Consortium for Educational Communication

Module

on

Fermented Dairy Products

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TEXT

1. Introduction

Milk is the normal mammary secretion of milking animals obtained from one or more milkings without either addition to it or extraction from it, intended for consumption as liquid milk or for further processing. Milk is highly perishable and many methods have been developed to preserve it. The earliest method which has been used for many thousand years is *fermentation*. Milk can be fermented by inoculating fresh milk with the appropriate bacteria and keeping it at a temperature, which favors bacterial growth. As the bacteria grow, they convert milk sugar (lactose) to lactic acid. The lower pH caused by lactic acid preserves the milk by preventing the growth of putrefactive and/or pathogenic bacteria which do not grow well in acid conditions.

Fermentation is defined biochemically as the catabolism of glucose (or other sugars) in which the terminal hydrogen acceptor is an *organic* molecule (carbon containing). During the breakdown of sugar, known as glycolysis, excess hydrogen atoms are generated and must be deposited somewhere. In lactic acid bacteria, they "dump" excess hydrogens on to pyruvic acid, the end product of glucose. This turns pyruvic acid into lactic acid. In all fermentation, NADH gives up its hydrogen to produce NAD, which is required for further glycolysis. Yeast too performs fermentation, but with different terminal hydrogen acceptors (acetaldehyde) and products (CO_2 and ethanol). Alcoholic fermentation is also an anaerobic process. Since the terminal hydrogen acceptor in each of these microbiological processes is an organic molecule, they are, by definition, fermentation.

Fermented dairy products are products that can be produced via fermentation of lactose by microorganisms naturally found in milk. These are made from milk of almost all domesticated milch animals. These products are popular due to their differences in taste and their favorable physiological effects. There are two main species of bacteria that are involved in milk fermentation; these are Lactic acid bacteria and Bifidobacteria. Historically, fermentation process involved unpredictable and slow souring of milk caused by these organisms. However, modern microbiological processes have resulted in the production of different fermented milk products of higher nutritional value under controlled conditions. Various dairy products which are different in name but similar in content can be found and those products are an important part of human diet. However consumer's interest in fermented milk products isgaining momentum due to the development of new foodprocessing techniques, changing social attitudes; scientificevidence of health benefits of certain ingredients. Some cultured dairy foods such as bioghurt, yakult,actimel etc. are already marketed as therapeutic and dieteticproducts.

Table 1 lists some famous fermented dairy products, their country of origin and the groups of microorganisms involved in the process.These fermented dairy products feature a number of advantages:

- They offer a high degree of hygienic safety.
- They have an increased shelf life compared to the raw product.
- Raw materials are refined by improving quality-determining properties.
- Manufacture requires only basic technology and low energy consumption.
- They meet a demand for natural and organic food.

Table 1. Fermented dairy products, countries and microorganisms based on fermentation types (Yilmaz, 2006).

Fermentation types		*Product names*	*Countries*	*Microorganisms*
Lactic Acid Fermentation	Mesophilic	aetmjolk Fimljolk Lattfil Ymer	Scandinavian Countries Scandinavian Countries Scandinavian Countries Denmark	*Lactococcus lactis* susp. *lactis*, *Lactococcus lactis* susp. *biovar* *diacetylactis*, *Leuconostoc mesenteroides* spp. *Cremoris*
	Thermophilic	Yoghurt Laban ve Zabadi Skyr Ayran Mil-Mil E Miru-Miru	Various countries Egypt Japan Japan	*L. bulgaricus, S. thermophilus* *B. bifidum, L. bulgaricus,* *S. thermophilus* *L. bulgaricus, L. casei,* *S. thermophilus* *L. bulgaricus,* *S. thermophilus* *B. bifidum, B. breve,* *L. acidophilus* *L. casei, L. acidophilus,* *B. breve*
	Therapeutic	Aco-yoghurt Acidophilous milk AB-fermented milk AB-yoghurt Biogarde Bioghurt Bifighurt Yakult	Switzerland Various countries Denmark Denmark Germany Germany Germany Japan	*L. acidophilus, L. bulgaricus,* *S. thermophilus* *L. acidophilus* *L. acidophilus, B. Bifidum* *L. acidophilus, B. bifidum,* *L. bulgaricus, S. thermophilus* *L. acidophilus, B. bifidum,* *S. thermophilus* *S. thermophilus,* *L. acidophilus* *B. bifidum, S. thermophilus* *L. casei*
Yeast-Lactic Acid Fermentation		Kefir Koumiss Acidophilous- Yeast	Russia Russia Russia	Lactic Acid Bacteria, Acedic acid bacteria and yeast *L. bulgaricus,* *L. acidophilus,* *Torula koumiss, Sacc. Lactis* *L. acidophilus*, lactose fermenting yeast
Fungus-Lactic Acid Fermentation		Viili	Finland	*Lactococcus lactis* susp. *lactis*, *Lactococcus lactis* susp. *biovar* *diacetylactis,* *Leuconostoc mesenteroides* subsp. *cremoris, Geotricum candidum*

