

UNIT – 2 Industrial Dairy fermentations

❖ Taxonomy of lactic acid bacteria (LAB) present in fermented products

Lactobacillales or **lactic acid bacteria (LAB)** are an order of Gram-positive, low-GC, acid-tolerant, generally nonsporulating, nonrespiring, either rod- or coccus-shaped bacteria that share common metabolic and physiological characteristics. These bacteria, usually found in decomposing plants and milk products, produce lactic acid as the major metabolic end product of carbohydrate fermentation. This trait has, throughout history, linked LAB with food fermentations, as acidification inhibits the growth of spoilage agents. Proteinaceous bacteriocins are produced by several LAB strains and provide an additional hurdle for spoilage and pathogenic microorganisms. Furthermore, lactic acid and other metabolic products contribute to the organoleptic and textural profile of a food item. The industrial importance of the LAB is further evidenced by their generally recognized as safe (GRAS) status, due to their ubiquitous appearance in food and their contribution to the healthy microflora of human mucosal surfaces. The genera that comprise the LAB are at its core *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Lactococcus*, and *Streptococcus*, as well as the more peripheral *Aerococcus*, *Carnobacterium*, *Enterococcus*, *Oenococcus*, *Sporolactobacillus*, *Tetragenococcus*, *Vagococcus*, and *Weissella*; these belong to the order Lactobacillales.

Brief facts

- *Lactic Acid Bacteria (LAB)* or *Lactics* constitute a diverse group of microorganisms associated with plants, meat, and dairy. They are used in the manufacture of dairy products such as acidophilus milk, yogurt, buttermilk, and cheeses. The Lactics are also important commercially in the processing of meats (sausage, cured hams), alcoholic beverages (beer, fortified spirits), and vegetables (pickles, and sauerkraut). Although the LAB have beneficial effects in the food industry, they can be a nuisance as contaminants by producing off-flavors and contributing to spoilage.
- The Lactics have been characterized primarily by their ability to form various isomers of lactic acid from the fermentation of glucose. Lactic acid may be extracted from the fermentation product and a determination

made of the ability to optically rotate light. If the rotation is to the right, it is termed *Dextrorotary (D-Lactate)*; if to the left, it is termed *Levorotary (L-Lactate)*, or, if there is a mixture of both D and L, it is termed *racemic*

- The Lactic Acid bacteria are grouped as either *Homofermenters* or *Heterofermenters* based on the end product of their fermentation. The *Homofermenters* produce lactic acid as the major product of fermentation of glucose. The *Heterofermenters* produce a number of products besides lactic acid, including carbon dioxide, acetic acid, and ethanol from the fermentation of glucose.
- In general, the LAB may be characterized as Gram-positive, aerobic to facultatively anaerobic, asporogenous rods and cocci which are oxidase, catalase, and benzidine negative, lack cytochromes, do not reduce nitrates to nitrite, are gelatinase negative, and are unable to utilize lactate.
- Orla and Jensen also divided the Lactobacilli into the three groups (the *Thermobacteria*, *Streptobacteria*, and the *Betabacteria*) based on growth temperature and biochemical reactions. Although those three groups have been replaced for the most part, the three names are still in common use and are defined according to growth temperature, ability to ferment pentoses, ability to produce carbon dioxide from glucose or gluconate, requirement for thiamine, formation of lactic acid as a major product of fermentation, Homofermentative or Heterofermentative type of fermentation, reduction of fructose to mannitol, and hydrolysis of arginine.
- The many properties of LAB - ability to provide health benefits, their roles in the food industry (spoilage or starter cultures), potential pathogenicity - are strain-specific. Numerous strains are continuously screened for desirable characteristics.

1. ***Genus Lactobacillus***
2. ***Genus Leuconostoc***
3. ***Genus Streptococcus***

1. Genus *Lactobacillus*

These cultures are Gram positive, rod-shaped bacteria, which are homo or hetero fermentative. Biochemical characteristics of various group of lactobacilli has been shown in Table.

Characteristics	Lactobacilli		
	Group I Obligately Homofermentative	Group II Facultatively Heterofermentative	Group III Obligately Heterofermentative
Orla-Jenson Group	Thermobacterium	Streptobacterium	Batabacterium
Growth at 45 C	+	–	±
Growth at 15 C	–	+	+
Fermentation of ribose	–	+	+
Gas from gluconate	–	+	+
Presence of Aldolase	+	+	–
Presence of phosphoketolase	Absent	Inducible	Constitutive
Examples	<i>Lb. delbrueckii</i> subsp. <i>delbrueckii</i> <i>Lb. delbrueckii</i> subsp. <i>bulgaricus</i> <i>Lb. delbrueckii</i> subsp. <i>lactis</i> <i>Lb. helveticus</i> <i>Lb. acidophilus</i>	<i>Lb. casei</i> subsp. <i>casei</i> <i>Lb. casei</i> subsp. <i>paracasei</i> <i>Lb. rhamnosus</i> <i>Lb. plantarum</i> <i>Lb. curvatus</i>	<i>Lb. kefir</i> <i>Lb. fermentum</i> <i>Lb. brevis</i> <i>Lb. reuteri</i>

2. Genus *Leuconostoc*

These organisms are coccoid in shape, single or in pairs, short chains, Gram positive, microaerophilic, heterofermentative lactic acid fermentation, produce D(–)-lactate, CO₂ and aroma compounds from lactose, mesophilic (optimal growth 20 to 30°C).

Flavour producers

Leu. mesenteroides subsp. cremoris

Leu. mesenteroides subsp. dextranicum

Leu. lactis

3. Genus *Streptococcus*

Streptococcus were reclassified into *Lactococcus*, *Enterococcus*, *Vagococcus*, and *Streptococcus* based on biochemical characteristics, as well as molecular features.

Lactococci (formerly Lancefield group N streptococci) are used extensively as fermentation starters in dairy production. These cultures are spherical or ovoid cocci, 1.0 µm in diameter, chains or pairs, Gram positive, microaerophilic, homofermentative lactic acid, produce L (+)-lactate from lactose, mesophilic (absence of growth at 45°C).

L. lactis subsp. lactis

L. lactis subsp. cremoris

L. lactis subsp. lactis biovar diacetylactis

Both *L. lactis* subspecies (*L. l. lactis* and *L. l. cremoris*) are widely used as generic LAB models for research. *L. lactis* ssp. *cremoris*, used in the production of hard cheeses, is represented by the laboratory strains LM0230 and MG1363. In similar manner, *L. lactis* ssp. *lactis* is employed in soft cheese fermentations.

