Unit- 4 Fermented foods and probiotics

1) Cheese Production and Classification

Cheese comes in many varieties. The variety determines the ingredients, processing, and characteristics of the cheese. Cheese can be made using pasteurized or raw milk. Cheese made from raw milk imparts different flavors and texture characteristics to the finished cheese. For some cheese varieties, raw milk is given a mild heat treatment (below pasteurization) prior to cheese making to destroy some of the spoilage organisms and provide better conditions for the cheese cultures.

Cheese can be broadly categorized as **acid or rennet cheese**, and **natural or process cheese**.

Acid cheeses are made by adding acid to the milk to cause the proteins to coagulate.

Fresh cheeses, such as cream cheese or queso fresco, are made by direct acidification. Most types of cheese, such as cheddar or Swiss, use rennet (an enzyme) in addition to the starter cultures to coagulate the milk.

Natural cheese is an industry term referring to cheese that is made directly from milk.

Process cheese is made using natural cheese plus other ingredients that are cooked together to change the textural and/or melting properties and increase shelf life.

Ingredients

The main ingredient in cheese is milk. Cheese is made using cow, goat, sheep, water buffalo or a blend of these milks.

The type of coagulant used depends on the type of cheese desired. For acid cheeses, an acid source such as acetic acid (the acid in vinegar) or gluconodelta-lactone (a mild food acid) is used. For rennet cheeses, calf rennet or, more commonly, rennet produced through microbial bioprocessing is used. Calcium chloride is sometimes added to the cheese to improve the coagulation properties of the milk.

Flavorings may be added depending on the cheese. Some common ingredients include herbs, spices, hot and sweet peppers, horseradish, and port wine.

Bacterial Cultures

Cultures for cheese making are called **lactic acid bacteria (LAB)** because their primary source of energy is the lactose in milk and their primary metabolic product is lactic acid. There is a wide variety of bacterial cultures available that provide distinct flavor and textural characteristics to cheeses. Starter cultures are used early in the cheese making process to assist with coagulation by lowering the pH prior to rennet addition. The metabolism of the starter cultures contribute desirable flavor compounds, and help prevent the growth of spoilage organisms and pathogens.

Typical starter bacteria include

Lactococcus lactis subsp. *lactis* or *cremoris,*

Lactobacillus delbruckii subsp. *Bulgaricus*

Lactobacillus helveticus.

Streptococcus salivarius subsp. *thermophilus,*

Adjunct cultures are used to provide or enhance the characteristic flavors and textures of cheese. Common adjunct cultures added during manufacture include

Lactobacillus casei and *Lactobacillus plantarum* for flavor in Cheddar cheese, *Propionibacterium freudenreichii* for eye formation in Swiss.

Adjunct cultures can also be used as a smear for washing the outside of the formed cheese, such as the use of *Brevibacterium linens* of gruyere, brick and limburger cheeses.

Yeasts and molds are used in some cheeses to provide the characteristic colors and flavors of some cheese varieties. Torula yeast is used in the smear for the ripening of brick and limberger cheese. Examples of molds include *Penicillium camemberti* in camembert and brie, and *Penicillium roqueforti* in blue cheeses.

Enzymes

Rennet / Rennin is a complex of enzymes produced in the stomachs of ruminant mammals. Chymosin, its key component, is a protease enzyme that curdles the casein in milk. This helps young mammals digest their mothers' milk. Rennet can also be used to separate milk into solid curds for cheese making and liquid whey. In addition to chymosin, rennet contains other important enzymes such as pepsin and a lipase. Rennet is used in the production of most cheeses

General Manufacturing Procedure- The temperatures, times, and target pH for different steps, the sequence of processing steps, the use of salting or brining, block formation, and aging vary considerably between cheese types. The following flow chart provides a very general outline of cheese making steps.

