UNIT 1  STARTER CULTURES AND NUTRITIONAL IMPORTANCE OF FERMENTED MILKS

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1.0  OBJECTIVES

After reading unit we should be able to:

- understand the importance of starter cultures in preparation of fermented milk products.
- know the role of starter cultures in manufacture of fermented products.
- know the type of starters.
- classify starters.
- enumerate various factors which affect fermentation process of starters.
- prepare starter cultures.
- learn maintenance and preservation of starters.

1.1  INTRODUCTION

Fermented milks have a history of thousands of years. They are enjoyed everywhere in the world for their characteristics refreshing acid taste. Most of them use the lactic acid fermentation by lactic acid bacteria, but some also use additional alcohol fermentation due to yeasts or lactic fungi to prevent bacterial and mould contamination.
and improve storage properties. This characteristic as a preserved food is one reason for their success, but with the diversification of the diet in recent years and their image as health foods, not only plain fermented milks, but also fermented milks in liquid or frozen form, and with all kinds of fruit juice or added fruit, have appeared in the market.

Research into the health-giving effects of fermented milks has flourished since Metchnikoff developed his hypothesis on longevity, and the physiological significance of fermented milk and of lactic acid bacteria is continuing to become clearer. Clinical trials are also already showing beneficial results, and fermented milks are now recognized as health foods as well as being pleasant preserved foods. The starter cultures of today used for bringing about fermentative changes in milk in the form of a variety of fermented milks comprise special types of lactic acid bacteria. This useful group of bacteria include lactococci, lactobacilli, Leuconostocs, pediococci, and propionic acid bacteria along with some common yeasts and moulds which are now extensively used in the preparation of several types of fermented products.

1.2 ROLE OF STARTERS IN FERMENTED FOODS

The primary function of almost all starter culture is to develop acid in the products. The secondary effects of acid production include coagulation, expulsion of moisture, texture formation, and initiation of flavour production. In addition to these, the starters also help in imparting pleasant acid taste confirming protection against potential pathogens and spoilage causing microorganisms and providing a longer shelf life to the product.

A starter culture consists of food grade microorganism(s) that on culturing in milk produce predictable attributes. The common starter culture used in manufacture of fermented milk products throughout the world are presented in table 1.1

<table>
<thead>
<tr>
<th>Starter culture</th>
<th>Role/ function</th>
<th>Fermented dairy product</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactococcus lactis</em> subsp. <em>lactis</em></td>
<td>Acid</td>
<td>Cultured butter milk, sour cream, cottage cheese, all types of cheeses (Domestic and foreign), starter culture etc.</td>
</tr>
<tr>
<td><em>Lactococcus lactis</em> subsp. <em>Cremoris</em></td>
<td>Acid and flavour</td>
<td>Sour cream, ripened cream, butter, cheese, butter milk, starter cultures etc.</td>
</tr>
<tr>
<td><em>Lactococcus lactis</em> subsp. <em>diacetilactis</em></td>
<td>Acid and flavour</td>
<td>Sour cream, ripened cream, butter, cheese, butter milk, starter cultures etc.</td>
</tr>
<tr>
<td><em>Streptococcus thermophilus</em></td>
<td>Acid</td>
<td>Emmental, Cheddar and Italian cheese and yoghurt.</td>
</tr>
<tr>
<td><em>S. durans, S. faecalis</em></td>
<td>Acid and flavour</td>
<td>Soft, Italian, Cheddar and some Swiss cheese varieties.</td>
</tr>
<tr>
<td><em>Leuconostoc citrovorum, Leuco, dextranicum</em></td>
<td>Flavour</td>
<td>Cultured butter milk, sour cream, cottage cheese, ripened cream, butter and starter cultures.</td>
</tr>
<tr>
<td><em>Lactobacillus delbrueckii</em> subsp. <em>bulgaricus</em>, <em>L. lactis, L. helveticus</em>.</td>
<td>Acid and flavour</td>
<td>Bultarian butter milk, yoghurt, kefir, kumiss, Swiss, Emmental and Italian cheese</td>
</tr>
<tr>
<td><em>L. acidophilus</em></td>
<td>Acid</td>
<td>Acidophilus butter milk</td>
</tr>
<tr>
<td><em>Propionibacterium shermanii</em></td>
<td>Flavour and eye formation</td>
<td>Emmental and Swiss cheese</td>
</tr>
</tbody>
</table>
Generally, three different types of starter cultures are used in dairy industry for the manufacture of a variety of fermented products.

In general, organisms that grow at ambient temperatures are termed mesophillic cultures and those growing at higher temperatures are called thermophillic cultures. Dahi and other Indian fermented milk products use mesophillic starter cultures whereas, yoghurt uses thermophillic cultures. The characteristics of mesophillic and thermophillic cultures used in manufacture of different fermented milk products are presented in tables 1.2 and 1.3.