Mesophilic lactic acid bacteria	Major Metabolite
<i>Lactococcus lactis</i> ssp. <i>lactis</i>	L (+) Lactate
<i>Lactococcus lactis</i> ssp. <i>diacetylactis</i>	L (+) Lactate, Diacetyl
<i>Lactococcus lactis</i> ssp. <i>cremoris</i>	L (+) Lactate
<i>Leuconostoc mesenteroides</i> ssp. <i>mesenteroides</i>	D (–) Lactate, Diacetyl
<i>Leuconostoc mesenteroides</i> ssp. <i>cremoris</i>	D (–) Lactate, Diacetyl
<i>Leuconostoc mesenteroides</i> ssp. <i>dextranicum</i>	D (–) Lactate, Diacetyl
<i>Pediococcus acidilactici</i>	DL Lactate
Thermophilic lactic acid bacteria	
<i>Streptococcus thermophilus</i>	L(+)Lactate, Acetaldehyde
<i>Lactobacillus delbrueckii</i> ssp. <i>delbrueckii</i>	D (–) Lactate
<i>Lactobacillus delbrueckii</i> ssp. <i>bulgaricus</i>	D (–) Lactate, Acetaldehyde
<i>Lactobacillus delbrueckii</i> ssp. <i>lactis</i>	D (–) Lactate
<i>Lactobacillus fermentum</i>	DL Lactate, CO ₂
<i>Lactobacillus helveticus</i>	DL Lactate
<i>Lactobacillus kefir</i>	DL Lactate, CO ₂
<i>Lactobacillus kefirianofaciens</i>	DL Lactate, CO ₂
Therapeutic lactic acid bacteria	
<i>Lactobacillus acidophilus</i>	DL Lactate
<i>Lactobacillus paracasei</i> ssp. <i>paracasei</i>	L (+) Lactate
<i>Lactobacillus paracasei</i> ssp. <i>biovar. Shirota</i>	L (+) Lactate
<i>Lactobacillus rhamnosus</i>	L (+) Lactate
<i>Lactobacillus reuteri</i>	DL Lactate, CO ₂
<i>Bifidobacterium adolescentis</i>	L(+) Lactate, acetate
<i>Bifidobacterium bifidum</i>	Lactate, acetate
<i>Bifidobacterium breve</i>	L(+) Lactate, acetate
<i>Bifidobacterium infantis</i>	Lactate, acetate
<i>Bifidobacterium longum</i>	L(+) Lactate, acetate

Various dairy starter cultures with their metabolites

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❖ Acid fermented milks (acidophilus milk, yoghurt).

Fermented milk products, also known as **cultured dairy foods**, **cultured dairy products**, or **cultured milk products**, are dairy foods that have been fermented with lactic acid bacteria such as *Lactobacillus*, *Lactococcus*, and *Leuconostoc*. The fermentation process increases the shelf life of the product, while enhancing the taste and improving the digestibility of milk. There is evidence that fermented milk products have been produced since around 10,000 BC.

The general process by which fermented milk products are made begins with a preliminary treatment of milk which may include clarification, fat separation and standardization, and evaporation. Processing follows next, with de-aeration, homogenization, and pasteurization. The milk is then cooled to the appropriate fermentation temperature and starter cultures are added.

Starter cultures differ for each product. They consist of microorganisms added to the milk to provide specific characteristics in the finished fermented milk product in a controlled and predictable manner. The primary function of lactic acid starters is to ferment lactose into lactic acid, but they may also contribute to flavour, aroma and alcohol production, while inhibiting spoilage microorganisms. A single strain of bacteria may be added, or a mixture of several microorganisms may be introduced. The bacteria, yeasts and moulds work at different temperatures as well. Thermophilic lactic acid fermentation favour hot temperatures (40-45°C) while mesophilic lactic acid fermentation occurs at cooler temperatures (25 and 40°C).

As the starter cultures grow within the milk, fermentation takes place. Fermentation is the chemical conversion of carbohydrates into alcohols or acids. In fermented milk products both alcohol and lactic acid may be produced, like in kefir and koumiss, or just lactic acid, like in sour cream. The bacteria ingest the lactose (milk sugar), and release lactic acid as waste causing the acidity to increase. This rise in acidity causes the milk proteins to denature (unfold) and tangle themselves into masses (curds) while also inhibiting the growth of other organisms that are not acid tolerant.

Functional Properties

Fermented milk products have numerous functional properties:

- **Preservation:** bacteria are inhibited from growing through pH reduction when lactic acid is formed, and shelf life is increased
- **Flavour Enhancement:** the sour characteristic of fermented milk products comes from fermentation products (lactic acid, diacetyl, carbon dioxide, ethanol); these products act as excellent flavour carriers for herbs, spices and other flavourings
- **Texture Enhancement:** some fermented milk products (sour cream or crème fraîche) can add body and thickness to sauces, dips or vinaigrettes
- **Reducing Caloric Content:** many fermented milk products come in low fat or fat free varieties and can be used to substitute for higher fat ingredients
- **Emulsification:** milk proteins help stabilize fat emulsions in salad dressings, soups and cakes
- **Foaming and Whipping:** crème fraîche is capable of being whipped like whip cream
- **Nutritional benefits:** fermented milk products may contain probiotics (bacteria that are beneficial to health) as well as many vitamins and minerals.

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❖ Acidophilus milk

Acidophilus milk, sometimes called sweet acidophilus milk, has *Lactobacillus acidophilus* bacteria added to it, giving it a tangy flavor and thickened texture. This cultured product is usually low in fat and has a longer shelf life than ordinary milk. Many people believe it can benefit digestion and prevent allergies due to the activity of the acidophilus bacteria in the intestines.

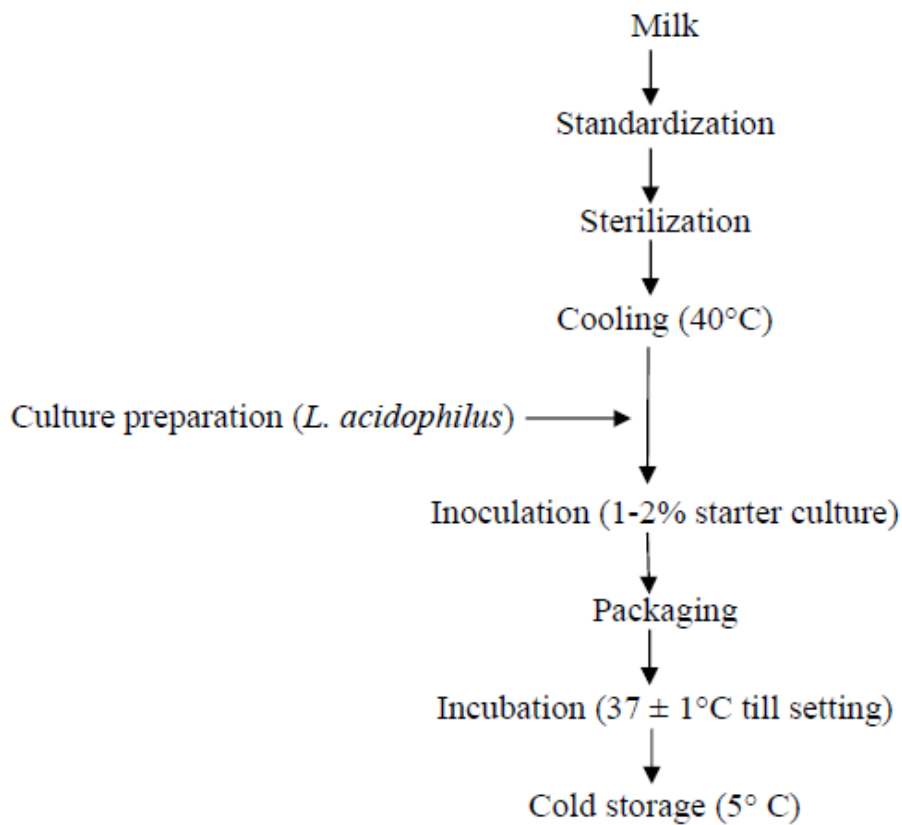


Starter culture

Starter cultures that contain *L. acidophilus* are available, although they often contain other strains, including *Streptococcus thermophilus* and *Lactobacillus bulgaricus*,