2 Dairy Starter Culture

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.

2.1 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.

2.2 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 flavoring 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:

a)It should be quick and steady in acid production.

b) It should produce product with fine and clean lactic flavor.

c) It should not produce any pigments, gas, off-flavor and bitterness in the finished products.

d)Should be associative in nature in product development.

1.3 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 intermediatory or end products, which give typical attributes to fermented milk. The roles played by starters during fermentation of milks are:

a)Produce lactic acid

b) Bring about coagulation of protein and form gel

c)Produce volatile flavor compounds like diacetyl, acetaldehyde and several intermediate compounds.

d)Possess proteolytic and lipolytic activities.

e)Produce other compounds like CO_2, alcohol, propionic acid, which are essential in products like kefir, Swiss cheese.

f)Control the growth of pathogens and spoilage organisms.

g)Some dietary cultures like *Lb. acidophilus*, give health benefits and produces antibacterial substances.

h)Help in texturizing and ripening of cheese.

2.3.1 Carbohydrate metabolism

Lactose is the major carbohydrate of milk, which is utillsed 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 CO_2, 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 mechanisms to take up lactose from the medium and its subsequent hydrolysis:

a)Most of the Lactobacilli, Leuconostocs and *S. thermophilus* take up lactose through a specific permease enzyme located in cell membrane. The lactose inside the cell is then splitted by enzyme β-galactosidase into glucose and galactose. The galactose is converted to glucose and together with glucose is fermented by glycolysis. Fig.1

b)Lactococci and a few lactobacilli like *Lb. casei*, take up lactose and galactose by the action of phosphoenol pyruvate dependant phosphotransferase system, which involves catalytic activity of four specific proteins. The lactose is phosphorylated while in transportation and is hydrolysed by β-phosphogalactosidase into glucose and galactose-6-phosphate. 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.