### Table 1.2 Attributes of mesophillic starters (lactic acid bacteria) used in fermented milk products

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Lactococcus lactis subsp. lactis</th>
<th>Lactococcus lactis subsp. cremoris</th>
<th>Lactococcus lactis subsp. lactis biovar diacetylactis</th>
<th>Leuconostoc mesenteroides subsp. cremoris</th>
<th>Leuconostoc mesenteroides subsp. dextranicum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell shape and configuration</td>
<td>Cocci, pairs, short chains</td>
<td>Cocci, pairs, short/long chains</td>
<td>Cocci, pairs, short chains</td>
<td>Cocci, pairs, short/long chains</td>
<td>Cocci, pairs, chains</td>
</tr>
<tr>
<td>Catalase reaction</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Growth temperature (°C)</td>
<td>28-31</td>
<td>22</td>
<td>28</td>
<td>20-25</td>
<td>20-25</td>
</tr>
<tr>
<td>Optimum</td>
<td>8-10</td>
<td>8-10</td>
<td>8-10</td>
<td>4-10</td>
<td>4-10</td>
</tr>
<tr>
<td>Minimum</td>
<td>40</td>
<td>37-39</td>
<td>40</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>Maximum</td>
<td>40</td>
<td>37-39</td>
<td>40</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>Incubation temperature (°C)</td>
<td>21-30</td>
<td>22-30</td>
<td>22.28</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>Heat tolerance (60°C/ 30 min)</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lactic acid isomers</td>
<td>L (+)</td>
<td>L (+)</td>
<td>L (+)</td>
<td>D (−)</td>
<td>D (−)</td>
</tr>
<tr>
<td>Lactic acid produced in milk (%)</td>
<td>0.8-1.0</td>
<td>0.8</td>
<td>1.0</td>
<td>0.1-0.3</td>
<td>0.1-0.3</td>
</tr>
<tr>
<td>Acetic acid production (%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.2-0.4</td>
<td>0.2-0.4</td>
</tr>
<tr>
<td>Gas (CO₂) production (%)</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>Proteolytic activity</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>Lipolytic activity</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>Citrate fermentation</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavour/aroma</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Mucor polysaccharide production</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>No dextran from sucrose</td>
<td>Dextran from sucrose</td>
</tr>
<tr>
<td>Hydrogen peroxide production</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
</tbody>
</table>
**Table 1.3. Attributes of thermophilic starters used in yoghurt**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Streptococcus thermophilus</th>
<th>Lactobacillus delbrueckii subsp. bulgaricus</th>
<th>Lactobacillus delbrueckii subsp. lactis</th>
<th>Lactobacillus acidophilus</th>
<th>Lactobacillus casein subsp. casei</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell shape and configuration</td>
<td>Spherical to ovoid, pairs to long chains</td>
<td>Rods with round ends, single, short chains, metachromatic granules</td>
<td>Rods with round ends, metachromatic granules</td>
<td>Rods with round ends, single, pairs short chains, no metachromatic granules</td>
<td>Rods with square ends, short/ long chains</td>
</tr>
<tr>
<td>Catalase reaction</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Growth temperature (°C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Optimum</td>
<td>40-45</td>
<td>40-45</td>
<td>40-45</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>Minimum</td>
<td>20</td>
<td>22</td>
<td>22</td>
<td>20-22</td>
<td>15=20</td>
</tr>
<tr>
<td>Maximum</td>
<td>50</td>
<td>52</td>
<td>52</td>
<td>45-48</td>
<td>40-45</td>
</tr>
<tr>
<td>Incubation temperature (°C)</td>
<td>40-45</td>
<td>42</td>
<td>40-45</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>Heat tolerance (60°C/30 min)</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lactic acid isomers</td>
<td>L (+)</td>
<td>D (-)</td>
<td>D (-)</td>
<td>DL</td>
<td>L (+)</td>
</tr>
<tr>
<td>Lactic acid produced in milk (%)</td>
<td>0.7-0.8</td>
<td>1.5-4.0</td>
<td>1.5-3.0</td>
<td>0.3-2.0</td>
<td>1.2-1.5</td>
</tr>
<tr>
<td>Acetic acid production (%)</td>
<td>Trace</td>
<td>Trace</td>
<td>Trace</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gas (CO₂) production</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Proteolytic activity</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>Lipolytic activity</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>Citrate fermentation</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flavour/aroma</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>Mucor polysaccharide production</td>
<td>±</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hydrogen peroxide production</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alcohol production</td>
<td>-</td>
<td>Trace</td>
<td>Trace</td>
<td>Trace</td>
<td>Trace</td>
</tr>
<tr>
<td>Salt tolerance (% max)</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>6.5</td>
<td>2.0</td>
</tr>
</tbody>
</table>
1.3 TYPES OF STARTERS

In general, three different types of starter cultures are used in dairy industry for the manufacture of a variety of fermented products.

i. Single Strain Starters

A single strain starter is a pure culture of lactic acid bacteria such as *Lactococcus lactis* subsp. *lactis* or *L. lactis* subsp. *cremoris*, etc. This type of culture, if found satisfactory in vigor and flavour, it can give a steady acid production and thereby, a predictable quality of fermented milk product. However, there is a serious disadvantage with this type of starter as during its application, if it gets attacked by a phage or fails due to any other reason, the quality of the resultant product can be adversely affected.

ii. Mixed Strain Starters

These consist of two or more strains or species and thus, may be more variable in behaviour. The mixed strain starters are generally combinations of *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* and the gas and aroma producing mesophillic lactic acid bacteria (*L. lactis* subsp. *diacetilactis* and *Leuconostoc* spp.). Mixed starters are considered safe because if one strain is attacked by a phage, the others usually continue to work because of high phage specificity. A wider tolerance to other factors like temperature and pH, changes, etc. may be an additional advantage.

iii. Multiple Strain Starters

Multiple strain starters are mixture of known compatible, non-phage related, carefully selected strains which give generally consistent products when used commercially for production of fermented milk products. Although, their overall phage relationships may be known, the number of individual phage relationships among the strains in these cultures to relatively unknown. A multiple strain starter culture consists of known number of single strains so that the starter can be used for an extended period of time.

**Thermophilic lactic acid bacteria (LAB)**

Thermophilic LAB (37-45°C) are also used in dairy industry for the manufacture of some fermented products like yoghurt, acidophilus milk and high temperature scalded cheese such as Swiss cheese. The examples of thermophillic LAB are *S. thermophilus* and the *Lactobacillus* species. These starters produce lactic acid at faster rate at high temperature and the rate of acid production is further enhanced if symbiotic relationships exist between different species. The such typical example is of yoghurt culture namely, *S. thermophilus* and *L. delbrueckii bulgaricus*. Similarly, the combined activity of mesophillic and thermophillic LAB and yeasts leads to lactic acid/ alcohol fermentation in milk during the manufacture of Kefir and Kumiss.

1.4 CLASSIFICATION OF LACTIC STARTER CULTURE

Tamine (1981) has proposed an overall classification of lactic starter cultures, which has been illustrated in Fig. 1.1
Fig. 1.1 Classification of Dairy Starter Cultures
**Characteristics of a good starter culture**

The following desirable characteristics must be looked into for exploring them for full fermentation potentials while selecting a particular LAB starter culture, either single or combination with others for manufacture of fermented milk products.

i) The lactic starter culture must produce sufficient lactic acid at desirable rate to suit the plant schedule and produces high quality product.

ii) Good LAB culture must continue acid production over the appropriate range of temperatures, at which it is likely to be used during the processing of milk into production of fermented dairy products.

iii) The LAB starters should be resistant to antibiotics and bacteriophages. They should also be active in the presence in inhibitory substances as well as residual amounts of chemical, sanitizers and detergents in milk.

iv) A good starter culture should not produce bacteriocins and bacteriocin like substances or any other antibiotic type of substance inhibiting other strains in the mixed culture.

v) A good LAB starter culture should produce desirable flavour, aroma, consistency, body and texture in the fermented products.

vi) The use of starter cultures producing certain defects like ropy body, malty or any other undesirable flavour and similar other defects should be immediately discontinued.

vii) In case of mixed cultures, the individual cultures must be able to synergize maximum aroma and flavour production without inhibition of acid production.

viii) The associative action of mixed cultures must be quite stable and contributes towards the development of a good starter culture even after repeated sub-cultures.

**Check your Progress I**

1) What is role of starter culture in fermented foods?

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2) What are common starter cultures used for preparation of fermented dairy products?

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...................................................................................................................
...................................................................................................................
3) Describe types of starter.

4) Discuss characteristics of a good starter.

5) Give classification of dairy starter cultures.

