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1. Introduction

Quite when humans first harvest the seeds of grasses, learned to grind them into flour, mix the flour with water and bake the resulting dough to give bread are events understandably lost in antiquity. So also the discovery that, by allowing the dough to stand and become infected with gas-and acid-producing microorganisms probably a mixture of yeasts and lactic acid bacteria, the baked bread had a much more attractive texture than flat unleavened bread. Tradition has it that this discovery was made in Egypt several thousand years ago. Evidence comes from the exquisitely preserved model of a bakery found in a 4000-year-old tomb discovered at Thebes, the ancient capital of Upper Egypt. Moreover, the Book of Exodus, in its vivid account of the aspirations of the Jews On their flight from Egypt, makes special note of the fact that they had to subsist on unleavened bread, having fled without yeast barm.

To retain and perpetuate the dough fermentation, one surmises that a portion of each leavened but unbaked dough was retained so that it could be mixed with the next dough to be prepared. How long this practice continued is not, nor will ever be, known It would appear, however, that it was later replaced by the use of yeast skimmed from brewery and wine fermentations. No doubt, the physical similarity between dough being leavened and cereal extracts and grape juice being fermented to give beer and wine prompted this modest advance in baking technology. The Romans seem to have been particularly adept at improving this technique in bread manufacture Witness that Pliny, in his Naturalis Historiae states that "the bread is there lighter than elsewhere". Presumably this was because the top skimming or barm from beer fermentation was already being used to raise dough. However, brewer's yeast had many disadvantages when used to leaven doughs. Its fermentative activity in dough was often low and variable, added to which it was often sour by the time it became available for use. Moreover, as a semi-liquid product, it was inconvenient to handle and transport. Nevertheless, it would seem that spent brewer's yeast or barm was used to ferment bread doughs for many centuries. It is not absolutely certain when yeast was first propagated specifically to be used in bread-making. Kiby (1912),in his handbook on pressed-yeast manufacture, states that the year 1781 was probably the first occasion on which baker's yeast was propagated as a separate entity, and that this took place in Holland with the result that the propagation came to be known as the Dutch process. Its efficiency was, however, low and according to Jorgensen (1948), gave a yield of only 4-6% by weight of pressed yeast based on the weight of grain raw material employed; approximately 25% (w/w) of ethanol was produced at the same time. The next major advance came around 1860 with the introduction of the Vienna processor Wienerverfahren. In this process, the yield of yeast biomass grown anaerobically in a grain mash was considerably improved by passage of a gentle stream of air through the mash. Based on the amount of grain supplied, the pressed yeast biomass yield was around 14% although heat of ethanol was still in the region of 30%.

Around this time, Pasteur (1858) made the important discovery that the amount of yeast biomass obtained from a fixed quantity of sugar was very much greater if the culture was vigorously aerated. However, applying this discovery in baker's yeast propagation was a relatively slow process, probably because of the lack of availability of efficient aeration devices. The first person to make use of the discovery, at least as far as can be gleaned from published accounts, was the Dane Eusebius Brunnin 1881.In the following years, the discovery was further exploited by several German chemists. The last major technological advance in baker's yeast propagation came shortly after the end of the First World War. During that war, because of a shortage of grain, beet and cane molasses were used as substitutes in media supplemented with phosphate and ammonia. Just after the end of the war, the classical batch process, in which all nutrients were included in the starting medium, was replaced by the fed-batch processor Zulaufverfahren. This involves adding the carbohydrate supply (molasses) incrementally during the period of yeast growth. It was patented almost simultaneously by Soren Sak in Denmark (Sak, 1919) and F.F.Hayduck in Germany (Hayduck,1919). Anyone interested in the patent controversy which this discovery engendered should refer to the pamphlet by Wagner (1936). Adoption of the Zulau F process (often known simply as the Z-process) increased the yield of moist pressed-yeast biomass considerably, initially to around 75% (w/w) based on the weight of molasses used and subsequently, as refinements were introduced, up to virtually 100%. The commercial processes used to day in the manufacture of baker's yeast are essentially based on the Zulau F process. However, two further innovations have been examined. Continuous production of yeast biomass using the chemist at principle (Pirt,1975), which one would believe is ideally suited top reduction of baker's yeast, has been tried but abandoned (Olsen,1960,1961;Sher,1961,1969).The degree of contamination, particularly with wild yeasts, cannot be controlled because the doubling time of wild yeasts, such as Candidakrusei, is considerably shorter than that of Saccharomyces cerevisiae under commercial conditions (Burrows,1970). The second innovation is the production of active-dried yeast (ADY), which is described later in this chapter.

Production of baker's yeast is now a very large and stable industry worldwide. All developed countries in the world have one or more plants producing this commodity. Production and use of baker's yeast have regularly come under the reviewer's scrutiny in recent years. The biochemistry and microbiology of baker's yeast, along with processes used in its manufacture, have been overviewed by, among others Burrows (1970, 1979), Trivedi *et al* (1986), Rosen (1987), Beudeker *et al* (1989) and Evans (1990). Oura *et al* (1982) concentrated more on the use of yeast in baking breads.

2. Role of yeast in bread-making

It is generally recognized that yeast has three major and one minor role in the making of bread. The first major role is to increase the volume of the dough by evolving carbon dioxide gas as a result of the alcoholic fermentation of sugars in the dough. The second

major function is to bring about a change in structure and texture in the dough as a result of stretching caused by formation of carbon dioxide bubbles. Thirdly, the presence of yeast in the dough during bread-making has a significant effect on the flavour of the bread. Finally, it is sometimes stated that yeast makes a contribution to the nutritional status of bread. Although yeast contains nutritionally valuable proteins and other compounds, their contribution to the nutrient value of bread must be marginal, simply because of the low proportions of yeast included in bread doughs. Each of the three major roles is discussed in the following subsections.