General Cheese Processing Steps

1. Standardize Milk
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2. Pasteurize/Heat Treat Milk
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3. Cool Milk
↓
4. Inoculate with Starter & Non-Starter Bacteria and Ripen
↓
5. Add Rennet and Form Curd
↓
6. Cut Curd and Heat
↓
7. Drain Whey
↓
8. Texture Curd
↓
9. Dry Salt or Brine
↓
10. Form Cheese into Blocks
↓
11. Store and Age
↓
12. Package

Processing Steps in Cheddar Cheese Production:

The times, temperatures, and target pH values used for cheddar cheese will depend on individual formulations and the intended end use of the cheese. These conditions can be adjusted to optimize the properties of Cheddar cheese for shredding, melting, or for cheese that is meant to be aged for several years.

1. Standardize Milk

Milk is often standardized before cheese making to optimize the protein to fat ratio to make a good quality cheese with a high yield

2. Pasteurize/Heat Treat Milk

Depending on the desired cheese, the milk may be pasteurized or mildly heat-treated to reduce the number of spoilage organisms and improve the environment for the starter cultures to grow. Some varieties of cheeses are made from raw milk so they are not pasteurized or heat-treated. Raw milk cheeses must be aged for at least 60 days to reduce the possibility of exposure to disease causing microorganisms (pathogens) that may be present in the milk.

3. Cool Milk

Milk is cooled after pasteurization or heat treatment to 90°F (32°C) to bring it to the temperature needed for the starter bacteria to grow. If raw milk is used the milk must be heated to 90°F (32°C).

4. Inoculate with Starter & Non-Starter Bacteria and Ripen

The starter cultures and any non-starter adjunct bacteria are added to the milk and held at 90°F (32°C) for 30 minutes to ripen. The ripening step allows the bacteria to grow and begin fermentation, which lowers the pH and develops the flavor of the cheese.

5. Add Rennet and Form Curd

The rennet is the enzyme that acts on the milk proteins to form the curd. After the rennet is added, the curd is not disturbed for approximately 30 minutes so a firm coagulum forms.

6. Cut Curd and Heat

The curd is allowed to ferment until it reaches pH 6.4. The curd is then cut with cheese knives into small pieces and heated to 100°F (38°C). The heating step helps to separate the whey from the curd.

7. Drain whey

The whey is drained from the vat and the curd forms a mat.

8. Texture curd

The curd mats are cut into sections and piled on top of each other and flipped periodically. This step is called **cheddarin**g. Cheddaring helps to expel more whey, allows the fermentation to continue until a pH of 5.1 to 5.5 is reached, and allows the mats to "knit" together and form a tighter matted structure. The curd mats are then milled (cut) into smaller pieces.

9. Dry Salt or Brine

For cheddar cheese, the smaller, milled curd pieces are put back in the vat and salted by sprinkling dry salt on the curd and mixing in the salt. In some cheese varieties, such as mozzarella, the curd is formed into loaves and then the loaves are placed in brine (salt water solution).

10. Form Cheese into Blocks

The salted curd pieces are placed in cheese hoops and pressed into blocks to form the cheese.

11. Store and Age

The cheese is stored in coolers until the desired age is reached. Depending on the variety, cheese can be aged from several months to several years.

12. Package

Cheese may be cut and packaged into blocks or it may be waxed.

- **Types of cheeses**

These are classified on the basis of Aging, Texture, Process and methods of making, Fat content , Kind of milk, Region and country.

i) Fresh

These cheeses are *uncooked* and *unripened*, as well as mild and very moist with a soft texture. Some examples of fresh cheeses include: feta, ricotta, cream cheese, and cottage cheese.

ii) Semi-Soft

Semi-soft cheeses have a very *buttery* and *smooth* taste and are mild and moist. Examples include: fontina, gorgonzola, and Gouda.

iii) Soft

These cheeses are event softer than fresh cheeses and are known for their *creamy* texture and ability to be *spread easily*. Boursin, brie, and belpaese are three examples of soft cheese.

iv) Natural Rind

Natural rind cheeses have a rind that naturally form as the cheese comes in contact with air during the aging process. Their texture is *dense* and they are usually aged for a longer period of time. Stilton and Tomme de Savoie are two of the varieties.

