

Food and Industrial Microbiology

Microbiology of Fermented Dairy Products

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September 2006 (Revised)

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Dairy starter cultures, Lactic acid bacteria, Fermented milk products, Probiotics, Therapeutic value

Dairy Starter Culture

The preservation of food by fermentation is one of the oldest methods known to mankind. A typical example is lactic acid fermentation, which is widely used for the preparation of several fermented milk products, such as dahi (curd), yoghurt, acidophilus milk, shrikhand and various varieties of cheeses. In the modern dairy industry, dairy starter cultures are pre-requisite for the production of safe products of uniform quality. Lactic acid bacteria are often called dairy starter cultures, which are used for the production of various fermented milk 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 (Plate 1).

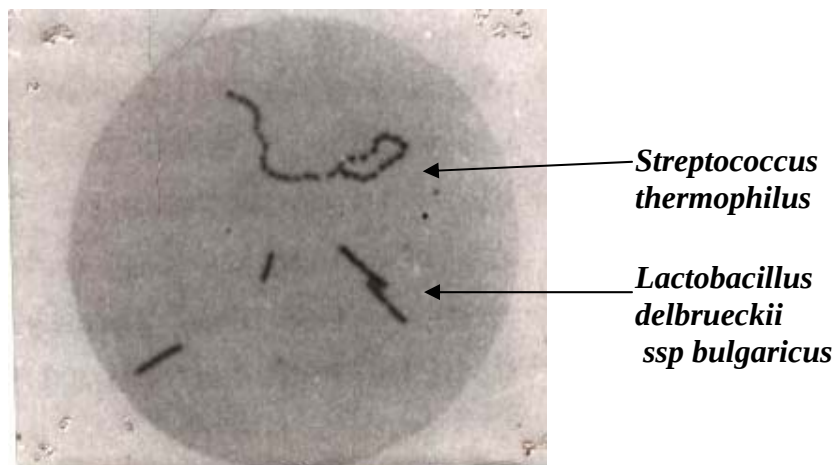


Plate 1 : Yoghurt Cultures

Thermophilic starter cultures are microaerophilic and fresh heated milk should be used to achieve a better growth of the culture since heat treatment reduces 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*.

Characteristics of selected lactic starter culture

1. Genus Lactobacillus

These cultures are Gram positive, rod-shaped bacteria, which are homo or hetero fermentative (Plate 2). Biochemical characteristics of various group of lactobacilli has been shown in Table 1.



Plate 2: Microscopic examination of *Lactobacillus acidophilus* culture

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>

Table-1: Biochemical characteristics of different groups of Lactobacilli

2. Genus *Lactococcus*

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*

3. 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).

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 kefiranoferiens</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

Table 2: Various dairy starter cultures with their metabolites

Flavour producers

Leu. mesenteroides subsp. cremoris
 Leu. mesenteroides subsp. dextranicum
 Leu. lactis

List of various dairy starters with their metabolites are shown in Table 2.

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 intermediary 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

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 (Fig. 1), 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.

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

1. 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. Fig.1.

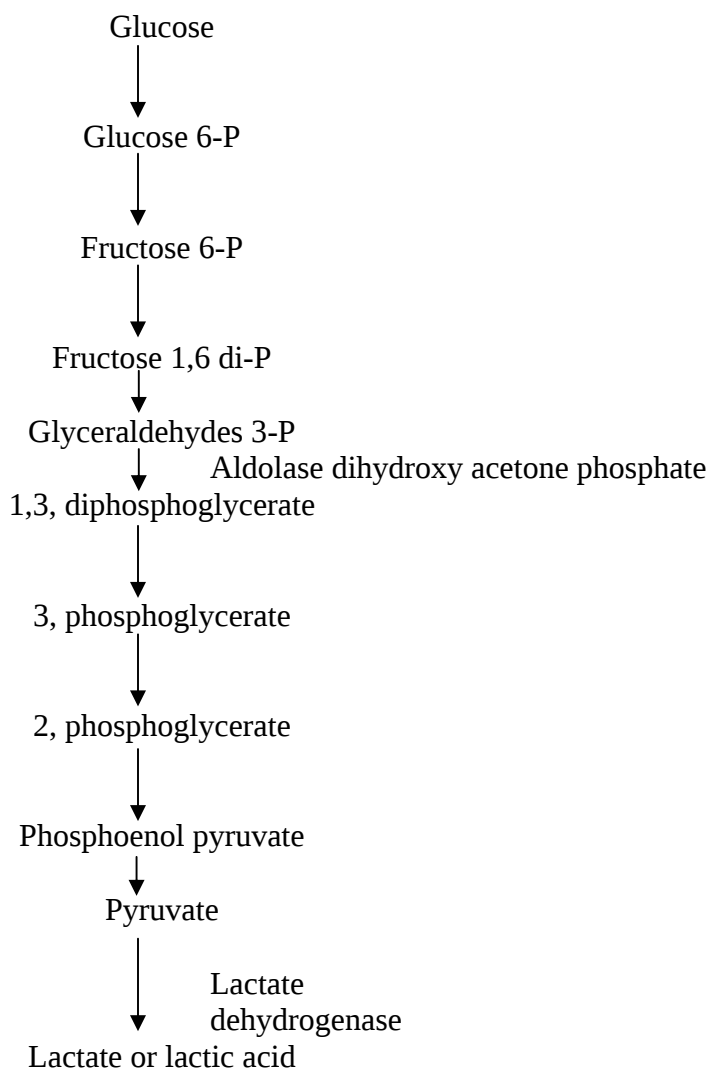


Fig. 1: Glycolytic pathway of homo fermentative lactic acid bacteria

2. 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. 2) pathways for lactose and galactose utilization by different lactic acid bacteria has been depicted in Fig. 3.

Propionibacteria ferment lactic acid, carbohydrates and polyhydroxy alcohols to propionic acid, acetic acid and carbon dioxide (CO₂). Conversion of lactic acid to propionic acid gives characteristic sweet flavour in Swiss cheese, while CO₂ helps in eye formation, a typical regular holes in cheese body, which is essential in Swiss cheese.

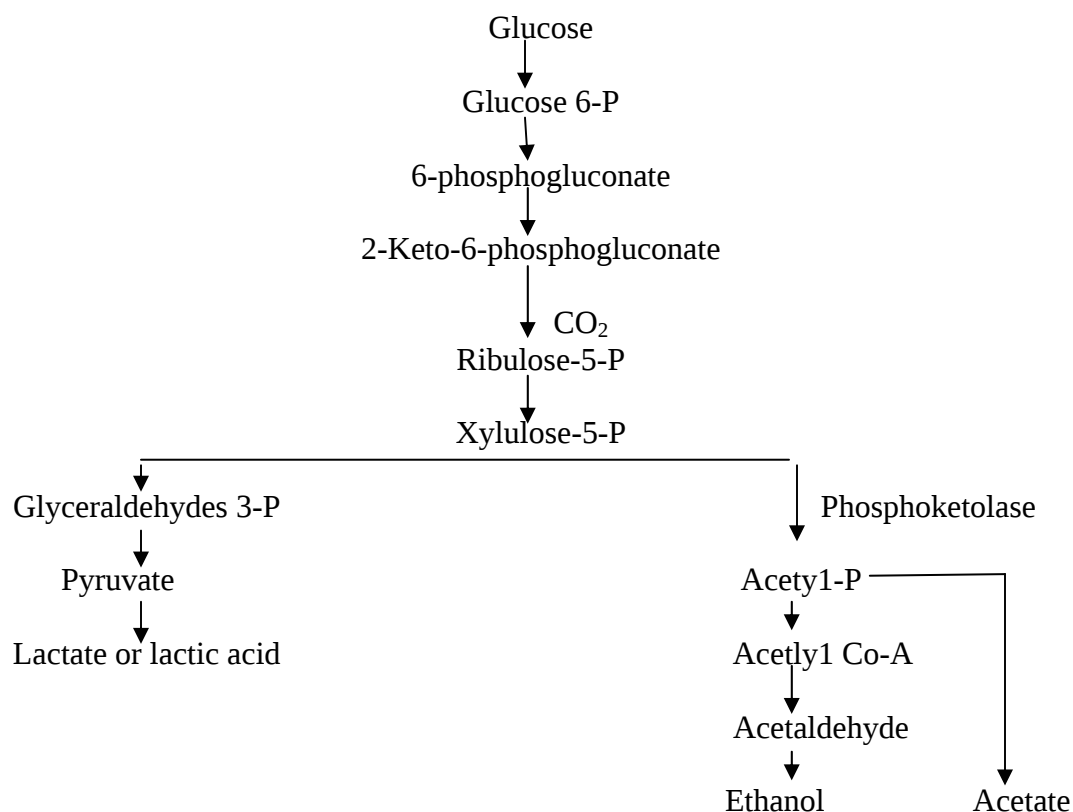


Fig. 2 Metabolic pathway of heterofermentative lactic acid bacteria

2. Citrate metabolism

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. 4 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 (Fig. 5).

