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5. Industrial Application of Fungi in Ethanol, Beverage and Food Productions

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5.1 Introduction:

Beverages and fermentation technology is one of the most popular and well established area for food technology since long, when the tombs, stone and wooden sculptures and other artifacts of the ancient Egyptian civilization carry numerous depictions of life of almost 6000 years ago. Among these depictions are scenes of bakeries, breweries, vineyards and wine presses. This is perhaps some of the first documentary evidence of the use of fungi in large-scale food and beverage production. Current discovery of the ice man in the foothills of the Alps with cereal grain and mushrooms in his pouch suggest that bread making and mushroom use may go back 10,000 years. Yet the discovery of the organism responsible for fermentation use in bread and wine making was not until 1680 when Antoine van Leeuwenhoek first discovered yeast cells under his microscope. It was more than 200 years later before Louis Pasteur concluded that through anaerobic respiration (fermentation) sugar was converted into carbon dioxide and alcohol by such tiny yeast, while yeast is the central to the baking and brewing industries. Many fungi are used in the production of beverages and a wide array of exotic foods (Table 5.1). It was examined that first the use of fungi in brewing and baking, then in the production of cheeses, other exotic fermented foods and beverages.

Table 5.1: Fungi used in Food & Beverage Production

Product Type	Fungal species	Raw material	Process	Commercial product
Industrial product	*Saccharomyces cereveciae*		Synthesis of invertase	Cordial cherries

Product Type	Fungal species	Raw material	Process	Commercial product
	Aspergillus niger	Starch	Aerobic metabolism	Citric acid
	Aspergillus sp	Starch	Synthesis of Amylase	Bread making and textile fibre
	Penicillum notatum	Corn starch solution	Aerobic metabolism	Penicillin
	Fusarium moniliforme	Corn starch solution	Aerobic synthesis	Gibberellins
	Penicillium roqueforti	Milk curd	Aerobic metabolism	Camembert and Limburger Cheese
Alcoholic Beverages	*Saccharomyces sp*	Germinated grain (malt)	Natural fermentation	Beer
		Fruit juice	Natural fermentation	Wine
		Rice	Natural fermentation	Sake
		Grain mash	Fermentation and distillation	Whiskey
		Fruit juice	Fermentation and distillation	Brandy
		Molasses	Fermentation and distillation	Rum
		Potatoes	Fermentation and distillation	Vodka
		Agave	Fermentation	Tequila
Asian Foods	*Aspergillus oryzae, A. soyae, Saccharomyces rouxii, Candida etchellsii*	Soybean	Fermentation	Miso
	Aspergillus soyae, A. oryzae, Saccharomyces rouxii, Candida versatilis,	Soybean	Fermentation	Soy sauce
Coffee	*Saccharomyces sp*	Coffee bean	Fermentation	Flavour bean
Bakery products	*Saccharomyces sp*	Dough	Fermentation	CO2 production to cause dough rise

Now yeast i.e. *Saccharomyces cerevisiae* is used in brewing and baking industry for commercial production and home baking. Yeast is now available in market in different forms which is stated bellow.

- **Compressed Yeasts:** It is commonly refered to as "yeast cakes" or wet yeast. It contains about 70% moisture and has a shelf life of about 3-4 weeks. Such yeast is perishable and should be stored under refrigeration (4-5º C).
- **Crumbled Yeast:** It is similar to yeast cakes but with less water and in a crumbled condition. This type is often sold in 50lb bags.
- **Active Dry Yeast:** Dry yeast is prepared similar to compressed yeast but is dried under specially controlled conditions. The moisture content is 6-8%, which enables them to be stored for a few months. Dry yeast should be revived in water at 105-110º F.
- **Instant Dry Yeast:** Through a quick drying process under controlled conditions, more porous yeast can be produced that become active immediately on rehydration. This is probably the most popular yeast used in home baking.

5.2 Ethanol Production:

For large-scale production of ethanol, an organism that is tolerant to high concentrations of ethanol and osmotic pressure is essential. Species of *Saccharomyces* are the most tolerant to ethanol. Wine yeasts can tolerate levels up to 20%, whereas, baker's yeast used in beer making and bakeries can tolerate levels at from 4 to 6%. Most organisms are inactivated in concentrations of ethanol above 15%. Extensive research has been done to improve strains that will be effective in utilizing various substrates and tolerate high concentrations of glucose or sucrose and ethanol above 15%. They must also be resistant to certain metabolites that may be toxic. Ethanol tolerance is genetically controlled by a large number of genes (Ismail & Ali, 1971).

