PRODUCTION OPTIMIZATION OF HONEY BEER

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CERTIFICATE

This is to certify that the work entitled, "PRODUCTION OPTIMIZATION OF HONEY BEER " submitted by YATIN SHARMA AND TARUN RAGHAV in partial fulfillment for the award of degree of Bachelor of Technology in Biotechnology of Jaypee University of Information Technology has been carried out under my supervision. This work has not been submitted partially or wholly to any other University or Institute for the award of this or any other degree or diploma.

SUPERVISOR:

MRS. RUNNI MUKHERJII

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ABSTRACT

A fermenting strain of *Saccharomyces cerevisiae* was utilized for the production of honey beer. The strain used here is S5. A YPD media is used for the growth of the yeast Saccharomyces Cerevisiae .A YPD media consists of yeast extract ,peptone and dextrose. After yeast is grown then we carry out the sugar estimation of honey i.e to check the amount of sugar present in honey. Thus after mixing honey ,water and various growth factors we keep the solution for few months for the production of honey beer . Once the beer is produced we carry out the optimization steps at different temperatures , pH, sugar concentration etc. To get the beer that is best in taste, color , aroma , and smell as compared to beer that are available in the market . Thus our main aim is to produce a beer that is better in color , aroma , taste and smell as compared to the one's available in the market and also to make a better beer in laboratory that is relatively cheap as compared to the one's made in industries.

CHAPTER 1

INTRODUCTION

Honey is a mixture of sugars and other compounds . With respect to Carbohydrates , honey is mainly fructose (about 38.5%) and glucose (about 31.0%) making it similar to the synthetically produced inverted Sugar syrup which is approximately 48% fructose , 47% glucose , and 5% sucrose thought to function as antioxidants as antioxidants including chrysin chrysinpinobaksin vitamin c catalase and pinocembrin . The specific composition of any batch of honey depends on the flowers available to the bees. Honey's remaining carbohydrates include maltose , sucrose , and other complex carbohydrates . Honey contains trace amounts of several vitamins and minerals.

As with all nutritive sweeteners , honey is mostly sugars and is not a significant source of vitamins or minerals . Honey also contains tiny amounts of several compounds produced the honey .

Typical honey analysis:

Fructose: 38.%

Glucose: 31.3%

Sucrose: 1.3%

Maltose: 7.1%

Water: 17.2%

Higher Sugars: 1.5%

Ash: 0.2%

Other/ undetermined: 3.2%

Honey has a density of about 1.36 kilograms per litre (36% denser than water)

RECENT SEARCHES ABOUT HONEY BEER:

TYPE OF HONEY THAT WORKS BEST IN MICROBREWED BEER:

From a technical standpoint, virtually any type of honey can be used in the brewing process. The United States produces over 300 types of honey, with the colors ranging from water white to dark amber and the tastes from delectably mild to distinctively bold. Each type of honey contributes something different in terms of end – product color, aroma, rounding effect and flavor. In lagers, brewers tend to prefer mild honeys such as clover honey. Other floral sources such as alfalfa, wildflower, buckwheat, sage or citrus are excellent ingredients in porters, stouts and herb or spice beers .From a marketing standpoint, brewers may also consider using honeys which are unique to their regions or that will specially appeal to specific.

WAYS BY WHICH BREWERS USE HONEY IN BREWING PROCESS

Brewers generally add honey to the kettle toward the end of fermentation and avoid exposing honey to high temperatures for an extended period of time. This is done to prevent the loss of honey volatiles which contribute to the flavour of the final products .

HONEY CAN BE CONSIDERED AS A BREWING ADJUNCT

Brewing adjuncts are added , among other reasons , to extend capacity and they contribute little to the quality of the product . For this reason , microbrewers generally avoid brewing adjuncts . Honey does contribute fermentable sugars but it also contributes a flavor and aroma of its own and adds value to beers by increasing their consumer appeal . Honey beer is often lighter and " crisper " than all - malt beer , but it does not lack character . In the United States , there is a consensus among brewers : they consider honey to be a high – value functional ingredient , not just a brewing adjunct .

AFFECT OF HONEY ON FLAVOUR OF MICROBREWED BEERS

Through several mechanisms: first , honey contributes its own flavour , Second , honey has an impact on how the four basic tastes are perceived And third , honey has a " smoothing " or " rounding " effect on the overall flavour profile . Obviously , the extent to which honey affects the flavour of beers depends upon the type of honey selected (floral source) , the amount of honey added and the brewing technique used . Sensory Research conducted for the National Honey Board has shown that honey can decrease the perception of sourness and bitterness . When added to beer , this means that honey tends to reduce the bitterness provided by hops (to which some consumers may object) without masking the desirable flavour components that hops provide . Brewers say that honey gives a nice " roundness " to the beer which is very desirable .

MELONEL AND CYSER ARE MOST COMMONLY USED HONEY BEERS

Melomel is a type of mead which is flavored with fruits other than apple Cyser is a variety of melomel made with honey and apple juice . These products are types of meads , which are fermented beverages made from honey . Meads are sometimes called " honey wines " or " honey beers "(in the case of sparkling mead) , but they are not truly beers that are grain – based .