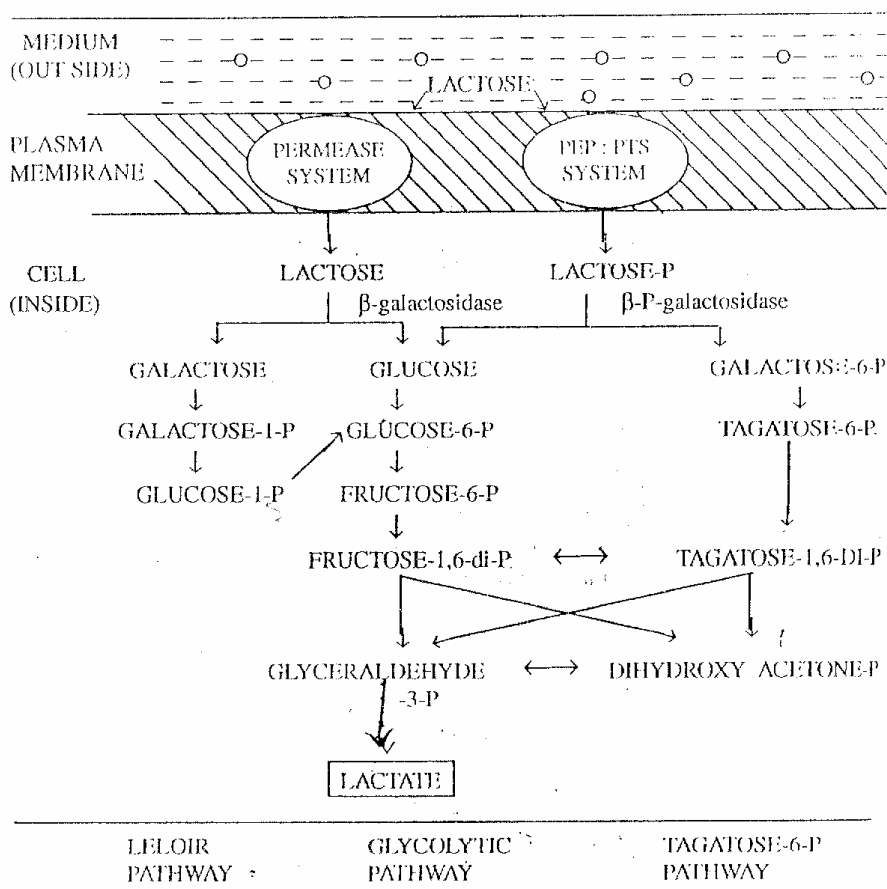


Fig. 3: Lactose and galactose utilization by different lactic acid bacteria

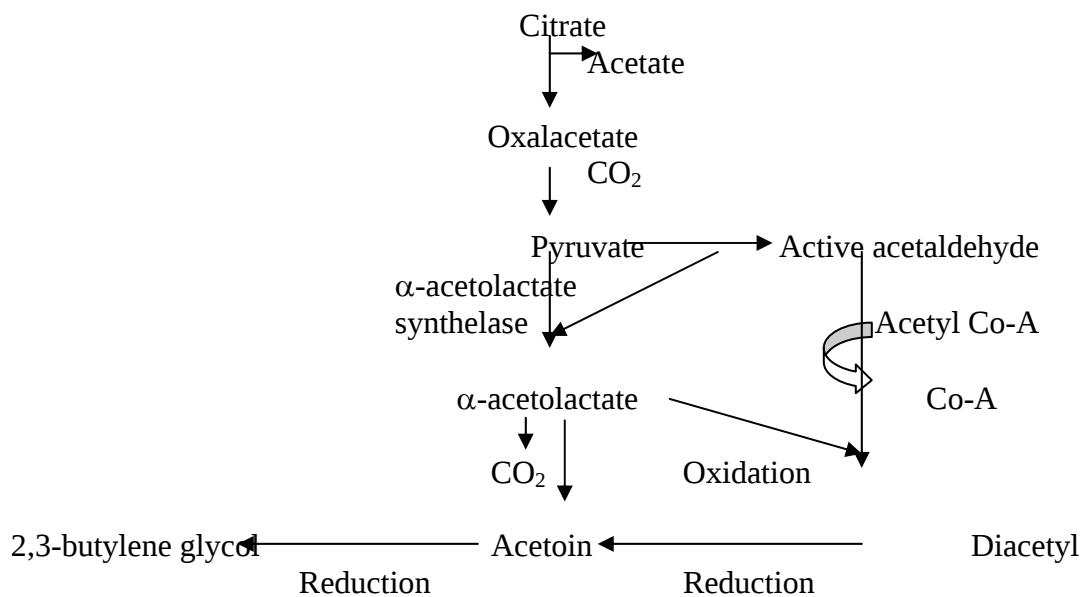


Fig. 4: Production of flavouring compounds by lactic acid bacteria through citric acid metabolism

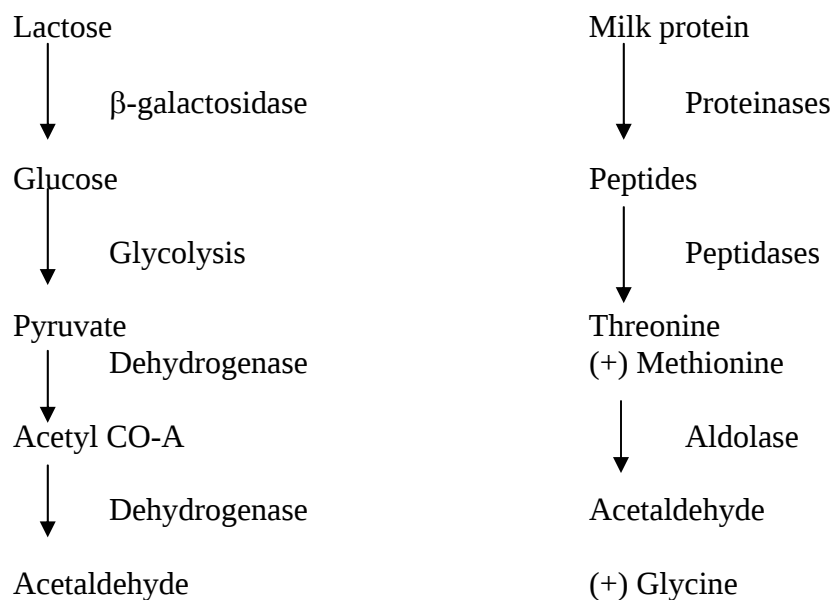
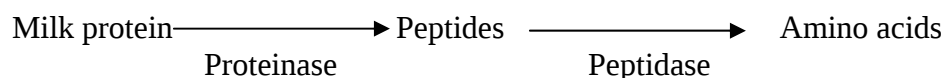


Fig. 5: Production of acetaldehyde by lactic starter cultures

4. Protein metabolism

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 and
- (iii) Peptides and amino acids act as flavour precursors.