1.5 FACTORS AFFECTING FERMENTATION PROCESS OF STARTER CULTURES

The fermentation process of LAB starters can be influenced by several factors such as temperature, pH, strain capability, growth medium, inhibitors, bacteriophage, incubation period, heat treatment of milk, etc. There is a need to take adequate precautions to achieve the optimal activity of lactic acid bacteria during the manufacture of fermented milks. Some of the important factors affecting the growth and activity of lactic starter cultures as discussed below:

i. Temperature

The performance of lactic acid bacteria during milk fermentation is influenced by a number of factors. Temperature is one of the important factors, which directly affect the growth of microorganisms. Although different type of lactic acid bacteria have different optimal temperatures for their growth, the majority of lactic starters such as *L. lactis subsp lactis*, *L. lactis subsp cremoris*, etc. grow optimally at 27-32°C. On the other hand, *S. thermophilus* and some lactobacilli grow best at 37-42°C temperature range. However, Leuconostocs have their optimal temperature at 20-30°C. This variation in optimal temperature requirement may be a very important factor in strain dominance in mixed and multiple starters.

ii. pH

The pH is one of the most important factors which can influence the growth and metabolic activity of lactic starter cultures during milk fermentations. Although milk
is considered to be nearly the perfect food for man as well as bacteria, it still needs some enrichment for use as a bulk starter medium. Lactic acid bacteria produce lactic acid at the level of more than 10 per cent of their weight per minute after their growth in milk and thus, pH of milk is lowered. The extreme acidic pH could be detrimental for the viability of LAB. Hence, control of pH in milk during propagation of starters is very important. This fact is generally ignored during the commercial production of bulk starters. Both externally and internally pH controlled media used for the preparation of bulk starters. Another method for controlling pH is consisting of diffusion culture technique for the removal of toxic end products of metabolism of starter cultures by dialysis. In this context, the preparation of starter culture concentrates in low lactose milk is also promising as this can eliminate the possibility that acid from lactose could be detrimental to their own viability and survival. When starter cultures are allowed to grow till the pH falls below 5.0, considerable injury to the cells results, which could adversely affect the performance of these organisms. In externally pH controlled whey medium, the pH drop is controlled by an insoluble buffer so that the lactic acid produced is immediately neutralized. Another promising and practical approach to tackle this problem could be through genetic manipulation of these organism by introducing a pH sensitive promoter for regulation of structural genes involved in acid production.

iii. Strain Compatibility

Mixed starters have been used for the preparation of several fermented dairy products. However, maintenance of mixed strain starters in cheese factories is not much practiced any longer, partially because repeated subculture of mixed strains of lactococci may result in decrease in number or loss of all but one of the strains that eventually a single strain remains in the mixed starter preparation. Some of the factors responsible for strain dominance or overgrowth in a mixed culture by one strain include differences in generation times, acid sensitivities, the production of antibiotics or bacteriocins by the component strains, differences in optimum temperature and rates of plasmid loss etc. The main objective of using a mixture of strains in mixed culture is that even if one strain become infected by a specific bacteriophage, the other strains would be unaffected and enable the culture to produce adequate acid during bulk culture preparation. Same thing may hold good for antibiotic resistance of some strains in the mineral cultures which will not be affected by the presence of inhibitory substances in milk and the milk fermentation cultures also plays a very important role in bringing about the desirable changes in milk.

iv. Growth Medium

The media used for the cultivation of LAB are quite complex. The most widely used growth media are MRS, M-17 and Lactic or Elliker’s medium. Although Elliker’s medium is of choice, M-17 is also an excellent growth medium and is extensively used for the growth of lactococci. Several plating media have been described for differentiation of different species of lactococci, namely, *L. lactis subsp. lactis*, *L. lactis subsp. cremoris* and *L. lactis subsp. diacetylactis*. Another medium on which Lactococci can be differentiated is one to distinguish between fast and slow growing cultures. The medium is designated as Fast Slow Differential Agar (FSDA). Fast colonies (lac+, prt+) on this medium appear 1-3 mm in diameter, shiny white, convex and surrounded by a red zone against the blue background of the medium indicating lactose fermentation. Slow colonies (lac+, prt+) are 0.2-0.5 mm in diameter, translucent and flat.

Apart from these media, lactic acid bacteria also grow very well in milk. However, it is pointed out that during specific seasons of the year, more inoculum may be
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needed during milk fermentation in order to achieve the same type of acid production obtained with lower inoculum rates at other times of the year.

v. Inhibitory Substances

The growth and activity of the starter cultures in milk is adversely affected due to the presence of residual antibiotics and sanitizers in milk as well as the production of antibiotic-like substances (bacteriocins) by certain wild strains of *Lactococcus lactis subsp. lactis* and other lactic cultures in raw milk. Antibiotics such as penicillin or streptomycin may enter milk as a result of their indiscriminate use in the treatment of mastitis or udder diseases. Hence, milk must be thoroughly monitored for the presence of residual antibiotics before addition of starter cultures. The methods based on immunological reactions as well as isotopic tracer dilution procedures (Charm test) are very effective.

vi. Bacteriophages

Bacteriophages are considered as one of the single most important factors causing slow acid production by lactic acid bacteria in the commercial environment. The most promising solution to this problem could be by replacing the phage sensitive strains with phage resistant one. The use of defined single strains and their phage resistant mutants is becoming increasingly popular throughout the world for the manufacture of various fermented foods.

vii. Incubation Period

The period of incubation is another important factor, which can affect the growth of lactic acid bacteria. The higher the temperature up to a certain limit, the faster does the culture pass through its growth phases. Normally, 16-24 hr incubation is adequate for the maximal growth of these organisms at their optimal temperatures. However, storage of the ripened starters for about 18 hrs at low temperature does not appear to affect their activity, although over-ripened cultures are adversely affected on prolonged storage.

viii. Heat Treatment of Milk

Heat treatment of milk usually improves its value as a medium for starter organisms and other lactic acid bacteria. Adequate heat treatment of milk drives out the dissolved oxygen, brings about formation of sulphydryl compounds (acting as growth factors), destruction of inhibitory substances naturally present in milk and killing of antagonistic bacteria. However, with more severe heating, slight protein breakdown may occur with the formation of peptides and amino acids, which act as nutrients. In addition to these, different species of lactic acid bacteria appears to behave differently in heat-treated milks. For example, the growth of *S. thermophilus* appears to be favored, while *L. lactis subsp. cremoris* disfavored by drastically heat treatment of milk.

ix. Degree of Aeration

The lactic acid bacteria appear to be indifferent to aeration of the medium or slightly preferring a reduced oxygen tension. It can be stated that acid production is faster at the bottom of the container or under controlled oxygen tension condition (i.e. reduced oxygen tension than that of atmosphere). Possibly, a reduced level of oxygen tension is favorable for the initiation of growth as it affords energy for growth by a mechanism somewhat more efficient than the lactic fermentation, which releases only a small fraction of the energy available in the lactose. Aeration, agitation and the surface culture usually depress the activity of lactic acid bacteria. However, agitation is obviously a vague condition and may sometimes accelerate souring. It clearly includes
two quite different factors, namely, oxygenation and movement of the medium, which appear to have opposing effects on the starter cultures. Although excessive aeration may be the cause of slow starters, its effects may be neutralized by heating milk or by adding sulphydryl compounds.