v) Hard

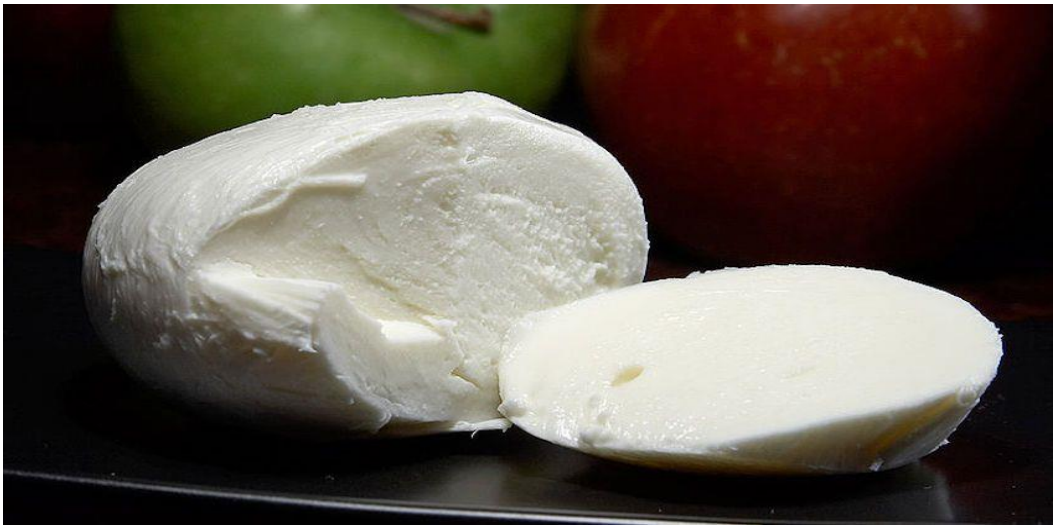
Hard cheeses are aged for a very long time and are the *driest* variety. They normally have a very *strong flavor* and are mostly consumed in a *grated* form to add to the flavor of several dishes. Asiago, parmigiano-reggiano, and pecorino romano are the most common types of hard cheeses.

vi) Blue

Blue cheeses are known for their moldy appearances. This is because they are injected with a specific strand of mold called *penicillium roqueforti* or *penicillium glaucum.* They have a very *distinctive* taste and smell, and people who like this type of cheese often have an acquired taste for it. St. Agur and Big Woods Blue are two types of this moldy cheese.

1. Mozzarella

Mozzarella is one of the most popular cheeses which is found almost all over the world. The cheese has its origin from Italy and has a very mild flavour and it is soft and chewy. Mozzarella is traditionally made from Italian buffalo's milk. However, in recent times, mozzarella is being also produced from cow, goat and sheep's milk. It is available in two main varieties that are fresh and dried. The fresh version is mostly used in salads and sandwiches. The dried mozzarella is used for culinary purposes, which is most commonly used in pizza, lasagna and other baked dishes.



2. Parmesan

Parmigiano Reggiano which is commonly referred to as parmesan also originates from Italy. The cheese has a very strong texture with a robust flavour. It is mostly used as a condiment and adds a lot of flavour to foods.

3. Ricotta

Ricotta is another Italian cheese which is made from sheep, cow, goat or Italian water buffalo milk whey left over from the production of cheese. Ricotta can be eaten smooth and mixed with condiments as well as sugar,

cinnamon. It is also commonly used in savoury dishes like ravioli, lasagne, ravioli and calzone, and more commonly in salad.

4. Blue Veined Cheese

Blue veined cheese which is also known as blue cheese is a term used to describe a cheese produced with cow, sheep or goat's milk. The final product is characterized by green, grey, blue or black veins or spots of mould throughout the body.

5. Cheddar

It is possibly the most popular variety of cheese which comes from the village of Cheddar in Somerset, England. There are different kinds of Cheddar cheese and the cheese comes in many different strengths also. The different strengths of Cheddar depend on how long the cheese was aged. Mild cheese has a softer and smooth texture and a creamy flavour too. The vintage Cheddar is hard and crumbly and also has a sharp tangy flavour.

6. Gouda

Gouda is a Dutch cheese originally from the town of Gouda in the Southern Netherlands. The texture, taste and flavour of the cheese can vary a lot. Younger Gouda cheese is mild in flavour with a smooth, semi-hard texture. Whereas older Gouda cheese is hard and crumbly with a strong and deeper flavour.