Table below summarizes the flavor and color profiles of some typical American honeys . Variations may occur within a floral source , depending upon the geographic origin of the honey:

Floral Source	Typical Color	Typical Flavor	Suggested Use in Beers

Clover	**Light**	**Mild**	**Herb beers,spice beers**
Alfalfa	**Light**	**Mild**	**Ales,Lagers**
Sage	**Light**	**Mild**	**Pale Ales**
Tupelo	**Light**	**Distinct,delicate**	**Ales,Lagers**
Orange blossoms	**Light**	**Mild,heavy bodied**	**Spice beers Holiday beers Light beers**
Raspberry	**White to light**	**Delicate**	**Ales Spice,fruit beers**
Blueberry	**Medium to dark**	**Distinct,fruity**	**Spice,fruit beers Stouts**
Wildflower	**Medium to dark**	**Medium to strong**	**Pale ales,speciality beers**
Buckwheat	**Dark**	**Strong**	**Stout,porters**

CHAPTER 2

OBJECTIVE OF THE PROJECT

The main objective of the project is:

1) Production optimization of honey beer by altering the pH,temperature,sugar concentration,so as to get a honey beer improved in taste,aroma,colour and alcohol concentration.

2)To produce a beer at lab scale level.

3)To produce a beer at low cost.

CHAPTER 3

LITERATURE REVIEW

3.1 INTRODUCTION

Honey is a food made of certain insects using nectar from flowers. The variety produced by honey bees is the one most commonly reffered to and is the type of honey collected by beekeepers and consumed by humans.

Honey produced by other bees and insects has distinctly other bees and insects has distinctly different properties . Honey bees form nectar into honey by a process of regurgitation and store it as a food source in wax honeycombs. Beekeeping practices encourage overproduction of honey so that the excess can be taken without endangering the bee colony . Honey gets its sweetness from the monosaccharides fructose and glucose and has approximately the same relative sweetness as that of granulated sugar (74% of the sweetness of sucrose a disaccharide) . It has attractive chemical properties for baking and a distinctive flavour which leads some people to prefer it over sugar and other sweeteners . Most micro – organisms do not grow in honey because of its low water activity of 0.6. However honey sometimes contains dormant endospores of the bacterium *Clostridium botulinum* , which can be dangerous to infants as the endospores can transform into toxin – producing bacteria in the infant's immature intestinal tract , leading to illness and even death. Honey has a long history as a comestible and is used in various foods and beverages as a sweetener and flavouring . It also has a role in religion and symbolism . Flavors of honey vary based on the nectar source , and various types and grades of honey are available . It is also used in various medicinal traditions to treat ailments . The study of pollens and spores in raw honey (melissopalynoloy) can determine floral sources of honey. Because bees carry an electrostatic charge , and can attract other particles , the same techniques of melissopalynology can be used in area environmental studies of radioactive particles , dust , or particulate pollution .

Fig.1: A jar full of honey

3.2 MODERN USES:

As a food and in cooking

The main uses of honey are in cooking, baking , as a spread on , and as an addition to various beverages such as tea and as a sweetener in some commercial beverages. According to international food regulations , " honey stipulates a pure product that does not allow for the addition of any other any other substance ... this includes , but is not limited to , water or other sweeteners , honey barbecue and honey mustard are common and popular sauce flavours .Honey is the main ingredient in the alcoholic beverage Mead , which is also known as "honey wine" or "honey beer" .

Historically , the Ferment for mead was honey's naturally – occurring yeast . Honey is also used as an adjunct in beer .Its glycemic index ranges from 31 to 78 , depending on the variety .

3.3 CLASSIFICATION:

Honey is classified by its floral source , and there are also divisions according to the packaging and processing used . There are also regional honeys . Honey is also graded on its color and optical density by USDA standards , graded on a scale called the Pfund scale , which ranges from 0 for " water white " honey to more than 114 for " dark amber " honey .

Floral source

Generally , honey is classified by the floral source of the nectar from which it was made . Honeys can be from specific types of flower nectars , From indeterminate origin , or can be blended after collection . Most commercially available honey is blended , meaning that it is a mixture of two or more honeys differing in floral source , color , flavour , density or geographic origin .

Polyfloral

Polyfloral honey , also known as wildflower honey , is derived from the nectar of many types of flowers . The taste may vary from year to year , and the aroma and the flavor can be more or less intense , depending on which blooming are prevalent .

Monofloral

Monofloral honey is made primarily from the nectar of one type of flower . Different monofloral honeys have a distinctive flavor and color because of differences between their principal nectar sources . In order to produce monofloral honey beekeepers keep beehives in an area where the bees have access to only one type of flower . In practice , because of the difficulties in containing bees , a small proportion of any honey will be from additional nectar from other flower types . Typical examples of North American monofloral honeys are clover , orange blossom , sage , tupelo , buckwheat , and sourwood . Some typical European examples include thyme , thistle , heather , acacia , dandelion , sunflower , honeysuckle , and varieties from lime and chestnuttrees . In North Africa , such as Egypt , examples include clover , cotton , and citrus , mainly orange blossoms .