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

Glucose

↓

Glucose 6-P

↓

Fructose 6-P

↓

Fructose 1,6 di-P

↓

Glyceraldehydes 3-P

↓ Aldolase dihydroxy acetone phosphate

1,3, diphosphoglycerate

↓

3, phosphoglycerate

↓

2, phosphoglycerate

↓

Phosphoenol pyruvate

↓

Pyruvate

↓ Lactate dehydrogenase

Lactate or lactic acid

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

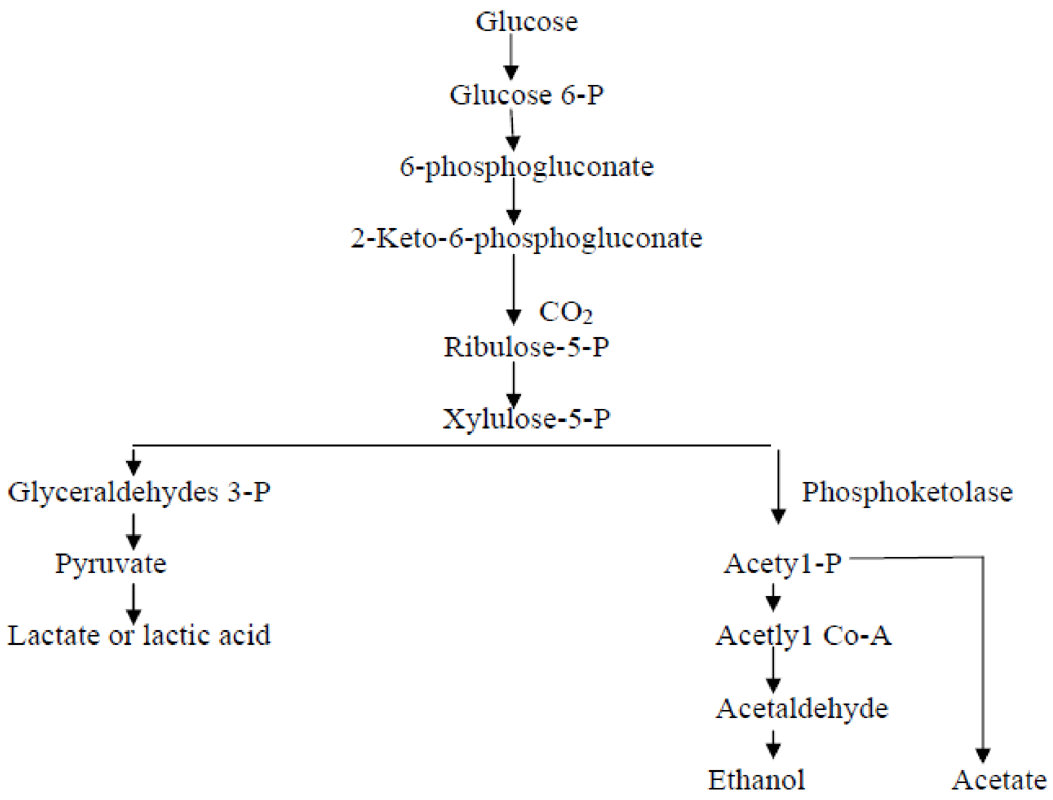

Fig. 2 Metabolic pathway of heterofermentative lactic acid bacteria

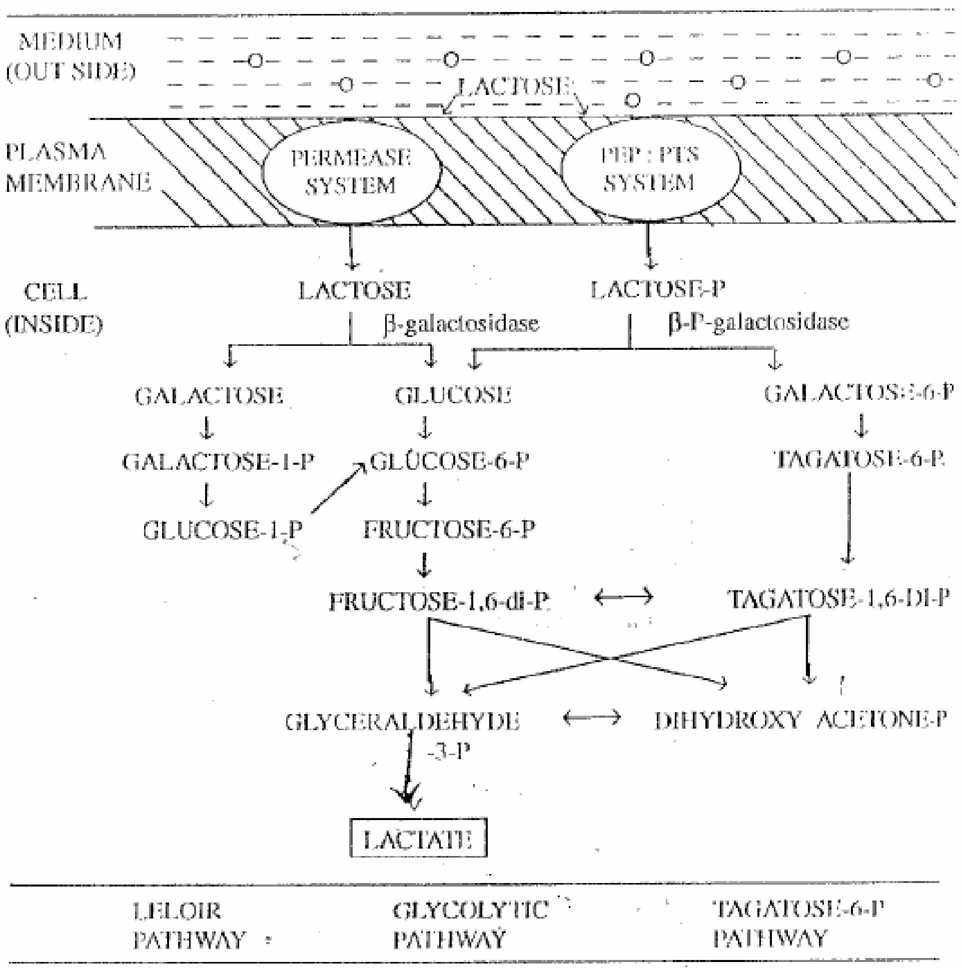

Fig. 3: Lactose and galactose utilization by different lactic acid bacteria

2.3.2 Citrate metabolism