5. Lipid metabolism

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.

6. *Vitamins metabolism*

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.

7. *Production of bacteriocins*

Lactic acid bacteria are exerting antagonistic effect against several other organisms, due to production of several antimicrobial substances. These include lactic acid, acetic acid, other organic acids, hydrogen peroxide, diacetyl, reduced pH and EH and a number of bacteriocins. Bacteriocins are the proteins produced by the bacteria that are inhibitory to closely related species. However, some of the bacteriocins of lactic acid bacteria have shown wide spectrum activities. The exact mechanism for synthesis and other characteristics of many bacteriocins are still not clear. However, the nisin is the only one, which is fully characterised and used as food preservative, other bacteriocins produced by lactic acid bacteria are Acidophilin, Lactocidin, Brevicin, Helveticin, etc.

Propagation and maintenance of starter culture

(a) Propagation of starter culture

Large quantities of starter culture in active and pure form are essential to the success of starter in product manufacture. This can be achieved by careful propagation of cultures. A typical flow diagram for propagation of starters in dairy industry is given in Fig. 6.

The propagation is required to maintain activity of the culture and also to increase the number of cells and volume of the culture for inoculation into the milk for product manufacture. Strict aseptic conditions are required to maintain the purity of starters during propagation. Similarly, the medium (milk) should be free from antibiotic residues or any other substances harmful to the starter. Control of temperatures during pasteurization of milk, incubation and cooling are also important factors affecting the activity of culture.

The starter preparation techniques described below can be used for all types of fermented milk products. The starter is produced in several steps.

Master culture	:	Starter available commercially from Laboratories.
Mother culture	:	Starter inoculated with master culture.
Intermediate/Feeder culture:		Larger quantities of mother culture.
Bulk Culture	:	Culture used in production.

Following is the flow diagram for the propagation of starter culture:

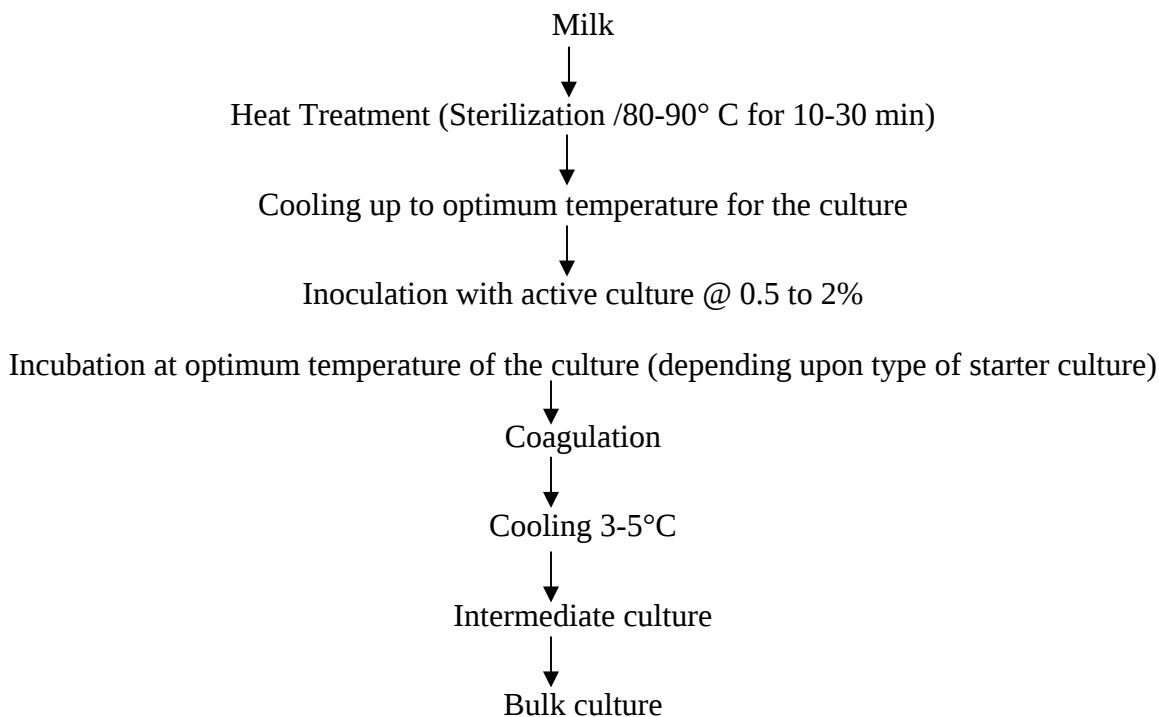


Fig.6: Method of propagation of starter cultures

This traditional method of starter propagation is time consuming, laborious, requires skilled personnel and is more prone to contamination. The other method, make use of concentrated culture sufficient to inoculate large quantity of milk for bulk starter production or directly for product manufacture. In recent years to prevent the contamination during starter propagation and bulk starter production, especially from bacteriophage, several new techniques have been developed. These include Lewis system, Jones system and Alfa-Laval system which employ mechanical and chemical control measures in design of equipment and bulk starter tank to prevent the entry of contaminants during propagation. Starter propagation in specially designed media such as devoid of calcium ions which help in controlling phage infection. Such media are called phage inhibitory media (PIM) or phage resistant media (PRM).

Now-a-days, several laboratories supply DVS or DVI (Direct-vat-set or Direct-vat-Inoculation) cultures, which are highly concentrated cultures, having cell population of about 10^{10} to 10^{12} cells per gram which are used directly for setting the vat or for the preparation of bulk culture. These cultures omit the need of culture propagation at the factory and reduce the risk of failure of milk fermentation for product development.

(b) Maintenance of starter cultures

The usual method of maintenance of these dairy starter cultures is by regular transfer into a small quantity of clean, previously well-sterilized and cooled milk. The cultures can be stored in the refrigerator and sub-cultured once in 2 or 3 days. During such frequent transfers, cultures are likely to get contaminated, if proper precautions are not taken. The best method of preservation and maintenance of cultures is to maintain them in ampules or vials under freeze-dried condition

and sealed under vacuum. The desirable characteristics and activity of these cultures can be maintained satisfactorily under this condition and transported over long distances with ease. Now, the freeze dried starter culture powder and converted culture have also been converted in the form of DVI or DVS culture so that these can be handled with ease and convenience to maintain fermentation operations in dairy industry.

(c) Mechanically Protected Systems for Propagation of Starter Culture

This technique that is known as Lewis System consists of using re-usable polythene bottles (115 and 850 g capacity) for mother and feeder cultures, respectively. These bottles are fitted with Astell rubber seals, and the growth medium, i.e. 10-12% reconstituted antibiotic free skim-milk powder, is sterilized in these bottles. The starter culture transfers are carried out by means of two-way hypodermic needles, and the overall technique is illustrated schematically in Fig 7. In this system, which is quite popular, a pressurized bulk starter tank is required where the growth medium is heat treated inside the sealed vessel.