Preparation of acidophilus milk

The milk for this product can be skimmed from full cream milk but because *L. acidophilus* does not grow well in milk and would be easily overgrown by usual microflora, the base milk has to be virtually sterile when the culture is added. The milk is then left to incubate at 37°C for 12-16 h or till the acidity of the product reaches around 0.8 to 0.9 per cent (as lactic acid). Consequently, the optimum acidity is achieved by cooling the milk to 5°C or less and halting any further activity by the culture. The culture could generate up to 1.0 to 2.0 per cent lactic acid, but the impact of such levels on cell viability over 2-3 weeks can be devastating in a low solid product. After cooling, the acidophilus milk is bottled and consumed under chilled conditions. Acidophilus milk has shelf life of two weeks under refrigeration. Steps involved in preparation of acidophilus milk are given in Fig.



Nutritional Information

Generally speaking, acidophilus milk is comparable to regular milk of the same type. Most of the commercially available products have about 1% milkfat, providing about 110 calories per 8 ounces (236 mL); regular 1% milk has about 102 calories. Both have about the same amount of protein (8 grams) and calcium (about 30% of the recommended daily allowance).

Storage

Like other milk products, acidophilus milk should be checked regularly for changes in texture, color, or smell. If the milk begins to smell different or change color, it should be discarded. Active bacteria in the milk can continue reproducing as the product ages, causing it to become dangerous to consume. It is important to keep this type of milk chilled to minimize bacterial activity. When homemade, this milk should be used within a week; commercial products may have a longer expiration date.

Health Benefits

Acidophilus bacteria is considered a probiotic, meaning that they are usually beneficial to human health. Some people believe that consuming products that contain live active cultures can help to treat digestive problems and yeast infections. Acidophilus bacteria is said to increase the number of beneficial intestinal and vaginal bacteria when eaten. Dairy cultures typically contain a smaller amount of probiotic bacteria when compared to acidophilus supplements in pill or powder form.

This beverage is used in place of regular cow's milk for some individuals who are lactose intolerant. During fermentation, the bacteria feed on the lactose sugar in the milk, breaking some of it down. For people who are lactose intolerant, that means that their bodies may have an easier time digesting this milk. Acidophilus milk is only lightly fermented, however, so it does still contain milk sugar; this can cause gas and bloating in some people.

Acidophilus milk is also sometimes recommended for use with infants who are old enough to drink cow's milk. One theory holds that, when consumed by infants, acidophilus milk might reduce the chances of the child of developing allergies later in life. There is no proof that drinking this milk will cure an already existing allergy.

Some studies also suggest that this type of milk may help lower cholesterol levels. The bacteria in the intestines may help prevent cholesterol in food from being absorbed so it does not enter the bloodstream.

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❖ Yogurt

Yogurt is a fermented milk product that contains the characteristic bacterial cultures *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. All yogurt must contain at least 8.25% solids not fat. Full fat yogurt must contain not less than 3.25% milk fat, lowfat yogurt not more than 2% milk fat, and nonfat yogurt less than 0.5% milk.

The two styles of yogurt commonly found in the grocery store are set type yogurt and swiss style yogurt. Set type yogurt is when the yogurt is

packaged with the fruit on the bottom of the cup and the yogurt on top. Swiss style yogurt is when the fruit is blended into the yogurt prior to packaging.

Ingredients

The main ingredient in yogurt is milk. The type of milk used depends on the type of yogurt – whole milk for full fat yogurt, lowfat milk for lowfat yogurt, and skim milk for nonfat yogurt. Other dairy ingredients are allowed in yogurt to adjust the composition, such as cream to adjust the fat content, and nonfat dry milk to adjust the solids content. The solids content of yogurt is often adjusted above the 8.25% minimum to provide a better body and texture to the finished yogurt. The CFR contains a list of the permissible dairy ingredients for yogurt.

Stabilizers may also be used in yogurt to improve the body and texture by increasing firmness, preventing separation of the whey (syneresis), and helping to keep the fruit uniformly mixed in the yogurt. Stabilizers used in yogurt are alginates (carageenan), gelatins, gums (locust bean, guar), pectins, and starch.

Sweeteners, flavors and fruit preparations are used in yogurt to provide variety to the consumer. A list of permissible sweeteners for yogurt is found in the CFR.

Bacterial Cultures

The main (starter) cultures in yogurt are *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. The function of the starter cultures is to ferment lactose (milk sugar) to produce lactic acid. The increase in lactic acid decreases pH and causes the milk to clot, or form the soft gel that is characteristic of yogurt. The fermentation of lactose also produces the flavor compounds that are characteristic of yogurt. *Lactobacillus bulgaricus* and *Streptococcus thermophilus* are the only 2 cultures required by law (CFR) to be present in yogurt.

Other bacterial cultures, such as *Lactobacillus acidophilus*, *Lactobacillus subsp. casei*, and Bifido-bacteria may be added to yogurt as probiotic cultures. Probiotic cultures benefit human health by improving lactose digestion, gastrointestinal function, and stimulating the immune system.



General Manufacturing Procedure

1. Adjust Milk Composition & Blend Ingredients
2. Pasteurize Milk
3. Homogenize
4. Cool Milk
5. Inoculate with Starter Cultures
6. Hold
7. Cool
8. Add Flavors & Fruit
9. Package

1. Adjust Milk Composition & Blend Ingredients

Milk composition may be adjusted to achieve the desired fat and solids content. Often dry milk is added to increase the amount of whey protein to provide a desirable texture. Ingredients such as stabilizers are added at this time.

2. Pasteurize Milk

The milk mixture is pasteurized at 185°F (85°C) for 30 minutes or at 203°F (95°C) for 10 minutes. A high heat treatment is used to denature the whey (serum) proteins. This allows the proteins to form a more stable gel, which prevents separation of the water during storage. The high heat treatment also further reduces the number of spoilage organisms in the milk to provide a better environment for the starter cultures to grow. Yogurt is pasteurized before the starter cultures are added to ensure that the cultures remain active in the yogurt after fermentation to act as probiotics; if the yogurt is pasteurized after fermentation the cultures will be inactivated.

3. Homogenize

The blend is homogenized (2000 to 2500 psi) to mix all ingredients thoroughly and improve yogurt consistency.

4. Cool Milk

The milk is cooled to 108°F (42°C) to bring the yogurt to the ideal growth temperature for the starter culture.

5. Inoculate with Starter Cultures

The starter cultures are mixed into the cooled milk.

