STUDY ON THE QUALITY OF COMPRESSED
BAKER'S YEAST IN HASAHISSA FACTORY

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DEDICATION

TO MY DEAR FAMILY
FATHER, MOTHER, BROTHERS
AND SISTERS
TO MY DEAR FRIENDS AND
COLLEAGUES

WITH LOVE AND RESPECT

ISAM
ACKNOWLEDGEMENTS

I WOULD LIKE TO EXPRESS MY GRATITUDE AND THANKS TO MY SUPERVISOR DR. HASAN ALI MUDAWI FOR HIS HELPFUL AND CONSTRUCTIVE SUPERVISION WHICH WAS VITAL TO THE SUCCESS OF THE RESEARCH.

MY THANKS AND RECOGNITION ARE EXTENDED TO MY CO-SUPERVISOR DR. MYMOUNA EL MUBARK OSMAN

I WOULD LIKE TO THANK ALL THE STAFF MEMBERS OF HASAHISSA YEAST FACTORY AND THE STAFF OF INDUSTRIAL RESEARCH AND CONSULTANCY CENTRE FOR THEIR UNLIMITED HELP.

MY THANKS AND APPRECIATION ARE EXTENDED TO EVERY BODY WHO HELPED ME DURING THIS STUDY.

ABOVE ALL, MY THANKS AND PRAISE TO ALLAH WHO GAVE ME PATIENCE AND WILL TO ACCOMPLISH THIS WORK.
ABSTRACT

THIS STUDY FOR COMPRESSED BAKER'S YEAST WAS CARRIED OUT AT HASAHISSA YEAST FACTORY IN SUDAN.

THE RAW MATERIALS USED IN THE PRODUCTION OF THE COMPRESSED BAKER'S YEAST WERE ANALYZED (MOLASSES, UREA, AIR, WATER AND ORIGINAL STRAIN). CHEMICAL, PHYSICAL AND MICROBIOLOGICAL ANALYSIS WERE CARRIED OUT DURING PROCESS.

CHEMICAL AND PHYSICAL ANALYSIS FOR TREATED MOLASSES SHOWED A DECREASE IN TOTAL SUGARS (47.1 – 25.3), TOTAL SOLUBLE SOLIDS (77 – 40), SPECIFIC GRAVITY (1.493 – 1.170) AND pH VALUE (5.4 – 4.5) COMPARED WITH THE RAW MOLASSES. BUT DURING PROCESS THERE WAS DECREASE IN pH (3.81 – 3.53) AND BRIX (12.5 – 3.2) TO APPLIED BATCH PROCESS SYSTEM, BUT DURING APPLYING CONTINUOUS PROCESS THE pH AND BRIX VALUES WERE INCREASED WITH INCREASING TIME pH (3.2 – 5.9) BRIX (0.8 – 9.1) RESPECTIVELY.

THE CONCENTRATION OF CREAM (GPL) AND YEAST CELL CONCENTRATION (YCC) WERE INCREASED DURING FERMENTATION TO SPECIFIC TIME FOR BATCH AND CONTINUOUS PROCESS SYSTEM GPL (10.6 – 162.8), YCC LOG10 7.1 – 9.46 CFU/ML.

ALCOHOL CONTENT WAS HIGHER IN THE STARTER CULTURE 1.02% COMPARED WITH THE COMMERCIAL BATCH 0.63%. THE CHEMICAL COMPOSITION OF THE FINAL PRODUCTS SHOWED A HIGH MOISTURE CONTENT 70.5%, PROTEIN 14.3%, ASH 1.66%, FAT 1.2% AND CARBOHYDRATE 12.2%.
THE ACTIVITY OF YEAST WAS GOOD AS IT HAD PRODUCED VIABILITY $\log_{10} 9.98$ CFU/G, $\text{CO}_2$ PRODUCTION WAS $175\% / 3\text{HR}$, AND THE TIME TAKEN TO LEAVEN DOUGH WAS 75 MINUTES AND BAKING TEST GAVE 2.9 SPECIFIC VOLUME.

MICROBIOLOGICAL TESTS SHOWED NO CONTAMINATION FOR ORIGINAL STRAIN, BUT HIGH CONTAMINATION OF AIR IN THE SURROUNDINGS OF THE PLANT AND FACTORY CHAMBERS EXCEPT CULTURE ROOM.

THE TOTAL BACTERIAL COUNT (TBC) (NIL TO $\log_{10} 2.49$ CFU AFTER 30 MINUTES EXPOSURE), WILD YEAST (WY) (NIL TO $\log_{10} 7.25$ CFU AFTER 30 MINUTES EXPOSURE), AND MOLDS (NIL TO $\log_{10} 1.56$ CFU AFTER 30 MINUTES EXPOSURE).

CONTAMINATION IN TREATED MOLASSES AND WATER WAS NIL, BUT UREA WAS CONTAMINATED, TBC $\log_{10} 2.38$ CFU/G, WY $\log_{10} 1$ CFU/G AND MOLD WAS NIL.

ALSO MICROBIAL CONTAMINATION WAS RATHER LOW IN STARTER CULTURE THAN IN THE COMMERCIAL BATCH. TBC (NIL TO $\log_{10} 4\text{CFU/ML}$), WY (NIL TO $\log_{10} 7.25 \text{CFU/ML}$) AND MOLD (NIL TO $\log_{10} 5.95 \text{CFU/ML}$).

THE FINAL PRODUCT SHOWED A DEGREE OF CONTAMINATION TBC ($\log_{10} 4.4$ TO $\log_{10} 4.5\text{CFU/G}$), WY ($\log_{10} 5.4$ TO $\log_{10} 9.07\text{CFU/G}$), AND MOLDS ($\log_{10} 5$ TO $\log_{10} 8/G$).
بسم الله الرحمن الرحيم
خلاصة الأطرامة

ﺇﺠﺭﻴﺕ ﺑﺫﻩﺍ ﺍﻟﺩﺭﺍﺴﺔ ﻓﻲ ﺍﻟﺴﻭﺩﺎﻥ ﺑﻤﺼﻨﻊ ﺍﻟﺨﻤﻴﺭﺓ ﺑﺎﻟﺤﺼﺎﺤﻴﺼﺎ ﺡﻴﺙ ﺗﻡ ﺗﺤﻠﻴل ﺍﻟﻤﻭﺍﺩ ﺍﻟﺨﺎﻡ ﻓﻲ ﺍﻟﻌﻤﻠﻴﺔ ﺃﺠﺭﻴﺔ ﻓﻲ ﺍﻟﺩﺭﺍﺴﺔ ﻓﻲ ﺍﻟﺴﻭﺩﺎﻥ ﺑﻤﺼﻨﻊ ﺍﻟﺨﻤﻴﺭﺓ ﺑﺎﻟﺤﺼﺎﺤﻴﺼﺎ ﺡﻴﺙ ﺗﻡ ﺗﺤﻠﻴل ﺍﻟﻤﻭﺍﺩ ﺍﻟﺨﺎﻡ ﻓﻲ ﺍﻟﻌﻤﻠﻴﺔ ﺃﺠﺭﻴﺔ ﻓﻲ ﺍﻟﺩﺭﺍﺴﺔ ﻓﻲ ﺍﻟﺴﻭﺩﺎﻥ ﺑﻤﺼﻨﻊ ﺍﻟﺨﻤﻴﺭﺓ ﺑﺎﻟﺤﺼﺎﺤﻴﺼﺎ ﺡﻴﺙ ﺗﻡ ﺗﺤﻠﻴل ﺍﻟﻤﻭﺍﺩ ﺍﻟﺨﺎﻡ ﻓﻲ ﺍﻟﻌﻤﻠﻴﺔ ﺃﺠﺭﻴﺔ ﻓﻲ ﺍﻟﺩﺭﺍﺴﺔ ﻓﻲ ﺍﻟﺴﻭﺩﺎﻥ ﺑﻤﺼﻨﻊ ﺍﻟﺨﻤﻴﺭﺓ ﺑﺎﻟﺤﺼﺎﺤﻴﺼﺎ ﺡﻴﺙ ﺗﻡ ﺗﺤﻠﻴل ﺍﻟﻤﻭﺍﺩ ﺍﻟﺨﺎﻡ ﻓﻲ ﺍﻟﻌﻤﻠﻴﺔ ﺃﺠﺭﻴﺔ ﻓﻲ ﺍﻟﺩﺭﺍﺴﺔ ﻓﻲ ﺍﻟﺴﻭﺩﺎﻥ ﺑﻤﺼﻨﻊ ﺍﻟﺨﻤﻴﺭﺓ ﺑﺎﻟﺤﺼﺎﺤﻴﺼﺎ ﺡﻴﺙ ﺗﻡ ﺗﺤﻠﻴل ﺍﻟﻤﻭﺍﺩ ﺍﻟﺨﺎﻡ ﻓﻲ ﺍﻟﻌﻤﻠﻴﺔ ﺃﺠﺭﻴﺔ ﻓﻲ ﺍﻟﺩﺭﺍﺳﺔ ﻓﻲ ﺍﻟﺴﻭﺩﺎﻥ ﺑﻤﺼﻨﻊ ﺍﻟﺨﻤﻴﺭﺓ ﺑﺎﻟﺤﺼﺎﺤﻴﺼﺎ ﺡﻴﺙ ﺗﻡ ﺗﺤﻠﻴل ﺍﻟﻤﻭﺍﺩ ﺍﻟﺨﺎﻡ ﻓﻲ ﺍﻟﻌﻤﻠﻴﺔ ﺃﺠﺭﻴﺔ ﻓﻲ ﺍﻟﺩﺭﺍﺳﺎ
7.25 \times 10^10 \text{ CFU} / \text{ML} \text{ Salmonella, and } 10 \times 10^7 \text{ CFU} / \text{ML} \text{ for } E. coli.

In the producibility test, the proportion of the total number of salmonella was also 10\%.

In the final product, the proportion of the total number of salmonella was also 10\%.
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CHAPTER ONE

1. INTRODUCTION

Man used yeast before he knew how to write. Hieroglyphics suggested that the ancient Egyptian civilization were using living yeast and the processes of fermentation to rise their bread over 5000 years ago. Of course, they didn’t know what was responsible for the leavening process. At that time a small portion of the dough was used to start or leaven each new bread dough. Later scientific research found that yeast is a microorganism (visible only with a microscope).

Specially selected strains of *Saccharomyces cerevisiae* are employed for the production of baker's yeast (Prescott and Dunn, 1959).

Several factors serve to make yeast an ideal source of microbial food. Their nutritive value is high and they are easily cultivated using in expensive raw materials.

Experience already gained in brewing and in the manufacture of baker's yeast has proved useful in developing the yeast industry (Rose, 1961).
Yeast was produced as food on a large scale on Germany during the First World War (Stanpury et al., 1995).

The manufactures of baker’s yeast in Sudan was started at 1982 in Sudanese fermentation industry (S.F.I) Hasahissa yeast factory is owned by Sudanese Development Corporation (SDC).

At that time the factory produced active dry yeast and after one year the factory was closed because of lack of proper control for temperature and the marketing was also not effective.