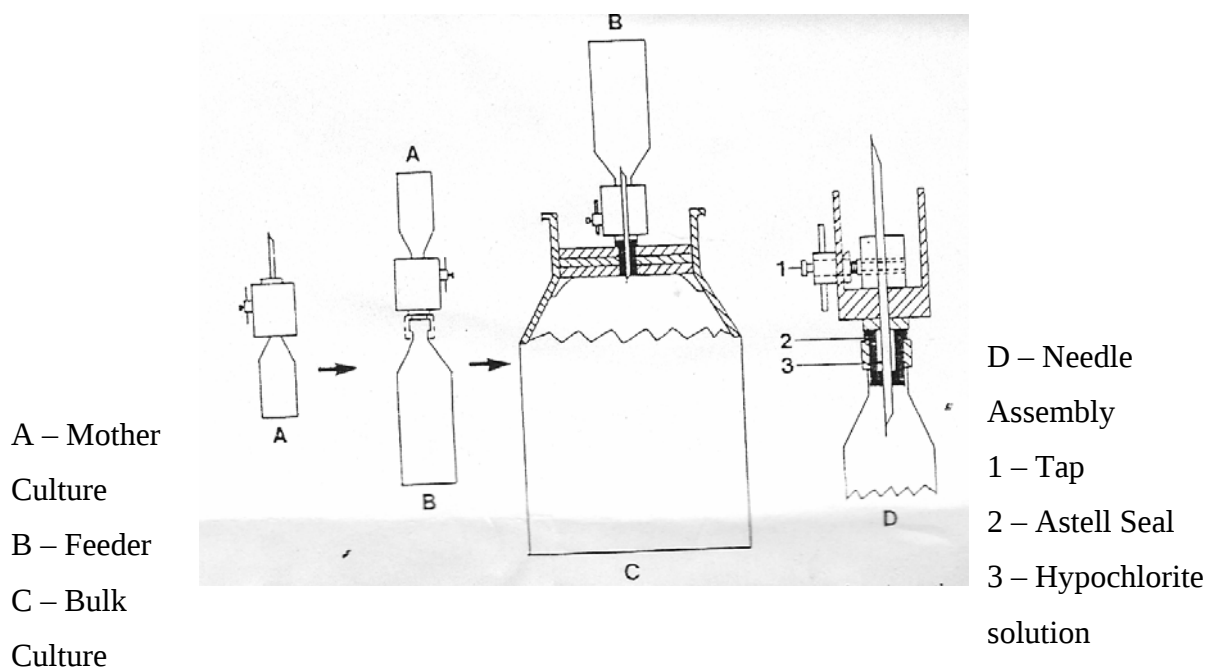


Fig. 7: Lewis System of propagation of starter cultures

(Source: Dairy Microbiology by R.K. Robinson)

It is worthwhile pointing out that, during the heating or cooling of the milk, no air escapes from or enters the tank. The top of the tank is flooded with sodium hypochlorite solution ($100 \text{ mg litre}^{-1}$), so that the transfer of the starter inoculum from feeder to bulk starter milk medium is through a sterile barrier. Special lids can be fitted in the Lewis system, so that it can be used successfully for the production of smaller volumes of starter culture too. In the Lewis system, the starter transfer from one container to another relies on squeezing the polythene bottle to eject the culture. However, in another system, which is known as Alfa-Laval system filter-sterilized air, under pressure, for transferring the culture are required. The filter consists of a special

hydrophobic fibre paper fitted with a prefilter on each side, and the whole unit is enclosed in a protective casing.

Fermented Milk Products

Fermented milks are sour milk products prepared from milk, whole, partially or fully skimmed, concentrated milk or milk substituted from partially or fully skimmed dried milk, homogenized or pasteurized or sterilized and fermented by means of specific dairy starter cultures.

The origin of cultured dairy product is obscure and it is difficult to be precise about the date when they were first made. In the early part of the century, Metchnikoff (1845-1916) claimed that owing to lactic acid and other products present in sour milks, fermented by lactic acid bacteria, the growth and toxicity of anaerobic, spore-forming bacteria in the large intestine are inhibited. Lactic acid is biologically active and capable of suppressing harmful microorganisms, especially putrefactive ones and so has a favorable effect on human vital activities. Metchnikoff's theory of longevity considerably influenced the spread of fermented milk products to many countries, particularly in Europe. He also promoted extensive studies concerning biochemical and physiological properties of fermented milks.

Milk fermentation for processing of milk into fermented milk products for increasing the shelf-life and having different flavour and texture characteristics have been practiced in different parts of the world. Milk has been processed into cheese, yoghurt, acidophilus milk, kefir, dahi, kumiss and various other fermented products. In the preparation of various fermented milk products, lactic starters occupy the key position as the success or failure of such products is directly related to the types of starter used. Fermented milk products prepared by various starter cultures are given in Table 3.

Spoilage of fermented milk products on storage also takes place due to non-lactic contaminants, such as sporeformers, micrococci, coliform, yeast and molds. These undesirable organisms rapidly increase in number when the starters are weak and the ratio of non-lactic to lactic organisms is high. Containers having a large surface of air in contact with the fermented milk accelerate the process of spoilage. Fermented milk products are generally spoiled by yeasts and molds and also by lactic acid bacteria which may cause sour, bitter and cheesy flavour.

Nutritional and therapeutic value of fermented dairy products

From dietary point of view, sour milk products, such as yoghurt, dahi, acidophilus milk, kumiss and other fermented milks are far more valuable than milk. During fermentation of milk, the composition of the minerals remains unchanged, while those of proteins, carbohydrates, and vitamins and to some extent fat constituents, change which produce special physiological effects. Dietary and therapeutic qualities of sour milk products are determined by microorganisms and substances formed as a result of biochemical process accompanying milk souring. These substances are lactic acid, alcohol, carbon dioxide, antibiotics and vitamins.

Fermented product	Microorganisms responsible for fermentation	Description
Sour cream	<i>Lactococcus</i> sp., <i>Leuconostoc</i> sp.	Cream is inoculated and incubated until the desired acidity develops.
Cultured buttermilk	<i>Lactococcus</i> sp., <i>Leuconostoc</i> sp.	Made with skimmed or partly skimmed pasteurized milk.
Bulgarian buttermilk	<i>Lactobacillus bulgaricus</i>	Product differs from commercial buttermilk in having higher acidity and lacking aroma.
Acidophilus milk	<i>Lactobacillus acidophilus</i>	Milk for propagation of <i>L. acidophilus</i> and the milk to be fermented are sterilized and then inoculated with <i>L. acidophilus</i> . This milk product is known for its medicinal therapeutic value.
Yoghurt	<i>Streptococcus thermophilus</i> , <i>Lactobacillus delbrueckii</i> ssp. <i>bulgaricus</i>	Made from milk in which solids are concentrated by evaporation of some water and addition of skim milk solids. Product has consistency resembling thick custard.
Dahi	<i>Lactococci</i> , <i>Leuconostoc</i> sp., <i>Lactobacillus delbrueckii</i> ssp. <i>bulgaricus</i> , <i>S. thermophilus</i>	Popular Indian fermented milk product, has slightly sour taste and prepared by using mixed lactic starter. Generally product is dominating with diacetyl flavour.
Kumiss	<i>Lactobacillus delbrueckii</i> ssp. <i>bulgaricus</i> , <i>Lactobacillus acidophilus</i> , <i>Saccharomyces lactis</i> , <i>Torula Kumiss</i>	Originally made from Mare's milk, product is prepared by mixed fermentation by using acid and alcohol producing starter culture. It is widely consumed in Eastern Europe.
Kefir	<i>Lactobacillus kefir</i> , <i>Lactobacillus brevis</i> , <i>Lactobacillus acidophilus</i> , <i>Lactococcus lactis</i> ssp. <i>Lactis</i> , <i>Leuconostoc</i> sp., <i>Yeast</i> , <i>Acetic acid bacteria</i>	It is self-carbonated fermented milk product. Fermentation is carried out by the Kefir grains which are embedded with many microorganisms. Finished product is effervescent drink containing 0.1 – 1.1% lactic acid and 0.5 – 1.0% alcohol.

Table 3 Types of fermented milk products

Following biochemical processes make fermented milk products more nutritive than milk:

(a) Proteolysis in milk

Proteolysis in milk takes place by exo- or endo-peptides 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.

(b) Hydrolysis of lactose

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.

(c) Lipolysis

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.

(d) Changes in vitamins

There is more than two fold increase in vitamins of B-group especially thiamine (B_1), riboflavin (B_2) and nicotinamide as a result of biosynthetic process during milk fermentation. Subsequently, vitamin B_2 , ascorbic acid and vitamin B_1 decrease by approximately one half as they are utilized by the bacteria present in milk. However, the increase or decrease in vitamin content depends on the type of culture.