x. **Effect of Carbon Dioxide**

A minimum concentration of carbon dioxide is essential for the initiation of bacterial growth. Complete removal of carbon dioxide from a medium results in extended lag phase until the bacteria have slowly produced sufficient carbon dioxide to sustain normal growth. For most of the lactic acid bacteria, the optimum initial concentration of CO$_2$ varies from 0.2 – 2.3% by volume. Sterilized skim milk may contain only 0.3-0.5% of CO$_2$ and this may account for the prolonged lag phase of the given starter culture. However, incorporation of yeast extract in milk at a concentration of 0.5% can get rid of this problem.

xi. **Storage conditions**

Storage of lactic acid bacteria is yet another important factor affecting their performance during the manufacture of fermented milk products. It is desirable not to store the mature cultures in presence of acid that they have produced during growth. Storage under these conditions will result into cellular injury and promote the loss of plasmids, which may increase the proportion of slow cells in the population. Such cultures will become slow and can no longer be useful for the preparation of fermented products. Therefore, it is important that during the maintenance of such cultures, they should be transferred to fresh milk and in refrigerator without incubation. When a new culture is needed, it is removed from the refrigerator, incubated and transferred to fresh medium and placed in the refrigerator again. However, mature cultures may also be stored at 2-5°C either in milk with added calcium carbonate or in stabs of selective media. Cultures can also be frozen and stored at -40°C or below in the freeze-dried state. Frozen storage in liquid nitrogen (-196°C) in the form of starter concentrates also affords stability to cultures.

### 1.6 PREPARATION OF STARTERS

The propagation and preparation of LAB starter cultures is one of the most important operations in a dairy plant required for manufacture of fermented products. Since the quality of a starter has a direct bearing on the quality of the final product, starter must be pure, active and in good shape. The good quality starter cultures could only be prepared by employing certain essential factors such as selection of good quality milk, necessary heat treatment of milk, well cleaned and sanitized utensils, optimum amount of inoculum, aseptic transfer of culture, incubation at appropriate temperature and time, cooling and properly storing till its use.

i. **Selection of milk**

The milk selected for preparation of starter must be from healthy animals that are secreting normal and clean milk, free from inhibitory substances. The abnormal milk, mastitic milk and milk with unusually high lipolytic activity and low in total solids should not be used for preparation of starter cultures as well fermented products.

ii. **Heat treatment of milk**

Milk to be used for starter propagation should be given adequate heat treatment to inactivate the normal germicidal effects and also to destroy the majority of the microorganisms present as normal flora or contaminants. To achieve best results milk should be heated to minimum of 71.1°C for 30 min. Sometimes sterilized milk
is recommended for starter propagation. However, more drastic heat treatment results in the inhibition of culture and the texture and body of the curd formed is soft and the scorched flavour developed therein hinders the accelerate assessment flavoring and aroma development. The steaming and boiling of milk for 30-60 min for mother culture is preferred. After heat treatment, immediately milk is cooled to incubation temperature to minimize the physico-chemical changes in it.

iii. Containers/Utensils

Glasswares, stainless steel, aluminium, enamelled wares are the most appropriate material for starter propagation. The copper and copper alloys should not be used. The surface of containers should be smooth and free from crevices. The container should be thoroughly cleaned and sterilized by steaming for at least 30 min at 100°C.

iv. Level of Inoculum

The level of inoculum used for propagation of starter cultures may also affect the performance of the starter. This depends upon the characteristics of starter culture, activity and condition of the culture at the time of inoculation, time and temperature of incubation employed. Normally 0.5 to 2.0% inoculum is used for starter preparation. The amount of inoculum may vary according to type of starter culture.

v. Culture Transfer

The starter culture should be properly and aseptically transferred avoiding all the changes of contamination. The strict aseptic conditions are needed to avoid aerial contamination with bacteriophages, molds and other undesirable microorganisms. For this purpose, a separate culturing room should be constructed and adequately equipped with laminar flow, UV lamp and provision for spraying cleaners and sanitizers. For transfer of cultures, all the accessories required should be thoroughly cleaned and sterilized.

vi. Incubation period

Normally, after inoculation the majority of milk starter cultures are incubated at 21.1°C to 30°C and time of incubation is determined depending upon the inoculum size and the activity of individual culture. In general incubation time of 14-16 hours appears to be ideal when the inoculum level used is 1 percent. The maintenance of proper temperature is very important and hence, it should be carefully monitored.

vii. Cooling

After attaining the desirable growth of the starter culture, it should be immediately cooled to stop further growth so that it should be in sound and active condition for further use. The cultures are stored under refrigeration including mother cultures until further use.

1.7 METHOD OF PROPAGATION AND PRODUCTION OF STARTERS

For the best performance and maintenance milk should be sterilized by heating at 90°C for 1 hour. The breakdown products of milk by employing heat treatment act as bacterial growth factors. This is probably one of the reasons why lactic acid bacteria grow more rapidly in heated than unheated milk. After giving sufficient heat treatment milk is cooled to 22-25°C and inoculated with appropriate inoculum size. After inoculation, the culture is incubated at 22-25°C until clotting takes place, and thereafter it is stored in a refrigerator. Further, a small aliquot of the culture is re-
inoculated into a similar container containing sterilized milk for storage of mother culture. The remaining culture is inoculated into the starter can or vessel containing sterilized milk. The purity of mother culture however is very essential. The inoculum generally is added at the rate of 1 percent from a culture having approximately 0.8 percent acidity. The acidity should not exceed 0.9 percent lactic acid. In case of poor performance including slow activity, any visible abnormality in behaviour, flavour, and colour and appearance of the starter, it should be immediately discarded and fresh starters should be used.

The purity and activity of the starter culture should be maintained by any means to achieve desirable fermentation efficiently in the manufacture of cultured dairy products. The starter culture must contain maximum number of viable organisms and must be very active under production conditions of the plant. For the preparation of fermented dairy products like cheese, dahi, yoghurt, etc. starter cultures are often maintained in milk, heated to 90°C for 1 hour and bulk starters may be grown in milk held at 90°C for 30 min. This heat treatment is adequate to kill phages and all other vegetative bacterial cells.

i. Preparation of Mother Culture

The preparation of mother culture is a very important step in the production of bulk starters for manufacture of fermented milk products at large scale. The mother cultures are generally maintained in narrow neck polyethylene bottles, which are thoroughly cleaned and sterilized using steam jets. Sometimes these bottles are sanitized with 0.1 per cent -hypochlorite solution. The milk is pasteurized in these bottles at 72-73°C for 45 minutes and the bottles then submerged in the cooling water, and thereafter, these milk bottles are stored in a refrigerator for future use. The milk-containing bottle is inoculated by injecting few drops of the desired culture by means of a hypodermic needle, which can pierce the seals of caps. Thereafter, the content is incubated at desired temperature in a BOD incubator.