Distillation is involved the conversion of a substance into a vapor that is subsequently condensed to the liquid state. The process goes back to the time of Aristotle (around 350-380 BC). Distillation is used in particular to separate mixtures of liquids in which the boiling points of the liquids differ. The fermented alcoholic substrates contain water, alcohol, oils and other liquids. The boiling point of alcohol is 79.3°C and that of water is 100° C; thus the alcohol will vaporize at much lower temperature than water. In the production of distilled alcoholic beverages, the fermented substrates are vaporized at 79.3°C and the vapor is then condensed in a cooling coil to separate from the other liquids. Fungal species of *Saccharomyces* sp and *Schizosaccharomyces* sp are used to produce alcoholic beverages.

5.3 Industrial Ethanol for Automobiles:

It is produced in the U.S. using corn as the primary substrate. In Brazil, sugarcane is used for large-scale alcohol production for automobiles. With the decreasing of world fossil fuel reserves, production of ethanol from plant products is more attractive. With industrial ethanol, flavor and other qualities are not as important as consumable products. Therefore, the yeast strains used are selected for their efficiency to digest substrates and in their tolerance to high levels of ethanol.

5.4 Beverages:

Alcoholic beverages are made from a large variety of starchy or sugary products. Alcoholic beverages are of different types and these are produced using different agricultural products. Some of them are-

- **Whiskey:** It is made largely from barley, but sometimes from other grains. They are essentially aged grain alcohol that starts out as clear but becomes colored when aged inside of charred barrels.
- **Bourbon:** It is a form of whiskey made from fermented corn, or occasionally other grains.
- **Vodka:** It is produced largely from potatoes in Russia and other east European countries because these are cheap. They often use grains as well.
- **Rum:** It is a distilled spirit made from sugar-containing substrates like molasses and cane juice. It is initially clear, but like bourbon, it is darkened by storage in wooden barrels or in recent years adding caramel after distillation. It is popular in tropical and subtropical countries where sugarcane is grown.
- **Cognac:** It is made from distilled grape wine and gets its name from Conacais, France where it was first made. British and Dutch merchants began to distill wine to prevent spoilage in shipment.
- **Brandy:** It is made in a similar way as cognac, initially from grape wine but later in North America from fermented juices of pears and peaches.
- **Tequila:** It is briny liquor made exclusively in Mexico from fermented juice of the *Agave* cactus. The Mexicans were making a fermented drink 1000 years before they were invaded by Spain. The Spaniards brought to Mexico with them the art of distillation; hence, tequila came into existence.

Wine: Wine in the strict sense is the fermented juice of grapes. However, wines are now made of many juices. The making of wine is known from the earliest history of man (perhaps 5000 BC). Talk about a vintage! Scientists say they have found the oldest evidence of wine residue at the bottom of a squat, 7,000-year old pottery jar. Traces of two chemicals in the jar, found in the Zagros Mountains of Iran, extend the known history of wine-making of 2,000 years. Wines are named after the type of grapes or the geographic area or specific village where they were first produced. For example, Burgandy, Bordeaux, Champagne, and Alsacs are important wines of France. During the 17th century, wine makers in the Rhine valley found that grapes allowed to rot on the vine gave the wine a sweeter taste. There are three basic types of wines, (1) table wines, (2) fortified wines and (3) sparkling wines.

Table wines are made from pressed grapes fermented in vats with the addition of sugar, yeasts, sulfur dioxide. *Saccharomyces ellipsoideus* is the common yeast used in the fermentation process. The alcohol content is around 12-15%. Sulfur dioxide is used to keep down the vinegar producing bacterium *Acetobacter*. Wine may be chilled in vats to cause sedimentation and the free run wine is decanted.

Fortified wines receive an addition of alcohol, brandy or other alcoholic beverages, and the final alcohol level is from 16-23%. A common fortified wine is port wine which gets it name because sailors who used to come into port would purchase wine spiked with brandy or liquors to give a greater alcohol level.

Sparkling wines such as champagne go through a double fermentation in which the alcoholic content reaches 20%. Some sparkling wines have a natural effervescence; others are made effervescent by bubbling them with carbon dioxide. All natural wines are below 20% alcohol because beyond that level, the fermenting yeast would be killed.

There are also red, white, and pink (rosé) wines. Red wines are made from black grapes in which the husks (mush or skins) are left in contact with the juice throughout fermentation.

Most white wines are made from green colored grapes or from black grapes in which the husks are removed soon after pressing.