(e) Antibacterial activities

The bactericidal properties of fermented milk products are determined by antibiotic activity of bacteria growing in the product. The antibiotic properties are generally associated with lactobacilli in yoghurt and materials responsible for such antibacterial actions are described as lactic acid, hydrogen peroxide and other substances such as antibiotics and bacteriocins.

(f) Changes in Minerals

In fact there is not any significant changes in minerals in milk after or during fermentation process by lactic acid bacteria and the nutritional values of fermented milk products remain intact.

II. Therapeutic Value

Fermented milk products are well known for “long life” and “cure all” properties due to their nutritional, therapeutic and prophylactic values. The main advantages of regular intake of fermented milk products, such as yoghurt, dahi, acidophilus milk, kefir are:

- These products are easily absorbed and better assimilated than sweet whole milk. Assimilation of milk is 32 per cent in one hour, while that of fermented milk products is 91 per cent in the same period. Better assimilation of fermented milk product is due to partial peptonization and intensity of secretion of ferment by digestive tract glands.
- They stimulate appetite due to their pleasant, refreshing and pungent taste.
- These products also improve central nervous and respiratory system.
- Curd consists of a sufficient amount of indispensable amino acid methionine, which removes excessive fat from the liver. In case of arteriosclerosis, methionine improves the general condition of the patient. Aged people should take curd.
- Gastric juice secreted by the action of fermented milk product and the desirable ratio of calcium and phosphorus induced leads to a high digestive capability.
- Fat-free curd is necessary for those who suffer from heart disease, arteriosclerosis, hypertension and chronic inflammation of the liver.
- Fermented milk products improve the immune response and leads to longevity.

Yogurt

Yogurt (also spelled jugurt or yoghurt) is a semisolid fermented milk product, which originated centuries ago in Bulgaria. Its popularity has grown and is now consumed in almost all parts of the world. Although the consistency, flavour and aroma may vary from one region to another, the basic ingredients and manufacturing processes are consistent. Yogurt is strictly defined as a milk product produced by the action of two bacteria – *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. In addition, yogurt may contain bifidobacteria and supplementary flora like *Lactobacillus acidophilus* for improving its therapeutic significance.

Although milk of various animals has been used for yogurt production in various parts of the world, most of the industrialized yogurt production uses cow's milk, whole milk, partially skimmed milk, skim milk or cream may be used. In order to ensure the development of the yogurt culture, the following methods for preparation of yoghurt must be followed:

(a) Preparation of Yoghurt

A good quality milk is clarified and then standardized to achieve the desired fat content. The various ingredients are then blended together in a mix tank equipped with a powder funnel and an agitation system. The mixture is then pasteurized for 30 min at 85°C or 10 min at 95°C. These heat treatments, which are much more severe than fluid milk pasteurization are necessary to:

- Produce a relatively sterile and conducive environment for the starter culture
- Denature and coagulate whey proteins to enhance the viscosity and texture

The mix is homogenized using pressures of 2000 to 2500 psi before final heat treatment. Besides thoroughly mixing the ingredients, homogenization also prevents creaming and wheying

off during incubation and storage. Stability, consistency, body and texture are enhanced by homogenization. After the final heat treatment the mix is cooled to an optimum growth temperature and inoculated with the yoghurt starter culture.

A ratio of 1:1 of *Str. thermophilus* and *Lb. bulgaricus* inoculation is added to the jacketed fermentation tank. A temperature of 42°C is maintained for about 4 h without agitation, till the milk sets. This temperature is suitable for the two microorganisms. The titratable acidity is carefully monitored until the titratable acidity is 0.85 to 0.90 per cent. At this time, chilled water is circulated in the jacket and agitation begins, both of which slow down the fermentation. The coagulated product is cooled to 5-22°C, depending on the product. Fruit and flavour may be incorporated at this time, and then packaged (Plate 3). The product is now cooled and stored at refrigeration temperatures (5°C) to slow down the physical, chemical and microbiological degradation.



Plate 3 : Fruit and Flavored Yoghurt

(b) Types of Yogurt

There are two types of plain yogurt: (i) stirred yogurt and (ii) set yogurt.

The above description is essentially for the manufacturing procedures for stirred style. In set style, the yogurt is packaged immediately after inoculation with the starter and is incubated in the packages. Other yogurt products include fruit and flavored yoghurt, frozen yoghurt, liquid yoghurt. Main steps involved in preparation of stirred yoghurt are indicated in Fig.8.

Acidophilus Milk Products

Acidophilus milks are sour milk products in which milk is allowed to ferment under conditions that favour the growth and development of larger number of *Lactobacillus acidophilus* alone or in combination with other lactic acid bacteria or lactose fermenting yeasts.

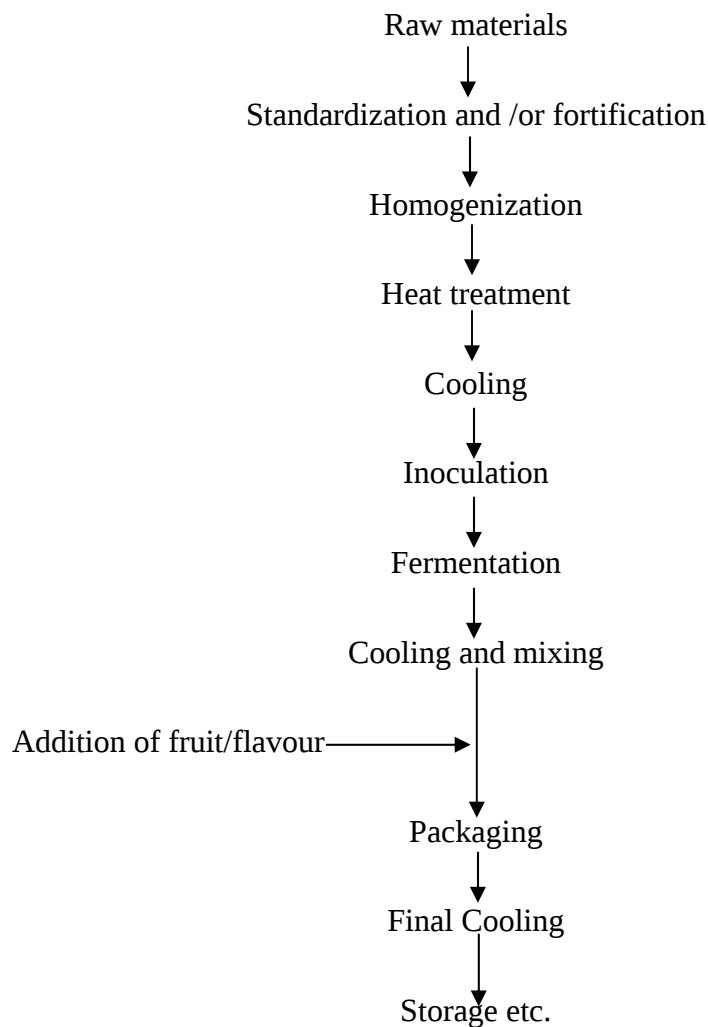


Fig. 8: Flow diagram of the main steps involved in preparation of stirred yoghurt

Types of acidophilus milks

Acidophilus sour milk

Acidophilus yoghurt

Bioghurt

Acidophilus yeast milk

Acidophilin

Acidophilus concentrates

Acidophilus paste

Dried acidophilus

Lyophilized form of milk

Organisms

Lb. acidophilus

Lb. acidophilus + *S. thermophilus*

Lb. acidophilus, *L. bulgaricus*, *S. thermophilus*

Lb. acidophilus + Lactose fermenting yeast

Lb. acidophilus, *Lc. lactis*, Kefir fungi

Lb. acidophilus

Lb. acidophilus

Lb. acidophilus

(a) *Therapeutic importance of acidophilus milk products*

- Possesses significant nutritional and prophylactic properties.
- Control gastrointestinal disorders, such as diarrhoea, constipation, dyspepsia, flatulence and colitis.
- Acidophilus yeast milk, which is rich in alcohol and CO₂, excite respiratory and central nervous system.
- Induction of *L. acidophilus* into the intestine return to normalcy in the intestinal microflora and body comforts
- Possible lowering of blood cholesterol.
- Possible improvement in immune status.
- Lower proliferation of cancerous cells.