2.1. Carbon dioxide evolution in doughs

Flours contain sugars that can be fermented by strains of Sacch.cerevisiae, through the Embden-Meyerhof-Parnas glycolytic pathway to produce ethanol and carbon dioxide. The immediately available sugars are glucose, fructose, sucrose, maltose and levosins (better known as gluco fructans), the least of these being a mixture of polysaccharides containing residues of glucose and fructose and which might beat least partially hydrolysed by invertase present in yeast. Dry flours contain about 2% (w/w) of these utilizable sugars, glucose fructans accounting for the bulk of this value. Additional maltose enters the dough as soon as the flouris wetted. This arises from the action of α- and ß-amylases on starch contained in damaged starch granules in the flour. This additional maltose accounts for about 2.8% of the weight of the flour, and makes a major contribution to the carbon dioxide-producing capacity of the yeast. The immediately available sugars would support a typical dough fermentation for only a few minutes. Some flours after milling do not contain sufficient α- amylase. Under these conditions, flours at the mill or doughs at the bakery may be supplemented with additional enzymes, often in the form of commercially available fungal enzymes or barley malt. Particularly in North America and in the Far East, doughs may be heavily supplemented with sucrose or glucose to a concentration which can reach up to 25-30% of the weight of the dough flour. High-fructose corn syrups are not often used in North America for this purpose. This additional sugar makes a contribution to the substrate for evolution of carbon dioxide in the dough, and also to the taste of the bread. When doughs are not supplemented with sugars, they are frequently referred to as “lean”, particularly in North America. The temperature at which the dough is processed clearly has an effect on carbon dioxide evolution, so that a careful control over dough temperatures is essential. Traditionally, doughs were processed at around 25°C, thereby leading to long fermentation times since this is well below the optimum temperature for carbon dioxide production by Sacch. Cerevisiae. Modern dough-processing techniques use higher temperatures,in the region of 35°C. During the early stages of the baking process, some fermentation by yeast continues, although strains of Sacch.cerevisiae are rapidly killed at temperatures much above 50°C.

2.2. Changes in dough structure and texture

Explanations for the role of yeast in changes that take place in the structure and texture of dough during processing have been many and. During this processing, the dough becomes more elastic and better equipped to retain the carbon dioxide evolved in the form of small bubbles. It is now thought that yeast contributes to the rheological changes by causing the protein (gluten) molecules to be stretched as a result of carbon dioxide evolution. The glutathione and cysteine, which may under certain circumstances be excreted by yeast, could affect protein- disulphide bonds in gluten. However, this contribution by yeast is probably minimal in modern-day bread making.

2.3. Contribution to bread flavor

An important contribution to bread flavour is made by compounds derived from yeast. The flavour components produced by the yeast in bread making include organic acids, aldehydes, ethanol, higher alcohols, esters and ketones. Some of these, alcohols for example, escape during baking. Others react with each other and with other compounds found in the dough to form new and more complex flavor compounds. These reactions occur primarily in the crust and the resultant flavor diffuses into the crumb of the baked bread.

3. Raw materials

3.1. Baker yeast

- ✓ ***Scientific name***: Saccharomyces cerevisiae
- ✓ ***Common name***: Brewer's yeast/ Baker's yeast
- ✓ ***Habitat***: Saccharomyces when translated means "sugar fungus". That is what this yeast uses for food. They are found in the wild growing on the skins of grapes and other fruits

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

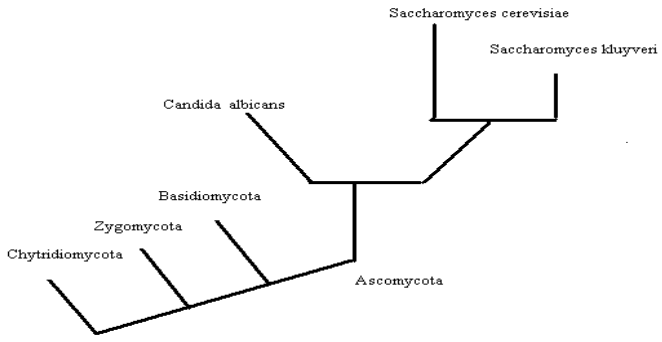


Figure 1.1. Classification of yeast

✓ ***Adaptations***: Saccharomyces cerevisiae has adapted in several important ways. One is the fact that they are able break down their food through both aerobic respiration and anaerobic fermentation. They can survive in an oxygen deficient environment for a period. Another adaptation they have is their ability to have both sexual and asexual reproduction. Very few other Ascomycota can do both processes. And very few organisms can do all four of these processes. This allows this species to live in many different environments

✓ ***Life Cycle***: Saccharomyces cerevisiae has both asexual and sexual eproduction. In asexual reproduction the haploid of the yeast under goes mitosis and forms more haploid yeasts. There is an a and ά strain of these haploids. Then these haploid yeasts, one from each strain, can fuse together and become on cell. Then the nuclei of both cell fuses together and this cell is now the zygote. These diploid cells can go through mitosis, which they call budding, and four more zygotes or they can under go meiosis and from

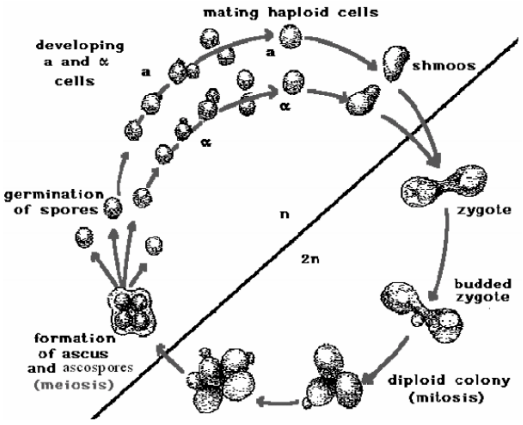


Figure 1.2. life cycle of yeast

an ascus which will split into four ascospores. These haploids can then under go germination and become haploid yeast again.

Virtually all yeasts used to leaven bread doughs are strains of Sacch. cerevisiae. No serious attempt has been made to consider replacing strains of this yeast with other yeast species in bread-making. Therefore, it is worth pondering awhile on the reasons for the supremacy of Sacch. cerevisiae as a baker's yeast. Bread is a staple item in the human diet, and it follows that any commodity used in bread-making must be toxicologically acceptable.

Saccharomyces cerevisiae has proved its complete acceptability as a dietary item since, in the form of beers and wines, it has been consumed albeit in small amounts for several thousands of years with, we have every reason to believe, absolutely no adverse effects. Moreover, this yeast has proved time and time again that it is a very sturdy organism. There is no better testimony to its toughness than the realization that it has survived, apparently for thousands of years, for making beers by being transferred, as a skimming or barm, from one fermentation to the next, initially in extremely hot Middle-East climates that were hardly conducive to retention of viability in any micro-organism.

The capacity of Sacch. cerevisiae to survive starvation conditions can be expressed quantitatively in the form of the adenylate charge. This value is a measure of the extent to which cellular AMP is charged with additional high-energy phosphate bonds in the form of ADP and ATP. On a scale of 0.0-1.0, vigorously growing micro-organisms have an adenylate charge of 0.7-0.8. When starved of nutrients, almost all micro-organisms rapidly die as their adenylate charge drops to a value of 0.4 or thereabouts. Not so Sacch. cerevisiae, the adenylate charge of which has been observed to decline to a value of 0.15 while the yeast cells remain.