6. Hold

The milk is held at 108°F (42°C) until a pH 4.5 is reached. This allows the fermentation to progress to form a soft gel and the characteristic flavor of yogurt. This process can take several hours.

7. Cool

The yogurt is cooled to 7°C to stop the fermentation process.

8. Add Fruit & Flavors

Fruit and flavors are added at different steps depending on the type of yogurt. For set style yogurt the fruit is added in the bottom of the cup and then the inoculated yogurt is poured on top and the yogurt is fermented in the cup. For swiss style yogurt the fruit is blended with the fermented, cooled yogurt prior to packaging.

9. Package

The yogurt is pumped from the fermentation vat and packaged as desired.

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❖ **Cultured butter milk (Slightly acid fermented milks)**

Buttermilk refers to a number of dairy drinks. Originally, buttermilk was the liquid left behind after churning butter out of cream. This type of buttermilk is known as *traditional buttermilk*.

The term *buttermilk* also refers to a range of fermented milk drinks, common in warm climates

This fermented dairy product known as *cultured buttermilk* is produced from cow's milk and has a characteristically sour taste caused by lactic acid bacteria. This variant is made using one of two species of bacteria either *Lactococcus lactis* or *Lactobacillus bulgaricus*, which creates more tartness.

The tartness of buttermilk is due to acid in the milk. The increased acidity is primarily due to lactic acid produced by lactic acid bacteria while fermenting lactose, the primary sugar in milk. As the bacteria produce lactic acid, the pH of the milk decreases and casein, the primary milk protein, precipitates, causing the curdling or clabbering of milk. This process makes buttermilk thicker than plain milk. While both traditional and cultured buttermilk contain lactic acid, traditional buttermilk tends to be less viscous, whereas cultured buttermilk is more viscous.

Buttermilk can be drunk straight, and it can also be used in cooking. Soda bread is bread in which the acid in buttermilk reacts with the rising agent, sodium bicarbonate, to produce carbon dioxide which acts as the leavening agent. Buttermilk is also used in marination, especially of chicken and pork, whereby the lactic acid helps to tenderize, retain moisture, and allows added flavors to permeate throughout the meat.



Traditional buttermilk

Originally, buttermilk referred to the liquid left over from churning butter from cultured or fermented cream. Traditionally, before cream could be skimmed from whole milk, the milk was left to sit for a period of time to allow the cream and milk to separate. During this time, naturally occurring lactic acid-producing bacteria in the milk fermented it. This facilitates the butter churning process, since fat from cream with a lower pH coalesces more readily than that of fresh cream. The acidic environment also helps prevent potentially harmful microorganisms from growing, increasing shelf-life. However, in establishments that used cream separators, the cream was hardly acidic at all.

Cultured buttermilk

Commercially available cultured buttermilk is milk that has been pasteurized and homogenized (with 1% or 2% fat), and then inoculated with a culture of *Lactococcus lactis* plus *Leuconostoc citrovorum* to simulate the naturally occurring bacteria in the old-fashioned product. Some dairies add colored flecks of butter to cultured buttermilk to simulate residual flecks of butter that can be left over from the churning process of traditional buttermilk.

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Kefir (Acid alcoholic fermented milk)

Kefir or **kephir** is a fermented milk drink made with kefir "grains", a yeast/bacterial fermentation starter. It is prepared by inoculating cow, goat, or sheep milk with kefir grains.

Traditional kefir is fermented at ambient temperatures, generally overnight. Fermentation of the lactose yields a sour, carbonated, slightly alcoholic beverage, with a consistency and taste similar to thin yogurt.

The kefir grains initiating the fermentation are a combination of lactic acid bacteria and yeasts in a matrix of proteins, lipids, and sugars. This symbiotic culture of bacteria and yeast forms "grains" that resemble cauliflower. A complex and highly variable community of lactic acid bacteria and yeasts can be found in these grains, although some predominate; *Lactobacillus* species are always present.

Kefir grains contain kefiran, a water-soluble polysaccharide, which imparts a creamy texture and feeling in the mouth. The grains range in color from white (the acceptable color of healthy grains) to yellow; the latter is the outcome of leaving the grains in the same milk during fermentation for longer than the optimal 24-hour period, and continually doing so over many batches. Grains may grow to the size of walnuts, and in some cases larger.

During fermentation, changes in composition of nutrients and other ingredients occur. Lactose, the sugar present in milk, is broken down mostly to lactic acid (25%) by the lactic acid bacteria, which results in acidification of the product. Propionibacteria further break down some of the lactic acid into propionic acid (these bacteria also carry out the same fermentation in Swiss cheese). A portion of lactose is converted to kefiran, which is indigestible by gastric digestion. Other substances that contribute to the flavor of kefir are pyruvic acid, acetic acid, diacetyl and acetoin (both of which contribute a "buttery" flavor), citric acid, acetaldehyde and amino acids resulting from protein breakdown.

The slow-acting yeasts, late in the fermentation process, break lactose down into ethanol and carbon dioxide. Depending on the process, ethanol concentration can be as high as 1–2% (achieved by small-scale dairies early in the 20th century), with the kefir having a bubbly appearance and carbonated taste. This makes kefir different from yogurt and most other sour milk products where only bacteria ferment the lactose into acids.



Nutritional composition

Kefir products contain nutrients in varying amounts from negligible to significant, including dietary minerals, vitamins, essential amino acids, and conjugated linoleic acid, in amounts similar to unfermented cow, goat or sheep milk. At a pH of 4.2 - 4.6, Kefir is composed mainly of water and by-products of the fermentation process, including carbon dioxide and ethanol.

Typical of milk, several dietary minerals are found in kefir, such as calcium, iron, phosphorus, magnesium, potassium, sodium, copper, molybdenum, manganese, and zinc in amounts that have not been standardized to a reputable nutrient database. Also similar to milk, kefir contains vitamins in variable amounts, including vitamin A, vitamin B₁ (thiamine), vitamin B₂ (riboflavin), vitamin B₃ (niacin), vitamin B₆ (pyridoxine), vitamin B₉ (folic acid), vitamin B₁₂ (cyanocobalamin), vitamin C, vitamin D, and vitamin E. Essential amino acids found in kefir include methionine, cysteine, tryptophan, phenylalanine, tyrosine, leucine, isoleucine, threonine, lysine, and valine, as for any milk product

Probiotics

Probiotic bacteria found in kefir products include *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, *Streptococcus thermophilus*,

Lactobacillus delbrueckii subsp. bulgaricus, *Lactobacillus helveticus*, *Lactobacillus kefirifaciens*, *Lactococcus lactis*, and *Leuconostoc* species.

Production

The traditional or artisanal method of making kefir is to add 2–10% kefir grains directly to milk in a sealed goatskin leather bag, which is traditionally agitated one or more times a day. Today the leather bag is replaced with a corrosion-resistant container such as a glass jar. It is not filled to capacity, allowing room for some expansion as the carbon dioxide gas produced causes the liquid level to rise. Non-lightproof containers are stored in the dark to prevent degradation of light-sensitive vitamins. After a period of fermentation lasting around 24 hours, ideally at 20–25 °C (68–77 °F), the grains are strained from the liquid using a corrosion-resistant (stainless steel or plastic) utensil, and kept as the starter for another batch.