(b) *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. 9.

Dahi

Dahi or curd is an Indian fermented milk product which is equally known for its palatability, refreshing taste and therapeutic importance as claimed in the ayurvedic literature. Some of its characteristics are similar to other fermented milk products such as yoghurt and acidophilus milk but it differs with regard to heat treatment of milk, starter culture, chemical composition and taste. In addition, dahi also has antibacterial properties against pathogenic and non-pathogenic organisms.

(a) *Types of Dahi*

Some of the fermented milks and different types of dahi consumed throughout India have been categorized as follows:

North Zone	:	Dahi, Lassi
South Zone	:	Dahi, Buttermilk (Mattha)
East Zone	:	Payodhi or Lal dahi or Mishti dahi
West Zone	:	Shrikhand, Chakka, Chhash, Dahi

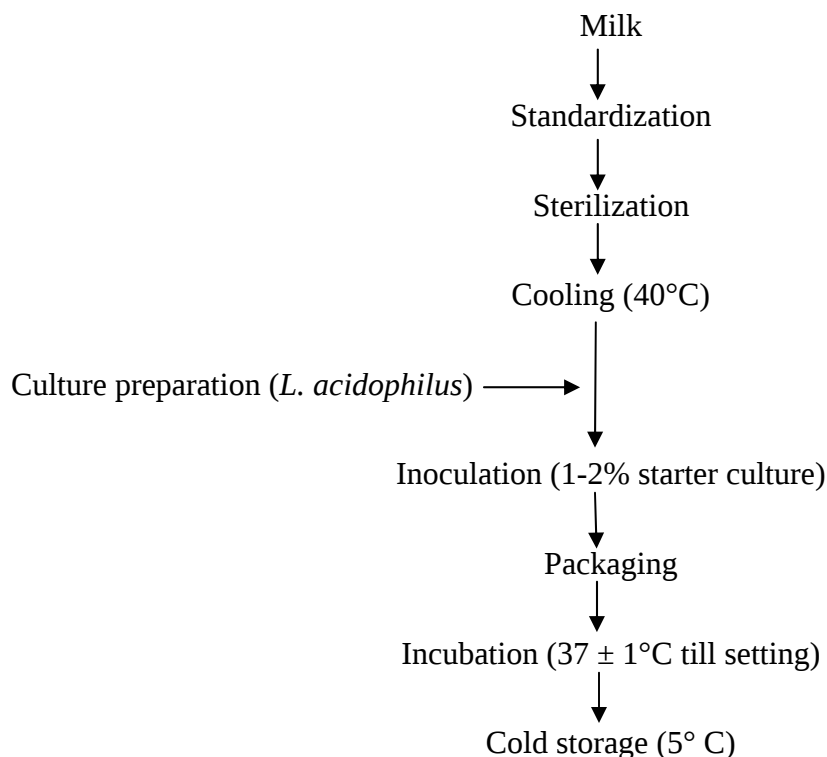


Fig. 9. Flow sheet for the preparation of acidophilus milk

Based on the acidity level (% lactic acid), dahi has been classified into categories such as sweet dahi with a maximum acidity of 0.7 per cent and sour dahi with 1.0 per cent acidity. Starter culture used in the preparation of dahi is normally dahi left over from previous day. The composition of microflora varies from one household to another and from one place to another. In general, it has been found that dahi culture is dominated by streptococci and lactobacilli. In sour dahi, however, lactobacilli predominate. For commercial manufacture by organized dairy, single starter culture (*Lactococcus lactis* subsp. *diacetylactis*) or mixed culture is used. The raw materials used are cow and/or buffalo milk, standardized milk, skim milk and reconstituted skim milk powder.

(b) Techniques of preparation of dahi

The traditional method for preparation of dahi invariably involves a small scale, either in consumers' household or in the sweet makers shop in urban areas. In the household, milk is boiled, cooled to about 37°C and inoculated with 0.5 – 1 per cent of starter (previous day's dahi or butter milk) and allowed to set overnight. It is then stored under refrigeration and consumed.

The standardized method for the preparation of dahi is given below in a flow diagram (Fig. 10).

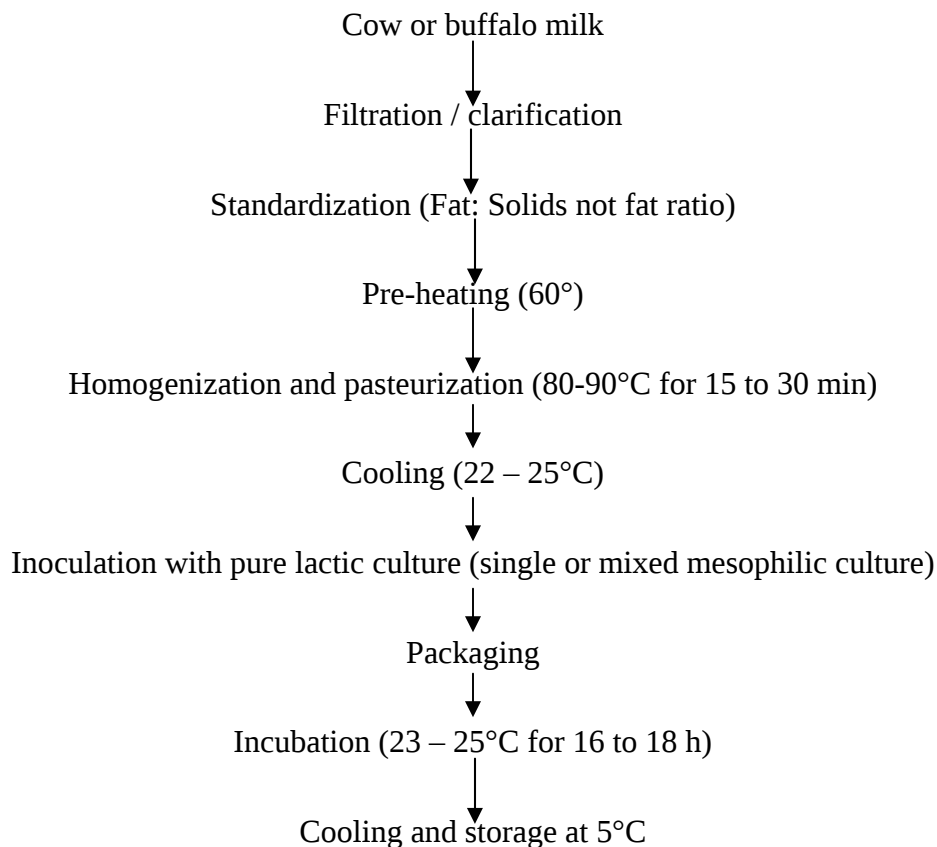


Fig. 10 Flow Diagram for the Preparation of Dahi

(c) Properties and Composition of Dahi

According to Bureau of Indian Standards (1978), specifications for fermented milk, dahi, should have a pleasing flavour and a clean acid taste, devoid of undesirable flavour, should have firm, solid body and texture and be uniform with negligible whey separation. Other characteristics should be as follows:

Characteristics	Sweet dahi	Sour dahi
Acidity (% lactic acid)	0.7	1.0
Yeast and molds (per gram) Max.	100	100
Coliforms (per gram) Max.	10	10
Phosphatase test	Negative	Negative

A good quality dahi made from whole milk has a cream layer on the top, the rest being made up of a homogenous body of curd and the surface being smooth and glossy, while the cut surface should be firm and free from cracks of gas bubbles and it should have a pleasant acid taste with sweetish aroma. Composition and quality of dahi vary widely from one locality to another as it is being prepared under different domestic conditions as well as milk, with variable chemical and bacteriological quality used for the preparation. However, the chemical composition of dahi has been reported as fat ranging from 5 to 8 per cent, protein 3.3 to 3.4 per cent, ash 0.75 to 0.79 per cent and lactic acid 0.5 to 1.1 per cent.