Later at 2003 the factory was again opened after rehabilitation of the machines and the establishing of the cooling system, but till now the problem of marketing is not solved.

Now the factory in Hasahissa produce compressed baker’s yeast, the maximum capacity is 10 ton/day, but the actual production not exceed 2 – 2.5 ton/day.

Objective of the research work:

The work carried out in Hasahissa yeast factory. The aim is to evaluate the fermentation process behavior for starter culture and commercial batch.
This study is particularly oriented to investigate the followings:

1- To evaluate the hygienic precaution of air condition.

2- Analysis for major raw materials: molasses, urea, water and original strain (these strain brought from India).

3- Carry out: physical, chemical and microbiological tests during fermentation process in all fermenters.

4- Study of the differences in the fermentation steps between batch and continuous process system.

5- Test the activity of compressed baker’s yeast as final product.
CHAPTER TWO

2. LITERATURE REVIEW

2.1. BIOLOGY OF BAKER’S YEAST:

All types of baker’s yeast are strains of species Saccharomyces cerevisiae and they belong to the genus Saccharomyces and the family Saccharomycetaceae (Bronn, 1982).

Yeast are widely distributed in nature as they are found in sweet fruits, in soil, in the eliminatory tract of man and animal. However, the most important genera of the yeast are Saccharomyces and Candida, these genera are of commercial interest specially Saccharomyces cerevisiae which involved in baking and alcohol production (Looder, et al., 1958).

Yeast can be differentiated from bacteria by their larger cell sizes and their oval, elongate, elliptical, or spherical cell shaped (Osman, 1997).

Jay (1992) mentioned that the typical yeast cell range from 5 to 8mm in diameter, with some being even larger.
The average of cell size is $5 \times 8$ mm, volume $10^{-10}$ cm$^3$, cell dry weight $0.3 \times 10^{-10}$ g, and number of cell per gram compressed yeast $10^{10}$ (Bronn, 1982).
2.2 Uses of yeast:

2.2.1 Production of alcohol:

The first published work done in the Sudan on ethanol fermentation was by Agab (1978). In this work Agab isolated yeast from nature's habitats in the Sudan and used them together with commercial yeast strain for ethanol production.

Also Hamad (1986) has isolated thermo tolerant yeast from habitats in Sudan and tested them for ethanol production.

Ahmed (2003) used commercial yeast for the fermentation of sweet sorghum juice, and the result gave the highest amount of ethanol.

2.2.2 Medical uses:

Yeast have been reported to be used for treating nutritional anemias in rats, children and guinea pigs. Compressed yeast was considered as a household remedy for many diseases (Jayaraman, 1973).

2.2.3 Production of microbial biomass:

The commercial production of microbial biomass may be divided into two major processes.
THE PRODUCTION OF THE YEAST TO BE USED IN THEIR BAKING INDUSTRY AND THE PRODUCTION OF MICROBIAL CELLS TO BE USED AS HUMAN OR ANIMAL FOOD WHICH IS REFERRED TO AS SINGLE CELL PROTEIN (SCP) (STANPURY ET AL., 1995).

THE AYLWARD AND JUL (1975) MORE RECENT INVESTIGATIONS HAVE BEEN CONCERNED WITH THE GROWTH OF YEAST ON MOLASSES TO GIVE THE SO CALLED SINGLE CELL PRODUCTION, ALSO YEAST IS A GOOD SOURCE OF PROTEIN 50%.

2.2.3.1 BAKER’S YEAST IN THE BAKING INDUSTRY:

Many microorganisms ferment sugars with the released carbon dioxide, bakers yeast is best adapted for leavening of bakers products (BANWART, 2002).

The amount of dry yeast 2.5% produced 350ml CO₂ in one hour (REED AND PEPPLER, 1973).

FRAZIER (1967) MENTIONED THAT THE RATE OF GAS PRODUCTION BY THE YEAST IS INCREASED BY THE ADDITION OF MORE YEAST.

The production of carbon dioxide and alcohol from the sugars pre-existing in the hour and from those formed during fermentation is dependent upon the action of certain enzymes contained in the yeast.
These enzymes are maltase, invertase, and zymase. Maltase hydrolysis the maltose to glucose, the invertase produces glucose and fructose from the sucrose and the complex enzyme system zymase is the actual fermenting agent that acts on glucose and fructose and produces the alcohol and the carbon dioxide that distends the dough (Johnes and Amose, 1967).
2.3 **Microbiological Control:**

The production of yeast is not fully aseptic in the true sense, stringent precaution to minimize infection must be taken if satisfactory yeast is to be obtained. Three dangers must be guarded against. Provided that proper care is taken, bacterial infection should be kept under control during the propagation stage. At the acid use, it can be expected that the rate of growth of the yeast will exceed that of invading bacteria.

The second danger is from mold. Although the presence of small numbers of mold organisms does not, as general rule affect the activity of the yeast as leavening agent, the appearance of the mold colonies on the surface of yeast blocks is considered unesthetic.

The most serious infection for the yeast manufacture is wild yeast. Accidentally introduced wild yeast will often grow more vigorously than the culture yeast and may largely replace the culture yeast by the need of the successive propagation stages. Since wild yeast is almost always of indifferent performance dough, the effect on the

2.3.1 Sterilization of Plant:

LEE (1950) mentioned that, in most instances the propagation vessels and other plant equipment and pipe line in yeast factories are sterilized before the start of propagation by means of steam, and antiseptics. Alkalis, hydrochloride, phenol, and formaldehyde have all been employed.

2.3.2 Process Water:

COOK (1958) stated that in the early seed stage of commercial yeast propagation, the molasses after being suitability diluted with water is sterilized by heat as in laboratory microbiological practice. In later stage, when the scale of operation has increased and the seed yeast is suspended in a dilute aqueous media, called the “START DIP” prior to the exceptional addition of the sterilized nutrient wort, it is usually found safe to use tap water adequately chlorinated.

2.3.3 Air Disinfection:
COMPLETE STERILIZATION OF THE LARGE QUANTITIES OF AIR REQUIRED IN THE MANUFACTURE OF YEAST IS DIFFICULT. IN GENERAL PRACTICE, THEREFORE, THE AIR OF USE IN THE LARGE SCALE COMMERCIAL STAGE OF PROPAGATION IS MERELY FILTERED. USUALLY BY PASSAGE THROUGH A HEAD PACED WITH LESSING RINGS COATED WITH GLYCEROL OIL (WALTER, 1940).

STERILIZATION OF AIR BY HEAT REQUIRES A TEMPERATURE OF APPROXIMATELY 500ºC, IT IS TO BE EFFECTIVE WITHIN A REASONABLY SHORT TIME (LEE, 1950).

COOK (1958) MENTIONED THAT STERILIZATION BY MEANS OF ULTRAVIOLET IRRADIATION HAS ALSO BEEN FOUND EXPENSIVE AND INEFFECTIVE FOR LARGE VOLUMES.

2.4 YEAST NUTRITION

2.4.1 SOURCE OF CARBON:

BAKER’S YEAST FERMENT GLUCOSE, FRUCTOSE AND MANNOSE, AND THIS APPLIED ONLY IN GLUCOSE CONCENTRATION OF 1-10%, AND FRUCTOSE CONCENTRATION OF 2-8%, ALSO THE RATE OF FERMENTATION OF MANNOSE IS 20-25% (ROSE AND HARRISON, 1971).

MOLASSES IS A GOOD MEDIUM FOR PRODUCTION OF BAKER’S YEAST (AL-MUDHAFFAR, 1978).

2.4.2 PHOSPHATE:
PHOSPHORUS MAY BE ADDED AS CALCIUM SUPER PHOSPHATE, AMMONIUM PHOSPHATE OR PHOSPHORIC ACID, ACCORDING TO AVAILABILITY, PURITY AND CONVENIENCE.

IF PURE AMMONIUM PHOSPHATE IS AVAILABLE THIS IS PROBABLY MOST SUITABLE (MARKHAM AND BYRNE, 1968).

2.4.3 NITROGEN:

THORNE (1954) REPORTED THAT THE AMMONIUM SULPHATE, AMMONIUM CHLORIDE, AMMONIUM SOLUTION AND UREA ARE ALL SUITABLE SOURCES OF ASSIMILABLE NITROGEN FOR INDUSTRIAL USE.

2.4.4 OXYGEN:

ALTHOUGH LIMITED GROWTH OF YEAST MAY BE OBTAINED UNDER ANAEROBIC CONDITION, A VERY RAPID SUPPLY OF OXYGEN IS REQUIRED FOR MAXIMUM GROWTH AND EFFICIENT UTILIZATION OF SUBSTRATE, OXYGEN IS, IN FACT, A MAJOR NUTRIENT, ABOUT 1.0 G OXYGEN BEING REQUIRED FOR THE PRODUCTION OF 1.0 G YEAST DRY MATTER (ROSE AND HARRISON, 1970).

2.4.5 OTHER ELEMENTS:

OLSON AND JOHNSON (1949) REPORTED THAT POTASSIUM, SULPHER AND MAGNESIUM ARE ESSENTIAL, AND ARE GENERALLY SUPPLIED AS INORGANIC SALTS. AN EXCESSES DO NOT APPEAR TO HAVE
ANY DETERMINED EFFECTS UNLESS PRESENT IN SUFFICIENT CONCENTRATION TO CAUSE OSMOTIC DAMAGE.

MOST OF THE MINOR ELEMENTS KNOWN TO BE ESSENTIAL FOR PLANT AND MICROORGANISMS HAVE, AT ONE TIME OR ANOTHER, BEEN STATED TO BE NECESSARY FOR YEAST GROWTH, ONLY THREE, COPPER, IRON AND ZINC ARE STATED.

2.4.6 GROWTH FACTORS (SUBSTANCES):

2.5. CULTURE REQUIREMENTS:

2.5.1 TEMPERATURE:

**IT IS POSSIBLE TO GROW YEAST REASONABLY SATISFACTORYLY**
at any temperature in the range of 20-40°C (White, 1954) but in
commercial practice it is normally restricted to 25-35°C
(Rose and Harrison, 1970).

Whoollen (1969) stated that the yeast is most active at
temperature of about 40°C, and at a temperature of 50 to 60°C
the yeast is killed.

2.5.2 HYDROGEN ION CONCENTRATION:

White (1954) mentioned that the most strains of baker’s
yeast grow well at pH values between 3.5 and 7.0, normally
values between 3.5 and 4.5 are employed. Since under this
condition the growth of many kinds of bacteria which may be
present as contaminants is restricted.

Adsorption by the yeast cell wall of dark coloured
suspended matter from molasses occurs below pH 5.0 and for
this reason high values are sometimes favoured.

2.5.3 YEAST CONCENTRATION:

Provided that the necessary conditions aeration,
agitation, temp, and pH value can be maintained yeast will
Grow satisfactorily over a wide range of cell concentration.

Good quality yeast can be grown up to a concentration of 50 g dry matter per liter of medium (Rose and Harrison, 1970).

2.5.4 Preparation of media:

Agab and Dirar (1980) mentioned that, two media were used for both enrichment and isolation of yeasts. The urea-molasses medium was containing molasses equivalent to 8% total sugars, 0.1% sodium dihydrogen phosphate and 0.35% urea.