Citrate or citric acid is present in milk in low concentration (average 0.16%) and is metabolized only by flavor producing species of mesophilic cultures, i.e., *Lc. lactis* biovar *diacetylactis* and *Leuconostoc* spp. The metabolites produced i.e. diacetyl, acetoin, acetate and CO_2 are important flavor 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 di-acetyl. 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.

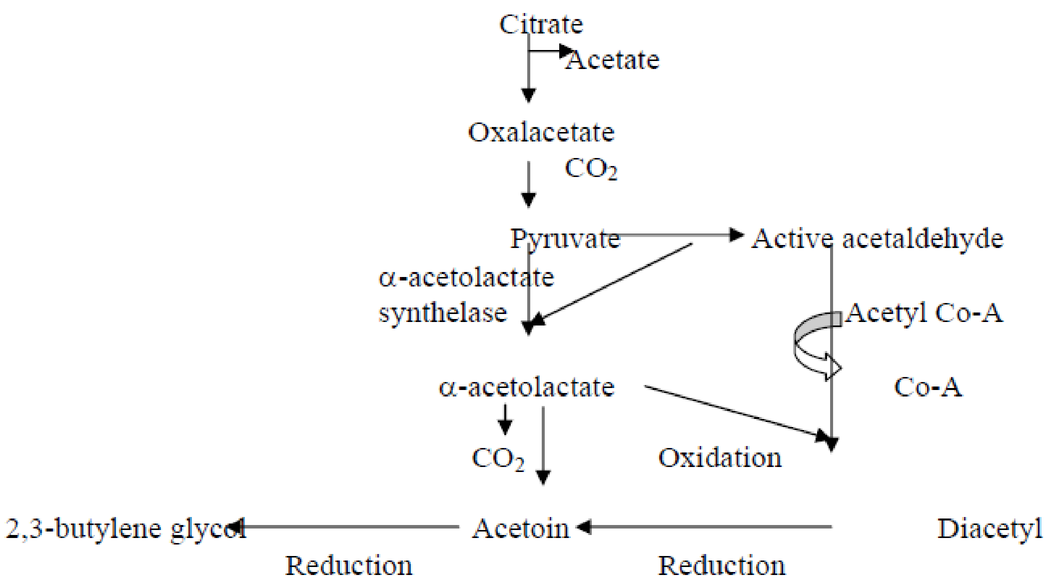

Fig. 4: Production of flavouring compounds by lactic acid bacteria through citric acid metabolism

2.3.3 Acetaldehyde production

Acetaldehyde is one of the important flavor compounds produced by starter cultures in fermented milks and is a major flavor 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).