Quality of dahi can be improved with regard to increase in riboflavin and folic acid by incorporating propionic acid bacteria such as *Propionibacterium shermani* along with dahi starter culture. Regarding palatability and therapeutic importance of dahi, it has been known to create relish for food, promote the appetite, increases strength and leads to longevity.

Cultured Butter Milk

Buttermilk is really the liquid left from butter making. However, cultured buttermilk is a fermented milk product made from pasteurized skim milk low fat milk in which mesophilic lactic acid bacteria is added as starter.

(a) Starter culture for cultured buttermilk

Starter cultures are typically mixtures of flavour and acid producers *Leuconostoc spp.* and *Lactococcus lactis* subsp. *diacetylactis* produces diacetyl, the flavour most commonly associated with flavored butter and *Lactococcus lactis* is used to produce lactic acid which contributes to the acidic flavour typically associated with cultured butter milk.

(b) Preparation of cultured butter milk

The starting ingredient for buttermilk is skim or low-fat milk. The milk is pasteurized at 82° to 88°C for 10 - 30 minutes. This heating process is done to destroy all naturally occurring bacteria and to denature the protein in order to minimize wheying off (separation of liquid from solids). The milk is then cooled to 22°C and starter cultures of desirable bacteria, such as *Lactococcus lactis*, *Lac.cremoris*, *Leuconostoc citrovorum* and *Leu. dextranicum* are added to develop buttermilk's acidity and unique flavour. These organisms are used in proper combination to obtain the desired flavour.

The ripening process takes about 12 to 14 hours (overnight). At the correct stage of acid and flavour, the product is gently stirred to break the curd, and it is cooled to 7.2°C (45°F) in order to stop fermentation. It is then packaged and stored under refrigeration.

Butter or Makhan

Butter or Makhan is a fat rich product obtained from cow or buffalo milk or a combination thereof or curd obtained from cow or buffalo milk or a combination thereof without the addition of any preservative, including common salt, any added colouring matter or added flavouring agent. It should be free from other animal fats, wax and vegetable oil and fats. It should contain not less than 76.0 per cent of milk fat by weight.

(a) *Types of Butter and Manufacturing Processes*

- (i) Traditionally, butter is manufactured from ripened cream produced by natural souring. Now-a-days, ripened cream butter is made by the careful use of specific starter bacteria (mesophilic starter) added to the cream, which produces lactic acid and various flavour compounds such as diacetyl and then churned to get butter.
- (ii) In another method in which cream is not ripened but is churned and sweet cream buttermilk is drained off.

i) Butter Preparation from Ripened cream by lactic starter cultures

In this method of preparation, after pasteurization of cream it is cooled to 16 – 21°C and inoculated with about 4 per cent of a mixed starter culture containing the acid producers *Lactococcus lactis* and/or *Lac. Cremoris*, and the flavour producers *Leuconostoc cremoris* and/or *L. dextranicum* and *Lac. lactis* subsp. *diacetylactis*. The temperature of ripening depends on the season, and may vary from 16 to 18°C in summer or 19 to 21°C in winter. Higher temperatures favour rapid ripening, while the lower levels result in easier cooling subsequently.

The ripening process is always carried out in two or three stages in order to facilitate the cooling of the highly viscous ripened cream. Cooling of the cream can be used to modify the hardness characteristics of the final butter. To produce a firm butter, following process is used:

1. Cool the cream, after pasteurization, to 19°C and inoculate with the required amount of starter, and hold at 19°C until the pH falls to 5.2;
2. Cool to 14 – 16°C and hold for 2 h;
3. Cool to churning temperature.

To produce soft butter, following process is used:

1. Cool the cream to 6 – 8°C after pasteurization, inoculate with starter and hold for 2 to 3 h to form an intensive crystal network in the fat;
2. Carefully warm (using warm water at 25°C) to 19°C, this stage aims to melt the small crystals and create a network of predominantly large crystals, hold at 19°C until the pH falls to about 4.9;
3. Cool to 15 – 16°C.
4. Before manufacture, cool to churning temperature.

A week's collection of butter is converted into ghee. Butter is used for direct consumption on bread and chapaties. It is also used for the preparation of ayurvedic and unani medicines. Butter contains important sources of vitamins such as vitamin A, which is essential for a good eye sight and vision.

Cheese

A dairy product prepared from cow, buffalo, goat or sheep's milk that is set aside to thicken until it separates into a liquid, called whey, and semisolids, called curd. The whey is drained off and the curd is formed into the shape as per specification of cheese. It is packaged immediately, making it a fresh cheese like Ricotta cheese or cottage cheese, or it is aged using various curing methods.

(a) Types of cheeses

There are 400 varieties of cheeses, of which 18 are distinct. Important varieties of cheeses are given below:

1. Cheddar

Cheddar is a hard variety with about 40% moisture and has a diverse selection of tastes that range from mild to sharp. This is dependent upon the age of the cheese. Mild Cheddar is perfect for sandwiches because it has a mellow balance of flavors. Sharp Cheddar is good for cooking because its flavour is released when heated and it shreds well with other cheeses.

2. Mozzarella

Mozzarella has a mild, milky taste and is more of a cooking cheese due to its good binding properties, moist texture and ability to melt. It is a “stretched-cured” cheese meaning that during the manufacturing process the curd is pulled, kneaded and shaped while it is still pliable. Therefore, it absorbs the flavors and juices of the ingredients surrounding it and is perfectly designed for cooking. Mozzarella is also low in fat; therefore, it is ideal to use even when dieting. Mozzarella is an ideal cheese for Pizza making.

3. Swiss

Swiss cheese, which is also known as Emmental or Schweizer, is a firm cheese with a sweet, mildly nutty flavour. This cheese is known for the holes or eye formation that develop as it ripens. These holes or eyes range in diameter from ½ inch to 1 inch and begin forming when the cheese is about 3 weeks old.

4. Camembert

Camembert has a soft texture with a buttery taste and mushroom smell. It tastes best when it is at room temperature and the center becomes soft and it is a mold-ripened cheese.

5. Processed Cheeses

It is prepared by melting one or more pressed, cooked or uncooked cheeses, and adding milk, cream, butter and sometimes flavouring agents. One or several ripened cheeses are heated and mixed, then pasteurized at high temperature (130-140°C) after other dairy products, such as liquid or powdered milk, cream, butter, casein, whey, and seasoning have been added (Plate 4)



Plate 4: Processed Cheese

(b) Technology of Cheese Making

Cheese manufacture is essentially a dehydration process in which the casein, fat and colloidal salts of milk are concentrated 6 to 12 fold (approx. 10 fold in Cheddar cheese) with removal of 90 per cent water of milk and almost all of the lactose, whey proteins and soluble milk salts. Concentration is achieved by coagulating the casein by (a) enzyme modification (rennet cheeses), (b) acidification to isoelectric point (pH 4.6) by starter or addition of acid, or (c) acidification to pH 5.2 – 5.4 by starter or addition of acid and heating to 70–80°C. Concentration of the total colloidal phase of milk by ultrafiltration is now used commercially in the manufacture of a few varieties, e.g., Feta and Quarg. Rennet cheeses are usually ripened after manufacture when the typical characteristics of the individual cheese develops. Acid cheeses are generally consumed fresh. All rennet cheese are produced by a common mechanism as shown in Fig. 11.