Honeydew honey

Instead of taking nectar , bees can take honeydew , the sweet secretions of aphids or other plant sap – sucking insects . Honeydew honey is very dark brown in color , with a rich Fragrance of stewed fruit or fig jam and is not sweet like nectar honeys . Germany's Black Forest is a well known source of honey dew – based honeys , as well as some regions in Bulgaria . In Greece , pine honey (a type of honeydew honey

) constitutes 60 – 65 % of the annual honey production . Honeydew honey is popular in some areas , but in other areas beekeepers have difficulty selling the stronger flavoured product .

The production of honeydew honey has some complications and Dangers . The honey has a much larger proportion of indigestibles than light floral honeys , which can cause can cause dysentery to the bees , resulting in the death of colonies in areas with cold winters . Good beekeeping management requires the removal of honeydew prior to winter in colder areas . Bees collecting this resource also have to be fed protein supplements , as honeydew lacks the protein - rich pollen accompaniment gathered from flowers .

Bastard Honey

Bastard honey is honey whose floral origins are unknown . Compared to monofloral honey , honey from one type of plant , Or polyfloral honey , honey that has been mixed , bastard honey's origins are either unknown , undisclosed , or something else of the sort . The idea of bastard honey comes from the idea of illegitimate honey , whose ancestry is unknown .

Brewer's yeast

Brewer's yeast refers to the yeast used in the making of beer, as well as the deactivated yeast produced as a by-product of beer fermentation. However, it can also be used to make nutritional or taken as a nutritional supplement itself. Brewer's yeast is extracted from hops and grains.[3] Studies have been conducted that raise the possibility of brewer's yeast helping to control cholesterol and blood sugar levels, but no conclusive research has been completed at this point in time.

Brewer's yeast is also sometimes known as baker's yeast, and is a probiotic containing living microbial species. Probiotics help the human body maintain a healthy bacterial balance, promoting the proliferation of friendly flora in the digestive system (yogurt is perhaps the most widely known source of probiotics, as it contains such live cultures such as *lactobacilli*).

Brewer's yeast may be used to treat digestive maladies such as diarrhea, and is often recommended to treat irritable bowel syndrome or reduce the negative effects of lactose intolerance.

Brewer's yeast is often used as a source of B-complex vitamins, chromium, and selenium. The B-complex vitamins in brewer's yeast include B1 (thiamine), B2 (riboflavin), B3 (niacin), B5 (pantothenic acid), B6 (pyridoxine), B9 (folic acid), and H or B7 (biotin). These vitamins help break down carbohydrates, fats, and proteins, which provide the body with energy. They also support the nervous system, help maintain the muscles used for digestion, and keep skin, hair, eyes, mouth, and liver healthy. However, brewer's yeast does not contain vitamin B12, an essential vitamin found in meat and dairy products; vegetarians sometimes take brewer's yeast mistakenly believing that it provides B12, which can be lacking in their diet.

A number of Saccharomyces *species* are used to brew beer, depending on whether it is a top or bottom fermenting beer. Some brewers use a different genus of yeast, especially for specialty beers like Belgian wheat beers. To brew beer, brewer's yeast is added to hops and malted barley and allowed to ferment the hops and barley into alcohol, made bubbly by the carbon dioxide. The beer ferments for several weeks at varying temperatures, depending on the type of beer being made, and then is ready to bottle.

In addition to being used for beer, deactivated brewer's yeast is also used as a nutritional and flavor supplement, and is often found labeled as "nutritional yeast" in grocery stores. It is important to make sure that you purchase an inactive form of yeast for a nutritional supplement, because active yeast will flourish in your intestines, potentially causing health problems. Some consumers sprinkle brewer's yeast on food simply because it tastes good, but many people, including vegetarians, also use brewer's yeast to eat a balanced diet.

Brewer's yeast is extremely nutritionally rich, and contains protein, amino acids, vitamins, and minerals which can keep consumers healthy. Many folk remedies for poor skin include brewer's yeast, and some studies have suggested that consuming brewer's yeast can accelerate the healing time for cuts and similar injuries.

3.4 S.CEREVISIAE

Saccharomyces cerevisiae is a species of budding yeast. It is perhaps the most useful yeast owing to its use since ancient times in baking and brewing. It is believed that it was originally isolated from the skins of grapes (one can see the yeast as a component of the thin white film on the skins of some dark – colored fruits such as plums ; it exists among the waxes of the cuticle. It is one of the most intensively studied eukaryotic model in molecular and cell biology much like Escrechia coli as the model prokaryote. It is the microorganism behind the most common type of fermentation .*S*. cerevisiae cells are round to avoid, 5 – 10 micrometres diameter. It reproduces by a division process known as budding. Many proteins important in human biology were first discovered by studying their homologs in yeast ; these proteins include cell cycle proteins, signalling proteins, and protein - processing enzymes. The petite mutation in *S*. cerevisiae is of particular interest. Antibodies against *S*. cerevisiae are found in 60 – 70 % of patients with Crohn's syndrome and 10 – 15 % of patients with Ulcerative colitis.

" Saccharomyces " derives from Latinized crown and means " sugar mold " or " sugar fungus ", *saccharo* - being the combining form " sugar - " and *myces* being "fungus" *cerevisiae* comes from Latin and means " of beer ".