7. Cottage Cheese / Paneer

Cottage cheese is made by acidifying curds from milk with a bacterial starter culture. It is basically known as 'paneer' in India. It is very different from most of the other varieties. The nutrition profile is very different from other types of cheese due to a different production process. The cheese has a very low-fat content and has a mild salty taste. Cottage cheese is used by those who want a more protein-rich option.

8. Halloumi

Halloumi is a springy white cheese which is traditionally made from a mixture of goat and sheep milk. The texture of the cheese is similar to that of mozzarella or thick feta. But it has a strong, salty flavour. If you cook halloumi, it will remove all its saltiness and becomes more creamy instead. It can be easily grilled and fried because it has a high melting point. It is commonly used in salads.

9. Kalari (Traditional Kashmiri Cheese)

Kalari is a traditional ripened cheese indigenous to Rajouri, Poonch and Udhampur of Jammu province of Jammu & Kashmir in India. It is a very dense cheese which is usually sauteed and salted while serving.

10. Gorgonzola

Gorgonzola is one of the world's oldest kind of blue vein cheese. The cheese is mainly produced in the Northern Italian regions of Piedmont and Lombardy, Gorgonzola. Unskimmed cow's milk is used while preparing the cheese. It takes three to four months to attain full ripeness. The cheese has a soft texture with a nutty aroma.

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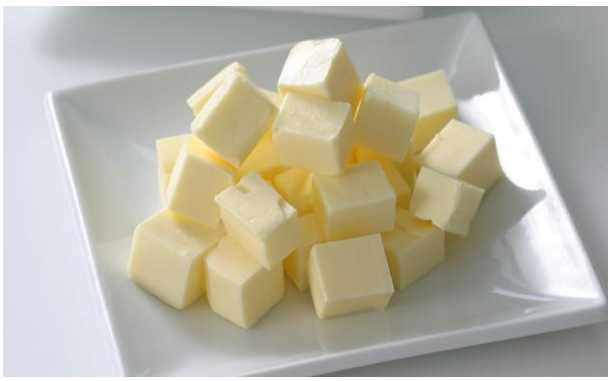
2) Butter

Butter is a dairy product made by churning fresh or fermented cream or milk, to separate the butterfat from the buttermilk. Butter consists of butterfat, milk proteins and water. Salt, flavorings, colours and preservatives are sometimes added to butter. Rendering butter produces clarified butter or *ghee*, which is almost entirely butterfat. It is generally used as a spread and a condiment, as well as in cooking, such as baking, sauce making, and pan frying. It is a good source of Vitamin A. Butter is a water-in-oil emulsion resulting from an inversion of the cream, an oil-in-water emulsion; the milk proteins are the emulsifiers. Butterfat is a mixture of triglyceride, a triester derived from glycerol and three of any of several fatty acid groups. Butter remains a solid when refrigerated, but softens to a spreadable consistency at room temperature, and melts to a thin liquid consistency at 32–35 °C.

It generally has a pale yellow color, but varies from deep yellow to nearly white. Its unmodified color is dependent on the animals' feed and is commonly manipulated with food colorings in the commercial manufacturing process, most commonly annatto or carotene. Annatto colour is extracted from the seeds of Bixa orellana.



Production of butter

Unhomogenized milk and cream contain butterfat in microscopic globules. These globules are surrounded by membranes made of phospholipids (fatty acid emulsifiers) and proteins, which prevent the fat in milk from pooling together into a single mass. Butter is produced by agitating cream, which damages these membranes and allows the milk fats to conjoin, separating from the other parts of the cream.

Commercial butter is about 80% butterfat and 15% water; traditionally made butter may have as little as 65% fat and 30% water.