There are many other examples which could be quoted to testify the rugged nature of Sacch. cerevisiae. We give just one, and that is acid washing. For many years, brewers, knowing that their pitching yeast has become contaminated with bacteria to a level that they consider unaccept-able, have resorted to washing it with acid. The procedure involves washing pitching yeast with 10% (v/v) phosphoric or sulphuric acid, a procedure which kills bacteria and, we venture to suggest, most other micro-organisms, but leaves Sacch. cerevisiae viable. If the supremacy of Sacch. cerevisiae as an ideal baker's yeast can be justified in terms already described, the situation is nicely compounded by know-ledge that the biochemistry and genetics of this yeast are well understood in modern-day terms, a situation which places the baker's yeast technologist in a position from which the use of this yeast in bread-making can enetically and phenotypically be optimized.

If strains of Sacch. cerevisiae are ideally suited to leavening bread doughs, the demands which are placed on this yeast are by no means uniform. The doughs which are used world-wide, as well as the types of bread demanded, are multifarious. So too are the additives which are included in doughs as well as the temperatures at which they are made and baked. This means that a variety of baker's yeasts, each with properties uniquely suited to a particular baking regime, is required. There is, as Evans (1990) aptly noted, no Holy Grail when it comes to strains of baker's yeast. This section describes the various properties which are looked for in strains used to make breads in various parts of the world.

- ✓ ***Morphology and size***

Yeast cells have been intensively investigated because of their practical importance. Microscopic investigations revealed the morphological characteristics of the cell, and later the fine structure of cell was studied by electron microscopy. Biochemists are now able to relate my metabolic functions to the ultrastructure of the cell. The shape of yeast cells varies from spherical to ovoid, lemon-shaped, pear-shape, cylindrical or even elongated. Parts of the structure which can be seen are the cell walls, cytoplasm, vacuoles of water or fat and granules. Electron micrographs show the membrane structures, the nucleus and structure of organelles.

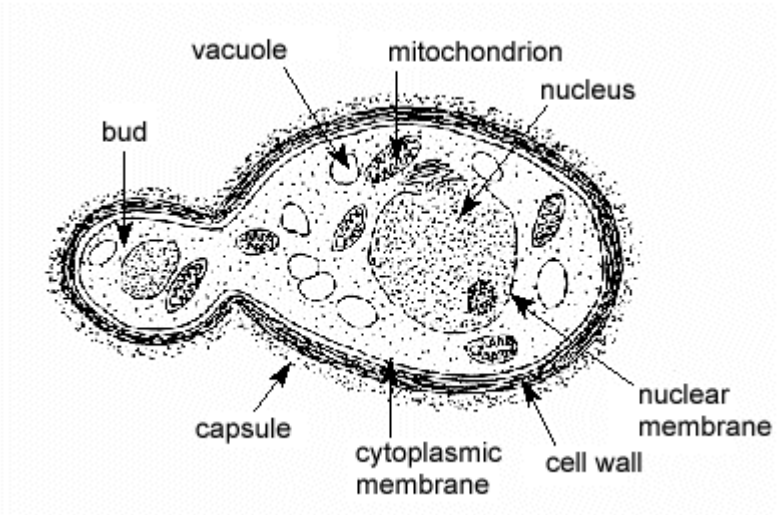


figure 1.3. structure of Sacch. cerevisiae

The size of baker yeast about 5-14 micrometers.

- ✓ ***Physiology***

Yeast differ considerably in their physiology.

The plenty supply of available moisture is one of the most important conditions of growth of yeasts. In comparison to the other two groups of micro-organisms important in food microbiology, it may be started that many yeasts grow better in presence of greater concentrations of solutes, like sugars and salts, than do most bacteria. This means that yeasts require less moisture than bacteria, but most yeasts need more moisture than moulds. The requirements are often expressed in values of water activity (a_w). Lower limits of water activity for ordinary yeasts tested thus far range from 0.88 to 0.94. It should also be kept in mind that the water activity values, mentioned above, may vary with the nutritive properties of the substrate, pH, temperature, availability of oxygen, and presence of inhibitory substances. The optimum range of temperature for growth of most yeasts is around 25 to 30ºC and the optimal range of activity is pH 4 to 5.5.

Although yeasts are classified by some specialists as plants, they lack chlorophyll and are unable to manufacture by photosynthesis, from inorganic substrates, the organic compounds required for energy supply and growth, so they need organic carbon sources. In general, sugars are the best nutrients for yeasts. All fermentative yeasts are able to ferment glucose to produce ethanol and carbon dioxide. This fermentation process is use in practice for producing beer, wine, industrial alcohol, and carbon dioxide produced by baker's yeasts accomplishes the leavening of bread. Some yeasts, e.g. film yeasts, can metabolize other organic carbon sources such as organic acids.

Yeasts can utilize both simple inorganic and organic compounds as a nitrogen source, but also amino acids, peptides, and polypeptides. In addition to sugars, the yeasts need several minor, biologically important compounds commonly known as growth factors.

The requirement of yeasts for an exogeneous source of vitamins varies widely. Some yeasts can synthesize all of their required vitamins, whereas other yeasts have multiple requirements. In some case the requirement is not absolute, the yeasts can growth without supplementation of medium with a given vitamin, but its growth is low. Biotin is the most commonly required vitamin to be supplemented in the medium whereas riboflavin and folic acid apparently synthesized in sufficient quantities by all yeasts. Addition of high levels of vitamins can enrich yeasts by taking advantage of their ability to concentrate vitamins, particularly vitamins of the B-group, from the medium into the yeast cell.

Eventual supplementation of medium with minerals depends on the type of medium. Generally phosphorus and sulfur is added.

3.2. Factors Affecting Yeast Growth

3.2.1. Sugar feed (media composition):

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

In respiration, pyruvate is decarboxylated inthe mitochondrion to acetyl- CoA which is completely oxidized in the citric acid cycle to CO2, energy and intermediates to promote yeast growth.

In anaerobic conditions, glucose is slowly utilized to produce the energy required just to keep the yeast cells alive. This process is called fermentation, in which the sugars are not completely oxidized yielding CO2and ethanol as final product.

When the yeast cell is exposed to high glucose concentration, catabolite repression occurs, during which gene expression and synthesis of respiratory enzymes are repressed, and fermentation prevails over respiration.

In industrial practice, catabolite repression (repression of gluconeogenesis, the glyoxylate cycle, respiration and the uptake of less preferred carbohydrates) by glucose and sucrose, also known as Crabtree effect, may lead to several problems, such as incomplete fermentation, development of off flavors and undesirable by products as well as decreased biomass and yeast vitality. Industrial production of Saccharomyces cerevisiaeis therefore, performed in aerobic, sugar limited fed-batch cultures .