Other names for the organism are :

- S. cerevisiae short form of the scientific name
- Brewer's yeast though other species are also used in brewing
- Ale yeast
- Top – fermenting yeast
- Baker's yeast
- Budding yeast

This species is also the main source of nutritional yeast and Yeast extract.

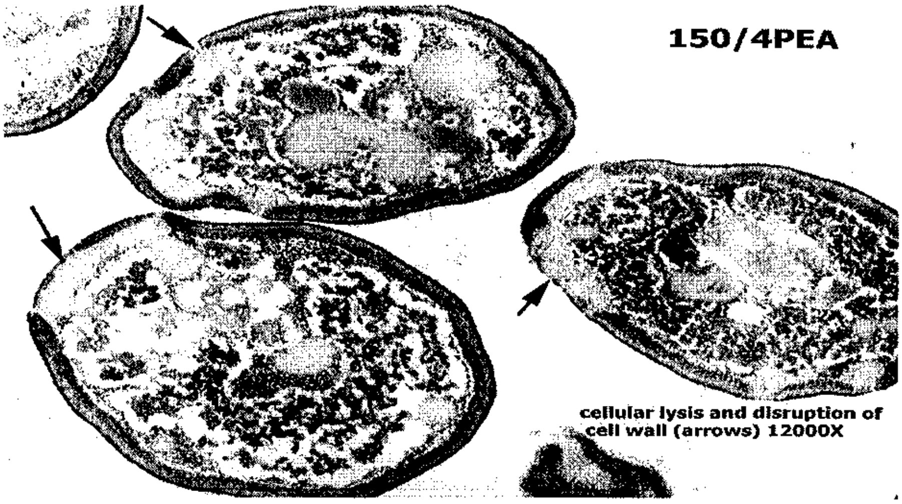

Fig.2: **S.cerevisiae**

Yeast in biological research

A model organism

When researchers look for an organism to use in their studies, they look for several traits. Among these are size, generation time, accessibility, manipulation, genetics, conservation of mechanisms, and potential economic benefit. The yeast species *S. pombe* and *S. cerevisiae* are both well studied; these two s pecies diverged approximately 300 to 600 million years before present, and are significant tools in the study of DNA damage and repair mechanisms. The alpha – factor of *S. cerevisiae*, has been compared to the liphophilic peptide created by the fungus *Tremella mesenterica*. *S. cerevisiae* has developed as a model organism because it Scores favourably on a number of these criteria. As a single celled organism *S. cerevisiae* is small with a short generation time (doubling time 1.5 – 2 hours @ 30 °C) and can be easily cultured. These are all positive characteristics in that they allow for the swift production and maintenance of multiple specimen lines at low cost.

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Genome sequencing

S. cerevisiae was the first eukaryotic genome that was completely sequenced. The genome sequence was released in the public domain on April 24, 1996. Since then, regular updates have been maintained at the *Saccharomyces*. Genome Database (SGD). This database is a highly annotated and cross- Referenced database for yeast researchers. Another important *S. cerevisiae* database is maintained by the Munich Information Center for Protein Sequences (MIPS). The genome is composed of about 12, 156, 677 base pairs and 6,275 genes, Compactly organized on

16 chromosomes . Only about 5,800 of these are believed to be true functional genes . It is estimated that yeast shares about 23% of its genome with that of humans .

Other tools in yeast research

The availability of the *S. Cerevisiae* genome sequence and the complete set of deletion mutants has further enhanced the power of *S. Cerevisiae* as a model for understanding the regulation of eukaryotic cells . A project underway to analyze the genetic interactions of all double deletion mutants through Synthetic genetic array analysis will take this research one step further. Approaches have been developed by yeast scientists which can be applied in many different fields of biological and medicinal Science . These include Yeast two - hybrid for studying protein interactions and tetrad analysis .

3.5 YEAST IN COMMERCIAL APPLICATIONS

Top-fermenting yeast

Saccharomyces cerevisiae is known as top - fermenting yeast , so called because during the fermentation process its hydrophobic surface causes the flocs to adhere to CO_2 and rise to the top of the fermentation vessel. It is one of the major types of yeast used in the brewing of ale , along with *Saccharomyces pastorianus* which is used in the brewing of lager .

Top – fermenting yeasts are fermented at higher temperatures than lager yeasts and the resulting ales have a different flavour than the same beverage fermented with a lager yeast . " Fruity esters " may be formed if the ale yeast undergoes temperatures near 21 °C (70 ° Fahrenheit) , or if the fermentation temperature of the beverage fluctuates during the process . Lager yeast normally ferments at a temperature of approximately

5 °C (40 °Fahrenheit) , where ale yeast becomes dormant . Lager yeast can be fermented at a higher temperature normally used for ale yeast, and this application is often used in a beer style known as " steam beer " .

Uses in aquaria

Owing to the high cost of commercial CO_2 cylinder Systems , CO_2 injection by yeast is one of the most popular DIY approaches followed by aquaculturists for providing CO_2 to underwater aquatic plants . The yeast culture is generally maintained in plastic bottles and typical systems provide one bubble every 3 – 7 seconds . Various approaches have been devised to allow proper absorption of the gas into the water .