Following are the steps of manufacturing butter on industrial scale

a) **Milk & cream collection**

b) **Ripening / Fermentation**
c) **Aging / Cooling**
d) **Churning**
e) **Draining & washing**
f) **Salting & working**
g) **Packing & storage**

a) Milk & cream collection

Butter is produced from the milk of buffalo, cow, camel, goat etc. Cream is separated from the milk. The cream should be sweet (pH greater than 6.6), not rancid, not oxidized, and free from off flavors. The cream is pasteurized at a temperature of 95°C or more to destroy enzymes and micro-organisms.

b) Ripening / Fermentation

Sometimes, cultures are added to ferment milk. Butter made from a fermented cream is known as **cultured butter**. During fermentation, the cream naturally sours as bacteria convert milk sugars into lactic acid. The fermentation process produces additional aroma compounds, including diacetyl, which makes for a fuller-flavored and more "buttery" tasting product. Today, cultured butter is usually made from pasteurized cream whose fermentation is produced by the introduction of *Lactobacilli* and *Leuconostoc* bacteria.

c) Aging / Cooling

Cream is held at cool temperatures to crystallize the butterfat globules, ensuring proper churning and texture of the butter. In the aging tank, the cream is subjected to a program of controlled cooling designed to give the fat the required crystalline structure. As a rule, aging takes 12 - 15 hours. From the

aging tank, the cream is pumped to the churn or continuous buttermaker via a plate heat exchanger which brings it to the requisite temperature.

d) Churning

Cream is agitated, and eventually butter granules form, grow larger, and come together. In the end, there are two phases left: a semisolid mass of butter and the liquid left over, which is the buttermilk.

e) Draining & washing

Thus the cream is split into two fractions: butter grains and buttermilk. In traditional churning, the machine stops when the grains have reached a certain size, whereupon the buttermilk is drained off. With the continuous buttermaker the draining of the buttermilk is also continuous.

After draining, the butter is worked (pressed) to a continuous fat phase containing a finely dispersed water phase. It used to be common practice to wash the butter after churning to remove any residual buttermilk and milk solids. This washing process would ensure that all the butter milk is washed out of the butter. Otherwise the butter would not keep and go rancid.

f) Salting, colouring & working

Salt is used to improve the flavor and the shelf-life, as it acts as a preservative. Most commonly annatto or carotene colours are added to improve texture. Annatto colour is extracted from the seeds of *Bixa orellana*. Further, the butter is worked to improve its consistency. The butter is spread over the butter worker, salt is sprinkled over the butter to prolong its keeping quality and improve its flavor. Easily soluble, fine grained worker has wooden corrugated roller. The object of working is to i) remove extra water ii) to render the butter compact and iii) to distribute the salt evenly.

g) Packing & storage

The butter is finally tapped into shape and then wrapped in waxed paper and then stored in a cool place. As it cools, the butterfat crystallizes and the butter becomes firm. Whipped butter , made by whipping air or nitrogen gas into soft butter , is intended to spread more easily at refrigeration temperatures.

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3) Idli

Idli is a south Indian savory cake popular throughout India. The cakes are usually two to three inches in diameter and are made by steaming a batter consisting of fermented black lentils (de-husked) and rice. The fermentation process breaks down the starches so that they are more readily metabolized by the body. The earliest mention of idli in India occurs in Tamil sangam literature. Also the aromatic flavour of Sambaar been mentioned in it around 6 CE. It is also found in Kannada writing of Shivakotiacharya in 920 CE.

Most often eaten at breakfast or as a snack, idlis are usually served in pairs with chutney, sambar, or other accompaniments

Preparation

To make idli, place two parts uncooked rice to one part split black lentil (minapa pappu, urad dal) in a pan and soak. Grind the lentils and rice to a paste in a heavy stone grinding vessel. Leave the paste to ferment overnight, until it has expanded to about 2½ times its original volume. In the morning, put the idli batter into the ghee-greased molds of an idli tray or "tree" for steaming. The perforated molds allow the idlis to be cooked evenly. The tree holds the trays above the level of boiling water in a pot, and the pot is covered until the idlis are done (about 10–25 minutes, depending on size).

Idlis are usually served in pairs with kobbari pachadi (chutney), sambar, karampodi with ghee. Kobbari pachadi and Karampodi are first used to eat in combination of idlis in Andhra Pradesh, specifically in Kostha Andhra Districts. Newer "quick" recipes for the idli can be rice- or wheat-based (rava idli). Besides the microwave steamer, electric idli steamers are available, with automatic steam release and shut-off for perfect cooking. Both types are non-stick, so a fat-free idli is possible.