This medium was also used for ethanol and biomass production. The molasses are heated and decanted before use in this media. The pH of each medium was not adjusted and remained between 5 and 5.4. To inhibit mold growth 0.025% calcium propionate was added to each medium.

2.6 Production of molasses in Sudan:

In the Sudan, the most suitable raw material available now for ethanol and baker’s yeast production is sugar cane molasses. The sugar factories working now in Sudan produce
AT MAXIMUM WORKING CAPACITY ABOUT 300,000 TONES OF MOLASS PER YEAR.


ONLY ABOUT 4% OF THE MOLASS PRODUCED ANNUALLY IS UTILIZED LOCALLY. THE REST IS EXPORTED (EL-KHIDIR, ET AL., 1992).

IN HASAHISSA YEAST FACTORY THE MAXIMUM UTILIZED MOLASSES IS ABOUT 10 TONS PER YEAR.

2.6.1 MICROORGANISMS IN MOLASSES:

HOING (1963) STATED THAT WITHOUT REGARD OF THE KIND OF MICRO FLORA, ALL THE FINAL MOLASSES CONTAINS 29-500 MILLION/G.

THE HARMFULL EFFECTS OF MICROORGANISMS CAN BE SUMMARIZED AS FOLLOWS:

- INFECTION OF WILD YEAST AND ACID FORMING BACTERIA DURING FERMENTATION.

- SUGAR LOSES BY HARMFUL MICROFLORA (ABOUT 4% MOLASSES SUGAR) IS LOST DURING MOLASSES STORAGE OR AT EARLY STAGE OF FERMENTATION.

2.7 CLARIFICATION OF MOLASSES PREPARATION FOR FERMENTATION INDUSTRY:
CHEMICAL AND MECHANICAL METHODS ARE APPLIED FOR MOLASSES CLARIFICATION WITH SPECIAL ATTENTION FOR MOLASSES PREPARATION FOR INDUSTRY.

REgardING THE FERMENTATION INDUSTRY PRACTICE, THE FOLLOWING TREATMENT STEPS ARE FOLLOWED:

- **Final molasses is diluted to total solids in the range of (40-45) around 20-25% total sugar and the pH is adjusted to the corresponding value.**

- **Dilute molasses are subjected to clarification by chemical and mechanical means.**

- **The molasses clarification results were obtained by the combined acid addition and pre-heating method by following the below diagram (Osman, 2002).**
Molasses clarification combined acid addition pre-heating

1. **Final Molasses**
2. **Conc** → **Molasses Dilution Brix (< 45)**
3. **Hot Molasses (80-90°C) and agitation 10-15min**
4. **Mechanical separation by means of decantation**
   - **Molasses**
   - **Clear Molasses**
The over reaction mechanism during molasses treatment is that the acid reacts with time with Ca(OH)$_2$ and Mg(OH)$_2$ already contained in molass solution to form:

$$\text{Ca(OH)}_2 + \text{H}_2\text{SO}_4 \xrightarrow{\Delta} \text{CaSO}_4 + 2\text{H}_2\text{O}$$

$$\text{Mg(OH)}_2 + \text{H}_2\text{SO}_4 \xrightarrow{\Delta} \text{MgSO}_4 + 2\text{H}_2\text{O}$$

Both CaSO$_4$ and MgSO$_4$ are coagulated and then precipitated.

Molass become sterilized due to inhibition of the harmful microflora i.e. wild yeast, (Osman, 2002)

2.9. Microbial growth kinetics:

Stanpury et al. (1995) mentioned that the fermentation may be carried out as batch, continuous and fed-batch process system.

The batch culture contains an initial limited amount of nutrient. The inoculated culture will pass through a number of phases as:

- **Lag phase:** In this phase no growth take place (time of adaptation). In a commercial process the length of the lag phase should be reduced.

- **Log or exponential phase:** The growth of the cells gradually increases till reach the maximum. Then the cells remain constant.
- **Stationary phase:** here the growth will decline to zero.
2.8.2 CONTINUOUS CULTURE:

STANPURY ET AL (1995) MENTIONED THAT EXPONENTIAL GROWTH IN BATCH CULTURE MAY BE PROLONGED BY ADDITION OF FRESH MEDIUM TO THE VESSEL. PROVIDED THAT THE MEDIUM HAS BEEN DESIGNED SUCH THAT GROWTH IS SUBSTRATE LIMITED, EXPONENTIAL GROWTH WILL PROCEED UNTIL THE ADDITIONAL SUBSTRATE IS EXHAUSTED. THIS EXERCISE MAY BE REPEATED UNTIL THE VESSEL IS FULL.

2.8.3 FED-BATCH CULTURE:

YOSHIDA ET AL. (1973) INTRODUCE THE TERM FED–BATCH CULTURE TO DESCRIBE BATCH CULTURE WHICH ARE FED CONTINUOUSLY WITH MEDIUM, WITHOUT THE REMOVAL OF CULTURE FLUID.

A FED–BATCH CULTURE ESTABLISHED INITIALLY IN BATCH MODE AND IS THEN FED THE MEDIUM.

2.9 MAIN STEPS IN THE PROCESS OF BAKER’S YEAST PRODUCTION:

ACCORDING TO BRONN (1982) THE MAIN STEPS IN THE PROCESS OF BAKER’S YEAST PRODUCTION ARE:

2.9.1 PREPARATION OF MEDIA:

MINERAL MEDIA ARE DISSOLVED IN WATER IN THE APPROPRIATE QUANTITIES AND THEN STERILIZED AND STORED IN THE FEEDING TANK.
2.9.2 Yeast Propagation:

The yeast is propagated in stainless steel fermentors. A seed culture is added to the mineral medium in the fermentors and molasses are fed to the fermentor in pre-calculated quantities. The fermentor is aerated and cooled, fermentation is run at about 30°C.

2.9.3 Harvest of the Yeast:

After completion of propagation the yeast is harvested by centrifugation and filtration, the resulting yeast contain about 27% dry matter and is called compressed yeast.

Rose (1961) mentioned that, at the end of propagation period, the yeast is recovered in a two stage process. The suspension is first of all passed through a centrifuge, and the separated yeast cream is mixed with water and again centrifuged.

The crop of yeast cells is separated by centrifugation and the yeast cream is washed with sterile water and cooled to 2°C. The paste is compressed to 10kg blocks (30, DM), and stored at 3-4°C (Vidal, 1978).

2.10 Brief description of yeast production in Sudan:
Production of compressed baker’s yeast in Hasahissa yeast factory is carried out using two types of fermentation processes, batch and continuous process system.

For the batch process all nutrient were added in one batch at the start of the process, but in the continuous process the nutrient were added frequently. In (S.F.I) there are three fermentors A, B and C to produce starter culture and commercial batches. Fermentor A: this is the smallest fermentor, the volume of which is 300 liter. The first step for the fermentation process to produce starter culture is from this fermentor and the system used by this fermentor is batch process system.

Fermentor B: this fermentor is bigger than fermentor A and its volume is 19,000 liter.

Fermentor B is the second step for fermentation process to produce starters culture, but some times it may be used as the first step for fermentation. Also, this fermentor use batch process system.

Fermentor C: this fermentor is bigger than fermentors A and B, the volume of this fermentor is 140,000 liters.
This fermentor represents the last stage of fermentation process to produce starter culture and also this fermentor is used to produce commercial batches directly.

Ingredients used in compressed baker's yeast process include the followings: molasses, urea, acids (H₂SO₄, H₃PO₄), sodium hydroxide (blashing powder), riboflavin, thiamin HCl, calcium-D-pentothenate, MgSO₄, and ZnSO₄.

Fermentation process was started in the culture room in which starting inoculum were prepared, then the inoculum was sent to the fermentor A and further to fermentor B and finally to the fermentor C, after the required fermentation period was completed, wort found in the fermentor C was separated, and the yeast cells were washed several times during separation and the separated cream was kept in cream collector tank at 4°C and it was considered as a starter culture for further commercial production.

The commercial batches were also produced in fermentor C mainly about four to five commercial batches were made from any starter culture batch depending on quality and quantity of the starter culture production.
AFTER COMMERCIAL BATCHES WERE SEPARATED, THE CREAM PRODUCED WAS SENT TO CREAM COLLECTOR TANKS AND KEPT AT 4°C.

THE CREAM WAS FILTERED IN A FILTER PRESS AND THE CAKE OBTAINED FROM THIS PROCESS WAS COMPRESSED AND MOLDED IN A MOLDING MACHINE AT A REQUIRED PACKING WEIGHT.

THE PACKED MATERIALS WERE KEPT IN COLD STORE AT 0°C FOR 24 HR BEFORE BEING SENT TO THE MARKET.
2.11 Yeast properties and quality control test:

2.11.1 Yeast activity:

The fermentative activity of yeast, and its behaviour in the bread making process are determined by methods which may involve test bakery equipment and normal bakery techniques. Many laboratory methods have been proposed for the routine determination of yeast activity and a critical appraisal of these has been given by (Burrows and Harrison, 1959).

These authors developed a method called the fermentometer test, based on gas volume measurement, in which the main source of error is inadequate temperature control. This can be reduced by using a small sample of dough which is mixed and fermented in a bottle immersed in water bath.

Gas–volume measurement gives a liable indication of dough—raising power inspite of the fact that appropriation of the gas produced in the dough escape.

A major source of variability in any yeast activity test based on dough fermentation, is the influence of a lock of consistency in the quality of the flour. Automatic activity
DETERMINATION USING GAS VOLUME METHOD IS QUITE FEASIBLE, AND MAY BE INCORPORATED IN A PROCESS STREAM OF YEAST CONCENTRATION THAT IS KNOWN AND STANDARDIZED (SHER, 1961).

2.11.2.2 OSMOSENSITIVITY:

IN THE PRESENCE OF HIGH CONCENTRATIONS OF SALTS AND SUGARS, THE YEAST CELLS SHRINKS OWING TO OSMOTIC EFFECTS. MANY ION AND LARGE UN-IONIZED MOLECULES DO NOT PENETRATE THE CELL WALL AND HENCE, UNLIKE SOME PLANT AND ANIMAL CELL ARE IN SUCH A SOLUTION.

UNDER THESE CONDITIONS THE FERMENTATIVE ACTIVITY OF THE YEAST IS SEVERELY REDUCED.

THE PRECISE REASON FOR THIS EFFECT IS NOT, HOWEVER KNOWN BUT POSSIBLE REASONS ARE:

(i) DEHYDRATION OF ENZYME SYSTEM IN THE CELL WALL.

(ii) LOSS OF WATER FROM THE CELL INTERIOR.

(iii) INHIBITION OF TRANSPORT OF SUBSTRATE INTO THE CELL.

FACTORS WHICH CONTROL YEAST PROPAGATION, SUCH AS GROWTH RATE, CAN INFLUENCE OSMOSENSITIVITY OF THE CELL BUT THE GENETICAL FACTORS ARE ALSO IMPORTANT (ROSE AND HARRISON, 1970)

2.11.3.3. KEEPING QUALITY:
Before tests for keeping quality are discussed, the extent of our knowledge of this property will be reviewed.