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Labneh / **Strained yogurt** / **Greek yogurt** / **yogurt cheese** / skyr (Iceland) / shrikhand (India) (Fermented milk production with extended self life)

Labneh is yogurt that has been strained to remove its whey, resulting in a thicker consistency than unstrained yogurt, while preserving yogurt's distinctive, sour taste. Like many types of yogurt, strained yogurt is often made from milk that has been enriched by boiling off some of its water content, or by adding extra butterfat and powdered milk. In Europe and North America, it is often made with low-fat or fat-free yogurt. In Iceland a similar product named skyr is produced.

Strained yogurt is sometimes marketed in North America as "Greek yogurt" and in Britain as "Greek-style yoghurt".

Due to the straining process to remove excess whey, even non-fat varieties of strained yogurt are much thicker, richer, and creamier than yogurts that have not been strained. Since the straining process removes the whey, or fluid, from the milk solids, it requires substantially more plain yogurt to produce a cup of strained yogurt, so the cost to make it is increased accordingly. Thickeners, such as pectin, locust bean gum, starches, guar gum, etc., listed in the ingredients indicate straining was not the method used to consolidate the milk solids.

Nutrition

Strained yogurt contains a higher protein density than regular yogurt. The protein in strained yogurt is largely casein protein. Labneh has 2.5 times higher protein content, 50% more minerals, and a considerably larger number of viable microorganisms than common yoghurt. In addition, the lactose concentration of labneh is low (approximately 6%) due to its fermentation into lactic acid, which makes it more suitable for use by lactose intolerant individuals.

Bacteria used

The genera *Lactobacillus* and *Bifidobacterium*,



Production

Yoghurt which has been strained in a cloth or paper bag or filter, traditionally made of muslin, to remove the whey, giving a consistency between that of yoghurt and cheese, while preserving yoghurt's distinctive sour taste. Like many yoghurts, strained yoghurt is often made from milk which has been enriched by boiling off some of the water content, or by adding extra butterfat and powdered milk.

The characteristic thick texture and high protein content are achieved through either or both of two processing steps. The milk may be concentrated by ultrafiltration to remove a portion of the water before addition of yogurt cultures. Alternatively, after culturing, the yogurt may be centrifuged or membrane-filtered to remove whey, in a process analogous to the traditional straining step.

The liquid resulting from straining yogurt is called "acid whey" and is composed of water, yogurt cultures, protein, a slight amount of lactose, and lactic acid.

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❖ Starter cultures for fermented dairy products

Definition

Dairy starter cultures are carefully selected microorganisms, which are deliberately added to milk to initiate and carry out desired fermentation under controlled conditions in the production of fermented milk products. Most of them belong to lactic acid bacteria (*Lactococcus*, *Lactobacillus*, *Streptococcus* and *Leuconostocs*). In some cases, few non-lactic starters (bacteria, yeast and mold) are also used along with lactic acid bacteria during manufacturing of specific fermented milk products, such as kefir, kumiss and mold ripened cheeses.

Functions of Starter Cultures

Starter cultures can be used as single strain, mixed strain and multiple strains depending upon the type of products to be prepared. The ability of starter culture to perform its functions efficiently during manufacture of fermented dairy foods depends primarily on purity and activity of starter cultures.

The major roles of starter culture during fermentation of milk are:

- a) Production of primarily lactic acid and few other organic acids, such as formic acid and acetic acid.
- b) Coagulation of milk and changes in body and texture in final products.
- c) Production of flavouring compounds, e.g., diacetyl, acetoin and acetaldehyde.
- d) Help in ripening of cheeses by their enzymatic activities.
- e) Produce antibacterial substances in the finished product.
- f) In addition, they may possess functional properties.

Thus, an ideal starter culture should be selected for the preparation of various fermented milks with the following characteristics.

1. It should be quick and steady in acid production.
2. It should produce product with fine and clean lactic flavour.
3. It should not produce any pigments, gas, off-flavour and bitterness in the finished products.
4. Should be associative in nature in product development.

Types of Starter Cultures

There are two major groups of starter cultures which are used in the preparation of fermented milk products classified on the basis of their

- (a) Physiological and growth characteristics, such as
 - (i) Mesophilic starter culture
 - (ii) Thermophilic starter culture
- (b) Biochemical characteristics such as
 - (i) Homofermentative lactic acid bacteria
 - (ii) Heterofermentative lactic acid bacteria.

Classification on the basis of physiological and growth characteristics.

Mesophilic starter culture

These cultures have optimum temperature for growth between 20 to 30°C and include *Lactococcus* and *Leuconostoc*. These mesophilic lactic cultures are used in the production of many cheese varieties where important characteristics are:

1. Acid producing activity
2. Gas production, and
3. Production of enzymatic activity for cheese ripening, e.g., proteases and peptidases enzymes.

The importance of fermented milk derived from mesophilic fermentation are consistency which is due to the lactic acid coagulation of the milk proteins and aroma and flavour produced by citric acid and lactose fermentation.

Thermophilic starter culture

These cultures have optimum temperature for growth between 37 to 45°C. Thermophilic cultures are generally employed in the production of yoghurt, acidophilus milk, swiss type cheese. Thermophilic cultures include species of *Streptococcus* and *Lactobacillus*. These cultures grow in association with milk and form the typical yoghurt starter culture. This growth is considered symbiotic because the rate of acid development is greater when two bacteria are grown together as compared to single strains.

Thermophilic starter cultures are microaerophilic and fresh heated milk should be used to achieve a better growth of the culture since heat treatment reduce amount of oxygen in the product. The important metabolic activities of thermophilic cultures in development of fermented milk products are:

- Acid production, e.g. lactic acid
- Flavour compounds, e.g., acetaldehyde
- Ropiness and consistency, e.g., polysaccharides
- Proteolytic and lipolytic activities, e.g., peptides, amino acids, fatty acids
- Possesses therapeutic significance, such as
 - (a) Improvement of intestinal organisms,
 - (b) Produce antibacterial substances, and
 - (c) Improve immunity.

Classification on the basis of biochemical activities

Homofermentative lactic starter

These lactic acid bacteria are characterized for their ability to ferment lactose almost exclusively to lactic acid while pentoses and gluconate are not fermented. The examples of these cultures are: *Lb. acidophilus*, *Lb. bulgaricus*.

Heterofermentative lactic starter

Main characteristics of these bacteria are ability to ferment hexoses and pentoses to lactic acid, acetic acid, alcohol and CO₂. The examples of these cultures are *Lb. brevis*, *Lb. fermentum*.

Streptococcus thermophilus, Lactobacillus bulgaricus

Streptococcus thermophilus also known as *Streptococcus salivarius* subsp. *thermophilus* is a gram-positive bacterium, and a fermentative facultative anaerobe, It tests negative for cytochrome, oxidase, and catalase, and positive for alpha-hemolytic activity. It is non-motile and does not form endospores.^[3] *S. thermophilus* is fimbriated. It has an optimal growth temperature range of 35 - 42 °C.