3.2.2. Aeration

Highly aerobic culture conditions are usedin the production of yeast specifically baker’s yeast to maximize cell growth. The modern technique of baker’s yeast production is based on applying the principle of the Pasteur reaction at the limit value

of its aeration. Pasteur defined fermentation as life without air. Its biochemistry involvesthe breakdown of carbohydrates only to the stage of ethanol.

Under aerobic conditions, however, maximum growth occurs and the efficiency of utilization of carbohydrate increases asrespiration and the breakdown of the carbohydrate to carbon dioxide and water becomes complete.

Generally oxygen has the following basic functions.

- Inhibits fermentation
- Increases respiration
- Agitation of the medium
- Removal of toxic end products
- Stimulation of vegetative growth

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

3.2.3. Temperature

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

3.2.4. pH

Yeasts grow well at acidic pH (acidophilic organisms). For industrial propagation low pH is helpful in restricting the development of many bacterial contaminations; however, the color of the yeast may be affected at low pH. The pH of the media is commonly adjusted by the addition of H_2SO_4, NH3, Na_2CO_3 or $NaHCO_3$ to the substrate.

3.3. Medium

- ✓ Molasses

Molasses is a by product of the sugar industry. It is residue after the crystallization of the main fraction. When no more sugar can be crystallized out of solution, the resulting

liquid (molasses), containing about 50% sucrose is eliminated. For every 100 Kg of plant, some 3.5 to 4.5 Kg of molasses may be obtained.

Based on its origin, it can be called cane molasses or beet molasses and is the cheapest source of carbohydrate. It contains 45-55% fermentable sugars including sucrose, glucose, fructose, raffinose, melibiose and galactose. The fact that molasses may be extracted from at least two sources of plant adapted to tropical and temperate climates permits the obtainment of molasses in a wide range of geographical locations. The use of molasses for the production of yeast biomass has simplified the manufacturing process in many ways. Its cost is reduced as compared with the use of grain and other raw materials.

The molasses is used as a source of carbon, energy and other essential nutrients. Molasses could not supply all the essential nutrients for yeast growth. Therefore, the addition of supplements such as (NH4)2SO4, urea, yeast extract or Peptone as nitrogen source, KH2PO4, H3PO4 as phosphorus source, other macro elements such as calcium in form of calcium salts, magnesium in the form of magnesium salts, microelements such as iron, zinc, copper, manganese are necessary for maximizing biomass yield of Saccharomyces cerevisiae or any other types of yeasts. Vitamins are also required for yeast growth (biotin, inositol, panthotenic acid and thiamine).

The composition molasses may vary quite widely depending on the location, soil type, the climatic conditions and the production process of each individual sugar factory

Table1. Percentage composition of cane and beet molasses

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

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

✓ Sunphat amon $(NH_4)_2SO_4$

A source of nitrogen for yeast cells, containing at least 21% nitrogen, moisture does not exceed 1.5%. Because ammonium sulphate is usually produced from sulfuric acid may be mixed FeS and $Fe_2(SO4)_3$ is toxic to yeast cells. Can handle with ammonium sulphate by letting in air to dry, 4 hours prior to fermentation system it must be dissolved into solution.

✓ Ammonia: used to adjust the pH and protein source for yeasts, contains 25% nitrogen

✓ Phosphoric acid: phosphorus sources, concentrations not less than 70%, with DAP can replace

✓ DAP: contains more than 50.5% P2O5

✓ MgSO4: types of engineering, freely soluble in water and MgO <16.3%, arcenic no more than 0.0003%.

✓ Chemical resistant infection, calcium chloride, formalin, NaOH: from 0.5-1% to prevent contamination of equipment and piping

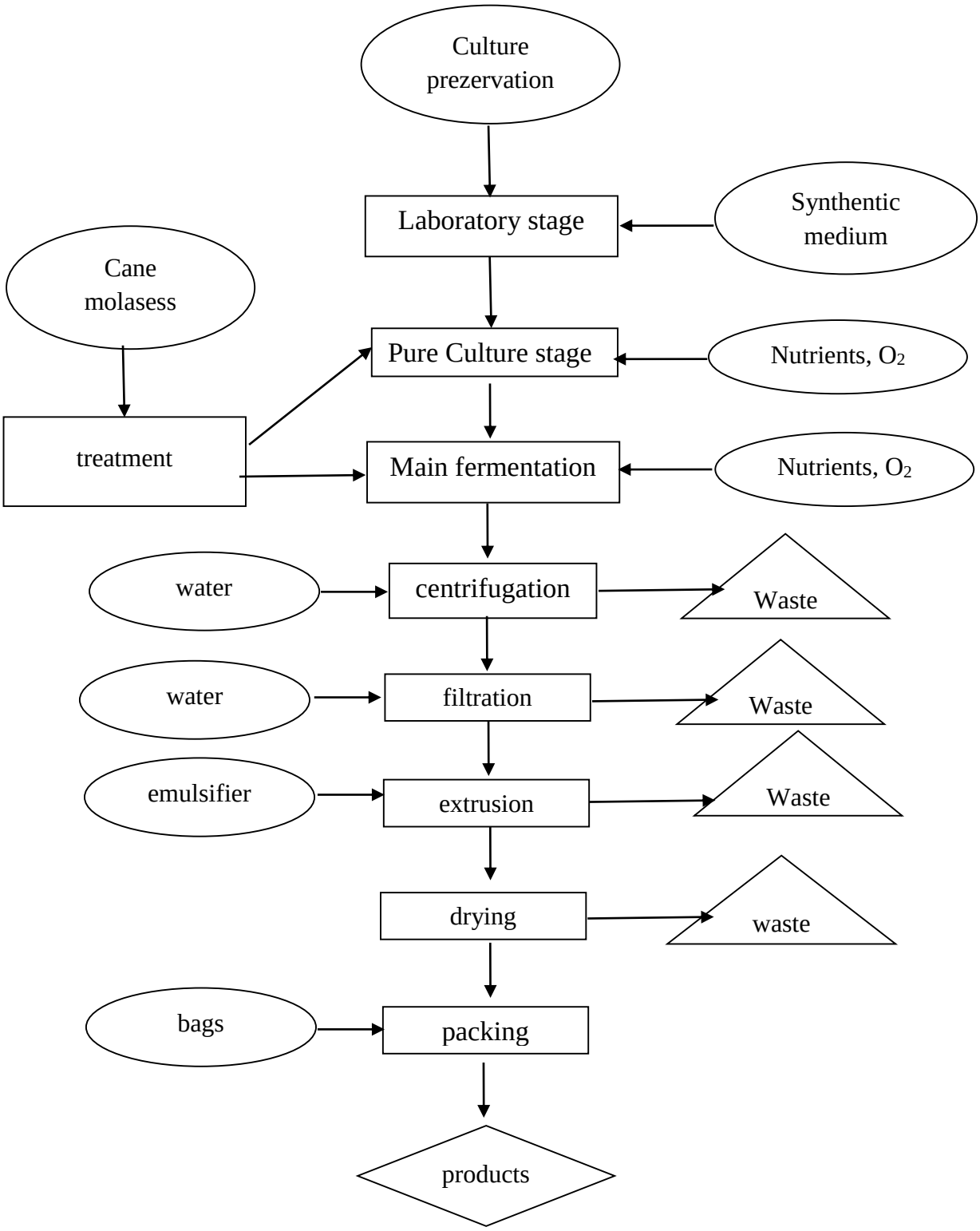
✓ Water: hardness 4-8º and turn from 8-12º (hard 1º corresponding to 10 mg CaO/ l), clean water, clear, colorless, odorless, content the salt is not more than mg/l: Cl^- = 0.5, $SO4^{2-}$ = 80, As = 0.05, Pb = 0.1, 5 = Zn, Cu = 3, FeO = 3, the concentration of microorganisms less than 20 E.coli/l.