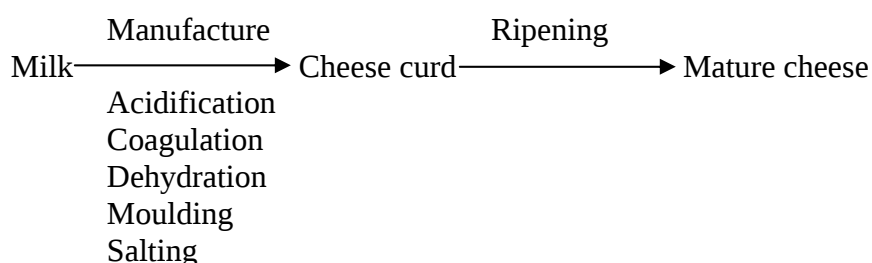


Fig. 11 Mechanism of cheese production

Microorganisms	Varieties of cheese
Mesophilic group	
Lactococcus lactis <i>Lact. lactis</i> subsp. <i>cremoris</i> <i>Lact. lactis</i> biovar. <i>diacetylactis</i> Leuconostoc cremoris	Cheddar, Cheshire, Caerphilly, Edam, Gouda, Camembert, Brie, Danish Blue, Stilton, Cottage and Cream cheese
<i>Propionobacterium</i> spp.	Emmental, Gruyere
Thermophilic group	
<i>Streptococcus thermophilus</i> <i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i> Lb. helveticus Lb. lactis	Parmesan, Romano, Emmental, Gruyere
Moulds	
Penicillium camemberti	Camembert, Brie
Penicillium roqueforti	Danish Blue, Roquefort, Stilton

Table 4: Some of the cheeses manufactured with different types of starter cultures (the precise combination of bacteria depends upon the variety of cheese and the inclination of the cheese maker)

Fast technological developments and ready market increased the production of cheese, which, in turn, resulted in a shortage of calf rennet. Search for suitable rennet substitutes gained momentum. Consequently, a number of rennet substitutes from animal and microbial sources are being used successfully in the cheese industry for Cheddar cheese. Modern cheese making, increasingly relies upon microbial sources of most important enzyme, the protease chymosin. It is now produced by recombinant microorganism.

For most of these cheeses, the process basically involves:

1. Pre-treatment of raw milk.
2. Formation of solid curd.
3. Removal of the liquid whey from curd.
4. Curd processing.
5. Ripening and aging.

Raw milk is first checked for various chemical and microbiological quality parameters and then pasteurized. The production of coagulated milk proteins or curd is then achieved by the activities of enzyme rennet and of lactic acid bacteria such as *Lactococcus lactis*, *Lc. cremoris* and *Str. thermophilus*. These bacteria have the ability to lower the pH through the fermentation of lactose to lactic acid, which facilitates protein coagulation. They also influence the flavour of the final product by producing specific flavour and aroma compounds and perform essential proteolysis and lipolysis in later maturation.

(c) Ripening of Cheese

Ripening is an expensive and time consuming process and Cheddar cheese is ripened for 6 to 9 months. Now emphasis has been focused towards the acceleration of cheese ripening because of the obvious economic and technological advantages. The principal methods employed to accelerate the ripening of Cheddar cheese are as follows:

1. Elevated ripening temperature
2. Use of elevated temperature combined with exogenous enzymes or mutant starters.
3. Use of modified starters such as lysozyme treated starters, heat or freeze shocked cell and mutant cultures.
4. Addition of enzymes or hydrolysed starter cells to cheese milk to stimulate the growth of starter cells.
5. Use of non-starter lactic acid bacteria as adjunct cultures.
6. Addition of exogenous enzymes such as proteinases / peptidases, encapsulated proteinases, -galactosidase and lipases.
7. Cheese slurries.

The principle biochemical changes involved in cheese ripening are glycolysis of residual sugars, lipolysis and proteolysis involving degradation of casein to lower molecular weight peptides and free amino acids. Acceleration of glycolysis is considered to be of no benefit in most or all

cheese varieties, while accelerated lipolysis may be beneficial in Blue or some Italian varieties, where it plays a major role in the development of characteristic flavour. The contribution of lipolysis to the flavour of Cheddar or Dutch cheeses is unclear and acceleration of lipolysis in these types is not usually undertaken as a means of enhancing flavour development. Proteolysis occurs in all cheese varieties and is considered to be a pre-requisite for good flavour development. By controlling manufacturing and ripening conditions it is possible to produce cheese of excellent quality.

Probiotic Cultures

Definition

Probiotic cultures are live microorganisms which when administered in adequate amounts confer a health benefit on the host. Lactobacilli and bifidobacteria maintain a healthy balance of intestinal flora by producing organic compounds, such as lactic acid, hydrogen peroxide, and acetic acid that increase the acidity in the intestine and inhibit reproduction of many harmful bacteria.

Characteristics of probiotics

- Probiotics must be alive.
- Probiotics must deliver a physiological benefit.
- Probiotics need not be restricted to food applications or oral delivery and can be used in pharmaceuticals.

Health benefits of probiotics

- Probiotics can increase the bioavailability of proteins and fats in the diet by breaking down these nutrients in the digestive tract. This is particularly important for infants, toddlers and patients who need building up during and after any illness.
- Probiotics can prevent diarrhoea of all kinds – eating yoghurt during traveling in strange countries can help prevent travellers' diarrhoea.
- May reduce chances of heart attack due to lowering of cholesterol
- Probiotics can help to reduce intestinal inflammation and hypersensitivity reactions in infants suffering from food allergies.
- Can reduce chances of cancer especially colon cancer due to antitumor activity.
- Probiotics may help to prevent liver damage caused by excessive alcohol intake.
- Probiotics may enhance immunity too.

Potential Probiotic cultures

Lactobacillus species

L. acidophilus, *L. casei*, *L. fermentum*, *L. gasseri*, *L. johnsonii*, *L. lactis*, *L. paracasei*, *L. plantarum*, *L. reuteri*, *L. rhamnosus* and *L. salivarius*

Bifidobacterium species

B. bifidum, *B. breve*, *B. lactis* and *B. longum*

Streptococcus species

S. thermophilus

Enterococci

Enterococci are part of the intestinal microflora in human and animals. *Enterococcus faecium* SF68 is a probiotic strain that has been used in the management of diarrhoea illness.

Saccharomyces

It belongs to yeast family, which include *Saccharomyces boulardii* as probiotic. *S. boulardii* is non-pathogenic yeast, which has been used to treat diarrhoea associated with antibiotic use.

Mechanism of action and desirable properties of probiotic organisms

Different mechanisms of action of probiotics have been described. However, they need to be ingested regularly for any health promoting properties to persist. Brief description of mechanisms and properties by which probiotics may protect the host against intestinal diseases are given below:

Desirable properties of probiotic strains

All the currently used probiotics have a long history, however, there are some criteria of assessing their selection. These criteria require a combination of *in vitro* and *in vivo* studies.

Viability of probiotic organisms

Probiotic bacteria must be viable and available in high concentration, (typically 10^6 cfu/g) of a product. The probiotic strain in use should be resistant to stomach acidity, pancreatic and bile secretion. Other factors resulting in a loss of viability include post-acidification of product during refrigeration, level of oxygen in products, oxygen permeation through the package, and sensitivity to the antimicrobial substances.

Biosafety

The probiotic strains should be safe with regards to human health. The microorganisms that are being used as probiotic should come under the category of GRAS (Generally Regarded As Safe). Any novel strain should be subjected to the appropriate legal approval.

Adherence properties

The adherence, colonization and multiplication in the intestine are the most important selection criteria for a probiotic culture. Colonization properties have been studied in *Bifidobacterium breve*, *B. longum*, *B. bifidum* and *B. infantis*. Probiotic strain, have also been found to be host specific, i.e., they colonize the compatible host. Change in viable population of gut may be measured to interpret the viability of probiotic strain and its effect on microbial population.

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