Where yeast is separated from its nutrient medium it quickly assumes a resting state. In this condition its fermentative activity decreases with time at a rate which is dependent on its metabolic state and on the ambient conditions.

This loss of activity may be due to:

(i) Loss of viability.

(ii) Intrinsic decrease of enzyme activity of the living cells.

(iii) Inhibition or lyses by its own products of metabolism or those of infecting organisms.

It is found in practice that in an uncontaminated and correctly growth yeast factors (i) and (ii) have negligible influence.

Keeping quality, as well as being dependent on the strain of yeast, is influenced by the method of preparation, thus anaerobically grown yeast has a much poorer keeping quality than that which is grow aerobically.
GENERALLY SPEAKING KEEPING QUALITY DECREASES AS FERMENTATIVE ACTIVITY INCREASES (ROSE AND HARRISON, 1970).

2.12 CHEMICAL COMPOSITION OF BAKER’S YEAST:

BRONN (1982) MENTIONED THAT THE CHEMICAL COMPOSITION OF COMPRESSED BAKER’S YEAST IS AS FOLLOWS:

THE CELL CONTAINS > 27% DRY MATTER (DM) AND 40–50% CRUDE PROTEIN IN DM.


THE CHEMICAL COMPOSITION OF DRY YEAST ACCORDING TO OSMAN (1997) IS AS FOLLOWS:

THE CARBOHYDRATE CONTENT 40 – 43%, THE PROTEIN CONTENT 42–46%, THE VIABLE COUNT $\log_{10} 9.0$ TO $\log_{10} 9.5$ CFU/G. AND BAKING RESULT WITH A SPECIFIC VOLUME 3.5 – 3.8 AND A RAISING TIME 45 TO 57 MINUTES.

2.13 MICROORGANISMS CONTAMINATION (IN PERCENT OF YEAST CELL VIABLE COUNT):
Less than 0.2% wild yeast, i.e. less than $10^7$ cell per gram of a compressed yeast, less than 0.2% bacteria, i.e. less than $10^7$ cell per gram of a compressed yeast (Shah, 1994).

The quality of some imported yeast is not always good. Routine test done at the Food Research Center (FRC) showed that some yeast types had very low activity and others were contaminated with bacteria (Mustafa, 1993).

Also it was found that yeasts having viable cell content above $10^9$ cell/gram will give good packing results and that ones of lower count will give bad results.
CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Materials

Molasses were used as raw materials for fermentation, samples from each fermentor were taken during fermentation, and samples of final products were used to evaluate the activity.

3.2 Methods:

3.2.1 Chemical analysis:

3.2.1.1 Determination of moisture content:

Moisture content was determined according to FAO (1986) methods: 3 g of compressed Baker’s yeast were weighed accurately in a clean preheated dish of known weight. The uncovered sample and dish were kept in an oven at 105°C and let to stay overnight. The dish was covered and transformed to a desiccator and weighted after reaching room temperature. The dish was again heated for two hours and was reweighed. This was repeated until constant weight was obtained.

The loss of weight was calculated as percentage of sample weight and expressed as moisture content.
\[ \text{MC} \% = \frac{(B - C) \times 100}{A} \]

WHERE:

MC: MOISTURE CONTENT

A: SAMPLE WEIGHT IN G

B: WEIGHT OF DISH + SAMPLE PRIOR TO DRYING

C: WEIGHT OF DISH + SAMPLE AFTER DRYING

(B – C): LOSS OF WEIGHT OF SAMPLE AFTER DRYING

3.2.1.2 DETERMINATION OF CRUDE PROTEIN:


EXACTLY 0.2 G OF COMPRESSED BAKER’S YEAST WAS DIGESTED IN A SMALL DIGESTION FLASK USING 0.4 G OF THE CATALYST, 3.5ML OF CONCENTRATED NITROGEN. FREE SULPHURIC ACID WAS ADDED TO THE FLASK AND THE CONTENT WAS DIGESTED FOR 2 HRS TILL A COLOUR LESS LIQUID WAS OBTAINED.

THE DIGEST WAS COOLED THEN DILUTED AND TRANSFERRED TO THE DISTILLATION UNIT USING MINIMUM VOLUME OF DISTILLED WATER AND MADE ALKALINE WITH 20ML OF 40% AQUEOUS NaOH SOLUTION. THE AMMONIA WAS DISTILLED INTO 10ML OF 2% BORIC ACID SOLUTION PLUS 3-4 DROPS METHYL RED INDICATOR FOR 5-10 MINUTES.
AFTER LOWERING THE RECEIVING FLASK CLEAR OF THE CONDENSER, THE APPARATUS WAS STEAMED FOR FURTHER 5 MINUTES TILL THE VOLUME IN THE RECEIVING FLASK REACHED 50-75ML. THE DISTILLATE WAS THEN TITRATED WITH 0.02N HCl

\[ N\% = \frac{T.F \times N \times 14.00 \times 100}{1000 \times wt \text{ of sample}} \]

% CRUDE PROTEIN = N% \times 6.25

WHERE:

T.F: ML HCl – ML BLANK

N: NORMALITY OF HCl

14: EACH ML OF HCl IS EQUIVALENT TO 14 MG NITROGEN.

1000: TO CONVERT FROM G TO MG

PROTEIN FACTOR : 6.25

3.2.1.3 DETERMINATION OF TOTAL ASH:

THE ASH CONTENT WAS DETERMINED ACCORDING TO FAO (1986) METHODS: A CRUCIBLE WAS WEIGHED EMPTY, THEN ACCURATELY 3 G OF SAMPLE WERE PUT IN IT. THE SAMPLE IN CRUCIBLE WAS PLACED IN A MUFFLE FURNACE AT 550ºC FOR 3 HRS OR MORE UNTIL WHITE GREY OR RED ASH WAS OBTAINED THEN THE CRUCIBLE WAS REMOVED FROM FURNACE AND PLACED IN A DISSCATOR
TO COOL then was weighed. The process was repeated until constant weight was obtained.
ASH CONTENT WAS CALCULATED USING THE FOLLOWING EQUATION:

\[
AC\% = \frac{W_2 - W_1}{W_s} \times 100
\]

WHERE:

\(AC\%\) : ASH CONTENT

\(W_1\) : WEIGHT OF EMPTY CRUCIBLE

\(W_2\) : WEIGHT OF CRUCIBLE WITH ASH

\(W_s\) : WEIGHT OF SAMPLE

3.2.1.4 Determination of Fat:

FAT CONTENT WAS DETERMINED ACCORDING TO FAO (1986) METHODS: FAT WAS DETERMINED BY EXTRACTION OF 4 G OF COMPRESSED BAKER’S YEAST BY HEXANE SOLVENT IN SOXHLET APPARATUS FOR 5 HOURS. THE EXTRACTED FAT WAS CALCULATED AS PERCENT FAT OF SAMPLE WEIGHT.

Calculation:

\[
\text{FAT} \% = \frac{W_2 - W_1}{S} \times 100
\]

WHERE:

\(W_1\) : WEIGHT OF EMPTY EVAPORATING DISH

\(W_2\) : WEIGHT OF EVAPORATING DISH + CONTENT AFTER DRYING

\(S\) : SAMPLE WEIGHT IN G.
3.2.1.5 **Estimation of total carbohydrates:**

Total carbohydrates was calculated by difference according to Person (1970). The total moisture, fat, protein, fiber, and ash were subtracted from 100 to obtain the difference that represent the total carbohydrates.

3.2.1.6 **Determination of sugars:**

Total sugars, invert sugars, and sucrose found in molasses were determined according to (Lane–Enyon) method Person (1970).

3.2.1.7 **Determination of alcohol content:**

Alcohol content was determined according to Bhandari (2002):

The steps:

1- **Taken 10 ml of sample in distillation flask.**

2- **Added a few pieces of pumice into distilling flask.**

3- **Added 25 ml of K₂Cr₂O₇ solution (42.59 gr/l) in receiving flask.**

4- **Added 10 ml H₂SO₄ conc in receiving flask.**

5- **Heated the sample till all alcohol content could transferen in receiving flask, at the end pointed the colour of K₂Cr₂O₇ changed to blacken.**
6- TRANSFERED QUANTITATIVELY THE CONTENT OF RECEIVER TO 1500 ML FLASK AND ADDED 8G KI THEN DILUTED THE SAMPLE UP TO ONE LITER BY DISTILLED WATER.

7- TITRATED THE SAMPLE BY 1N Na₂S₂O₃ TILL THE COLOUR IN ENDED POINT CHANGED FROM RED BROWN TO GREEN THEN STOPPED THE TITRATION AND ADDED 3-4 DROPS STARCH AND CONTINUED TITRATION TILL THE COLOUR BECOME BLUE.

Calculation:

\[
\% \text{ ALCOHOL CONTENT} = \left(25 - \frac{P}{q} \times 25\right) \frac{1}{10}
\]

Where:

- \( P \): TITRATION ML OF Na₂S₂O₃
- \( Q \): 21.8 (CONSTANT FACTOR)
- \( 25 \): VOLUME OF K₂Cr₂O₇
- \( 10 \): VOLUME OF H₂SO₄

3.2.1.8 Clarification of Molasses:

Molasses was diluted by hot water (1 : 1) and the pH value was adjusted to 4.5 – 5.0 by H₂SO₄ conc., and sterilized at 105°C (0.5bar) for 45 minutes, that time was the first retention time, then the molasses were transferred for another tank and took more retention time 60 minutes at 90°C. After one hour the pH, Brix, sp.Gr and sludge were checked.
When all the carried out tests were performed, the molasses were transferred to the receiver tank and the molasses were fed from fermentors A, B and C according to Bhandari (2002).

The molasses classification results were obtained following the below diagram:

**Molasses clarification in HasahiSSa yeast factory:**

```
RAW MATERIAL

HOT WATER
CONC. H₂SO₄

MOLASSES DILUTE BRIX (40-45) pH (4.5-5) ➔ STEA

HOT MOLASSES 106°C FOR 45MINUTE (FIRST RETENTION)

HOT MOLASSES 90°C FOR 1 HR (SECOND RETENTION)

TRANSFER CLEAR MOLASSES

MOLASSES RESIDUE ➔ CLEAR MOLASSES TO
```
3.2.2 Physical Analysis:

3.2.2.1 Determination of Total Soluble Solids:

Density in Brix was determined according to Indian standard methods (IS:1162-2000)

Apparatus:

Brix hydrometer was calibrated at 27.5°C and fitted with a thermometer. Immersion vessel, suitable for the hydrometer used.

(i) Procedure for raw molasses:

200 g of the raw molasses were weighed and 1400 ml of tap water was added and it was well mixed till a homogenous solution result.

The Brix value was determined using Brix hydrometer of suitable range and simultaneously the temperature of the solution was noted.

The temperature correction to the reading was applied using the table.

Calculation:

The corrected Brix reading was multiplied by 8. This gave the density of the molasses in the degrees Brix at 27.5°C.