3.6 USES OF S.CEREVISIAE:

The useful physiological properties of yeast have led to their Use in the field of biotechnology . Fermentation of sugars by Yeast is the oldest and largest application of this technology .Many types of yeasts are used for making many foods : baker's Yeast in bread production ; brewer's yeast in beer fermentation; Yeast in wine fermentation and for xylitol production . So-Called red rice yeast is actually a mold, *Monascus purpures*. Yeasts include some of the most widely used model Organisms for genetics and cell biology .Alcoholic beverages are defined as beverages that contain ethanol (C_2H_5OH). This ethanol is almost always produced by fermentation – the metabolism of carbohydrates by certain species of yeast under anaerobic or low – oxygen conditions . Beverages such as wine , beer , or distilled spirits all use yeast at some stage of their production .

"Brewer's yeast " (also known as " brewing yeast ") can mean any live yeast used in brewing . It can also mean yeast obtained as a by - product of brewing , dried and killed, and used as a dietary supplement for its B vitamin content. Brewers classify yeasts as top-fermenting and bottom-Fermenting. This distinction was introduced by the Dane Emil Christian Hansen. " Top-fermenting yeasts " are so called because they form a foam at the top of the wort during fermentation. They can produce higher alcohol concentrations and prefer higher temperatures, typically 16 to 24 °C (61 to 75 °F), producing fruitier, sweeter, ale-type beers. An example of a top-fermenting yeast is *Saccharomyces cerevisiae*, known to brewers as ale yeast. " Bottom – fermenting yeasts " are typically used to produce lager-type beers, though can also produce ale – type beers. These yeasts ferment more sugars, leaving a crisper taste, and grow well at low temperatures. An example of bottom fermenting yeast is *Saccharomyces pastorianus*, formerly known as *Saccharomyces carlsbergensis*.

For both types, yeast is fully distributed through the beer while It is fermenting, and both equally flocculate (clump together and precipitate to the bottom of the vessel) when fermentation is finished. By no means do all top-fermenting yeasts demonstrate this behaviour, but it features strongly in many English ale yeasts which may also exhibit chain forming (the failure of budded cells to break from the mother cell) which is technically different from true flocculation. In industrial brewing, to ensure purity of strain, a " clean " sample of the yeast is stored refrigerated in a laboratory. After a certain number of fermentation cycles, a full scale propagation is produced from this laboratory sample. Typically, it is grown up in about three or four stages using sterile brewing wort. The most common top-fermenting brewer's yeast, *Saccharomyces cerevisiae*, is the same species as the common baking yeast.

However, baking and brewing yeasts typically belong to different strains, cultivated to favor different characteristics: baking yeast strains are more aggressive, in order to carbonate dough in the shortest amount of time possible ; brewing yeast strains act slower , but tend to produce fewer off – flavours and tolerate higher alcohol concentrations (with some strains , up to 22%) .

Fermentation (wine):

Yeast is used in wine making where it converts the sugars present in grape juice or must into alcohol . Yeast is normally already invisibly present on the grapes . The fermentation can be done with this endogenous wild yeast , however , this may give unpredictable results depending on the exact types of yeast species present . For this reason a pure yeast culture is generally added to the must , which rapidly comes to dominate the fermentation . This represses wild yeasts and ensures a reliable and predictable fermentation . Most added wine yeasts are strains of *Saccharomyces cerevisiae* , though not all strains of the species are suitable . Different *S . cerevisiae* yeast strains have differing physiological and fermentative properties , therefore the actual strain of yeast selected can have a direct impact on the finished wine . Significant research has been undertaken into the development of novel wine yeast strains that produce a typical flavour profiles or increased complexity in wines .The growth of some yeasts such as *Zygosaccharomyces* and *Brettanomyces* in wine can result in wine faults and subsequent spoilage. *Brettanomyces* produces an array of metabolites when growing in wine , some of which are volatile phenolic compounds . Together these compounds are often referred to as *" Brettanomyces character "* , and are often described as *antiseptic* or *" barnyard "* type aromas . Brettanomyces is a significant contributor to wine faults within the wine industry .

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Baker's yeast:

Yeast, most commonly *Saccharomyces cerevisiae*, is used in baking as a leavening agent, where it converts the fermentable sugars present in dough into the gas carbon dioxide. This causes the dough to expand or rise as gas forms pockets or bubbles. When the dough is baked the yeast dies and the air pockets "set", giving the baked product a soft and spongy texture. The use of potatoes, water from potato boiling, eggs, or sugar in a bread dough accelerates the growth of yeasts. Salt, hot water and fats such as butter slow yeast growth. Most yeasts used in baking are of the same species common in alcoholic fermentation. Additionally, *Saccharomyces exiguous* (also known as *S. Minor*) is a wild yeast found on plants, fruits, and grains that is occasionally used for baking. Sugar and vinegar are the best conditions for yeast to ferment. In bread making the yeast initially respires aerobically, producing carbon dioxide and water. When the oxygen is depleted anaerobic respiration begins, producing ethanol as a waste product; however, this evaporates during baking.

