the second one is the net present value is $ 25,114,000 which is large value. Based on the results of this study the following conclusions could be drawn.

1. Raw material cane molasses is a potential resources for yeast production
2. Commercially available baker's yeast is the beasts strain for baker's yeast development comparing to wine and brewery yeast strains.
3. Low sugar concentration is more suitable for baker's yeast growth from cane molasses.
4. Addition of 2 % (w/v) ammonium sulphate in baker's yeast strain has protein enhancing effect.
5. The combined effect of pay back period and net present value shows industry scale baker's yeast production is economically attractive.

7.2 Recommendation

Molasses is not an adequate media for yeast growth. So that, it is necessary to use other nitrogen source chemical and nutrients which was not used on this thesis to enhance protein yield. In this thesis it was used only few ammonium salts and single concentration. It needs further experiments on other nitrogen source, vitamins and minerals on yeast growth and protein concentration with different concentration.

Though the experiments was optimized under different substrate concentration of molasses. It is necessary to carry over the research at large scale considering the following direction fur further study:

1. Identifying other growth promoter like phosphorous, urea, salts and vitamins
2. Selecting the appropriate concentrations.

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APPENDIX

Table A1. Standard glucose calibration data

Glucose (mg)	Absorbance at 540 nm
0.1	0.116
0.2	0.26
0.3	0.505
0.4	0.585
0.5	0.7
0.6	0.854
0.7	0.978
0.8	1.075
0.9	1.161
1	1.35

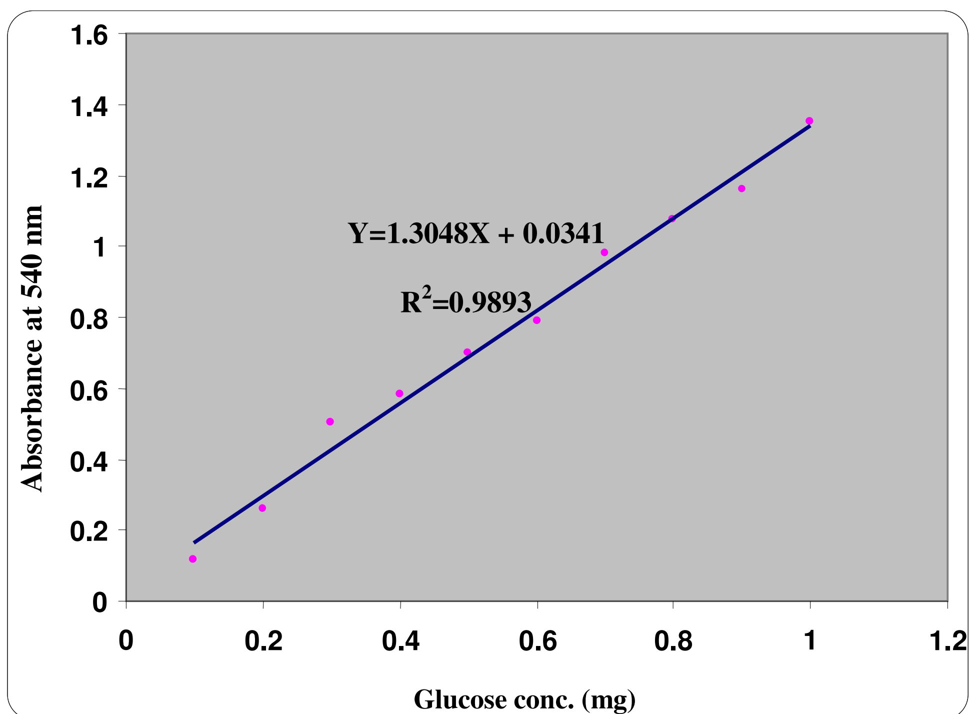

Fig A1. Standard glucose concentration reading curve.

Table A2. Standard protein calibration data.

BSA (mg)	Absorbance reading at 720 nm
0.1	0.302
0.2	0.662
0.3	0.863
0.4	1.24
0.5	1.32
0.6	1.87
0.7	2.03
0.8	2.387
0.9	2.87
1	3.33

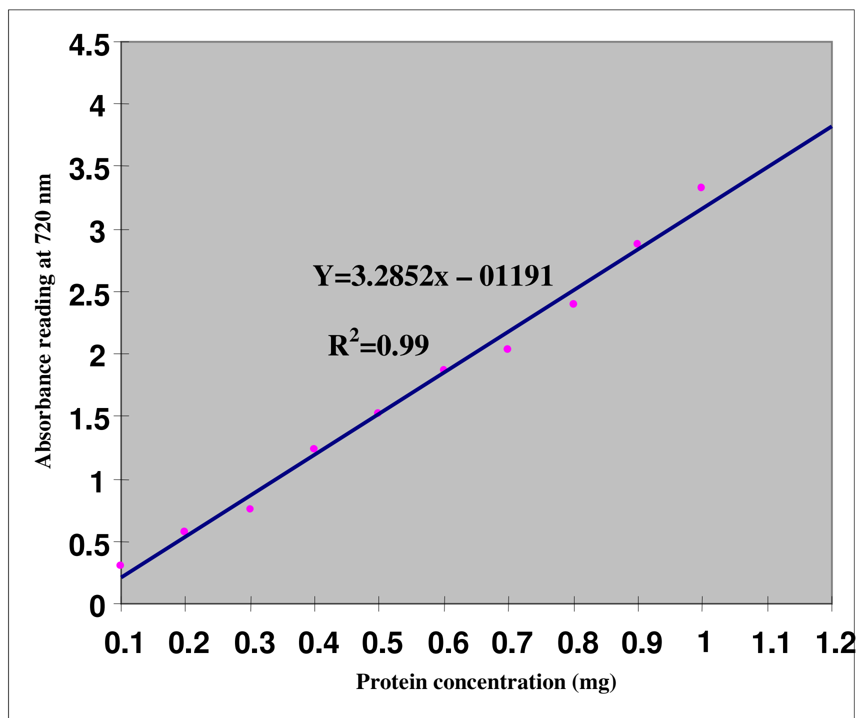

Fig A2. Standard protein concentration reading curve.