Pink Wines: Leaving the husks of black grapes in the fermenter for a short period of time produces a pink wine.

European wine production was almost devastated when in the 19th century imported American rootstock infested with a louse, *Phylloera*, which feeds on grape roots, destroyed more than 2,500,000 acres throughout France and surrounding countries. Many growers never recovered. Others imported resistant rootstock and the industry was soon reestablished.

5.5 Beer:

Beer is a beverage obtained by the alcoholic fermentation by yeasts of a malted cereal, usually barley malt with or without other starchy materials and to which hops have been added. Enzymes are often used in the early stages of brewing, i.e. the "mashing" stage, and later in the brewing process. Amylase produced by other fungi increases starch digestion and results in low carbohydrate, or "lite" beers. Several other carefully monitored enzymes are added during the brewing process. Without protease, for example, beers would become hazy, and Glucoamylase produced by certain species of *Aspergillus* are used to sweeten beers.

There is evidence that the brewing of beer developed independently in Egypt. Some have suggested that the Hebrews learned to use hops to flavor beer while they were in captivity in Babylon during the 8th and 9th century BC. The Greeks learned to make beer from the Egyptians and the Romans in turn learned from the Greeks. There are two types of beers- lager and ales.

a. **Lager Beer:** It employs bottom fermentation in which the "spent yeast" settles to the bottom and the green beer is aged. With aging, it mellows and is carbonated with CO_2.
b. **Ales Beer:** In the brewing of ales, the yeast selected is a top fermenter, forming foam that can be removed.

Four things are vital to brewing beer, barley malt, hops, water, and yeast. In the making of barley malt, barley is allowed to go into dormancy after harvest. During malting certain enzymes are formed within the grain. Before malting the barley is soaked in water to initiate germination, after which grains are dried to about 2% moisture level. Timing of germination, temperature and duration of drying and other factors affect the kind of malt that is produced. Corn, rice, and other grains may be added as adjuncts to add texture and flavor. These are closely guarded industry secrets. Hops are not essential for the manufacture of beer. The flowers of the female hops plant are used and only from unfertilized flowers. Pollinated hops will give the beer a bitter flavor. Hop growing in the U.S. is chiefly in Oregon, California, Idaho, and New York. The best hope for brewing come from Slovakia and the Czech Republic (formerly Czechoslovakia). Hops are added to mashed malt solution (wort) during boiling. Water quality is vital to beer production, especially pH. Alkaline or "hard" water gives poor results and water with sulphur, iron, or bacteria should be avoided.

Apparently, there must be ideal water in the mountains around Golden, Colorado! The yeast used in beer brewing is *Saccharomyces cerevisiae*.

The main stages of brewing are mashing, boiling, and fermenting. Prior to mashing, malt is crushed between rollers to form flour. Mashing involves adding water (100-150F), solubilizing the starch by enzyme action, and separating out the husk. After mashing, the "wort" is boiled in large copper tanks or kettles, stopping enzyme action. The wort is filtered and passed into a high-speed centrifuge to achieve clarity. It then passes into large coolers. Fermentation begins when yeast is added to the cooled wort at 1 lb/barrel. After about 8 days most all fermentable substrate has been converted into alcohol. The beer is then bottled, canned, or stored in large kegs for marketing. The following images show the various types of cookers, fermentation tanks and sediment tanks utilized in the manufacture of beer. The manufacture, distribution and sale of beer are major industries in the U.S. and in Florida.

5.6 Other Types of Fermented Products:

- **Rice Beer:** Rice beer is called sake in Japan, murcha or pachwai in India. It is made from rice using species of *Mucor* or *Aspergillus oryzea* with sugar.
- **Kanji:** A beerlike beverage made with carrots and beets fermented with *Hansenula*. Taste much like cherry wine.
- **Toddy:** Sweet fermented sap from tropical palms using *Saccharomyces cervisiae*. Many substitute organisms can be used.
- Others fermented products include Shoyu (=soy sauce). This is one fermented product referred to as Ketjap in Indonesia, has been produced in Japan for over 1000 years. The average Japanese consumes more than 3 gallons of soy sauce per year. Shoyu is prepared by soaking soybeans for 15hr and autoclaving it for 1hr. Clean, roasted wheat powder is added. After cooling, a starter, tane koji composed of *Aspergillus oryzae* or *A. soyae* is used as the inoculum. After fermentation for 3-4 months, or up a year, the material is combined with equal parts of brine and placed in tanks. The resulting mash is compressed and the liquid supernatant is retrieved sterilized and bottled.
- **Tea Fungus:** Perhaps one of the strangest fermentations encountered is the so called "tea fungus" of Eastern Europe and Asia sometimes referred to as Hongo. This drink is made from dry tea leaves steeped in a liter of water, and the leaves removed and 100 gm of sugar added. The solution is autoclaved and a "starter" culture is added and the material is allowed to ferment. Two yeasts and *Acetobacter* have been isolated from the starter.
- Anchu is an alcoholic drink in Taiwan made from red rice. Awamori is an alcoholic drink prepared from sweet potatoes in Japan. Braga is a fermented drink made from millet in Romania.