It is not known when yeast was first used to bake bread. The first records that show this use came from Ancient Egypt. Researchers speculate that a mixture of flour meal and water was left longer than usual on a warm day and the yeasts that occur in natural contaminants of the flour caused it to ferment before baking. The resulting bread would have been lighter and tastier than the normal flat, hard cake. Active dried yeast, a granulated form in which yeast is commercially sold. Today there are several retailers of baker's yeast; one of the best – known in North America is Fleischmann's Yeast, which was developed in 1868. During World War II Fleischmann's developed a Granulated active dry yeast, which did not require refrigeration and had a longer shelf life than fresh yeast. The company created yeast that would rise twice as fast, reducing baking time. Baker's yeast is also sold as a fresh yeast compressed into a square " cake ". This form perishes quickly, and must therefore be used soon after production. A weak solution of water and sugar can be used to determine if yeast is expired. In the solution, active yeast will foam and bubble as it ferments the sugar into ethanol and carbon dioxide. Some recipes refer to this as proofing the yeast as it " proves " [tests] the viability of the yeast before the other ingredients are added. When using a sour dough starter, flour and water are added instead of sugar this is referred to as proofing the sponge. When yeast is used for making bread, it is mixed with flour, Salt, and warm water or milk. The dough is kneaded until it is smooth, and then left to rise, sometimes until it has doubled in size. Some bread doughs are knocked back after one rising and left to rise again. A longer rising time gives a better flavour, but the yeast can fail to raise the bread in the final stages if it is left for too long initially. The dough is then shaped into loaves, left to rise until it is the correct size, and then baked.

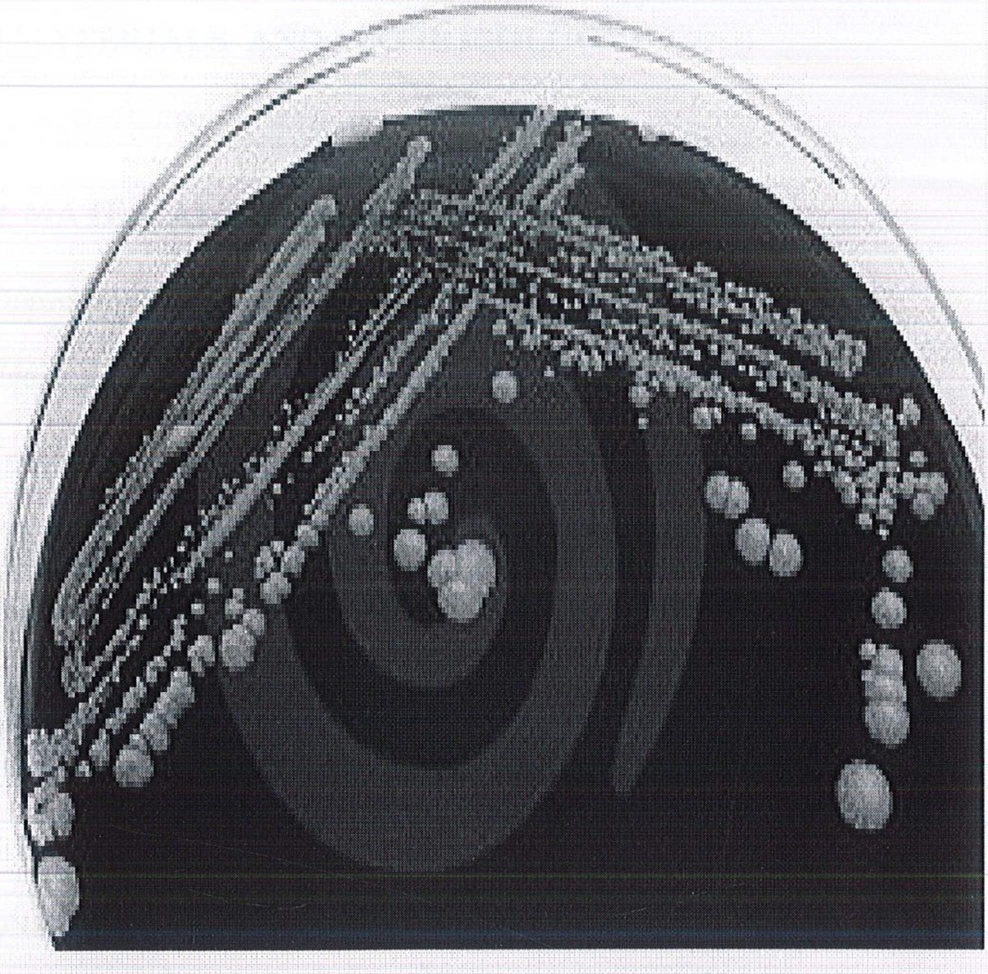


Fig 3:Baker's yeast

4.0 MATERIALS AND METHODOLOGY:

4.1 MATERIALS REQUIRED:

1) Brewer's yeast

2)Honey

3)Cotton disk

4)YPD media

5)Various chemicals

6)Laminar air hood

7)Shakers

8)Sulfite

9)DNS Reagent

10)Temperature probes

11)pH probes

4.2 PROCEDURE:

1) Media required for the growth of yeast

Media required for the growth of yeast is known as YPD media.