(ii) Procedure for treated molasses and WART:
BRIX HYDROMETER OF SUITABLE RANGE WAS USED DIRECTLY

(WITHOUT DILUTION) AND THE RESULTS WERE RECORDED.
3.2.2.2. **Determination of the Specific Gravity (Sp.Gr):**

**Sp.Gr** was determined according to Indian Standard Methods Method (IS : 1162-2000)

**Apparatus:**

**Specific Gravity hydrometer calibrated at 27.5° and fitted with a thermometer. Immersion vessel, suitable for the hydrometer used.**

**Procedure:**

The diluted molasses or treated molasses or wart were put in cylinder test (250ml/ vol.) and the Sp.Gr. Hydrometer was used directly and the results were recorded.

3.2.2.3 **Determination of pH Value:**

The potentiometer measurement of pH of the different samples was accomplished using pH meter.

3.2.2.4 **Determination of concentration of compressed Baker’s yeast (GPL):**

The determination of GPL was carried out according to (Bhandari, 2002): 80ml of sample (wart) divided in two centrifuge tubes was centrifuged (5000 rpm) for 10 minutes, the liquid moved to the centre and the obtained solid matter
MOVED TO THE EDGE OF CENTRIFUGE TUBE AND THEN THE SOLID
MATTER WITH CENTRIFUGE TUBE WAS WEIGHED.
Calculation:

\[ GPL = \frac{W_2 - W_1}{W_s} \times 1000 \]

Where:

GPL: Concentration of compressed baker’s yeast per gram

\( W_1 \): Weight of empty centrifuge tube

\( W_2 \): Weight of solid matter with centrifuge tube

\( W_s \): Volume of sample (40ml)

3.2.3 Determination of activity of compressed baker’s yeast:

3.2.3.1 CO\(_2\) production during dough fermentation or cylinder test:

\( \text{CO}_2 \) production was determined according to Bhandari, (2002):

Procedure:

100g flour plus 1.5 g sugar were taken and 58 ml tap water and 3 g compressed baker’s yeast were added then it was well mixed by kneading machine for about 4 minutes and the dough was kept in a measuring cylinder and the cylinder was put in water bath at 27°C for three hours.

Calculation:
THE INITIAL VOLUME OF THE DOUGH WAS READED AND
RECORDED AND ALSO AFTER 1, 2, AND 3 HOURS.

\[
\text{CYLINDER TEST} = \frac{\text{last volume} - \text{initial volume}}{\text{Initial volume}}
\]

WHERE:

CYLINDER TEST: CO₂ PRODUCTION PER ML

LAST VOLUME: VOLUME OF THE DOUGH IN CYLINDER AFTER 1, 2, 3 HOURS

INITIAL VOLUME: VOLUME OF THE DOUGH IN CYLINDER AT THE START TIME OR ZERO TIME

3.2.3.2 TIME TAKEN TO LEAVEN A DOUGH (PROOF TIME)

TIME TAKEN TO LEAVEN A DOUGH WAS DETERMINED ACCORDING TO (BHANDARI, 2002):

PROCEDURE:

300 G FLOUR PLUS 2G SALT WERE TAKEN AND 3ML OIL AND 3 G COMPRESSED BAKER’S YEAST AND 180 ML TAP WATER WERE WELL MIXED BY KNEEDING MACHINE FOR ABOUT 5 MINUTES AND THE DOUGH WAS PUT IN MOLD AND THE MOLD WAS KEPT IN INCUBATOR AT 37°C AND THE INITIAL TIME WAS RECORDED, WHEN THE DOUGH HAD
REACHED THE LAST LEVEL OF THE MOLD, THEN THE LAST TIME WAS
RECORDED.

CALCULATION:

**Time taken to leaven a dough per minute = last time – initial time**

WHERE:

**Last time:** time taken for the dough to reach the last level
of the mold

**Initial time:** this is the time when the dough was put in mold
and incubator.

3.2.3.3. Baking test:

The baking test was carried out according to the procedure described by Badi et al. (1976):

The formula used was as follows:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flour</td>
<td>250g</td>
</tr>
<tr>
<td>Yeast</td>
<td>2.5g</td>
</tr>
<tr>
<td>Salt</td>
<td>3.0g</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>80p.p.m</td>
</tr>
<tr>
<td>Water</td>
<td>variable</td>
</tr>
</tbody>
</table>
ALL INGREDIENTS MENTIONED ABOVE WERE WEIGHED AND MADE INTO A DOUGH IN A MONO–UNIVERSAL LABORATORY, DOUGH MIXTURE FOR 5 MINUTES AT MEDIUM SPEED.

THE DOUGH WAS ALLOWED TO REST FOR 15 MINUTES AT ROOM TEMPERATURE (38±2ºC), THEN SCALED TO THREE PORTIONS OF 120G EACH. THE THREE DOUGH PORTIONS WERE ROUGHLY MOULDED UP INTO ROUND BALLS AND ALLOWED TO REST FOR ANOTHER 15 MINUTES, THEN MOULDED UP INTO PANS AND PLACED IN THE FERMENTATION CABINET FOR FINAL PROOF (45MINUTES)

FINALLY THE FERMENTED DOUGH SAMPLES WERE BAKED INTO BREAD IN SIMON ROTARY TEST OVEN AT 220-250ºC (WITH SATURATION OF STEAM) FOR 20-25 MINUTES.

THE LOAVES WERE LEFT TO COOL, SLICED WITH AN ELECTRIC KNIFE AND SOME SLICES WERE KEPT CLOSED IN POLYETHYLENE BAGS AT ROOM TEMPERATURE.

3.2.3.3 BREAD QUALITY:

BREAD PRODUCED FROM THE WHEAT FLOUR WHERE LEFT TO COOL AT ROOM TEMPERATURE (38 ± 2ºC) FOR 1 HR AFTER BAKING, AND QUALITY WAS MEASURED ON TRIPLICATE LOAVES AS FOLLOWS:

BREAD VOLUME:
THE LOAF VOLUME EXPRESSED IN CUBIC CENTIMETERS WAS DETERMINED BY THE SEED DISPLACEMENT METHOD ACCORDING TO PYLER (1973). THE LOAF WAS PLACED IN A CONTAINER OF KNOWN VOLUME INTO WHICH SMALL SEEDS (MILLET SEEDS) WERE RUN UNTIL THE CONTAINER WAS FULL.

THE VOLUME OF SEEDS DISPLACED BY THE LOAF WAS CONSIDERED AS THE LOAF VOLUME.

BREAD SPECIFIC VOLUME:

THE SPECIFIC VOLUME OF THE LOAF WAS CALCULATED ACCORDING TO THE AACC METHOD (1986) BY DIVIDING VOLUME (CC) BY WEIGHT (G).

3.2.4 MICROBIOLOGICAL METHODS:

3.2.4.1 MATERIALS:

1 ML SAMPLES FROM THE ORIGINAL CULTURE AND FROM WATER USED FOR THE PROCESSING, WERE TAKEN, ALSO 1G SAMPLES FROM THE MOLASS AND UREA USED AS PROCESSING INGREDIENTS WERE TAKEN. OPEN PETRI DISHES PREPARED WITH MEDIA WERE EXPOSED TO AIR OF PLANT SURROUNDING AREA, AND MANY PARTS OF THE PLANT TO STUDY THE MICROBIOLOGICAL CONDITION OF THE AIR.

1 ML SAMPLES FROM EACH FERMENTOR WAS TAKEN BEFORE FERMENTATION PROCESS IMMEDIATELY, AT THE INITIAL
FERMENTATION TIME AND AFTER THE END OF FERMENTATION PROCESS IMMEDIATELY. ONE GRAM OF FINAL PRODUCT WAS USED TO EVALUATE MICROBIAL STATE.

3.2.4.2 Preparation of the media:

3.2.4.2.1 Yeast extract agar (Y.E.A):

YEAST EXTRACTagar MEDIUM OXOID WAS PREPARED BY SUSPENDING 10G (Y.E.A) MEDIUM IN 1000ML DW 121C COMPLETELY. pH WAS ADJUSTED TO 4.5–5 AND STERILIZED AT 15 IBS PRESSURE FOR 15 MINUTES.

2.3.4.2.1 Nutrient agar (NA):

NUTRIENTagar MEDIUM OXOID WAS PREPARED BY SUSPENDING 40G OF NUTRIENT AGAR IN 1000ML D.W, pH WAS ADJUSTED TO 7.3–7.5 AND STERILIZED AT 15 IBS PRESSURE (121ºC) FOR 15 MINUTES.

3.2.4.2.3 Lysine medium (Bhandari, 2002):

LYSINE medium was prepared by suspending 66G lysine medium plus 16G agar powder and 10% potassium and the lactate in 1000ML D.W, and boiled to dissolve the media completely. The pH was adjusted to 5.5 and sterilized at 121ºC/15MIN.
3.2.4.2.4 Fungus media (Bhandari, 2002):

The composition of fungus media is as follows:

- Ammonium succinate: 17 g/L
- Succinic acid: 7 g/L
- MgSO₄: 0.2 g/L
- Agar agar powder: 20 g/L
- Potassium dihydrogen orthophosphate: 5 g/L
- Distilled water: 1000 ml
- pH value: 5.5

After mixing these ingredients were boiled to dissolve media completely and the pH was adjusted then sterilized at 121°C/15 min.

3.2.4.2.5 Peptone water (Oxoid):

Peptone water was prepared by suspending 15 g media tryptone water in 1000 ml D.W, and boiled to dissolve the media completely and were sterilized at 121°C/15 min.

3.2.4.2.6 Cyclohexamide (Actidione):

Actidione was prepared by suspending 1.0 g of actidione in 1000 ml sterilized distilled water (Andrews, 1992).
3.2.4. Methods:

3.2.4.1 Preparation and Dilution of the Homogenate Sample:

The preparation of the samples and the chosen methods of analysis were carried out according to (Andrews, 1992).

25 g of sample were weighed into sterile microbiological bag. A dilution of 10 was obtained by adding 225 ml of buffer peptone water and mixing the contents by shaking. One ml of the bag contents was pipetted into separate tubes containing 9 ml of ¼ strength Ringer solution. The liquid was mixed carefully by aspirating 10 times with a sterile pipette. With the same pipette one ml was transferred to another dilution tube containing 9 ml of Ringer solution and mixed with a fresh pipette.

Dilutions of \(10^2\), \(10^3\), \(10^4\), \(10^5\) and \(10^6\) were obtained by repeating the steps of mixing and transferring.

3.2.4.2 Determination of Viability of Yeast:

3.2.4.2.1 By plating according to (Andrews, 1992):

One gram samples were taken and added to 9 ml peptone water. A series of dilutions up to \(10^8\) were made. Using the surface plate method, 0.1 ml from selected dilution was spread onto the surface of the solidified plates of yeast.
EXTRACT AGAR MEDIA CONTAINING 0.01% CHLORAMPHENICOL FOR THE SUPPRESSION OF BACTERIAL GROWTH. THE PLATES WERE INCUBATED AT 30°C FOR 3 DAYS.

ALL COLONIES WAS COUNTED BY USING COLONY COUNTER. THE NUMBER OF YEAST CELLS WAS COMPUTED PER ML BY MULTIPLYING THE RECIPROCAL OF THE DILUTION USED.