✓ Antifoam: have the effect of reducing the surface tension of solution, the pressure of carbon dioxide (CO2) broke foam out. Can be used oleic acid or peanut oil, rice bran oil, castor oil, a concentration of 0.005 -0.01% over the need to break the foam solution.

✓ Air: volume air used in manufacturing establishments yeast may contain a number of microorganisms significantly (up to several thousand/$1m^3$). This air must be filtered before use to produce.

4. Manufacture of dried Baker's yeast

4.1. Dried Baker’s yeast production process



Skema propagation for a batch:

Lab step: 10mL ⟶ 100mL ⟶ 1L ⟶ 10L

Industrial step: 100mL ⟶ $1m^3$ ⟶ $10m^3$ ⟶ $80m^3$ ⟶ $800m^3$

4.1.1. Medium preparation.

Cane molasses is the primary raw material for baker yeast production. It supplies all the sugar that yeast needs for growth and energy along with part of the needed nitrogen. Before it is fed to the yeast, concentrated molasses is diluted with water, clarified, and heat sterilized, diluted cane molasses: 3% w/w carbohydrate

Other constituents: ammonium sulphate 0.1-0.3%, urea 0.1-0.15%, DAP 0.2%, magnesium sulphate 0.05%, vitamin.

Water: before use, removal of suspended solids, colloids and microorganism is usually required, hard water is 4-8°, Cl^-<0.5, SO_4^{2-}<80, As<0.05, Zn<5, Cu<3, FeO<3.

Air: removal of dust, solid particles, microorganism.

Antifoam: acid oleic 0,01% .

4.1.2. Inoculum prepagation

❖ Laboratory stages.

The first fermentation stage takes place in the laboratory when a portion of the pure yeast culture is mixed with the molasses malt in a sterilized Erlenmeyer or Pasteur flask.

- *Objective*: yeast propagation
- *Transformations:*

- Physical: temperature increase during fermentation.
- Biological: metabolism of yeast cells, the yeast increase in number.
- Biochemical and chemical: the reactions in the yeast cells (hydrolysis, the Crebs, electron transport chain).
- Physical-chemical: pH increase, viscosity, turbidity increase. the solubility of O_2, CO_2 in the culture.

- *Technological factors:*

- Medium: contain 12 g KH_2PO_4, 120 g $(NH_4)_2SO_4$, 6 g $MgSO_4.7H_2O$, 12 g yeast extract were dissolved in 10 L distilled water,
- pH=5
- Temperature: 30°C
- Volume of O_2: $1.5m^3$ O_2 per $1m^3$ broth in one hour.
- Fermentation time: the yeast is allowed to grow in the flask for 3 days.

❖ Pure Culture Stage

- *Objective:* yeast propagation
- *Transformations:* similar to laboratory stage
- *Technological factors:*

- The capacities of the fermentation vessels used in this stage range 100 L.
- The pure culture fermentations are batch fermentations where the yeast is allowed to grow for 15 hours.
- pH= 5
- Temperature: 30°C
- Volume of O_2: $9m^3$ O_2 per $1m^3$ broth in one hour.
- Speed of stirring vanes are 30-35 rpm

- *Equipment :*

Fermenters have jacket to remove the heat produced from the production process and to cool the fermenter. In the horizontal, perforated pipe system, air is blown through a large number of horizontal pipes that are placed near the bottom of the fermenter.

The culture was agitated by stirring vanes.

Working principle is batch fermentation.

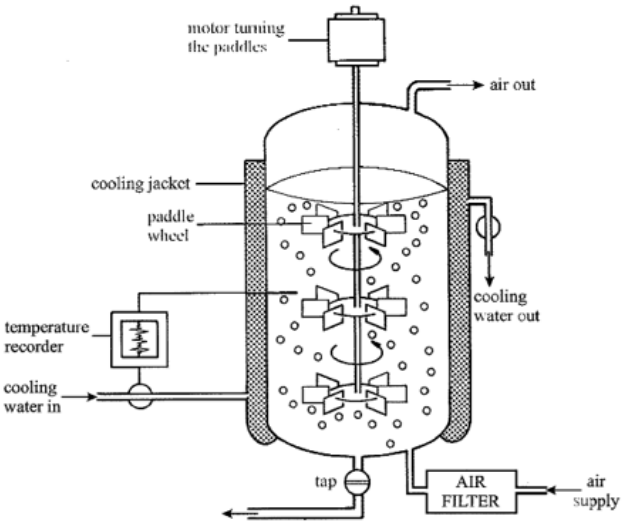


Figure 3.1. Batch fermenter

4.1.3. Main Fermentation Stages

- *Objective:* yeast propagation
- *Transformations:*

- Physical: temperature increase during fermentation.

- Biological: metabolism of yeast cells, the yeast increase in number, metabolites activities were decreased
- Biochemical and chemical: the reactions in the yeast cells (hydrolysis, the Crebs, electron transport chain), yeast synthesize trehalose.
- Physical-chemical: pH, viscosity, turbidity increase. the solubility of O_2, CO_2 in the culture.

Technological factors:

- Seed fermenter I: The capacities of the fermentation vessels used in this stage range 10000 L
- Seed fermenters II: there are 2 equipment, each of them have the capacities of the fermentation vessels used in this stage range 40,000 L
- Main fermenters: 2 equipments, each of them have the capacities of the fermentation vessels used in this stage range 400,000 L
- Time fermentation: about 15 hours for each stage.
- pH= 5
- Temperature: 30^oC
- Volume of O_2: 90m^3 O_2 per 1m^3 broth in one hour.
- Speed of stirring vanes are 30-35 rpm
- Don't provide the carbohydrate about 2.5 hours at the end of main fermentation stages so that yeast can synthesize trehalose.
- Yeast broth from the fermenter at about 5 percent solids.