Lactose
$\downarrow$ β-galactosidase
Glucose
$\downarrow$ Glycolysis
Pyruvate
$\downarrow$ Dehydrogenase
Acetyl CO-A
$\downarrow$ Dehydrogenase
Acetaldehyde

Milk protein
$\downarrow$ Proteinases
Peptides
$\downarrow$ Peptidases
Threonine
(+) Methionine
$\downarrow$ Aldolase
Acetaldehyde
(+) Glycine

Fig. 5: Production of acetaldehyde by lactic starter cultures

2.3.4 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.

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

a) Liberation of peptides and amino acids, which affect the physical structure of the product.

b) Many amino acids produced are essential for the growth of several cultures and

c)Peptides and amino acids act as flavor precursors.

2.3.5 Lipid metabolism

Starter bacteria are very weakly lipolytic and may possess lipases and esterase that can hydrolyze 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 flavor in fermented milk products.

2.3.5 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 B_{12}.

2.3.6 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.

1.4 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 fermented 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.

I. *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 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, tryptophan 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, etc, are:

a)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.

b)They stimulate appetite due to their pleasant, refreshing and pungent taste.

c)These products also improve central nervous and respiratory system.

d)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.

e)Gastric juice secreted by the action of fermented milk product and the desirable ratio of calcium and phosphorus leads to a high digestive capability.

f)Fat-free curd may help in prevention of heart diseases, arteriosclerosis, hypertension and chronic inflammation of the liver.

g)Fermented milk products improve the immune response and leads to longevity.

h) Possesses significant nutritional and prophylactic properties.

i)Control gastrointestinal disorders, such as diarrhoea, constipation, dyspepsia, flatulence and colitis.

j)Acidophilus yeast milk, which is rich in alcohol and CO_2, excite respiratory and central nervous system.

k)Induction of *L. acidophilus* into the intestine return to normalcy in the intestinal microflora and body comforts

l)Lower proliferation of cancerous cells.

2.5 Fermented food products and their preparation

2.5.1 Yogurt

Yogurt (also 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, flavor 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.

2.5.2 Preparation of Yoghurt

The 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 titrable 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 flavor may be incorporated at this time, and then packaged. The product is now cooled and stored at refrigeration temperatures (5°C) to slow down the physical, chemical and microbiological degradation.

2.5.3 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.6

Raw materials
↓
Standardization and /or fortification
↓
Homogenization
↓
Heat treatment
↓
Cooling
↓
Inoculation
↓
Fermentation
↓
Cooling and mixing
↓
Addition of fruit/flavour →
↓
Packaging
↓
Final Cooling
↓
Storage etc.

Fig 6. Flow diagram of main steps involved in preparation of stirred yoghur

2.6 Acidophilus Milk Products

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

2.6.1 Types of acidophilus milks Organisms

Acidophilus sour milk	*Lb. acidophilus*

Acidophilus yoghurt	*Lb. acidophilus* + *S. thermophilus*
Bioghurt	*Lb. acidophilus, L. bulgaricus, S. thermophilus*
Acidophilus yeast milk	*Lb. acidophilus* + Lactose fermenting yeast
Acidophilin	*Lb. acidophilus, Lc. lactis*, Kefir fungi

2.6.2 Acidophilus concentrates

Acidophilus paste	*Lb. acidophilus*
Dried acidophilus	*Lb. acidophilus*
Lyophilized form of milk	*Lb. acidophilus*.

2.6.3 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 devasting 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. 7.

Milk

↓

Standardization

↓

Sterilization

↓

Cooling (40°C)

↓ ← Culture preparation (*L. acidophilus*)

Inoculation (1-2% starter culture)

↓

Packaging

↓

Incubation (37 ± 1°C till setting)

↓

Cold storage (5° C)

Fig 7.Flow sheet for the preparation of acidophilis milk

2.7 Dahi

Dahi or curd is an Indian fermented milk product which is equally known for its palatability, refreshing taste and therapeutic importance. 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.

2.7.1 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

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.

2.7.2 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. 8).