3.2.4.2.3 BY HAEMOCYTOMETER:

THIS IS OTHER METHOD FOR DETERMINING VIABILITY OF YEAST:

ACCORDING TO BHANDARI (2002)

PRINCIPLE:

YEAST GROWS GRADUALLY AFTER BEING TAKEN FROM INCUBATED FLASK, EVERY HOUR, ONE CELL PRODUCE OTHER CELL, THE NEW CELL START AS BUD AT PRESENCE OF NUTRIENTS, OXYGEN AND WELL FEEDING.

THE RATE OF GROWTH INCREASES AT ANY HOUR DURING PROCESS OPERATIONS

APPARATUS:

1- SAMPLE OF YEAST OR WART
2- DISTILLED WATER
3- METHYLENE BLUE
4- MICROSCOPE
5- **HAEMOCYTOMETER**
6- **TEST TUBES**
7- **GLASS ROD**
8- **PIPETTE 10ML & 1ML**
**PROCEDURE:**

*One g of sample or 1ml of wart was taken and diluted by 9ml D.W in test tube and mixed well by glass rod using hand. Then 0.1ml of the dilution was taken suspended in 9.9ml D.W in another test tube and shaked well and one drop of the metheleene blue was put in test tube, and shaked again. One drop was put in squire haemocytometer and the haemocytometer was put under microscope. The number of life yeast cells was recorded.*

![Squire Haemocytometer Diagram](image)

**CALCULATION:**

\[
\text{Yeast cells count} = \text{AV} \times 2.5 \times 10^5 \times \text{DF}
\]

**WHERE:**

- \(\text{AV: average number for live squire } \left(\frac{x}{5}\right)\)
- \(2.5 \times 10^5: \text{constant factor}\)
**DF: DILUTION FACTOR**

3.2.4.2.3 *Enumeration of the total viable bacterial count in the raw materials, during processing and in the final products:*

**According to (Andrews, 1992)** one ml was pipetted from different dilution into Petri-dishes. Promptly, 15ml of nutrient agar medium was melted and allowed to cool to 45°C then poured into the petri dishes.

Immediately aliquots were mixed with agar medium by turning the dish to and fro 5 times in one direction, rotating it clockwise 5 times, turning it to and fro again 5 times in a direction at right angle to that used in the first time and rotating it counter clockwise 5 times.

After solidification, the petri-dishes were inverted and incubated at 36°C for 24 hours, colony counter was used to count all colonies. The number of presumptive colony forming units was computed per gram of specimen by multiplying by the reciprocal of the dilution used.

For the enumeration of the total viable bacterial count for any sample including yeast, nutrient agar was used with 0.01% cycloheximide or actidione as inhibitor to yeast growth.
3.2.2.4 Detection of wild yeast in the raw materials, processing line and final products:

According to Andrews (1992), one ml of each decimal dilution was pipetted into empty Petri-dishes. 15 ml of lysine medium, melted and allowed to cool to 45°C were promptly poured into the Petri-dishes, aliquots were immediately mixed with agar medium using the method described in 3.2.4.2.3.

After the agar had been solidified, the Petri-dishes were incubated at 25°C for three days.

All colonies were counted by using colony counter. The number of wild yeasts were computed per gram by multiplying the reciprocal of the dilution used.

3.2.4.2.5 Detection of molds in the raw materials, processing line and final products:

According to Andrews (1992), one ml of each decimal dilution was pipetted into empty Petri-dishes. 15 ml of fungus medium, melted and allowed to cool to 45°C were promptly poured into the Petri-dishes, aliquots were immediately mixed with the agar medium using the method described in 3.2.4.2.3.

After the agar had been solidified, the Petri-dishes were incubated at 24°C for three days.
All colonies were counted by using colony counter.

The number of molds were computed per gram by multiplying the reciprocal of the dilution used.
CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1. CHEMICAL AND PHYSICAL RESULTS:

4.1.1. MOLASSES BEFORE TREATMENT:

THE CHEMICAL AND PHYSICAL ANALYSIS OF MOLASSES BEFORE TREATMENT GAVE THE FOLLOWING RESULTS: THE TOTAL SOLUBLE SOLIDS (BRIX) WAS 77, SPECIFIC GRAVITY WAS 1.493, pH VALUE 5.4, TOTAL SUGARS 47.6% INVERT SUGARS 15.2% AND SUCROSE 32.4% (TABLE 1).

4.1.2. MOLASSES AFTER TREATMENT:

AS TABLE (2) SHOWED THERE WAS A DECREASE IN TOTAL SOLUBLE SOLIDS (FROM 77 TO 40), SPECIFIC GRAVITY (FROM 1.493 TO 1.170), TOTAL SUGARS (FROM 47.6 TO 25.3%), INVERT SUGARS (FROM 15.2 TO 9.4%) AND SUCROSE (FROM 32.4 TO 15.9%). THE DECREASE IN ABOVE MENTIONED PARAMETERS WAS MAINLY DUE TO ADDITION OF HOT WATER AND STEAM.

THERE WAS ALSO DECREASE IN THE pH VALUE (FROM 5.4 TO 4.5) DUE TO THE ADDITION OF SULPHURIC ACID.

THESE RESULTS ARE IN AGREEMENT WITH THE RESULTS OBTAINED BY OSMAN (2002).
**TABLE (1). PHYSICAL AND CHEMICAL COMPOSITION OF MOLASSES FROM EL-GUNEID FACTORY: (BEFORE TREATMENT, SEASON 2002/03)**

<table>
<thead>
<tr>
<th>COMPONENTS OF RAW MOLASSES</th>
<th>RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOTAL SOLUBLE SOLIDS (BRIX)</td>
<td>77</td>
</tr>
<tr>
<td>SPECIFIC GRAVITY (sp.Gr)</td>
<td>1.493</td>
</tr>
<tr>
<td>pH – VALUE</td>
<td>5.4</td>
</tr>
<tr>
<td>TOTAL SUGARS</td>
<td>47.6%</td>
</tr>
<tr>
<td>INVERT SUGARS</td>
<td>15.2%</td>
</tr>
<tr>
<td>SUCROSE</td>
<td>32.4%</td>
</tr>
</tbody>
</table>
**Table (2). Physical and Chemical Composition of Molasses from El-Guneid Factory: (After Treatment, Season 2002/03)**

<table>
<thead>
<tr>
<th>Components of Raw Molasses</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Soluble Solids (Brix)</td>
<td>40</td>
</tr>
<tr>
<td>Specific Gravity (Sp.Gr)</td>
<td>1.170</td>
</tr>
<tr>
<td>pH – Value</td>
<td>4.5</td>
</tr>
<tr>
<td>Total Sugars</td>
<td>25.3%</td>
</tr>
<tr>
<td>Invert Sugars</td>
<td>9.4%</td>
</tr>
<tr>
<td>Sucrose</td>
<td>15.9%</td>
</tr>
</tbody>
</table>
4.1.3. The results of experiments carried out during process:

4.1.3.1. Comparison between concentration of compressed baker’s yeast (GPL) and time of fermentation:

Usually the starter culture is produced from fermentor A., across fermentor B. ending in fermentor C. But some times it can be produced from fermentor B ending in fermentor C.

Fig. 1 presents the normal case of processing. It can be observed that the GPL increased weakly in the three fermentors A, B and C at the first four hours of fermentation.

Then in fermentor A the GPL showed negligible increase after 16 hours of fermentation, also there was no marked increase in fermentor B after 12 hours of fermentation. This indicate that running the fermentation process is not economical and it should stop.

Using the three types of fermentors mentioned above, it was noticed that the GPL increased with increasing fermentation time. That was also true for the commercial batch.

When fermentor B was used to produce the starter culture, the GPL was increased weakly in fermentor B and C at the first hours of fermentation, but fermentor B took
MUCH MORE TIME THAN FERMENTOR C, THIS MAY BE DUE TO THE USE OF HIGH CONCENTRATION OF SUGAR IN FERMENTOR B.

ALSO USING THE FERMENTORS B, C AND COMMERCIAL BATCH IT WAS NOTICED THAT AN INCREASE IN FERMENTATION TIME BROUGHT ABOUT AN INCREASE IN CONCENTRATION OF COMPRESSED BAKER'S YEAST (FIG. 2).
Fig. 1. Comparison between concentration of compressed baker's yeast and time of fermentation during process at the normal case
Fig. 2. Comparison between concentration of compressed baker's yeast and time of fermentation during process when the starter culture produced from fermentor B
4.1.3.2. pH VALUE OF DIFFERENT TYPES OF PROCESSES DURING FERMENTATION TIME:

WHEN THE STARTER CULTURE WAS PRODUCED FROM FERMENTOR A THE INITIAL pH WAS 3.81 AND DECREASED TILL REACHED 3.53 DURING FERMENTATION TIME, AND THE INITIAL pH VALUE FOR FERMENTOR B WAS 4.3 AND ALSO DECREASED TILL IT REACHED 3.5, BUT IN FERMENTOR C THE INITIAL pH VALUE WAS 3.6 AND INCREASED TILL REACHED 4.72. USINE BY COMMERCIAL BATCH THE INITIAL pH VALUE WAS 3.2 AND ALSO INCREASED DURING FERMENTATION TIME TILL REACHED 5.7 (FIG. 3).


THE DECREASE OF pH VALUE DURING FERMENTATION (FERMENTOR A AND B) WAS DUE TO THE APPLICATION OF BATCH PROCESS SYSTEM (CONSUMPTION OF NUTRIENTS AND METABOLITES ACTIVITY), BUT THE INCREASE IN THE pH VALUE DURING FERMENTATION (FERMENTOR C AND COMMERCIAL BATCHES) WAS DUE
TO THE USE OF CONTINUOUS PROCESS SYSTEM, BY THIS SYSTEM THE
NUTRIENTS WERE FED GRADUALLY.

THese results of pH value agreed with the results
obtained by White (1954).
Fig. 3. pH values of different types of processes during fermentation time at the normal case.
Fig. 4. pH values of different types of processes during fermentation time when the starter culture produced from fermentor B
4.1.3.3. **Total soluble solids (Brix) of different processes during fermentation:**

When the starter culture was produced from fermentor A the initial Brix value was 7.5 and decreased during fermentation till reached 3.2, in fermentor B the initial Brix was 11.3 and also decreased till reached 4.5, but in fermentor C the initial Brix was 1.45 and then increased during fermentation till reached 6.4 and the initial Brix of commercial batch was 0.8 and also increased till reached 7.9 during fermentation (Fig. 5).

When the starter culture was produced from fermentor B the initial Brix value was 12.5 and then decreased during fermentation till reached 5.2, but by the fermentor C the initial Brix was 1.4 and increased till reached 6.1, and the initial Brix of commercial batch was 1.0 and also increased till reached 9.1 during fermentation process time (Fig. 6).