Equipment:

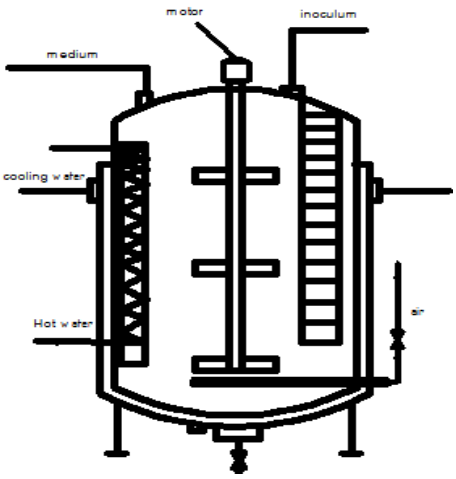


Figure 3.2. Fed-batch fermenter

Working principle: fed-batch fermentation.

The fermenters are usually constructed of stainless steel and are equipped with an incremental feed system. This incremental feed system may be a pipe that distribute the molasses over the entire surface of the fermenter liquid. The rate at which the molasses is fed is critical and may be controlled by a speed controller connected to a pump or by

a valve on a rotameter, which delivers a certain volume of molasses at regulated time intervals.

Fermenters have jacket to remove the heat produced from the production process and to cool the fermenter. In the horizontal, perforated pipe system, air is blown through a large number of horizontal pipes that are placed near the bottom of the fermenter.

The culture was agitated by stirring vanes.

4.1.4. Centrifugation

- *Objective:* dewatering and eliminate some other compounds

Isolate baker yeast from fermentation broth, concentration density of yeast.

- *Transformations:*

- Physical: volume reduction.
- Biological: nothing
- Biochemical and chemical: nothing
- Physical-chemical: viscosity, turbidity increase, increase dry matter contents.

- *Technological factors:*

- Using 2 machines, production yield 500-700kg/h per one, both machines are connected in parallel.
- The centrifuge bowl speed operated at 10,000 rpm.
- Add water to wash the broth (temperature of water is 20^{o}C)
- Yeast cream with a solids concentration of approximately 22%.

- *Equipment:*

Working principle: continuity

The feed generally enters at the top as well the clarified effluent. The solids that have accumulated against the wall are removed by a nozzle discharge system whereby the solids leave, with some of the fluid, in a concentrated solids phase. The nozzles should be angled tangentially backward to take advantage of the kinetic energy and reduce power consumption.

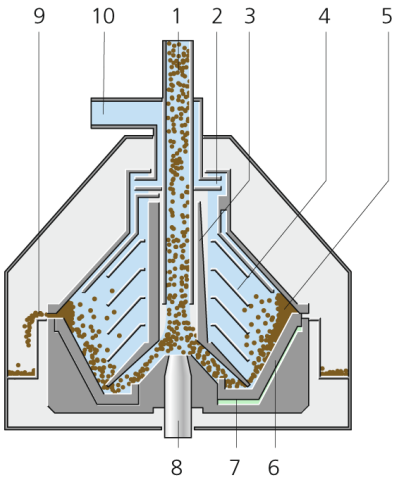


Figure 3.3. Yeast centrifuge

1. Product feed, 2. Centripetal pump, 3. Distributor, 4. Disk stack, 5. Solids holding space,6. Sliding piston, 7. Closing chamber, 8. Spindle, drive, 9. Solids ejection port, 10.Discharge, clarified phase.

4.1.5. Filtration

- *Objective:* dewatering and concentrate yeast scream
- *Transformations:*

- Physical: volume decrease because loss water and some others compounds.
- Biological: nothing
- Biochemical and chemical: nothing
- Physical-chemical: viscosity increase.

- *Technological factors:*

- Rotate 15 to 22 revolutions rpm.
- Concentration of dry matter is 35 percent after filtration.

- *Equipment:*

Using rotary vacuum filters

Working principle: continuous feed of yeast cream

Generally, the filter drum is coated with yeast by rotating the drum in a trough of yeast cream or by spraying the yeast cream directly onto the drum. The filter surface is coated with potato starch containing some added salt to aid in drying the yeast product. While the drum rotates, blades at the bottom of the drum remove the yeast. After a filter cake of yeast is formed and while the drum continues to rotate, excess salt is removed by spraying a small amount of water onto the filter cake.

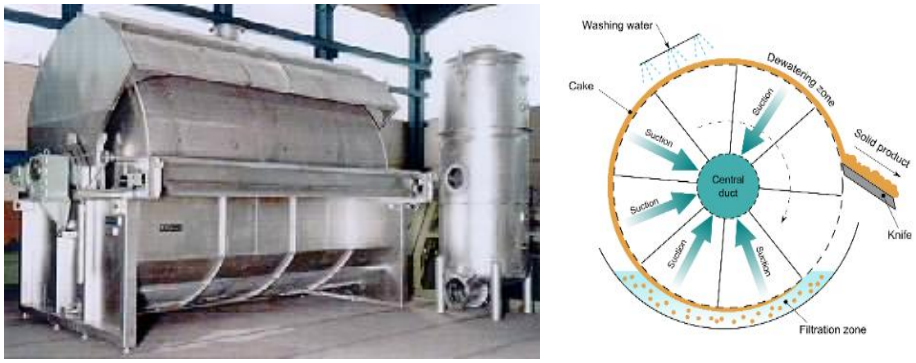


Figure 3.5. Rotary vacuum filter

4.1.6. Extrusion

- *Objective:* prepare for the drying stage.

The process of production of dried yeast involves mixing yeast with a number of additives, extruding the mixture to form very thin noodles, and then drying the noodles.

- *Transformations:*

- Physical: volume decrease because loss water and some others compounds, temperature increase.
- Biological: nothing
- Biochemical and chemical: nothing
- Physical-chemical: viscosity decrease.

- *Technological factors:*

- Rotate 30 rpm.
- Diameter of noodles are 3 mm.
- Concentration of dry matter is 38 percent after extrusion.