Microorganisms in idli batter formation

The microorganisms responsible for the characteristic change in the batter such as souring, as well as for gas production are *Leuconostoc mesenteroides*. In the later stages of fermentation, growth of *Streptococcus faecalis* and, still later, of *Pediococcus cerevisiae* becomes significant. The fermentation of idli demonstrates a leavening action caused by the activity of the heterofermentative lactic acid bacterium, *L. mesenteroides*.

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- **Criterion for Probiotics**

The word *probiotic* comes from the Greek word *pro*, meaning "promoting" and *biotic*, meaning "life." The Food and Agriculture Organization of the United Nations (FAO) defines probiotics as "live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host."

Our digestive system normally has what we would call "good" bacteria and "bad" bacteria. Maintaining the correct balance between the "good" bacteria and the "bad" bacteria is necessary for optimal health.

Probiotics are believed to protect us in two ways. The first is the role that they play in our digestive tract. We know that our digestive tract needs a healthy balance between the good and bad bacteria. When the digestive tract is healthy, it filters out and eliminates things that can damage it, such as harmful bacteria, toxins, chemicals, and other waste products.

The other way that probiotics help is the impact that they have on our immune system. probiotics help maintain a strong immune system. That may be because the immune system isn't being properly challenged by pathogenic organisms. Introducing friendly bacteria in the form of probiotics is believed to challenge the immune system in healthy ways."

Probiotics are most effective for:

- Treating childhood diarrhea
- Treating ulcerative colitis
- Treating necrotizing enterocolitis, a type of infection and inflammation of the intestines mostly seen in infants
- Preventing antibiotic-associated diarrhea and infectious diarrhea
- Preventing pouchitis, an inflammation of the intestines that can follow intestinal surgery
- Treating and preventing eczema associated with cow's milk allergy
- Helping the immune system
- Treating symptoms of irritable bowel syndrome
- Treating vaginitis

Probiotic products contain bacteria and/or yeasts that assist in restoring the balance in our gut.

1. Lactobacillus

There are more than 50 species of lactobacilli. They are naturally found in the digestive, urinary, and genital systems. Foods that are fermented, like yogurt, and dietary supplements also contain these bacteria. *Lactobacillus* has been used for treating and preventing a wide variety of diseases and conditions.

Some of the lactobacilli found in foods and supplements are *Lactobacillus acidophilus, L. acidophilus* DDS-1, *Lactobacillus blugaricus, Lactobacillus rhamnosus* GG, *Lactobacillus plantarium, Lactobacillus reuteri, Lactobacillus salivarius, Lactobacillus casei, Lactobacillus johnsonii,* and *Lactobacillus gasseri.*

2. Bifidobacteria

There are approximately 30 species of bifidobacteria. The make up most of the healthy bacteria in the colon. They appear in the intestinal tract within days of birth, especially in breastfed infants.

Some of the bifidobacteria used as probiotics are *Bifidobacterium bifidum, Bifidobacterium lactis, Bifidobacterium longum, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium thermophilum,* and *Bifidobacterium pseudolongum.*

3. Saccharomyces boulardii

This is also known as *S. boulardii* and is the only yeast probiotic. Some studies have shown that it is effective in preventing and treating diarrhea associated with the use of antibiotics and traveler's diarrhea. It has also been reported to prevent the reoccurrence of *Clostridium difficile*, to treat acne, and to reduce side effects of treatment for *Helicobacter pylori*.

4. Streptococcus thermophilus

This produces large quantities of the enzyme lactase, making it effective, according to some reports, in the prevention of lactose intolerance.

5. Enterococcus faecium

This is normally found in the intestinal tract of humans and animals.

- *E. faecium* SF68
- *E . faecium* M-74

6. Leuconostoc

This has been used extensively in food processing throughout human history, and ingestion of foods containing live bacteria, dead bacteria, and metabolites of these microorganisms has taken place for a long time. These bacteria produce typical curd flavors composed of diacetyl and acetyl methyl carbinol.