ii. Preparation of Bulk Starters

The vessels of different sizes are used for preparation of bulk starters. These vessels are closed completely air tight after the initial stage of filling with milk. These cans are filled with milk and heat treated by steam at 72-73°C for 45 min and cooled to incubation temperatures. The bulk culture is inoculated and incubated in the same manner as the mother cultures.

iii. Continuous Starter Production

Recently, this culturing technique has stimulated a lot of interest and dairy industry is striving for the introduction of this technique in the cheese plants with the advent of this technology, the manipulation and transfer of large bulk of starters are now avoided. Many cheese plants today may use a very huge amount of bulk starters every day. This has been possible only through continuous cultivation of the starters. The handling of large size cans for bulk starters is extensively cumbersome and inconvenient. Inspite of convenience and ease, the continuous starter production suffers from a serious problem. During the operation of starters, if the starter gets contaminated (attacked by a phage), the whole bulk and all the equipment get contaminated. Hence, the control of purity and activity is even more important in continuous starter production than in a batch method of production.

iv. Preparation of Master Culture

In case of preparation of master culture, the method of inoculation is the same as for mother culture. For this, litmus milk may be used in the polyethylene tubes. Specific
care must be taken to sterilize the chalk before adding to the milk as spores in chalk may be extremely resistant to heat. Litmus milk previously sterilized in glass bottles is filled into the tubes using a hypodermic needle. These are now ready for inoculation with the starter cultures. Such cultures may be maintained indefinitely with careful checking and testing after three months interval. Polyethylene containers are preferred over glass tubes and bottles as these are unbreakable, very light easily portable.

Check your Progress 2

1) Discuss the various factors affecting fermentation process of starter cultures.

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2) How will you prepare good quality starter cultures?

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3) Describe preparation of bulk starters.

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4) Describe preparation of master culture.

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...................................................................................................................
...................................................................................................................

1.8 MAINTENANCE AND PRESERVATION OF STARTER CULTURES

The stock and mother cultures are propagated in the laboratory, while the feeder and bulk cultures are produced at the starter room of the dairy. An active bulk starter culture must have the following characteristics.

- It must contain the maximum number of viable cells.
- It must be free from any contaminants, e.g. coliforms or yeasts and moulds.
- It must be active under processing conditions in the dairy and hence maintenance of the intermediate and other cultures is extremely important.

The mother and feeder cultures are grown in sterile media, mainly milk, under aseptic conditions and the activity of such cultures can be maintained by applying one of the following approaches. First, reducing or controlling the metabolic activity of the organisms by ordinary refrigeration; this is for short-term storage of a starter culture and it can be kept viable for up to a week. Second, concentration and separation of the organisms from their wastes, followed by re-suspension in a sterile medium and finally preservation by drying or freezing. The latter forms are used for extended
storage of the starter bacteria and such cultures may be obtained from stock collections available in dairy research establishments, colleges or culture bank organizations, or from commercial starter manufacturers.

**Methods of Starter Culture Preservation**

It is essential that starter cultures are preserved in order to maintain an available stock of these microorganisms for the production of bulk starter and in the case of a starter failure, some typical preserved cultures could be used for direct-to-vat inoculation (DVI). Also, successive culture transfers or sub-culturing, can induce mutants which may alter the overall behaviour and general characteristics of the starter. Furthermore, in the case of mixed starter cultures, successive sub-culturing could alter the balance or ratio of *S. thermophilus; L. delbrueckii subsp. bulgaricus;* in “bio: starters the counts of *Lactobacillus acidophilus* and *Bifidobacterium* spp. will be altered.

In general, dairy starter cultures may be preserved by one of the following methods:

- **Liquid starter.**

- **Dried starter:** (a) un-concentrated (spray dried or freeze dried/ lyophilized; these methods are rather old and not used at the present time), and (b) concentrated freeze-dried.

- **Frozen starter:** (a) frozen at –20°C (un-concentrated), (b) deep frozen at –40°C to –80°C (concentrated), and (c) ultra low temperature freezing at –196°C in liquid nitrogen (concentrated).

The main methods of starter culture preservation involve concentration of the bacteria, as well as various techniques of drying and freezing, and hence, the viability of a preserved culture may be dependent on:

- the basic growth medium,

- the presence of cryoprotective agents,

- rapid removal of metabolic compounds, e.g. lactic acid and carbonyl compounds,

- the nature of the suspending medium (if employed),

- conditions of freezing and/ or drying.

- Rate of thawing (deep frozen cultures),

- Methods of concentrations.

The latter aspect, sometimes referred to as cell biomass concentration, is of great importance; the number of bacterial cells per unit weight or volume is measured by counting the number of colonies produced after serial dilution, on an agar medium and the results are recorded by colony forming units (cfu) ml\(^{-1}\) or g\(^{-1}\). However, the cell biomass can be concentrated using different systems.

**i) Liquid starters:** Starter cultures can be preserved in a liquid form using one of two different growth media. The first type is reconstituted skimmed milk powder (SMP) (10-12 g 100ml\(^{-1}\) SNF (solids-not-fat), which is free from antibiotics. The milk is sterilized by autoclaving at 121°C for 10-15 min, and a sample is incubated for a week at 30°C to check its sterility. After inoculation, the milk is incubated at 30°C for 16-18 hours or at 42°C for 3-4 hours. At the end of the incubation period, the clotted culture must be cooled immediately.
and it can then be stored for up to a week at ordinary refrigeration temperature (e.g. <10°C). Alternatively, cool, autoclaved milk may be inoculated with a starter culture and then stored under refrigeration for incubation whenever it is required. It is worthwhile to note that successive sub-culturing is labour intensive, expensive and can induce mutant strains; furthermore, trained personnel are required to perform such duties in the laboratory. A maximum limit of 15-20 sub-cultures is recommended for the yoghurt starter bacteria to safeguard the proper ratio between cocci and the rods, and to reduce the effect of mutation. A slightly extended preservation of liquid cultures (i.e. reserved stock culture) can be achieved using litmus milk.

Starter culture activity is affected by the rate of cooling after incubation, level of acidity at the end of the incubation period and the temperature and duration of storage. Cooling is important to control the metabolic activity of the starter.

ii) **Dried starters:** An alternative method for the preservation of starter cultures is drying. The different drying methods used are:

- Vacuum drying (old methods not used at present time)
- Spray drying
- Freeze drying or lyophilization (widely used in the laboratory)
- Freeze drying of concentrated cultures (widely used commercially).

The main objectives behind these developments are first, to reduce the workload which is involved in maintaining liquid cultures, second, to improve the shelf life of the preserved cultures, and third, to facilitate the dispatch of cultures by post without any appreciable loss in their activity.

The drying process prior to 1950s was carried out under vacuum and the results were not encouraging (i.e. the preserved dried cultures contained only 1-2 per cent viable bacteria). To regain maximum activity several dried sub-culturing were required. In essence, this method of preservation consisted of taking an active liquid starter culture, adding lactose as a protective agent and calcium carbonate to neutralize the excess acid, followed by partial concentration of the mixture (i.e. removal of whey). The concentrated starters, which were by then in a granular form, were dried under vacuum.

iii) **Frozen starters:** Starter cultures can also be preserved in the frozen form and such cultures are produced by two different routes:

- Deep or subzero freezing (-30 to –80°C).
- Ultra low temperature freezing (-196°C) in liquid nitrogen.