2) Components of YPD media

i) Yeast extract- 1g/100ml water

ii)Peptone -2g/100ml water

iii)Dextrose -2g/100ml water

3) Preparation of YPD media

1)Take 500 ml of water in a measuring flask and into it add:

i)5g of yeast extract

ii)10g of peptone

iii)10g of dextrose

2)Then take 100 ml of the solution into five different flasks

3)Then autoclave these five flasks at temperature 120 degree celcius and pressure of 15 psi for 1 hour.

4)Then inoculate the yeast strain S5 into each of the five flasks in laminar air hood

5)After inoculation keep these flasks in shaker for 36 hours so that the growth of yeast takes place in these flasks.

4) Sugar Estimation of Honey

Sugar estimation of honey is necessary so that we can come to know the sugar content that is present in our honey. We carry out the sugar estimation using DNS reagent.

Components of DNS reagent include:

Sodium potassium tartarate -30 g

+

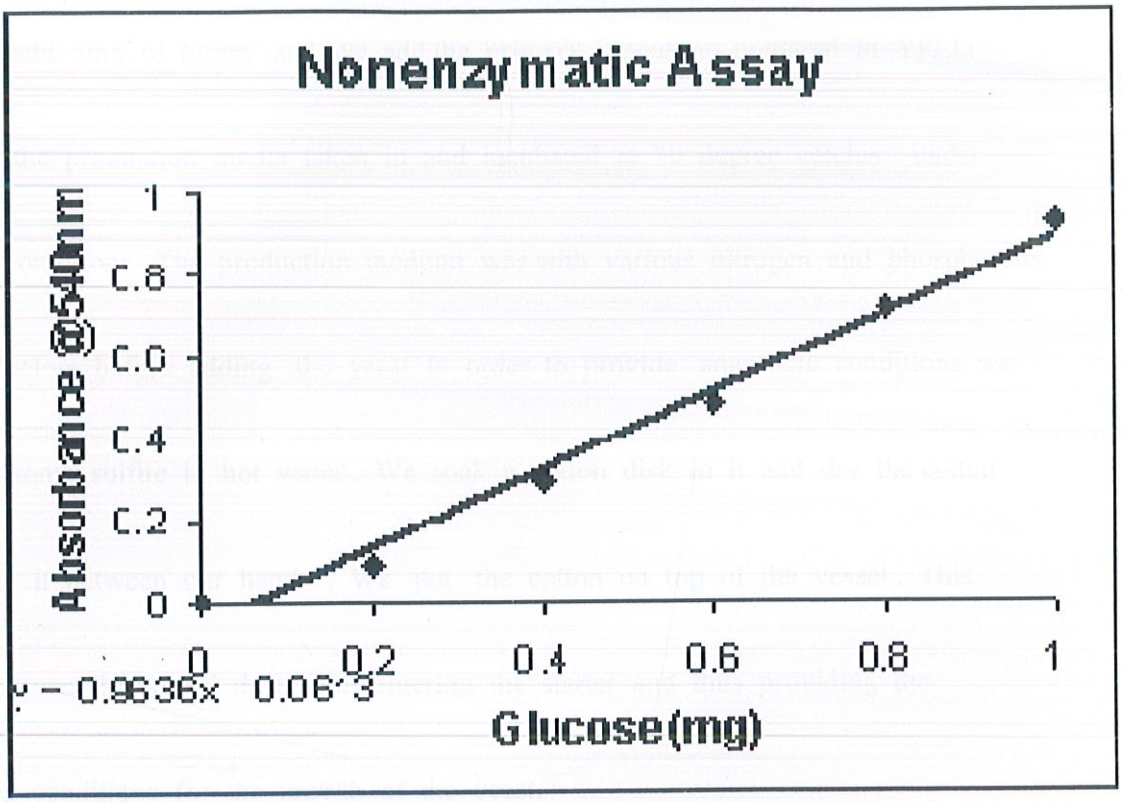
Sodium hydroxide -3.96g

+

Dinitro saliylic acid(DNS) -1.06g

In 100 ml of distilled water

STANDARD PLOT FOR SUGAR ESTIMATION:



Honey is a source of simple carbohydrates. Its composition on average, is 17.1 percent water, 82.4 percent total carbohydrate and 0.5 percent proteins, amino acids, vitamins and minerals. The average carbohydrate content is mainly fructose (38.5 percent) and glucose (31 percent). The remaining 12.9 percent of carbohydrates is made up of maltose, sucrose and other sugars.

5) Production of honey beer

For the production of honey beer we boil 1000 ml of water for 10- 15 minutes , then we add 5mg of honey and we add the primary inoculums prepared in YPED broth to the production media taken in and incubated at 30 degree celcius under shaking conditions . The production medium was with various nitrogen and phosphorous growth factors before adding the yeast .In order to provide anaerobic conditions we dissolve some sulfite in hot water . We soak a cotton disk in it and dry the cotton squeezing it between our hands . We put the cotton on top of the vessel . This should prevent bugs and dust from entering the starter and thus providing the anaerobic conditions for the growth of the yeast.