Several fermented Curd, yoghurt like dairy products and milk-based carriers containing these specially selected live probiotic microorganisms are now available. However, the actual mechanism underlying these activities has yet to be elucidated.

Curd production

Curds are traditional sour milk preparations that have become major dairy products. There are several variants, but commercial forms are usually prepared from whole milk. Raw milk is heated and then cooled prior to inoculation; the heating is necessary, otherwise later protein coagulation does not produce a smooth gel. Inoculation involves a mixed starter culture containing strains of *Streptococcus lactis,* Lactobacillus *acidopilus, Lactobacillus lactis, Leuconostoc specis.* Curd has peculiar smell due to production of diacetyl and acetyl methyl carbnol produced by Leuconostoc species.

Yoghurt production

Yoghurts are traditional sour milk preparations that have become major dairy products. There are several variants, but commercial forms are usually prepared from whole milk which may be supplemented with protein such as skim milk powder. This helps to form the protein-gel structure of the product. These raw materials are heated and then cooled prior to inoculation; the heating is necessary, otherwise later protein coagulation does not produce a smooth gel. Inoculation involves a mixed starter culture containing thermophilic strains of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* in a ratio of 1 : 1. The former produces mainly acid, whereas the latter generates more organoleptic compounds, particularly acetaldehyde. Their proteolytic enzymes and extracellular polymers also aid protein-gel formation. Yoghurt can be pasteurized to improve storage-life or remain 'live', the latter reputedly having probiotic qualities.

Curd

Yoghurt

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- **Mushrooms as Single Cell Proteins (SCP)**

Rapid developments in microbial protein production occurred during the 1960s and 1970s. Extensive research was conducted on a wide range of microorganisms as possible alternate protein sources, motivated by large increases in the price of conventional animal feed. It was during this period that the term single cell protein (SCP) was first coined at the Massachusetts Institute of Technology. SCP is not pure protein, but refers to the whole cells of bacteria, yeasts, filamentous fungi or algae, and also contains carbohydrates, lipids, nucleic acids, mineral salts and vitamins.

Mushrooms

Certain mushrooms and other fruiting bodies of filamentous fungi are edible and provide a good source of protein, whereas others have narcotic effects and some are highly toxic. A wide range has been traditionally used for food, but relatively few are grown commercially. In fact, of the hundreds of species that is edible, only about 10 are produced in any quantity. Mushroom production involves controlled non-axenic solidsubstrate fermentation. It is currently the only economically viable product from lignocellulose fermentation. Exploitation of such fruiting fungi for the generation of edible biomass has several advantages:

1. They represent examples of the most efficient conversion of plant wastes into edible food;
2. unlike many other single cell proteins, they are directly edible and many are considered to be food delicacies because of their characteristic texture and flavour;
3. harvesting of fruiting bodies is the easiest possible method of separating edible biomass from the substrate in a solid-state fermentation; and
4. compared with animal sources of protein, many have a far superior protein conversion efficiency per unit of land and per unit of time.

Agaricus bisporus

In Europe and the USA *Agaricus bisporus* (button mushroom) accounts for over 90% of total mushroom production value. Agarics are decomposers of cellulosic materials and are naturally found in meadows and woodlands, where they degrade plant debris. They are grown commercially in temperate regions using a

substrate of composted straw. A crop is produced within 6 weeks, whereas other mushrooms may take several months or even years to fruit. A closely related species, *Agaricus bitorquis*, is also grown in some areas. It is less prone to certain viruses and the bacterial blotch disease of mushrooms, caused by *Pseudomonas tolaasii*.

The *Agaricus* production involves the following stages--

1. Inoculum preparation: growth of spawn (inoculum) on sterilized cereal grains.
2. Solid-substrate preparation: composting of straw, manure and fertilizers at 60–70°C for 2 weeks.
3. Substrate 'sterilization', so-called 'peak heating' of compost for 5–7 days.
4. Spawn inoculation into 'sterilized' compost and mycelial growth, referred to as a 'run' at 25°C for 2–3 weeks.
5. Application of a casing (covering) layer of peat and chalk over the substrate.
6. Fruiting body production, fructification, in about four flushes (successive crops) over a period of 4–6 weeks.