Also the decrease of Brix value in fermentors A and B during fermentation was due to the use of batch process system (feeding once at the start of fermentation process), but the increase in the Brix value in fermentor C and commercial batches was due to the use of continuous process
SYSTEM, WHICH WAS LINKED WITH THE GRADUAL FEEDING OF THE
FERMENTORS.
Fig. 5. The total soluble solids (Brix) of different processes during fermentation time at the normal case.
Fig. 6. The total soluble solids (Brix) of different processes during fermentation time when the starter culture produced from fermentor B
4.1.3.4. **Comparison between number of yeast cell concentration (YCC) and fermentation during process (\(\log_{10}\) colony forming unit (\(\log_{10}\) CFU/ML)):**

**When the starter culture was produced from fermentor A** the initial yeast cell concentration (YCC) was \(\log_{10} 7.1\), and after 4 hours it was increased weakly till reached \(\log_{10} 7.12\), then increased more strongly till reached \(\log_{10} 8.16\) during 19 hours, but at the last 2 hours of fermentation the YCC increased again weakly till reached \(\log_{10} 8.23\), and the initial YCC for fermentor B was \(\log_{10} 7.8\), and also after 4 hours it was increased weakly till reached \(\log_{10} 7.84\), then increased more strongly till reached \(\log_{10} 8.61\) during 16 hours, and in fermentor C, the initial YCC was \(\log_{10} 8.04\) and after 4 hours it was increased weakly till reached \(\log_{10} 8.07\), then increased more actively till reached \(\log_{10} 8.98\) during 16 hours, but at the last 5 hours of fermentation the YCC increased again weakly till reached \(\log_{10} 9.04\) but in commercial batch the initial YCC was \(\log_{10} 8.51\) and increased more till reached \(\log_{10} 9.36\) during 16 hours (Fig. 7).

**When the starter culture was produced from fermentor B** the initial YCC was \(\log_{10} 7.27\) and after 4 hours
IT WAS INCREASED WEAKLY TILL REACHED $\log_{10} 7.3$, THEN INCREASED MORE TILL REACHED $\log_{10} 8.51$ DURING 21 HOURS, AND THE INITIAL YCC FOR FERMENTOR C WAS $\log_{10} 7.69$ AND AFTER 4 HOURS IT WAS INCREASED WEAKLY TILL REACHED $\log_{10} 7.74$ AND THEN INCREASED MORE TILL REACHED $\log_{10} 9.3$ DURING 23 HOURS, BUT IN COMMERCIAL BATCH THE INITIAL YCC WAS $\log_{10} 8.62$ AND INCREASED MORE TILL REACHED $\log_{10} 9.46$ DURING 19 HOURS (FIG. 8).

THE REASONS FOR LOW INCREASE IN YEAST CELL CONCENTRATION FROM FERMENTOR A, B AND C AT THE FIRST HOURS OF FERMENTATION WAS DUE TO ENTRANCE OF THE CELLS IN THE LAG OR ADAPTATION PHASE AND THE FOLLOWING MORE INCREASE IN NUMBER WAS DUE TO ENTRANCE OF THE CELLS IN THE LOG OR EXPONENTIAL PHASE, BUT THE LOW INCREASE IN NUMBER OF CELLS NEAR THE END OF FERMENTATION PROCESS WAS DUE TO THE CONSUMPTION OF NUTRIENTS AND NATURE OF THE STRAIN OF COMPRESSED BAKER'S YEAST USED AND WHETHER THEY REACHED THE MAXIMUM DOUBLICATE RATE.

BEFORE THE YEAST CELL ENTER THIS POINT, THE FERMENTATION PROCESS SHOULD BE STOPPED BECAUSE AFTER THIS TIME FURTHER PRODUCTION WAS UNECONOMICAL AND ALSO THE CELLS MAY ENTER STATIONARY PHASE AND DEATH PHASE.
Fig. 7. Comparison between number of yeast cells concentration (YCC) and fermentation time during process ($\log_{10}$ cfu/ml) at the normal case.
Fig. 8. Comparison between number of yeast cells concentration (YCC) and fermentation time during process ($\log_{10}$ cfu/ml) when the starter culture produced from fermentor B.
4.1.4. Alcohol content at the last stage of fermentation for the starter culture and commercial batch:

The alcohol content at the end of fermentation for starter culture was 1.02% and for commercial batch was 0.63%.

The increase of alcohol content for starter culture compared with commercial batch was due to the fact that the starter culture use high concentration of sugars in fermentor A and B at the initial stage of fermentation.

4.1.5. Chemical composition of compressed baker's yeast:

The chemical composition of compressed baker's yeast at Hasahissa Yeast Factory was as follows:

Moisture content 70.5%, protein content 14.4%, ash 1.66%, fats 1.2% and carbohydrates 12.2% (Table 3).

The results are comparable with the Egyptian standards for compressed baker's yeast (ES664042, 1963) and Sudanese standards and Metrology Organization (SSMO 511, 2004).
**Table 3. Chemical composition of compressed baker's yeast compared with Egyptian and Sudanese standards:**

<table>
<thead>
<tr>
<th>Types of Test</th>
<th>Results</th>
<th>Egyptian Standards for Compressed Baker's Yeast %</th>
<th>Sudanese Standards for Dried Baker's Yeast %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Moisture Content</strong></td>
<td>70.5</td>
<td>Not more than 72</td>
<td>Not exceed 8</td>
</tr>
<tr>
<td><strong>Protein Content</strong></td>
<td>14.437</td>
<td>Not less than 14</td>
<td>40 – 45</td>
</tr>
<tr>
<td><strong>Ash Content</strong></td>
<td>1.66</td>
<td>Not more</td>
<td>Not</td>
</tr>
<tr>
<td></td>
<td>THAN 2.5</td>
<td>EXCEED 8.5</td>
<td></td>
</tr>
<tr>
<td>---------------------</td>
<td>----------</td>
<td>------------</td>
<td></td>
</tr>
<tr>
<td><strong>FAT CONTENT</strong></td>
<td>1.2</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>CARBOHYDRATE</strong></td>
<td>12.2</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>CONTENT</strong></td>
<td></td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>
4.1.6. Activity of compressed baker's yeast:

4.1.6.1. CO₂ production during dough fermentation:

**Compressed baker's yeast in SFI has produced 128.5% CO₂ in the first hour of dough fermentation, after two hours the CO₂ produced was 160% and at the last hour or after three hours of dough fermentation the CO₂ produced was 175% (Table 4).**

Reed and Peppler (1973) reported that yeast produce 350 ml/CO₂ in one hour in the dough by 2.5% dry yeast. Oura et al., (1982) reported that the amount of sodium bicarbonate for baking should not exceed 6% of the weight of the dough, this amount gave not more than 214 ml CO₂/hr.

4.1.6.2. Time needed to leaven a dough:

The time needed to leaven a dough reached 75 minutes in a trial carried out in (SFI), but the baking test with specific volume has produced 2.9 (Table 5).

4.1.6.3. The viability of compressed baker's yeast:

**Viability in log₁₀ colony forming unit in one gram log₁₀ 9.98CFU/g. The Sudanese Standards Metrology Organization (SSMO)(2004) for active instant yeast (dry and compressed)**
STATED THAT THE VIABLE YEAST COUNT (VIABILITY) SHOULD BE ABOUT NOT LESS THAN \(1 \times 10^9\) CFU/G OF \textit{Saccharomyces cerevisiae}.

**Table 4. CO\(_2\) Production during Dough Fermentation (per hours)**

<table>
<thead>
<tr>
<th>Test</th>
<th>Time Needed to Leaven Dough</th>
<th>Standard Specification for SFI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cylinder Volume (ml)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>140</td>
<td>320</td>
</tr>
<tr>
<td>CO(_2) %</td>
<td>0</td>
<td>128.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Table 5. Time taken to leaven dough and specific volume during bread making using compressed baker's yeast:**

<table>
<thead>
<tr>
<th>Types of Test</th>
<th>Results</th>
<th>Standards Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time taken to leaven dough (proof time)</td>
<td>75 minutes</td>
<td>60 – 70 in (SFI)</td>
</tr>
<tr>
<td>Specific volume</td>
<td>2.9 cm³/gm</td>
<td>3 – 3.5 cm³/gm</td>
</tr>
</tbody>
</table>
4.2. MICROBIOLOGICAL RESULTS:

4.2.1. MICROBIAL RESULTS OF RAW MATERIALS: LOG CFU/G

4.2.1.1. MOLASSES BEFORE TREATMENT:

**The total viable bacterial count was** $\log_{10} 4.84$ CFU/G

while wild yeasts were $\log_{10} 2.47$ CFU/G and molds were $\log_{10} 2.00$ CFU/G. *(Table 6).*

4.2.1.2. MOLASSES AFTER TREATMENT:

**The total viable bacterial count, wild yeasts and molds**

were nil, this was due to sterilization process applied.

4.2.1.3. WATER BEFORE TREATMENT:

**The total viable bacterial count was** $\log_{10} 2.79$ CFU/ML

while wild yeasts were $\log_{10} 3.25$ CFU/ML and molds were $\log_{10} 1.48$ CFU/ML.

4.2.1.4. WATER AFTER TREATMENT:

All microbes were nil, because of the use of a dose of

chlorine (0.3 – 0.4 PPM)

4.2.1.5. UREA BEFORE TREATMENT:

**The total viable bacterial count was** $\log_{10} 2.23$ CFU/G

while wild yeasts were $\log_{10} 1.000$ CFU/G and molds were nil

*(Table 6).*

4.2.1.6. ORIGINAL YEAST CULTURE:
FREE OF CONTAMINATION.
### Table 6. Microbiological Analysis of Raw Materials: Molasses, Urea, Water Before Treatment, and Original Culture

<table>
<thead>
<tr>
<th>Contaminated</th>
<th>Samples</th>
<th>Molasses</th>
<th>Urea</th>
<th>Water</th>
<th>Original Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td></td>
<td>4.84</td>
<td>2.38</td>
<td>2.79</td>
<td>Nil</td>
</tr>
<tr>
<td>Wild Yeast</td>
<td></td>
<td>2.47</td>
<td>1.00</td>
<td>3.25</td>
<td>Nil</td>
</tr>
<tr>
<td>Mold</td>
<td></td>
<td>2.00</td>
<td>Nil</td>
<td>1.84</td>
<td>Nil</td>
</tr>
</tbody>
</table>
4.2.2. Evaluation of the microbiological contamination of the plant, surrounding air and plant units:

The contamination of the air surrounding the plant was too high, the total viable bacterial count (TBC) was $\log_{10} 2.49$ CFU, the wild yeast (WY) were $\log_{10} 1.56$ CFU and the molds were $\log_{10} 1.47$ after 30 minutes exposure, but the air inside the fermentors contains no microbes. Because the air before interning the fermentors was passed through blower of air which is an air filter, the size of this filter not allow the entrance of microorganisms, further more the filter should be cleaned continuously and treated by formalin and 95% alcohol.

The air inside the plant was also contaminated; the TBC of the fermentation unit was $\log_{10} 2.35$, WY were $\log_{10} 1.67$, molds were $\log_{10} 1.17$ and in filtration and packing units the TBC was $\log_{10} 1.86$, (WY) were $\log_{10} 1.73$ molds were $\log_{10} 1.39$, and in chemical analysis lab the TBC was $\log_{10} 2.16$, (WY) were $\log_{10} 1.17$ and molds were nil, but in the culture room the TBC, WY and molds were nil after 30 min exposure (Table 7).