- *Equipment:*

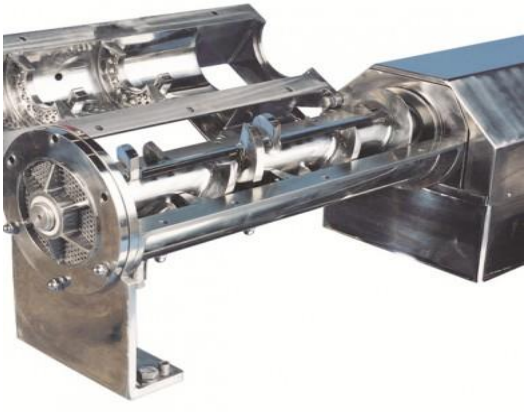


Figure 3.6. Yeast extruder

Working principle:

Material is fed into the housing at one end and forced forward by the rotating hammer until it comes in contact with the stationary anvil. The action of the anvil prevents the material from rotating with the shaft. Thus, the effect of the unit is to give a continuous kneading and mixing action on materials. The special designed shaft assembly is giving positive displacement to the product which is conveyed in direction of the outlet. A perforated orifice plate of various shapes and sizes can be used to extrude material into various sizes and shapes.

4.1.7. Drying

- *Objective:* dewatering and extend product shelf life.
- *Transformations:*

- Physical: volume decrease because loss water and some others compounds, increased dry matter content, temperature.
- Biological: metabolic activities of yeast decrease.
- Biochemical: metabolic activities of yeast's enzymes decrease.
- Physical-chemical: Evaporation of water and some others compounds.
- Chemical: nothing.

- *Technological factors:*

- Air temperature: first is 37.9ºC and then is 30ºC
- Humidity: 2 gH2O/kg air.
- Air quality: filtered F9.
- Concentration of dry matter is 95 percent after drying.
- Time is about 50 minutes per batch (include: charge, discharge and transport to silos).
- Particle size: 0.4 - 1.5 mm.

- *Equipment:*

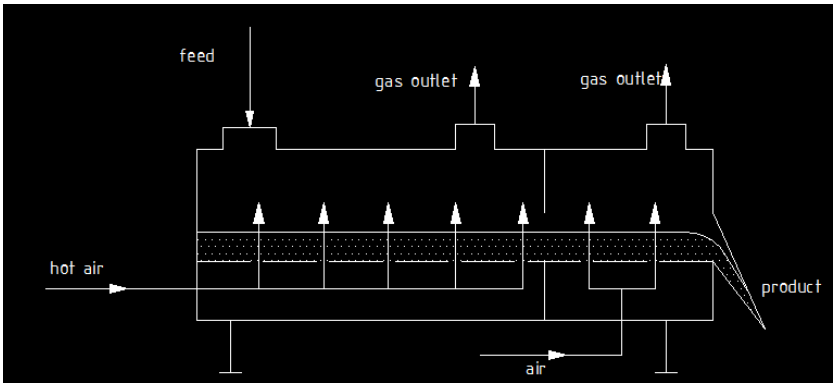


Figure 3.7. Fluidized bed dryer

Working principle:

- Drying phase (main drying step)

The humid wet yeast gets pre-dried at relatively high air temperatures (37.9ºC) with a high air flow. The “spaghetti” break into particles of approx. 0.4 to 1.5 mm length.

- Drying phase (final drying step)

The pre-dried yeast particles are further dried by a moderate temperature (30°C) with adsorption dehumidifier process air and are subsequently cooled.

4.1.8. Packing

- *Objective:* improvement the product
- *Transformations*: no significant change except for possible infection by microorganisms.
- *Technological factors:*
- Three bags size: 600g/bag, 5kg/bag, 20kg/bag
- *Equipment:*

Figure 3.8. Automatic vertical packaging machine

Working principle: The machine operate continuously and automatically.

4.2. Contaminations

The quality of commercial yeast depends not only on viability and fermentation activity, but also on the absence of gross contamination with other microorganisms. Commercial yeast production is not a completely aseptic process, often resulting in yeast product containing lactic acid bacteria (LAB), wild yeasts and moulds.

4.2.1. Wild yeasts and moulds

Yeasts and molds can be found in a wide variety of environments due to their capacity to utilize a variety of substrates and their tolerance of low pH values, low a_w values, and low temperatures. The term “wild yeast” refers to any other yeast that occurs in fermentation and processing of yeast product other than the culture strain used at the start of fermentation. Wild yeasts may enter the commercial yeast factory with the air, the water, and other raw materials, or they may be carried in by insects. The conditions under which S.cerevisiaeis propagated are also favourable for the growth of wild yeasts, which even at low levels can adversely affect the gassing power, stability and shelf-life of commercially manufactured yeast. Baker's yeast production is concerned with spoilage-causing wild yeast contamination, particularly with species such as Candida, Torulopsis, Geotrichum and Hansenula.

Air-borne contamination, especially with mould spores, becomes an increasing problem after the liquid yeast is passed through the separators. Many moulds (e.g. Penicillium and Fusarium) will grow readily on the surface of the yeast cake if not stored at low temperatures. However, moulds grow slowly in comparison to wild yeasts and bacteria, and therefore usually do not present problems in commercial yeast production, unless the product is stored for excessively long periods of time or at elevated temperatures.

4.2.2. Bacteria

Bacterial counts obtained from finished compressed yeast product generally fall between 10^4 and 10^8 bacteria per gram, which predominantly belong to the hetero fermentative lactic-acid producing genus Leuconostocor to the homo fermentative bacteria of the genus Lactobacillus. Other bacterial contaminants isolated from compressed yeast that belong to the LAB group include Pediococcus, Lactococcus and Enterococcus. In general, LAB can grow over a wide temperature and pH range , surviving both the processing and storage of commercial yeast. The types of LAB found as contaminants in the compressed yeast industry are most active up to ca. 50°C.

LAB may have negative effects on both the production and quality of commercial yeast by producing slime or gum which adversely affects the gassing power of the yeast. However, contamination of yeast with LAB may reduce the pH of the yeast product, making conditions less favorable for the growth of Enterobacteriaceae and potential foodborne pathogens. The metabolic activity of LAB during bread making has also contributes positively to leavening and flavour of bread.

Enterococci also belong to the group of LAB and are facultatively anaerobic, catalase negative, Gram-positive cocci arranged in pairs or short chains. Enterococcus spp. are resilient organisms and are characterized by their ability to survive and multiply in the presence of 6.5% NaCl, 40% bile salt, at 10°C or 45°C, and at pH 9.6 (Morrison et al., 1997; Bascomb and Manafi, 1998). Although the majority of Enterococcus spp. inhabit

the intestines of humans and other animals, some have been found free-living in soil, water,on plants or in dairy products. In food microbiology Ent. faecalis and Ent. faecium are the most commonly encountered. In general, Ent. faecalisis regarded as indicator of contamination from human sources, whereas Ent. faeciumand other species indicate contamination from non-human sources. Enterococcus spp. are among the most thermotolerant of non-sporulating bacteria, which results in their contamination of many fermented food products. Due to their ability to survive under adverse environmental conditions, they serve as a good index of hygiene and processing conditions. There is a need to determine the source of enterococci in commercially manufactured yeast as they may indicate the presence of other spoilage or pathogenic contaminants.