Sterile liquid milk freshly inoculated with an active starter cultures is deep frozen at –30 to –40°C to preserve the mother or feeder culture. Such frozen cultures can retain their activity for several months when stored at –40°C and this method of culture preservation became popular in the dairy industry because deep frozen cultures produced in centralized laboratories could be dispatched to a dairy in dry ice whenever required. These cultures are mainly packed in plastic containers and a typical example is the Astell-type plastic bottle.

### 1.9 COMPOSITION OF FERMENTED MILKS

The typical composition of fermented milks as per IDF (1981) is given below:
The compositional and organoleptic qualities of fermented milks depend on initial quality of milk, manufacturing conditions, types and levels of starter cultures and age of the products.

Fermented milks can be grouped into four types based on the acid content of these products.

<table>
<thead>
<tr>
<th>Type</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low acid</td>
<td>Cultured butter milk and cultured cream</td>
</tr>
<tr>
<td>Medium acid</td>
<td>Yoghurt and acidophilus milk</td>
</tr>
<tr>
<td>High acid</td>
<td>Bulgarian sour milk</td>
</tr>
<tr>
<td>Acid-alcohol</td>
<td>Kefir and Kumiss</td>
</tr>
</tbody>
</table>

1.10 TYPES OF FERMENTED MILKS

A variety of traditional as well as modern fermented milks are now in vogue in different parts of the world (Table 1.4). Originally, these were prepared from sheep and buffalo milk and to a lesser extent from that of goat, cow and mare milk and the fermentations were carried out in cloth or skin bags or wooden or earthen pots.

<table>
<thead>
<tr>
<th>Name</th>
<th>Country of origin</th>
<th>Milk types, conditions</th>
<th>Microflora</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dahi (Dadhi)</td>
<td>India, Persia</td>
<td>Cow’s or buffalo’s milk</td>
<td><em>L. lactis</em> subsp. <em>lactis</em>, <em>S. salivarius</em> subsp. <em>thermophilus</em>, <em>L. delbrueckii</em> subsp. <em>bulgaricus</em>, <em>plantarum</em>, lactose fermenting yeasts, Mixed culture (not defined)</td>
</tr>
<tr>
<td>Shrikhand (chakka)</td>
<td>India</td>
<td>Cow’s or buffalo’s milk</td>
<td><em>S. salivarius</em> subsp. <em>thermophilus</em>, <em>L. delbrueckii</em> subsp. <em>bulgaricus</em></td>
</tr>
<tr>
<td>Lassi</td>
<td>India</td>
<td>Cow’s or buffalo’s milk</td>
<td><em>S. salivarius</em> subsp. <em>thermophilus</em>, <em>L. delbrueckii</em> subsp. <em>bulgaricus</em></td>
</tr>
<tr>
<td>Cultured butter milk</td>
<td>Scandinavian and European</td>
<td>Cow’s or buffalo’s milk</td>
<td><em>L. lactis</em> subsp. <em>lactis</em>, <em>L. lactis</em> subsp. <em>diaeactilactis</em>, <em>Leuconostoc dextranicum</em> subsp. <em>citrovorum</em></td>
</tr>
</tbody>
</table>
### Fermented Products

<table>
<thead>
<tr>
<th>Aciphilus milk</th>
<th>Australia</th>
<th>Cow’s milk</th>
<th>L. acidophilus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yoghurt (bio-yoghurt)</td>
<td>Middle Asia, Balkans</td>
<td>Cow’s milk, goat’s or mixed milk</td>
<td>S. salivarius subsp. thermophilus, L. delbrueckii subsp. bulgaricus, Micrococcus and other lactic acid cocci, yeasts, molds</td>
</tr>
<tr>
<td>Kefir</td>
<td>Caucasus</td>
<td>Sheep’s, cow’s, goat’s or mixed milk, fermentation in skin bag or in wooden barrels</td>
<td>L. lactis subsp. lactis, Leuconostoc spp. L. delbrueckii subsp. caucasiciuc, Saccharomyces kefir, Torula kefir, micrococci, spore forming bacilli</td>
</tr>
<tr>
<td>Kumiss</td>
<td>Asiatic steppes</td>
<td>Mare’s, camel’s or asse’s milk, fermentation in skin bag</td>
<td>L. delbrueckii subsp. bulgaricus, L. acidophilus, Torula kumiss, Saccharomyces lactis, micrococci, spore forming bacilli lactis, micrococci, spore forming bacilli</td>
</tr>
<tr>
<td>Leben, Labneh</td>
<td>Lebanon and Arab</td>
<td>Goat’s or sheep’s milk, fermentation in skin bag/earthenware</td>
<td>L. lactis subsp. lactis, S. salivarius subsp. thermophilus, L. delbrueckii subsp. bulgaricus, lactose fermenting yeasts.</td>
</tr>
</tbody>
</table>

#### 1.11 NUTRITIVE VALUE

Studies on the nutritive value of fermented milks predominantly deal with yoghurt and are mainly carried out on animals. Determining the significance of the observed effects for human health is not easy. Following are the most important aspects when a fermented milk product is compared with plain milk.

**i. Composition**

**a) Lactose content:** Fermentation decreases the lactose content but not to such a low pH that any further sugar breakdown is impossible because the resulting product would become too acidic. At a lactic acid content of, say, 0.9 per cent the fermentation is often slowed down by cooling. About 20 per cent of the lactose in the milk has then been split, if both glucose and galactose are fermented. In yoghurt twice as much lactose is split since most of the yoghurt bacteria do not decompose galactose.

**b) Vitamin content:** Lactic acid bacteria often require certain B vitamins for growth, and can produce other vitamins. Accordingly, the properties of the culture involved largely determine the extent to which the concentrations of vitamins in the fermented milk differ from those in the original milk. In yogurt the level of most of the vitamins is somewhat reduced; the folic acid content may be increased but the utilisability by humans of the folic acid thus formed is not certain. The vitamin content in fermented products is also affected by the storage conditions and especially by the pretreatment of the milk. For instance, heat treatment of milk results in a decrease of vitamins B₁, B₁₂, C, and folic acid.
c) Other changes due to bacterial action are nutritionally insignificant.

d) Composition can be changed by such process steps as standardization and ultrafiltration, and by addition of skim milk powder, caseinate, stabilizers, flavourings, or fruit pulp.

ii. Nutritional Aspects

e) Edible energy: The fermentation process per se does not cause a substantial change of the energy content of milk. The conversion of lactose to lactic acid reduces the energy value by only a small percentage.

f) Digestibility

Protein and fat: The digestibility may be improved by a slight pre-digestion of the compounds by enzymes of the lactic acid bacteria. People with a weakened intestinal function may take advantage of the pre-digestion, but healthy people digest these compounds efficiently. In the stomach, the protein in fermented milk coagulates into finer particles than plain milk, which may increase digestibility. The gastric juice of babies contains little acid and, accordingly, sometimes (dextrorotatory) lactic acid is added to baby formulas.