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Media composition:

1)KH_2PO_4 0.1%

2)$(NH_4)_2SO_4$ 0.5%

3)$MgSO_4.7H_2O$ 0.05%

4)Yeast extract 0.1%

6) Measure of alcohol content present in honey beer

Once honey beer is produced we need to measure the alcohol content of the beer. We calculate alcohol content by method of specific gravity. The Specific Gravity of water at 60 degrees Fahrenheit is 1.000. Everything else is either more or less dense than water. Here we use a simple little tool called a Hydrometer to measure the Specific Gravity of a solution. It is essentially just a glass cylinder with a weight on the bottom and lines showing the different gravity points going up the side. We need to keep the beer at around room temperature in order to get a reasonably accurate reading. When brewers talk about Specific Gravity they will usually specify either the Original Gravity (OG), or Terminal Gravity (TG). The OG is a measurement of the beer before it Ferments, and the TG (also called Final Gravity – FG) is the measurement that is taken after fermentation is complete. The difference between the two tells us how much alcohol is in the brew So we took the OG and TG of our beer and found out that the OG was around 1.045 and TG was around 1.008.

Calculating the percentage of alcohol is as simple as plugging some numbers into the following equation.

% Alcohol = ((1.05 x (OG – TG)) / TG) / 0.79

So,

OG = 1.045

TG = 1.008

The equation would look like this:

$.0487 = ((1.05 \times (1.045 - 1.008))/1.008) / 0.79$

So, our beer had about 4.9% alcohol.

4.3 OPTIMIZATION OF HONEY BEER

Fermentation process carried out by yeast is known to vary with respect to temperature , pH and sugar concentration . It is therefore imperative to optimize the fermentation conditions for yeast cells so that the production efficiency increases.

EFFECT OF TEMPERATURE ON THE OPTIMIZATION OF HONEY BEER

To optimize the fermentation temperature, fermentation was carried out at 15, 20, 25, 30, and 35ºC. The samples were analyzed for alcohol content.

EFFECT OF pH ON THE OPTIMIZATION OF HONEY BEER

pH of 5.0, 6.0, 7.0 and 8.0 were tested for fermentation of produced honey beer with respect to various temperatures and sugar concentration.

EFFECT OF SUGAR CONCENTRATION ON THE OPTIMIZATION OF HONEY BEER

A sugar concentration of 35% is considered as standard for carrying out the optimization of honey beer with respect to different temperatures and pH.

CHAPTER 5

RESULTS AND DISCUSSIONS:

5.1 EFFECT OF TEMPERATURE ON THE OPTIMIZATION OF HONEY BEER

Temperature is one of the major constraints that determines the alcohol production. To know the optimum temperature for alcohol fermentation, the solutions were kept at 20, 25, 30 and 35°C with 35% sugar concentration. The samples were withdrawn after every 8 hours and the fermentation was carried out for 32 hours. A low ethanol yield of 6.8% was observed at 20°C in 32 hours. As shown in **Table** at 30°C alcohol yield was maximum and turned out to be 11%. However increasing the temperature beyond 30°C the growth as well as concentration of alcohol decreased. This decrease was pronounced at 35° C so 30° C was selected as optimum temperature for alcohol production.

TABLE 1: EFFECT OF TEMPERATURE ON THE OPTIMIZATION OF HONEY BEER

Time (hrs)	Growth(in O.D)				Alcohol(in %)			
	20^0C	25^0C	30^0C	35^0C	20^0C	25^0C	30^0C	35^0C
0	0.23	0.24	0.25	0.23	0	0	0	0
8	0.48	0.56	0.74	0.48	1.2	3	4.3	1.3
16	0.98	1.23	1.45	0.75	3	5.2	7.8	2.3
24	1.32	1.56	1.89	0.98	5.2	6.8	9.2	2.7
32	1.86	1.90	2.30	1.20	6.8	8.2	11.6	3

5.2 EFFECT OF pH ON THE OPTIMIZATION OF HONEYBEER

Initial sugar concentration of 20% and optimum temperature of 30^0c was selected for further studies and subjected to pH treatments 5 , 6 , 7 and 9 . The results are shown in table below. At pH 5 , fermentation took place but it gave low alcohol content . Best results were obtained at pH 6 where maximum ethanol production was noticed . An increase in alcohol concentration and productivity was observed with an increase in pH from 4.0 - 5.0 and found that the optimum pH range for *S.cerevisiae* strain S5 to be between pH 4.5 - 5.0 . Based on increase in concentration upon the optimization of honeybeer the pH 6 was selected as the best pH .

TABLE 2: EFFECT OF pH ON THE OPTIMIZATION OF HONEYBEER

Time (hrs)	Growth				Alcohol			
	pH5	pH6	pH7	pH9	pH5	pH6	pH7	pH9
0	0.23	0.23	0.26	0.29	0	0	0	0
8	0.46	0.89	0.79	0.89	2	3.4	2.6	3.4
16	0.98	1.56	1.36	1.56	3.6	7.8	4.9	6.4
24	1.32	2.1	1.98	2.1	4.8	9.2	7.6	8.1
32	1.56	2.56	2.12	2.56	7.9	11.6	8.6	9.7

CHAPTER 6

CONCLUSION:

The fermentation of honeybeer using *S.cerevisiae* (S5 strain) under optimized conditions i.e . pH 6 , sugar concentration 20% and temperature 30°C along with some growth nutrients revealed an increase in alcohol production with good fermentation efficiency.

CHAPTER 7

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