The contamination of the air surroundings the plant was due to the presence of plant in the industrial area, where the pollution is too high, but the contamination inside different parts of the plant was due to opening of the doors.
AND WINDOWS BY WORKERS AND ALSO DUE TO INSUFFICIENT CLEANING
AND GENERAL SANITATION.

THE LOW CONTAMINATION OF THE CULTURE ROOM UNIT WAS
DUE TO THE TREATMENT OF THIS ROOM BY FORMALIN.

<table>
<thead>
<tr>
<th>CONTAMINATED LOG$_{10}$ CFU AFTER 30 MINUTES EXPOSURE</th>
<th>SURROUNDING OF ATMOSPHERIC AIR</th>
<th>FERMENTATION UNIT</th>
<th>FILTER AND PACKING UNIT</th>
<th>CHEMICAL ANALYSIS LAB</th>
<th>CULTURE ROOM UNIT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TOTAL VIABLE BACTERIA L COUNT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.49</td>
<td>2.35</td>
<td>1.86</td>
<td>2.16</td>
<td>NIL</td>
<td></td>
</tr>
<tr>
<td><strong>WILD YEAST</strong></td>
<td>1.56</td>
<td>1.67</td>
<td>1.73</td>
<td>1.17</td>
<td>NIL</td>
</tr>
<tr>
<td>MOLD</td>
<td>1.47</td>
<td>1.17</td>
<td>1.39</td>
<td>Nil</td>
<td>Nil</td>
</tr>
</tbody>
</table>
4.2.3. Microbial Results of Starter Culture During Process:

The contamination (total viable bacterial count TBC, wild yeasts and molds) during production of starter culture from fermentors A and B before inoculation or before charge after inoculation or after charge and at the final fermentation stages were nil. Because the fermentors A and B use batch process system, and this system works under aseptic conditions, but in fermentor C the contamination had occurred in the final fermentation stage. Before charge (after preparation of the media), the TBC, wild yeasts and molds also were nil, then after charge (after transfer of the wort from fermentors B to C). The TBC, wild yeast and molds also were nil, but in the final fermentation stage the TBC was \( \log_{10} 2.3 \) CFU/ML, wild yeast were \( \log_{10} 6.0 \) CFU/ML and molds were \( \log_{10} 3.6 \)/ML and in the cream of compressed baker's yeasts after separation the TBC was \( \log_{10} 3.3 \) CFU/ML, WY were \( \log_{10} 6.3 \) CFU/ML and molds were \( \log_{10} 4.3 \) /ML (Table 8).

The contamination at the last fermentation stage and separated cream was due to the use of continuous process system and for this system the urea was added without treatment also the fermentor was opened frequently to add the nutrients, that means the contaminated air in process
UNIT CAN ENTER INSIDE THE FERMENTOR. FURTHERMORE, THE SEPARATOR WAS NOT CLOSED COMPLETELY DURING SEPARATION, THEN THE AIR ENTER INSIDE THE CREAM AND ALSO THE PROCESS OF SEPARATION WAS NOT HYGIENIC ENOUGH, THERE THE TECHNICIANS TOUCH THE WORKING UTENSILS BY THEIR HANDS.

Table 8. Microbiological analysis of starter culture during process

<table>
<thead>
<tr>
<th>ITEM</th>
<th>CONTAMINATION LOG_{10} CFU/ML</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TOTAL</td>
</tr>
<tr>
<td></td>
<td>BACTERIAL COUNT</td>
</tr>
<tr>
<td></td>
<td>WILD YEAST</td>
</tr>
<tr>
<td></td>
<td>MOLDS</td>
</tr>
<tr>
<td>Molasses after treatment</td>
<td>NIL</td>
</tr>
<tr>
<td>Fermentor A and B</td>
<td>NIL</td>
</tr>
</tbody>
</table>

113
<table>
<thead>
<tr>
<th>Category</th>
<th>Before Charge</th>
<th>After Charge</th>
<th>At Final Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fermentor A and B (after charge)</strong></td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td><strong>Fermentor A and B (at final stage)</strong></td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td><strong>Fermentor C (before charge)</strong></td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td><strong>Fermentor C (after charge)</strong></td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td><strong>Fermentor C (at the final fermentation stage)</strong></td>
<td>2.3</td>
<td>6.00</td>
<td>3.6</td>
</tr>
<tr>
<td><strong>Cream of Baker's Yeast after separation</strong></td>
<td>3.3</td>
<td>6.3</td>
<td>4.3</td>
</tr>
</tbody>
</table>
4.2.4. **Microbial results of commercial batch during process:**

**Concerning the contamination of commercial batch** during process it was found that the total bacterial count, wild yeasts and molds were nil for the periods before charge and after charge (after part of the starter culture was transferred to fermentor C).

The TBC was $\log_{10} 1.3$ CFU/ML, wild yeasts were $\log_{10} 3.17$ CFU/ML and molds were $\log_{10} 2.00$ CFU/ML and at the final fermentation stage the TBC was $\log_{10} 3.0$ CFU/ML, wild yeasts were $\log_{10} 6.14$ CFU/ML and molds were $\log_{10} 5.77$ CFU/ML and at the cream of compressed Baker's yeast after separation the TBC was $\log_{10} 4.0$ CFU/ML, wild yeasts were $\log_{10} 7.25$ CFU/ML and molds were $\log_{10} 5.95$ /ML (Table 9).

The contamination in the commercial batch was higher than that in the starter culture, because the initial commercial batch has used part of the starter culture, furthermore, the commercial batch has applied continuous process system.

In applying this system in Sudanese fermentation industry it was found that many ingredients were not
TREATED, THE FERMENTOR WAS OPENED CONTINUOUSLY TO ADD THE NUTRIENTS.
<table>
<thead>
<tr>
<th>ITEM</th>
<th>CONTAMINATION LOG\textsubscript{10} CFU/ML</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TOTAL BACTERIAL COUNT</td>
</tr>
<tr>
<td>FERMENTOR C (BEFORE CHARGE)</td>
<td>NIL</td>
</tr>
<tr>
<td>FERMENTOR C (AFTER CHARGE)</td>
<td>1.3</td>
</tr>
<tr>
<td>FERMENTOR C (AT THE FINAL FERMENTATION STAGE)</td>
<td>3.00</td>
</tr>
<tr>
<td>CREAM OF BAKER'S YEAST AFTER SEPARATION</td>
<td>4.00</td>
</tr>
</tbody>
</table>
4.2.5. Relation between contamination of compressed Baker's yeast as a final product and time needed for dough leavening:

In the final product the total bacterial count was log$_{10}$ 4.47 CFU/GR, wild yeasts were log$_{10}$ 5.3 CFU/GR and molds were log$_{10}$ 5.0 /GR, the time needed for dough leavening was 75 minutes.

In other experiment the TBC was log$_{10}$ 4.00 CFU/GR, wild yeasts were log$_{10}$ 7.25 CFU/GR and molds were log$_{10}$ 5.95/GR, the time needed was 80 minutes, and in a third experiment the TBC was log$_{10}$ 5.6 CFU/GR, wild yeasts were log$_{10}$ 7.7 CFU/GR and molds were log$_{10}$ 6.14/GR, the time needed was 85 minutes.

Till this point the production was consumable. But in the fourth experiment the TBC was log$_{10}$ 4.5 CFU/GR, wild yeasts were log$_{10}$ 9.07 CFU/GR and molds were log$_{10}$ 8.00/GR, the time needed to leaven a dough was 100 minutes. At this point the product was so highly contaminated, that the activity was nearly lost therefore the product was unconsumable (Table 10).

The contamination of the final product was due to the contamination of the commercial batch product.
FURTHERMORE, INSUFFICIENT CLEANING AND SANITATION OF FILTER PRESS, PACKING MACHINE, FILTER AND BACKING UNITS, AND WORKERS.

ALSO THE CAKE WAS KEPT OPEN IN THE FILTER PRESS AND BAKING UNIT TILL THE BAKING OPERATION WAS FINISHED, THE WORKERS MEANWHILE TOUCH THE CAKE WITH THEIR BARE HANDS WITHOUT USING GLOVES AND MASKS.

TABLE 10. RELATION BETWEEN CONTAMINATION OF COMPRESSED BACKER'S YEAST CREAM AND TIME NEEDED TO LEAVEN DOUGH

<table>
<thead>
<tr>
<th>CONTAMINATION LOG$_{10}$ CFU/GR</th>
<th>TIME NEEDED TO LEAVEN DOUGH (MIN)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TOTAL BACTERIAL COUNT</strong></td>
<td><strong>WILD YEAST</strong></td>
</tr>
<tr>
<td>4.47</td>
<td>5.30</td>
</tr>
<tr>
<td>4.00</td>
<td>7.25</td>
</tr>
<tr>
<td>5.6</td>
<td>7.79</td>
</tr>
<tr>
<td>4.50</td>
<td>9.07</td>
</tr>
<tr>
<td>------</td>
<td>------</td>
</tr>
</tbody>
</table>

CHAPTER FIVE

CONCLUSION

IN THIS STUDY WE CAN CONCLUDE THE FOLLOWING POINTS:

1. **All the raw materials used in the industry are suitable and good for the compressed baker's yeast production except urea which has shown some contamination, need to be treated.**

2. **The hygiene of the plant is not good enough.**

3. **The original culture of the compressed baker's yeast used is free of contamination and of high activity.**

4. **The plant uses two systems of production i.e. batch system for production of the starter culture and continuous system, but during the late there are some contamination.**

5. **Ultimately there are some problems concerning handling and storage of the compressed baker's yeast for this reason active dry yeast is more suitable to Sudan conditions.**
CHAPTER SIX
RECOMMENDATIONS

1. **The importance of hygienic guards for both plant and workers and the use of ultra violet sterilization techniques.**

2. **Raw materials should be sterilized.**

3. **Use of automatic feeding system for nutrients.**

4. **Use of computerizes machine to measure time of stopping fermentation process before reaching stationary phase.**

5. **Use of antibacterial and anti-mold materials during the fermentation process.**

6. **Applying use of cold transport technique from factory to consumers.**

7. **Study of shelf life of compressed baker's yeast according to Sudanese condition.**

8. **It is strongly recommended to manufacture dry yeast instead of compressed yeast to overcome bad weather conditions in the Sudan.**

9. **To increase the yeast production to meet the consumption in the Sudan.**
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APPENDIX
PLATE (2): TAKING SAMPLES FROM FERMENTOR A
PLATE (3): TAKING SAMPLES FROM FERMENTOR B
PLATE (4): TAKING SAMPLES FROM FERMENTOR C
Plate (5): Filter press stage for yeast cream
PLATE (6): PACKING STAGE OF COMPRESSED BAKER'S YEAST
Plate (7): Time taken to leaven dough (proof time)
PLATE (8). CO₂ PRODUCTION DURING DOUGH FERMENTATION
Plate (9): Final Product of Compressed Baker's Yeast
FLOW DIAGRAM FOR HASAHISSA YEAST FACTORY.

MOLASSES TREATED

FERMENTOR A

FERMENTOR B

FERMENTOR C

CHLORINATED WATER

SEPARATION

STARTER

COMMERCIAL

FILTER

COLD STORAGE

PACKING MACHINE

O2

O2

O2

HEAT

PLOWED