Lactobacillus delbrueckii subsp. *bulgaricus* (until 2014 known as *Lactobacillus bulgaricus*) is one of several bacteria used for the production of yogurt. It is also found in other naturally fermented products. It is a gram-positive rod that may appear long and filamentous. It is non-motile and does not form spores. It is regarded as aciduric or acidophilic, since it requires a low pH (around 5.4–4.6) to grow effectively.

Lactobacillus delbrueckii subsp. *bulgaricus* is commonly used alongside *Streptococcus thermophilus*^[2] as a starter for making yogurt. The two species work in synergy, with *L. d. bulgaricus* producing amino acids from milk proteins, which are then used by *S. thermophilus*.

Protocooperation and antibiosis are the most important interactions in the growth of the yogurt bacteria *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. Protocooperation can be understood as a mutual beneficial relationship. The associative growth of these two cultures results in a higher lactic acid production and development of flavour than what is possible from a single culture.

The *S. thermophilus* forms acid much slower, especially in milk, which lacks some amino acids. Hence *L. bulgaricus* liberates small peptides and amino acids, mainly valine, of which serves to enhance the growth of *S. thermophilus*. In return the cocci enhance the growth of *L. bulgaricus* by the formation of formic acid from pyruvic acid under anaerobic conditions. The rod shaped *L. bulgaricus* are more proteolytic (metabolically more efficient in the breakdown of proteins) than their symbiont counterpart. Under these protocooperative stimulations during combined growth of the yogurt bacteria, lactic acid is produced at a much faster rate than by that of individual pure cultures. Antibiosis is observed after certain acidity is reached. After this point, growth of *S. thermophilus* is halted. However, *L. bulgaricus* are less susceptible to acid and continues to grow. After about 3 hours post incubation, the relative amount of bacteria is equal. The more acid tolerant bacteria, *L. bulgaricus*, begin to overshadow its counterpart. *S. thermophilus* is inhibited at pH values between 4.2-4.4, while *L. bulgaricus* can tolerate pH values as low as 3.5-3.8.

Metabolism of starter cultures, biochemical changes in fermented milk, Metabolism in Dairy Starter Cultures

When starter culture grows in milk, it affects the constituents of milk and brings fermentative metabolic changes. It will produce different intermediates or end products, which give typical attributes to fermented milk. The roles played by starters during fermentation of milks are:

- Produce lactic acid
- Bring about coagulation of protein and form gel

- Produce volatile flavour compounds like diacetyl, acetaldehyde and several intermediate compounds.
- Possess proteolytic and lipolytic activities.
- Produce other compounds like CO₂, alcohol, propionic acid, which are essential in products like kefir, Swiss cheese.
- Control the growth of pathogens and spoilage organisms.
- Some dietary cultures like *Lb. acidophilus*, give health benefits and produces antibacterial substances.
- Help in texturizing and ripening of cheese.

1. Carbohydrate metabolism / Fermentation of lactose to lactic acid

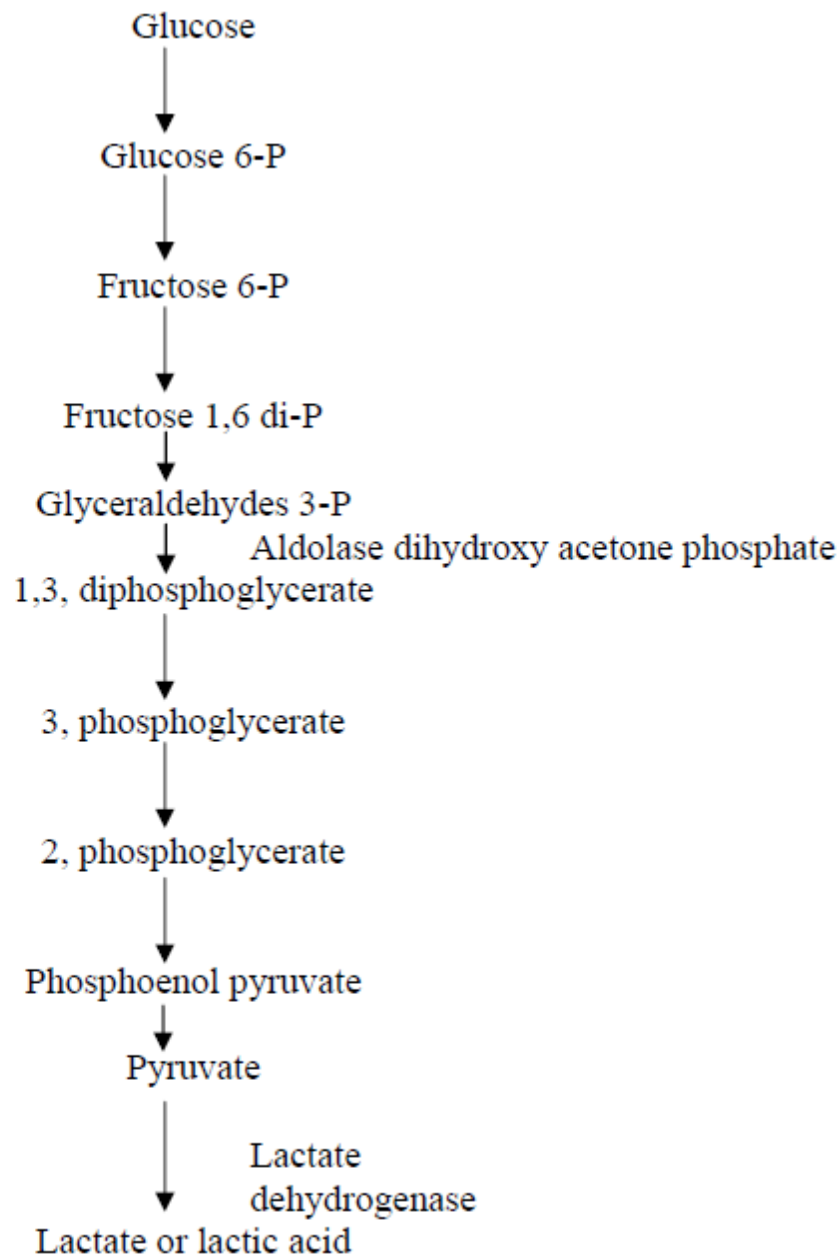
Lactose is the major carbohydrate of milk, which is utilised to varying extent by starters. The lactic acid bacteria containing aldolase, i.e., Streptococci, Lactococci, Pediococci and obligately homofermentative Lactobacilli carry out homolactic fermentation with production of only lactic acid as end product. The lactic acid bacteria containing phosphoketolase can be divided in 2 groups. The first i.e. Leuconostocs and obligately heterofermentative Lactobacilli, follow 6-P-gluconate pathway, with production of equimolar amount of CO₂, lactate and acetate, while Bifidobacteria, follow bifidus pathway, with formation of acetate and lactate in 3:2 molar ratio. An inducible phosphoketolase is carried by the facultative heterofermentative Lactobacilli, which makes possible for the production of lactate and acetate from pentoses.