Cow or buffalo milk

↓

Filtration / clarification

↓

Standardization (Fat: Solids not fat ratio)

↓

Pre-heating (60°)

↓

Homogenization and pasteurization (80-90°C for 15 to 30 min)

↓

Cooling (22 – 25°C)

↓

Inoculation with pure lactic culture (single or mixed mesophilic culture)

↓

Packaging

↓

Incubation (23 – 25°C for 16 to 18 h)

↓

Cooling and storage at 5°C

Fig 8. Flow diagram for the preparation of dahi

2.8 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.

2.8.1 Starter culture for cultured buttermilk

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

2.8.2 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 flavor. These organisms are used in proper combination to obtain the desired flavor.

The ripening process takes about 12 to 14 hours (overnight). At the correct stage of acid and flavor, 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.

2.9 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 coloring matter or added flavoring 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

2.9.1 Types of Butter and Manufacturing Processes

a) 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 (mesophilicstarter) added to the cream, which produces lactic acid and various flavor compounds such as diacetyl and then churned to get butter.

b)In another method in which cream is not ripened but is churned and sweet cream buttermilk is drained off.

2.9.2 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 flavor 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:

- 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;
- Cool to 14 – 16°C and hold for 2 h;
- Cool to churning temperature.

To produce soft butter, following process is used:

- 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;
- 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;
- Cool to 15 – 16°C.
- 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.

2.10 Cheese

A dairy product prepared from cow, buffalo, goat or sheep's milk that is set aside to thicken until it separates into 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.

2.10.1 Types of cheeses

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

2.10.1.1Cheddar

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 flavor is released when heated and it shreds well with other cheeses.

2.10.1.2 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.

2.10.1.3 Swiss

Swiss cheese, which is also known as Emmental or Schweizer, is a firm cheese with a sweet, mildly nutty flavor. This cheese is known for the holes or eye formation that develops 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.

2.10.1.4 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.

2.10.1.5Kalari

Kalari or *Kradi* traditionally known as "Maesh kraj" is a famous cheese found in Himalyan regions of Jammu & Kashmir, India. Traditionally, it is made by the tribes, locally called as "gujjars" from the acidified buttermilk released during churning of curd to release desi butter. Its production is limited and its manufacture is exclusively artisanal. No starter culture is added; fermentation is mostly carried out by microflora, naturally present in milk. The cheese exhibits bright, smooth and white color surface with slightly sour flavor and high moisture content.

2.10.1.6Processed Cheeses

It is prepared by melting one or more pressed cooked or uncooked cheeses, and adding milk, cream, butter and sometimes flavoring 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.

2.10.2 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 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 ultra-filtration 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 develop. Acid cheeses are generally consumed fresh. All rennet cheeses are produced by a common mechanism as shown in Fig. 9.

Milk $\xrightarrow{\text{Manufacture}}$ Cheese curd $\xrightarrow{\text{Ripening}}$ Mature cheese

Acidification
Coagulation
Dehydration
Moulding
Salting

Fig. 9 Mechanism of cheese production

2.11 Kefir

Kefir is a traditional popular Middle Eastern milk beverage. It originated in the Caucasus Mountains in the former Soviet Union, in Central Asia and has been consumed for thousands of years. It is the product of fermentation of milk with kefir grains and mother cultures prepared from grains. Kefir grains look like pieces of coral or small clumps of cauliflower, which contain a complex mixture of both bacteria (including various species of lactobacilli, lactococci, leuconostocs and acetobacteria) and yeasts (both lactose-fermenting and non-lactose-fermenting) such that beneficial yeast as well as friendly probiotic bacteria found in yogurt. Kefir grains or mother cultures from grains are added to different types of milk. It can be made from any type of milk; cow, goat or sheep, coconut, rice and soy but commonly cow milk is used. The grains cause its fermentation that results numerous components in the kefir. Kefir is characterized by its distinct flavor, typical of yeast, and an effervescent effect felt in the mouth. The main products of kefir fermentation are lactic acid, ethanol and CO_2, which confer this beverage viscosity, acidity and low alcohol content. Minor components can also be found, including diacetyl, acetaldehyde, ethyl and amino acids contributing to the flavor composition. This drink differs from other fermented dairy products because it is not the result of the metabolic activity of a single or a few microbial species.