Lactose: Lactose-intolerant users digest a sour milk product like yoghurt much better than plain milk due to decrease in the concentration of lactose in fermented milk. In addition, factors must exist in fermented product that lead to easier digestion of lactose. The lactase activity of the yoghurt bacteria as well as the stimulation of the lactase activity of the intestinal mucosal by yoghurt have been held responsible. Alternatively, the depletion of the stomach contents into the duodenum may be retarded when fermented milk is consumed; thereby, the contact time of lactose hydrolyzing enzymes with the substrate in the stomach would be extended, resulting in a better digestion of lactose.

g) pH adjustment: The consumption of fermented milks causes a smaller decrease of the pH of the stomach contents and thereby diminishes the risk of passage of pathogens. This is of particular importance for people suffering from a weakened secretion of gastric juice, e.g., many elderly people and babies.

h) Antimicrobial action: Lactic acid bacteria can form antibiotic compounds that injure pathogens in vitro. The in-vivo significance of these compounds in suppressing gastroenteritis is not quite clear.

i) Absorption of minerals: Due to the low pH of fermented milks, some minerals are better soluble than in plain milk; it is sometimes assumed that a better absorption of minerals is thus to be expected. However, the absorption of various elements, especially that of magnesium and zinc, is enhanced by lactose. The lactose content decreases during fermentation, causing the net absorption from sour milk to be lower. Animal tests with yoghurt have confirmed the effect; the absorption of phosphorus, which is less affected by lactose, proved to be somewhat increased. Clearly, as far as the uptake of minerals is concerned, the fermentation of milk offers no distinct nutritional advantages.

j) Some additional positive and negative effects:

- Intestinal flora: The consumption of living lactic acid bacteria through fermented milk is supposed to result in the implantation of a favourable flora of lactic acid bacteria in the large intestine; the flora might repress pathogens. The most probable effects result from bacteria that do not only survive the action of gastric juice in the gastrointestinal tract but can also colonize in the intestine, such as strains of the intestinal bacteria Lactobacillus acidophilus, L. Salivarius, and Bifidobacterium bifidum.
When yoghurt is eaten frequently, the common yoghurt bacteria survive the transport through the gastrointestinal tract but do not colonize. Thus far investigations have not permitted the conclusion that effects for humans are favourable.

- **Cholesterol level**: Some animal tests suggest that consuming fermented milk might contribute to decreased cholesterol content of the blood and, accordingly, could reduce the risk of heart and vascular diseases. However, even if this is true, the effect would be small. Consumption of fermented milk could further contribute to an increased resistance to pathogens by activating the immune system and to a decreased risk of colon cancer.

- **Dental caries**: Fermented milks have not been shown to cause caries due to damage to the enamel at low pH. The lactic acid bacteria of the mouth flora form non sticky dextrans from lactose and they consequently cause no dental plaque. Obviously, the saliva has adequate counteracting activity to prevent dental caries.

- **Contract**: Supposedly, the consumption of yoghurt can cause this eye disorder. Rats exclusively fed with yoghurt (made of concentrated milk) went blind because of the accumulation of galactitol in the eye lens. However, unlike the rat, humans can readily convert galactose to glucose; therefore, the galactose content of the blood does not increase and no galactitol is formed.

- **Lactic acid type**: The type of lactic acid formed has physiological significance. Two stereoisomers of lactic acid exist: detrorotatory L (+) lactic acid and levorotatory D(-) lactic acid. L (+) lactic acid can readily be metabolized in the body, but D(-) lactic acid at a slower rate. The latter acid is partly removed from the body through the urine. In traditional yoghurt some 40 per cent to 60 per cent of the lactic acid is levorotatory and is formed by *Lactobacillus delbrueckii* ssp. *bulgaricus*. Ingesting excessive quantities of (D-) lactic acid may cause acidosis, resulting in some tissue injury.

- The major constituents of yoghurt and health-promoting attributes of fermented milk are presented in Table 1.5 and 1.6, respectively

### Table 1.5. Typical values of the major constituents of milk and yoghurt

<table>
<thead>
<tr>
<th>Constituent/100 g milk</th>
<th>Milk</th>
<th>Yoghurt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whole</td>
<td>Skim</td>
</tr>
<tr>
<td>Water (g)</td>
<td>87.8</td>
<td>91.1</td>
</tr>
<tr>
<td>Energy values (kcal)</td>
<td>66</td>
<td>33</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>3.2</td>
<td>3.3</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>3.9</td>
<td>0.1</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>4.8</td>
<td>5.0</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>115</td>
<td>120</td>
</tr>
<tr>
<td>Phosphorus (mg)</td>
<td>92</td>
<td>95</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>55</td>
<td>60</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>140</td>
<td>150</td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>0.4</td>
<td>0.4</td>
</tr>
</tbody>
</table>
Table 1.6: Some of health-promoting attributes of yoghurt/fermented milks

<table>
<thead>
<tr>
<th>Action/ effect</th>
<th>Health benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td>In digestive tract</td>
<td>Active against Helicobacter pylori</td>
</tr>
<tr>
<td></td>
<td>Enhanced lactose digestion Stimulation of intestinal immunity.</td>
</tr>
<tr>
<td></td>
<td>Stimulation of intestinal peristalsis.</td>
</tr>
<tr>
<td>On intestinal microflora</td>
<td>Improves balance between microbial populations. Decrease in faecal enzyme activity. Colonization of intestinal tract. Reduced carrier time for Salmonella spp.</td>
</tr>
<tr>
<td>Other effects</td>
<td>Improved immunity to decrease. Suppression of some cancers. Reduction in serum cholesterol. Reduction in hypertension.</td>
</tr>
</tbody>
</table>

Check your Progress 3

1) What are the characteristics of a bulk starter culture?

2) List the methods of preservation of starter cultures.

3) Describe dried and frozen starters.

4) Give typical composition of fermented milks.

5) Discuss the nutritional aspects of fermented milks.
1.12 LET US SUM UP

Fermentation is one of the oldest methods practiced by human beings for the transformation of milk into products with an extended shelf life. About 400 generic names are applied to the traditional and industrialized fermented milk products manufactured throughout the world. Yoghurt, a popular fermented dairy product in Europe, North and South America resembles the Indian dahi. In India, several fermented milk products are manufactured such as dahi, lassi, chakka, shrikhand, mattha, chhach, misti dahi, etc. The typical characteristics of fermented milk products depend on the type of starter culture used in its preparation. The lactic starters must produce sufficient acid, resistant to bacteriophages, antibiotics and other inhibitory substance. They should produce desirable flavour, consistency, body and texture. In general, three different types of starter cultures are used in dairy industry for manufacture of fermented products viz. single strain, mixed strain and multiple strain starters. There are several factors affecting fermentation process of starter cultures such as temperature, pH, strain capability, growth medium, inhibitors, incubation period, heat treatment of milk, storage conditions, etc. which need to be controlled for optimum performance. All precautions should be taken to prepare good quality starters, as starters are considered to be the heart of fermented products. Starter cultures are required to be properly maintained and preserved by employing appropriate methods. The typical composition of products depends on the type of raw material used for its manufacture. Fermented milk products are nutritionally sound and possess health promoting attributes.