Lactose in milk is hydrolysed by metabolic activity of bacteria. Approximately 45-50% lactose; 16–20% galactose and 0.6-0.8% glucose are obtained from lactose hydrolysis on the basis of on average 5% lactose in milk. Lactose hydrolysis takes place due to β -galactosidase production by lactic acid bacteria. The importance of lactose is due to the lactic acid produced from the hydrolysis of lactose, which leads to a pH range in the bowel inhibiting the growth of putrefactants. In addition to this, lactic acid is important for organoleptic properties and calcium absorption.

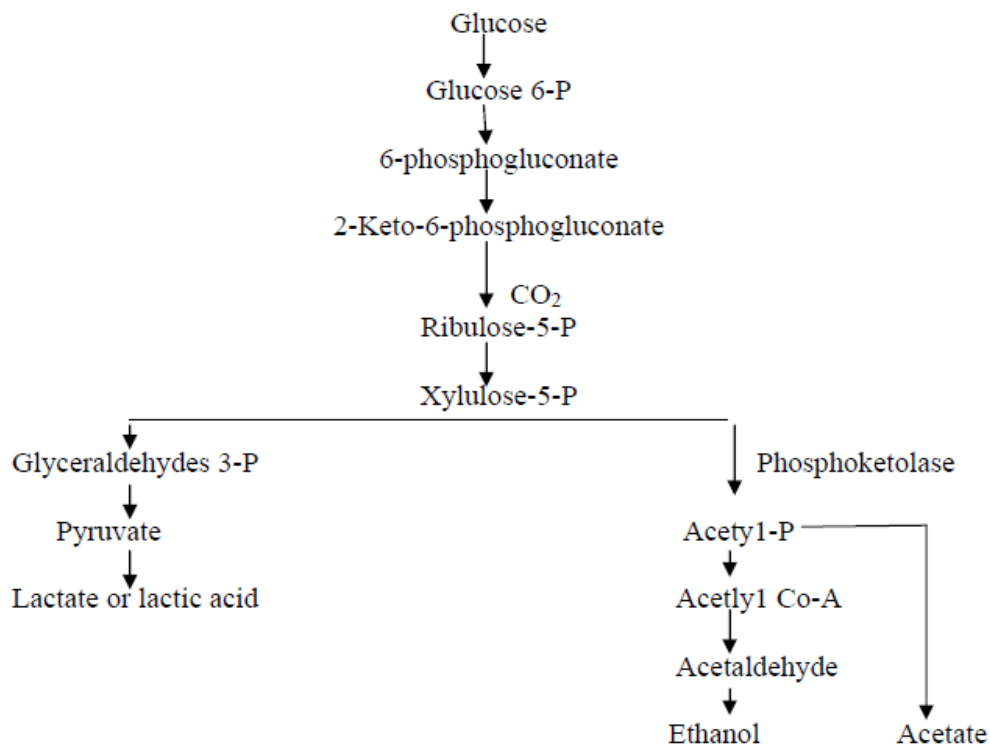
Lactic acid bacteria have two different mechanism to take up lactose from the medium and its subsequent hydrolysis:

A. Most of the Lactobacilli, Leuconostocs and *S. thermophilus* take up lactose through a specific permease enzyme located in cell membrane. The lactose

inside the cell is then splitted by enzyme B-galactosidase into glucose and galactose. The galactose is converted to glucose by 'Leloir pathway' and together with glucose is fermented by glycolysis.



Glycolytic pathway of homo fermentative lactic acid bacteria



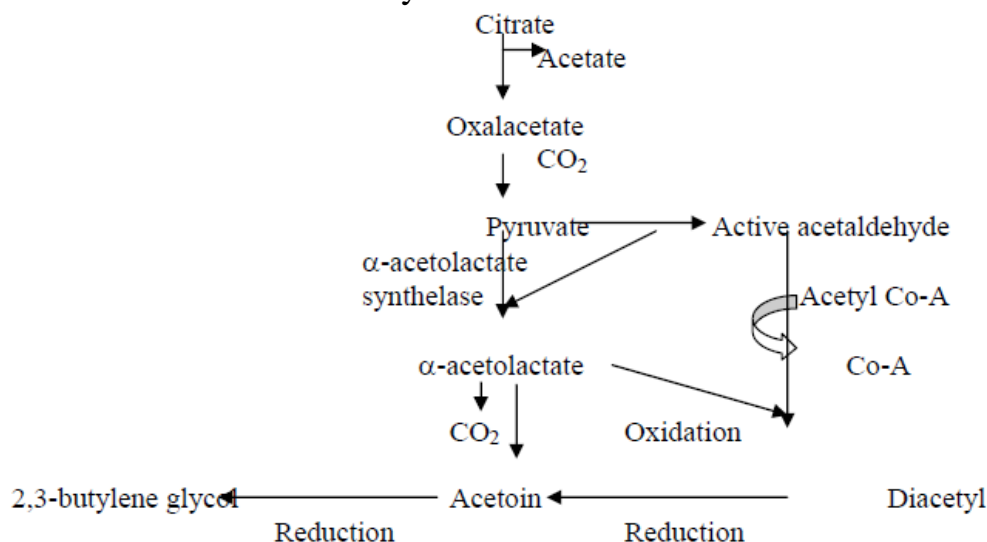
B. Lactococci and a few lactobacilli like *Lb. casei*, take up lactose and galactose by the action of phosphoenol pyruvate dependant phosphotransferase system, which involves catalytic activity of four specific proteins. The lactose is phosphorylated while in transportation and is hydrolysed by β -phosphogalactosidase into glucose and galactose-6-P. The galactose is utilized to lactic acid by tagalose-6-P pathway. *S. thermophilus*, *Lb. delbrueckii* subsp. *bulgaricus* and occasionally *Lc. lactis* do not metabolize galactose, but excrete out into the medium Fig. pathways for lactose and galactose utilization by different lactic acid bacteria has been depicted in Fig.

Propionibacteria ferment lactic acid, carbohydrates and polyhydroxy alcohols to propionic acid, acetic acid and carbon dioxide (CO_2). Conversion of lactic acid to propionic acid gives characteristic sweet flavour in Swiss cheese, while CO_2 helps in eye formation, a typical regular holes in cheese body, which is essential in Swiss cheese.

2. Citrate metabolism production of aromatic compounds / flavor compounds

Citrate or citric acid is present in milk in low concentration (average 0.16%) and is metabolized only by flavour producing species of mesophilic cultures, i.e., *Lc. lactis* biovar *diacetylactis* and *Leuconostoc* spp. The metabolites produced i.e. diacetyl, acetoin, acetate and CO₂ are important flavour compounds in fermented milks, cheese and ripened cream butter.

The pathway for citrate utilization by starters in Fig. indicates that there are two ways of producing diacetyl. It is formed by oxidative decarboxylation of α -acetolactate, which is exerted into the milk by bacterial cells. This is a chemical process-taking place in presence of lactic acid at low pH. The other theory states that the diacetyl is formed inside bacterial cell by reaction of acetyl-Co-A and active acetaldehyde.