Enterococcus spp. are also relatively resistant to chilling, and can be expected to survive the storage of commercial yeast products at low temperatures. In addition to their spoilage potential of food and beverage products, Ent.faecalis and Ent.faeciumhave been suspected as the causative agents of food borne illnesses, and have been classified as potential emerging food pathogens, receiving increased attention due to their potential role in serious human infections, such as endocarditis and bacteremia. Another concern and a contributing factor to the pathogenesis of enterococci is their emerging intrinsic resistance to a wide variety of antibiotics including penicillins, sulphonamides, carbapenems and other β-lactams; resulting in community-acquired and in hospital-acquired (nosocomial) infections.

Indicator microorganisms, such as coliforms, have been found to be suitable indicators of hygiene in fermented products such as sweetened yoghurt (pH 4 – 5), as long as the counts are determined in the first days after production. Similar to yoghurt, commercial yeast has a pH of 4 – 5 and contains high numbers of LAB, thus coliform organisms and Escherichia (E.) coli which have occasionally been found in commercial yeast can also be used as indicators of hygiene.

Coliforms are defined as facultatively anaerobic, Gram-negative, rod-shaped bacteria. Some coliforms are found in the intestinal tract of man and are indicators of fecal contamination, but most are found throughout the environment and have little sanitary significance. The coliform group includes species of the genera Escherichia, Klebsiella, Enterobacter and Citrobacter. Their presence in ready to eat foods is highly undesirable as they may indicate poor manufacturing practices and low hygiene standards (Jay, 1998). Screening food products for coliforms is not intended to detect fecal pollution but rather to measure the quality ofthe practices used to ensure proper processing and to minimize bacterial contamination on processed products.

E. coliis an oxidase negative, glucose fermenting bacterium belonging tothe coliform group. E.coliis commonly associated with soil, water, plants and the gastrointestinal tracts of humans and animals, and is used as anindicator of food safety. The presence of E. coliin processed products may indicate the presence of enteric pathogens such as

Salmonella and Shigella. However, some have argued that the role of E. colias an indicator organism should be subjugated to its role as a pathogen. E. coliis easily destroyed by heat (greater than 45°C) and is usually inactivated during freezing and cold-storage. Temperature is the main measure used to retard the growth of E. coliin Baker's yeast products by cold storage inactivation at 1 – 5°C.

Several studies have provided evidence that Baker's yeast can contain Bacillus spores and thus could serve as a vehicle for entry of Bacillus spores into baking environments. Bacillus strains are the causative agents of rope-spoilage of bread which is characterized by a sticky crumb and an odour like rotting pineapples. Spores of Bacillus spp. are heat resistant and survive the baking process 200 - 300°C, and after cooling of the bread, the spores germinate and grow as vegetative cells producing enzymes, which destroy the structure of bread. Rope-spoilage caused by Bacillus species, occurs mostly in wheat breads containing high concentrations of sugar, fat and/ or fruits. Although the addition of preservatives, such as calcium propionate in bread can be effective against mould as well as Bacillus species that cause rope, the presence of rope causing spores in Baker's yeast as an ingredient in commercial bread production is undesirable and should be minimized.

5. Standard of dried yeast's product

5.1. Metabolites activities

- The leavening ability of baker's yeast on dough ≤ 45 minutes.
- The stability of activity ≥ 72 hours.
- Zymase ≤ 45 minutes.
- Maltase ≤ 70 minutes.

5.2. Microbiological characteristics

- ***Coliforms*:** The content is below 1000 CFU/g following the NF ISO / 4832 *standard or an internal protocol compatible with this standard.*
- ***E.coli*:** The content is less than 10 CFU/g following the SDP 07/1 - 07/93 standard or an internal compatible protocol.
- ***Salmonella*:** Absence of Salmonella in a sample of 25g, following the NF ISO / FDIS 6579 standard or an internal protocol compatible with this standard.
- ***Listeria monocytogenes*:** The content is less than 100 CFU/g following the NF V08-55 standard or an internal protocol compatible with this standard.
- ***Staphyloccus aureus*:** The content is below 10 CFU/g following the NF ISO / 6888 standard or an internal protocol compatible with this standard.

5.3. Physico-chemical characteristics

- ***Fats / dry matter (%):*** The typical fat content on dry matter is 6% +/-2%, and is determined with an extraction method with appropriate solvents. In the case of instant dry yeast a food grade emulsifier (e.g. E491, E 472c,…) is used to protect the yeast during the drying process in order to maintain a good activity.
- ***Carbohydrates / dry matter (%):***The typical carbohydrate content on dry matter is 20% +/- 9%. Carbohydrates in the sense of regulation 1169/2011 means any carbohydrate which is metabolised by humans, and includes polyols.
- ***Fibre / dry matter (%):*** the typical fibre content on dry matter is 28% +/-5%.
- ***Proteins / dry matter (%):***The typical Kjeldahl protein content on dry matter is: 46% +/- 10%, as determined with the Kjeldahl method.
- See also the remarks made on the nitrogen content of yeast.
- ***Minerals / dry matter (%):*** The amount of minerals in yeast is highly dependent on the raw materials used in the preparation of the yeast. The variability in the levels of minerals in molasses thus explains the variability of minerals in yeast. Minerals are normally measured using Atomic Absorption Spectrometry (AAS).

Component	Typical content
Potassium	0.6% - 2.5%
Sodium	< 0.5%
Calcium	0.02% - 0.15%
Magnesium	0.03% - 0.25%
Iron	0.001% - 0.1%

- ***Vitamins / dry matter (%):*** Vitamins are determined by third party laboratories according to standard methods, often biological assays. Typical values for yeast are indicated in the table below.

Vitamin	Typical content	Units
B1 Thiamin	2 – 15	mg/100 g
B2 Riboflavin	2 – 8	mg/100 g
B6 Pyridoxin	0,5 – 6	mg/100 g
B8 Biotin	0.05 – 0.25	mg/100 g
B9 Folic acid	B9 Folic acid	mg/100 g
PP Nicotinic acid	10 – 60	mg/100 g

In the case of Instant Dry Yeast, ascorbic acid is sometimes added as an anti-oxidising agent for dough conditioning at a level of 0.1 – 0.5 %.

5.4. Others

- Heavy metals:
  - Lead : < 0.2 ppm
  - Arsenic : < 3 ppm
  - Cadmium : < 0.1 ppm
  - Mercury : < 0.1 ppm
- Micronutrients:
  - Copper : < 20 ppm
  - Zinc : < 200 ppm
  - Selenium : < 1 ppm

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