1.13 KEY WORDS

**Bulk starter** : Bulk starter refers to a large amount of culture prepared in starter vats for direct addition to milk in incubation tank.

**Concentrated cultures** : These cultures refer to liquid cultures which are concentrated through centrifugation.

**Concentrated freeze-dried cultures** : Concentration of liquid cultures is carried out through centrifugation which is followed by freeze-drying.

**Fermentation** : Fermentation is a biological process in which a chemical substrate is transformed into different end products by the action of microbial cells.

**Fermented foods** : Fermented foods (liquid, solid or semi-solid) are those that have been subjected to the action of microorganisms.

**Freeze-dried cultures (Lyophilized cultures)** : The freeze-dried cultures are prepared on the basis of liquid cultures being subsequently freeze dried. The advantage of these cultures consists in very good storage life, particularly under refrigeration.

**Intermediate culture** : Intermediate culture represents larger amounts of culture propagated in metal containers ranging in capacity from 4 to 20 litres or something more.
Liquid cultures: The liquid cultures contain viable bacteria ready to grow quickly on the first transfer if they have not been held too long or during transport at temperatures too high. Their keeping quality is limited due to the adverse effect of accumulated acid, particularly if exposed to high temperature.

Mesophillic cultures: In general, organisms that grow at ambient temperatures are termed mesophillic cultures.

Mixed strain starters: These consists of two or more strains or species and thus, may be more variable in behaviour.

Mother culture: Mother culture refers to the culture propagated in culture flasks of about half a litre to one litre capacity.

Multiple strain starters: Multiple strain starters are mixture of known compatible, non-phage related, carefully selected starters, which give generally consistent products.

Single strain starters: A single strain starter is a pure culture of lactic acid bacteria.

Thermophillic cultures: Organisms that grow at high temperatures are called thermophillic cultures.

1.14 SOME USEFUL BOOKS


1.15 ANSWERS TO CHECK YOUR PROGRESS

Your answer should include the following points

Check Your Progress 1

1. i. Primary role/ function of almost all starter cultures is to develop acid in the product.
   ii. Secondary effects of acid production include coagulation, expulsion of moisture, texture formation and flavour production.
   iii. Suppress the growth of potential pathogens and spoilage causing microorganism.
   iv. Enhance the shelf-life of the product.

2. Common starter cultures used for preparation of fermented dairy products are: 
   *Lactococcus lactis* subsp *lactis*, *L. lactis* subsp. *cremoris*, *L. lactis* subsp. *diacetylactis*, *streptococcus thermophilus*, *lactobacillus delbrueckii* subsp. *bulgaricus*, *L. helveticas*, etc.

3. Generally three different types of starter cultures are used in dairy industry for the manufacture of a variety of fermented products.
   - Single strain starters.
   - Mixed strain starters.
   - Multiple strain starters.

4. Characteristics of good starters:
   - Must produce sufficient lactic acid at appropriate range of temperature.
   - Resistant to inhibitory substances such as antibiotics, bacteriophages, chemicals, sanitizers, etc.
   - Should not produce bacteriocins, antibiotics, etc.
   - Produce desirable flavour, consistency, aroma taste, etc.
   - Should not produce any defect.

5. i. Classify dairy starters as: I. Bacteria, II. Moulds, III. Yeasts.
   ii. Further classify according to their physiological characteristics.
   iii. Give example of starter microorganisms.
Check Your Progress 2

1. i. Important factors affecting the growth and activity of starter culture: temperature, pH, capability of strain, growth medium/substrate, presence of inhibitory substances, heat treatment of milk, carbon dioxide, storage condition, etc.

   ii. Above points should be elaborated.

2. i. Essential factors for preparation of starters are: good quality milk, necessary heat treatment, well sanitized utensils, aseptically transfer of culture, proper incubation, storage etc.

   ii. Above points need to be elaborated.

3. i. Utensils to be used for bulk starters should be thoroughly cleaned and sanitized.

   ii. These utensils should be air tight to prevent contamination, Milk should be properly heat treated, proper inoculation of milk, incubation at appropriate temperature, and storing/using in product making.

4. i. In case of master culture preparation the method of inoculation is same as for mother culture.

   ii. For this litmus milk may be used in the polyethylene tubes.

   iii. Litmus milk is filled into the tubes using a hypodermic needle.

   iv. These tubes are inoculated with starter cultures. Such cultures may be maintained indefinitely and tested after each three months interval.

   v. Polyethylene containers are preferred over glass bottles as these are light, unbreakable and easily portable.

Check Your Progress 3

1. i. It should contain maximum number of viable cells.

   ii. It should be free from all kinds of contaminants including yeast, moulds, coliforms, etc.

   iii. It should be active during processing conditions and remain viable during storage under varied conditions.

2. i. In general, dairy starter cultures may be preserved by one of the following methods:

   i) Liquid starter.

   ii) Dried starter (freeze dried, lipolyzed spray dried)

   iii) Frozen starter (frozen and concentrated at −20°C, deep frozen – concentrated at −40°C – 80°C, ultra low temperature freezing at −196°C in liquid nitrogen, concentrated.

   ii. The above methods need to be elaborated.

3. i. Drying in one of the methods of preservation of starter cultures. The different drying methods used are:

   – Vacuum drying
Fermented Products

- Spray during
- Freeze drying or lyophilization.
- Freeze drying of concentrated cultures

ii. Starter cultures can also be preserved in the frozen form and such cultures are produced by (i) deep or subzero freezing (-30 to – 80°C), (ii) ultra low temperature freezing (-196°C) in liquid nitrogen.

4. i. The typical composition of fermented milks as per IDF (1981) is: TS 14-18, Fat 0.1 – 10, Protein 4-6, Lactose 2-3, and lactic acid 0.60 – 1.10 per cent.

ii. Composition of some of common fermented milk products may be given.

5. i. The most important aspects of fermented milk product as compared to plain milk should be given. This includes lactose and vitamin contents. Changes due to processing steps as standardization, ultra filtration and by addition of skim milk powder, caseinates, stabilizers, flavorings, or fruit pulp.

ii. The nutritional aspects such as edible energy, digestibility of different constituents such as proteins, fat, lactose, etc. should be discussed.

iii. Antimicrobial action of fermented milks, absorption of minerals and some additional positive and negative effects like on cholesterol level, intestinal flora, dental caries, may also be discussed.