2.11.1 Kefir production

There are three main ways of producing kefir (I) the artisanal process, (II) the commercial process by the Russian method and (III) the commercial process using pure cultures. Other substrates may also be used, such as milk from other animal species, coconut milk, soybean milk, fruit juices and/or sugar and molasses solutions.

The traditional artisanal production (Fig 10) involves milk inoculation with a variable amount of grains and fermentation for a period between 18–24 h at 20–25 °C. At the end of the fermentation process the grains are sieved and can be used for a new fermentation or kept (1–7 days) in fresh milk, while the kefir beverage is stored at 4 °C, ready for consumption. The initial inoculum concentration of the grains (grain/milk proportion) affects the pH, viscosity, final lactose concentration and the microbiological profile of the final product. Agitation during fermentation also influences kefir microbial composition, favoring the development of homofermentative lactococci and yeast. Incubation at temperatures above 30 °C stimulates the growth of thermophilic LAB, while being a disadvantage for yeast growth and mesophilic LAB.

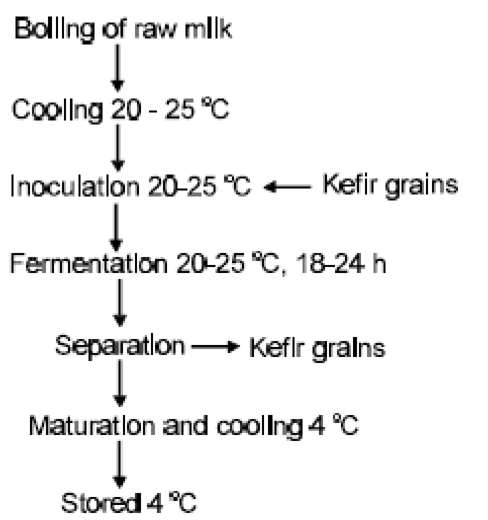

Fig 10.Traditional process of kefir making

The second method, known as the "Russian method", allows for the production of kefir on a larger scale, and uses a process of fermentation in series, from the percolate resulting from the first fermentation of the grains (fermented without the grains or mother culture).

Different methods can be used in the industrial process of kefir production, but all based on the same principle. The milk is inoculated with pure cultures isolated from kefir grains and commercial cultures. The maturation phase can be performed or not, consisting of maintaining the kefir at 8–10 °C for up to 24 h, to allow microorganism, primarily yeast, growth, contributing to the specific flavor of the product (Fig 11). Omission of this step is associated with development of atypical flavor in kefir.