5.7 Cheese:

The production of cheese is a multimillion dollar industry in the US and is also a dominant industry in other countries. It involves the fermentation of milk from different animals using various microorganisms and processing them under rigidly controlled conditions. Milk is coagulated, the liquid whey removed and the solid curd is preserved. Curd may be made in three ways: acid coagulation, heat coagulation and rennet coagulation. There are different forms of cheese such as block cheeses, powdered cheeses, soft cheeses and various processed cheeses. Ricotta and similar cheeses are made from heat coagulated curd.

Two of the most common cheeses in which filamentous fungi are used in fermentation are Camembert and Roquefort. Camembert cheese is originated in the town of Camembert in the Normandy district of France. Camembert cheese, also called Brie, obtains its flavor and texture from lipolytic and proteolytic activity of molds belonging to *Penicillium camemberti* and *P. casicolum*. The production of camembert cheese requires rigidly controlled conditions or else other fungi, particularly *Scopulariopsis brevicaulis*. For this reason, it has been very difficult to establish a successful industry in other countries. Camembert cheese has not gained wide popularity in the US. Roquefort Cheese is made from the fermentation of milk curd by *Penicillium roqueforti*, a species first isolated in 1904 and described by Charles Thom, USDA, and Peoria, IL. Charles Thom introduced the production of Roquefort cheese in the US in 1906. Thom and co-workers discovered the lipolytic and proteolytic enzymes of the mold released caproic, caprylic and capric acids which imparted flavor to Roquefort cheese. Other blue-veined cheeses similar to Roquefort are Dolce Verdi produced by *Penicillium expansum* and Ellischour with a red pigment produced by *Penicillium nalgiovensis*. Other blue cheeses include from age blue cheese in central France from sheep and cow milk; gorgonzola in northern Italy, stilton in England, cammelost in Norway and neufchatal in Germany.

5.8 Bread-Making Techniques:

Yeast fermentation is used in bread making. How to make a lot of dough? In the early years, bread making relied on natural fermentation. During the late 1700's, commercially available yeasts were used in large-scale production of bread. Strains of *Saccharomyces cerevisiae* have been selected in food processing for their ability to ferment sugar and produce valuable metabolites. When placing *S. cerevisiae* into bread dough, the cells hydrolyse sugars available in the environment and produce CO_2, and ethanol. Basic ingredients for bread making are flour, water, yeast and salt. Other ingredients such as sugars, eggs, butter, milk and other food grade supplements can be added to enhance the flavour, nutritive value, appearance and texture of the final product. There are several of bread making techniques and formulations. One of the main variables is the amount of added sugar. Some doughs are heavily sweetened with glucose, sucrose or glucose syrup. These types of bread dough are referred to as "sweet dough". In contrast, some dough is not sweetened at all, and they are termed "lean dough".

Baker's yeast can metabolise only a limited range of carbon sources. Closely related yeast strains differ greatly in their ability to utilise sugars because different strains carry different active sugar fermentation genes. *Saccharomyces cerevisiae* would preferentially utilise monosaccharides (transported into yeast via facilitated diffusion) and disaccharides (transported into yeast via active transport) compared to any other carbon sources. Sugars can also be inhibitory to various metabolic pathways. Out of the range of fermentable sugars, glucose is the most repressive. Glucose repression regulates the expression of fermentation genes at the transcriptional level. Glucose also affects transport of sugars into the cell and thereby affects the function of regulatory mechanisms for induction. In sweet dough, *Saccharomyces cerevisiae* utilise free sugars in the environment and produce ethanol and carbon dioxide. In lean dough, there is a limited amount of free sugars. The main carbon source for yeast to ferment in lean dough is maltose. Maltose is produced when water is added to flour and activates the plant amylolytic or diastatic enzymes alpha (a) and beta (B) amylase. The amylases act on damaged starch granules to form molecules of maltose. Maltose utilisation by an unadapted yeast cell requires an induction period after the free sugars level in the dough has been reduced to a negligible proportion. Some strains of *Saccharomyces cerevisiae* cannot utilise maltose immediately after the depletion of free sugars.