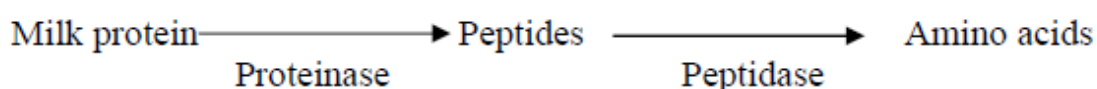


3. Acetaldehyde production

Acetaldehyde is one of the important flavour compounds produced by starter cultures in fermented milks and is a major flavour compound in yoghurt. In mesophilic cultures, the precursor of acetaldehyde is threonine while in thermophilic cultures, the precursor is sugar. Apart from this, the acetaldehyde may also be produced by lactic acid bacteria from nucleic acids, lipids and aromatic compounds in milks.

4. Protein metabolism / hydrolysis of proteins

Lactic acid bacteria are nutritionally fastidious in nature and require several amino acids and vitamins for their growth. Overall proteolytic system of lactic acid bacteria is very weak, but is sufficient to permit exponential growth in milk. The numbers, location and specificity of enzymes acting on milk proteins differ considerably in different strains. Casein is hydrolysed outside the cell-by-cell wall bound or excreted proteinases into oligopeptides. These are further hydrolysed to small peptides and amino acids by membrane bound peptidases as indicated below



Protein metabolism in lactic starters

The proteolytic activity of starter culture is important because it leads to

- (i) Liberation of peptides and amino acids, which affect the physical structure of the product.
- (ii) Many amino acids produced are essential for the growth of several cultures a
- (iii) Peptides and amino acids act as flavour precursors.

Proteolysis in milk takes place by exo- or endo-peptidases of lactic acid bacteria. The biological value of protein increases significantly from a value of 85.4 to 90 per cent. This increase is due to breakdown of protein into peptones, peptides and amino acids. The contents of essential amino acids such as leucine, isoleucine, methionine, phenylalanine, tyrosine, threonine, tryptophane and valine increase considerably which offer special advantages not only to healthy people but also particularly to the physically weak persons. Fermented milks (yoghurt, kefir, dahi) are having higher protein digestibility due to precipitating into fine curd particle by lactic acid that contributes to its higher nutritional value and capacity to regenerate liver tissue. During fermentation and storage the amount of free amino acids increases, particularly lysine, proline, cystine, isoleucine, phenylalanine, and arginine. Due to these biochemical changes in milk protein during fermentation make these products dietetic in nature.

5. Lipid metabolism / hydrolysis of lipids

Starter bacteria are very weakly lipolytic and may possess lipases and esterase that can hydrolyse triglycerides to lower fatty acids. Starters can produce certain volatile fatty acids, ($C_2 - C_6$) from amino acids too. This activity of starter contributes to the flavour in fermented milk products. The homogenization process reduces the size of fat globules, which become digestible. The production of free fatty acids as a consequence of lipolytic activity increases due to lactic acid bacteria as compared to milk. This leads to some physiological effects.

6. Vitamins metabolism / Vit. B content

Milk contains several water or fat-soluble vitamins. When starter cultures are growing in milk, some vitamins may be utilized by them, leading to their decrease. On the other side some vitamins may be synthesized also, leading to increased content in fermented milk. This increase or decrease depends greatly on the strain of starter. However, generally it is reported that yoghurt bacteria synthesize folic acid, niacin and vitamin B6. Propionibacteria are known to produce vitamin B12.

There is more than two fold increase in vitamins of B-group especially thiamine (B1), riboflavin (B2) and nicotinamide as a result of biosynthetic process during milk fermentation. Subsequently, vitamin B2, ascorbic acid and vitamin B1 decrease by approximately one half as they are utilized by the bacteria present.

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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*, *Streptococcus salivarius* subsp. *thermophilus*, *Lactobacillus delbruckii* subsp. *bulgaricus*, and *Lactobacillus helveticus*.

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, or the use of *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 limburger cheese. Examples of molds include *Penicillium camemberti* in camembert and brie, and *Penicillium roqueforti* in blue cheeses.

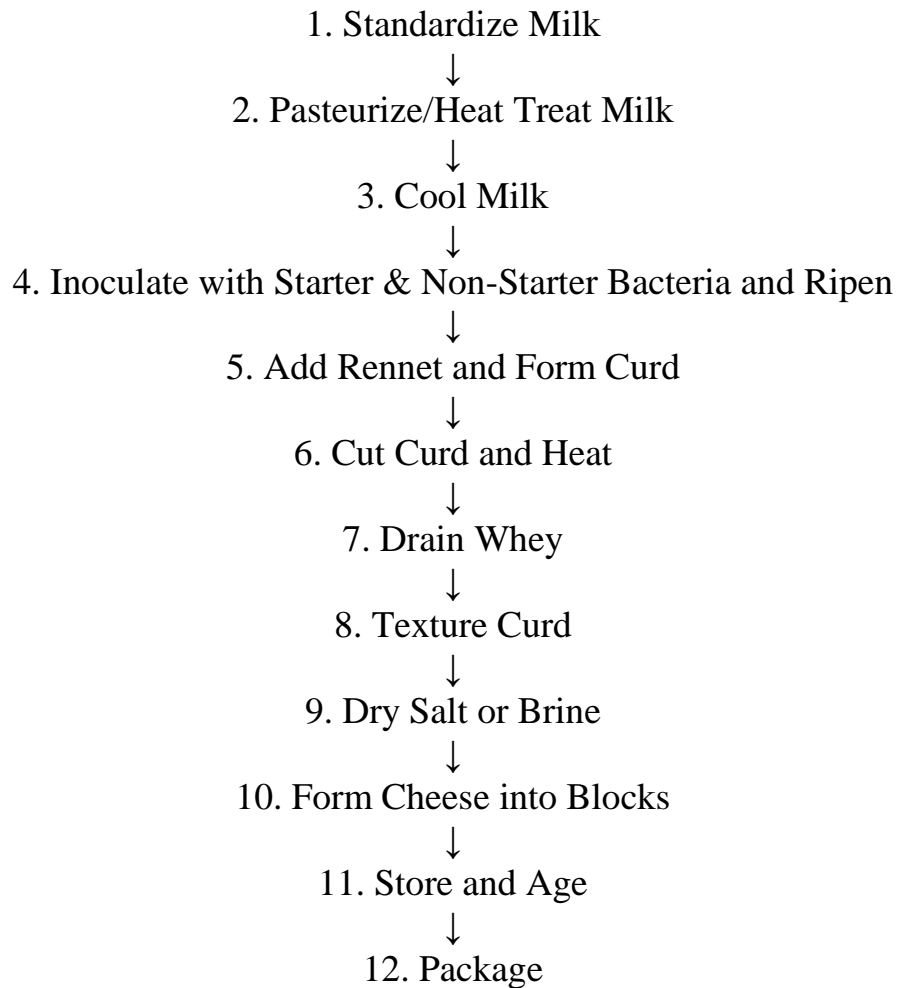
Enzymes

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 cheesemaking 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



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 **cheddaring**. 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.

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