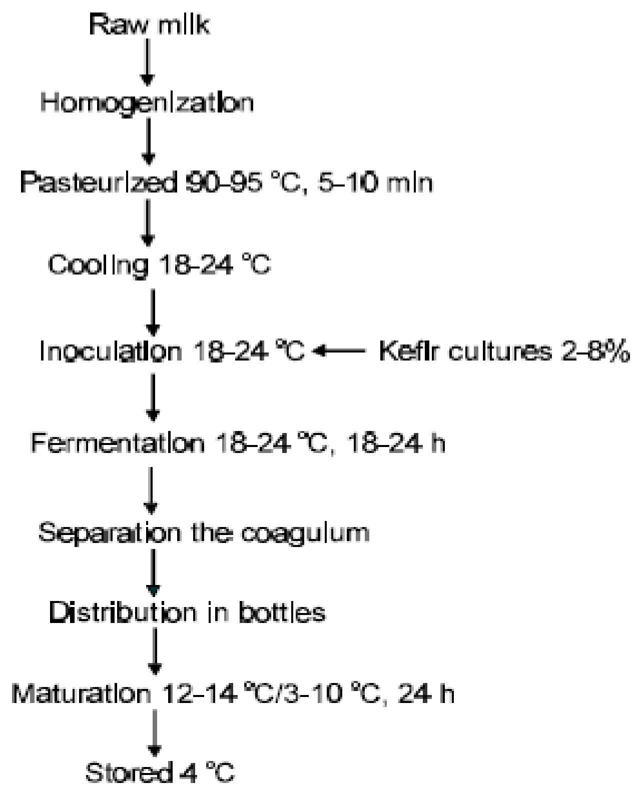

Fig 11.Industrial procedure for kefir production

2.12 Kumiss

Kumis is a fermented dairy product, traditionally made from mare's milk. *Kumis* issimilar to kefir, but is produced from a liquid starter culture, in contrast to the solid *kefir* "grains". Because mare's milk contains more sugars than cow's or goat's milk, when fermented, kumis has a higher alcohol content compared to *kefir*. Even in the areas of the world where *kumis* is popular today, mare's milk remains a very limited commodity. Industrial-scale production, therefore, generally uses cow's milk, which is richer in fat and protein, but lower in lactose than the milk from a horse. Before fermentation, the cow's milk is fortified in one of several ways. Sucrose may be added to allow a comparable fermentation.

2.12.1 Production of kumis

Kumis is made by fermenting raw unpasteurized mare's milk over the course of hours or days, often while stirring or churning. During the fermentation, lactobacilli bacteria acidify the milk, and yeasts turn it into a carbonated and mildly alcoholic drink.

Traditionally, this fermentation took place in horse-hide containers, which might be left on the top of a *yurt* and turned over on occasion, or strapped to a saddle and joggled around over the course of a day's riding. Today, a wooden vat or plastic barrel may be used in place of the leather container.

Other accountsfrom some cities in northern or western China have it that the skin, partially filled with mares' milk, is hung at the door of each home during the season for making such beverages, and passersby, who are familiar with the practice, give each such skin a good punch as they walk by, agitating the contents so they would turn into *kumis* rather than coagulate and spoil.

In modern controlled production, the initial fermentation takes two to five hours at a temperature of around 27 °C; this may be followed by a cooler aging period.The finished product contains between 0.7 and 2.5% alcohol(Fig 12).

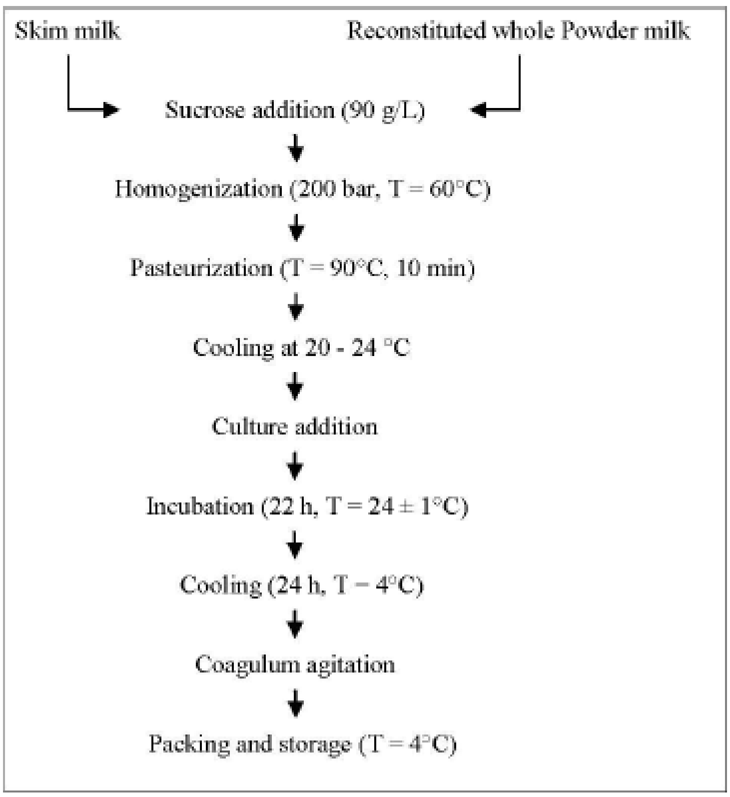

Fig12. Flow diagram for the production of artisanal kumis