When this happens, a decrease in gas production is observed. This phenomenon is termed maltose lag and the strain of yeast is called maltose-lagging strain. The maltose-lag period is entirely strain-dependent.

5.9 The purpose of Using Yeast in Bread Making is to:

- Produce CO2 during fermentation of free sugar in flour.
- Develop structure and texture by forming tiny gas chambers within the dough.
- Impart distinctive flavours.
- Enhance the nutritive value of bread.

Good strains of *S. cerevisiae* for bread making should be capable of producing active and regular fermentation in bread dough. Gas production by yeast depends on the activity of enzymes produced by yeast cells and fermentable sugars available. Consistent results should be achieved each time the baker places yeast in dough. Traits that yeast manufacturers and the consumers should look for in baker's yeast:-

- Growth rate and yield on medium containing different carbon sources.
- Fast leavening of the dough.
- Osmo-tolerance and cryo- tolerance.
- Consistency, colour and odour.
- Stability in dough and during storage.
- Keeping quality and extended shelf life.

As an industrial microorganism, the physiology of yeast determines the success of the organism in the market place. Different strains of yeast vary in their ability to convert sugar into biomass. Additionally, the selected organisms must exhibit a competitive performance in the market place. Yeast manufacturers must meet client's requirements in terms of yeast activity, stability and product consistency, and customer service. Specific aspects of yeast physiology that contribute to the competitiveness are carbohydrate metabolic pathways, catabolic repression of, and membrane synthesis.

Currently, different strains of *S. cerevisiae* are produced by yeast manufacturers to meet different bread making practices. Each strain of yeast is appropriate for only one or a few specific bakery application. This leads to the production of many yeast strains for different types of bakery products. Production of "all purpose" yeast will help the manufacturers save much time, space, resources and money. Addition of a gene encoding for alcohol dehydrogenase II isoenzyme into the maltose lagging strains of S. cerevisiae may reduce maltose lag by improving the maltose metabolic pathway of yeast. The end product is the reduction of the maltose lag phase during fermentation, whereby enhancing production of carbon dioxide and decreasing fermentation and leavening time.

5.10 Edible mushrooms:

Edible mushrooms are the fleshy and edible fruiting bodies of several species of macrofungi (i.e. fungi which bear fruiting structures that are large enough to be seen with the naked eye). They can appear either below ground (hypogeous) or above ground (epigeous) where they may be picked by hand.

Edibility may be defined by criteria that include absence of toxic effects on humans and undesirable taste and aroma. Edible mushrooms are consumed for their nutritional value and for their culinary value. Mushrooms used for as folk medicine are known as medicinal mushrooms.

The nutritional value of edible mushrooms is amazing as they contain high protein, fibre, vitamin and mineral contents and low-fat levels. They are very useful for vegetarian diets because mushroom provide essential amino acids like leucine, valine, glutamine, glutamic and aspartic acids. Mushrooms also have higher protein content than most of the fruits and vegetables. Edible mushrooms contain high amounts of ash, 80–120 g/kg of dry matter which contain mainly potassium, phosphorus, magnesium, calcium, copper, iron, and zinc. Carbohydrates are found in high proportions in edible mushrooms but low amount of fats. There are still more than 100 pharmaceutical chemicals originated from mushrooms and fungi and they are well known for antioxidant, anticancer, antidiabetic, antiallergic, immunomodulating, cardiovascular protector, anticholesterolemic, antiviral, antibacterial, antiparasitic, antifungal, detoxification, and hepatoprotective properties; they also protect against tumor development and inflammatory processes (Valverde et al., 2015).

While psychedelic mushrooms are occasionally consumed as recreational or entheogenic purposes, they can produce psychological effects, and are therefore not commonly used as food. Edible mushrooms include many fungal species that are either harvested as wild or cultivated form. Easily cultivated and common wild mushrooms are often available in markets. Before consuming any wild mushroom, it should be identified properly. Accurate determination and proper identification of an edible species is the only safe way to ensure edibility for wild mushroom and the only safeguard against possible accident. So, cultivated mushrooms are only recommended for edible purpose. Wild poisonous mushrooms that are frequently confused with edible mushrooms and responsible for many fatal poisonings include several species of the genus *Amanita*, in particular, *Amanita phalloides*, the *death cap*. It is therefore better to eat only scientifically recognized cultivated edible mushroom, rather than collected as wild form.

5.11 Reference:

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