The Microbiology of Milk and Milk Products

by J. Klaar

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THE MINISTRY OF THE MEAT AND MILK INDUSTRY
OF THE ESTONIAN S.S.R.

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CONTENTS

I. INTRODUCTION

Milk with its various contents of nutritive substances (proteins, milk sugar, also fat, mineral substances, microelements) is a favourable medium for the development of many microbial groups: the bacteria, the yeasts and the moulds. Most of the microbes, which develop in milk, are harmful for the quality of milk and milk products, but we use lactic acid fermenters for making milk products, such as sour cream butter, all kinds of natural cheese and sour milk products.

In the microbiology of milk we are dealing with microbes, which occur in milk and milk products. We get acquainted with the conditions of the external medium in which they reproduce and continue to live. We get to know their part in preserving of milk as technological raw-material and their influence on the quality and storage of milk products. Knowing the useful (from the point of view of milk technology) as well as the harmful physiological features of microbes, we are able to create the necessary conditions for reproduction of useful microbes, restraining at the same time the reproduction of harmful ones. Using the microbiological procedures, we are able to control the stages of the technological process and to estimate together with organoleptic features the quality of milk products.

In the Soviet Union the works of K.K. Happich, S.A. Korolev, A.F. Voitkevich, A.M. Skorodumova, V.M. Bogdanov, M.R. Gibshman have greatly influenced the development of microbiology of milk and milk products. Under the direction of K.K. Happich the Bacteriological Station was founded in 1895, by the Tartu Veterinary Institute, into whose programme was also included the microbiological investigation of milk and milk products. Through courses and exhibitions the above-mentioned Bacteriological Station became the leading centre of dairying development in Western Russia at the beginning of the present century.

II. THE MORPHOLOGY AND PHYSIOLOGY OF MICROBES USED IN THE TECHNOLOGY OF MILK AND MILK PRODUCTS

1. The lactic acid bacteria

The lactic acid bacteria represent a group of microbes, the fermentation process of which produces mainly lactic acid.

$$\left[\begin{array}{cccc} C_{12}H_{22}O_{11} & \xrightarrow[H_2O]{\text{lactose}} & C_6H_{12}O_6 & + \; C_6H_{12}O_6 \\ \text{lactose} & & \text{glucose} & \text{galactose} \end{array}\right.$$

$$C_6H_{12}O_6 \longrightarrow \underset{\text{lactic acid}}{2CH_3CHOH} \Big]$$

The lactic acid bacteria occur on all parts of green plants, they occur in the excrements of domestic and other animals and on the dairy utensils. In connection with the production of milk as food and making of milk products, certain lactic acid bacteria **were selected whose natural medium of reproduction became milk. Reproducing** in various environmental conditions **the morphological** characters and physiological properties of the lactic acid bacteria formed.

On the basis of the morphological characters lactic acid bacteria can be divided into two genera (g).

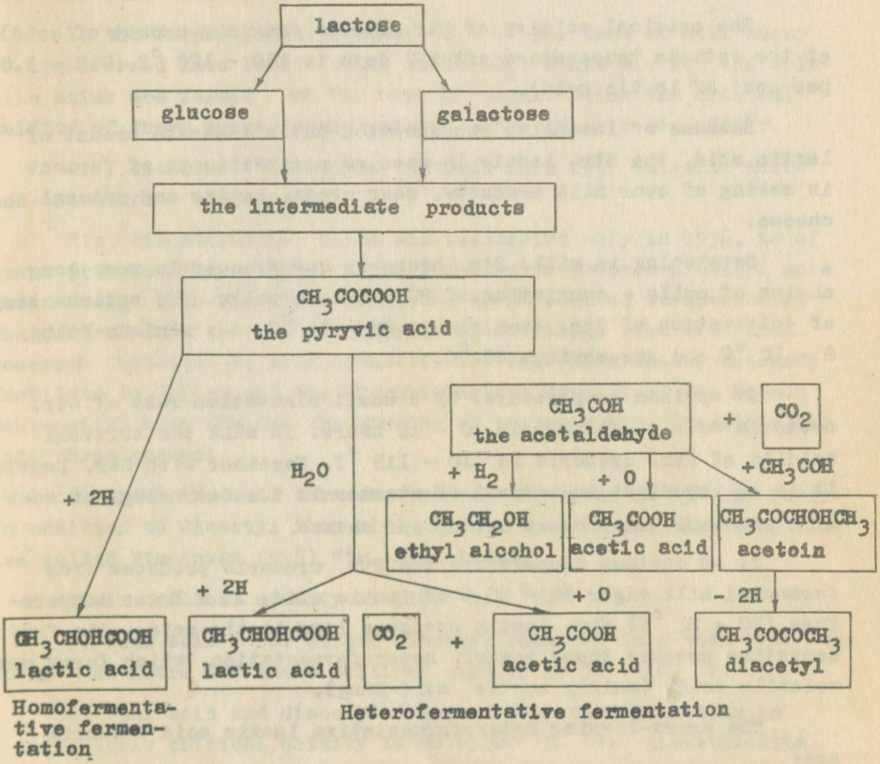
Those with spherical cells - genus streptococcus;
Those of rod-shaped cells - genus lactobacterium.

One part of the lactic acid bacteria has a typical lactic-acidic fermentation process, producing mainly lactic acid (homo-fermentative fermentation).

$$\left[C_6H_{12}O_6 \longrightarrow 2CH_3CHOHCOOH \right]$$

The other part of lactic acid bacteria generating lactic acid as the main product, produces at the same time volatile acids,

alcohols, carbon dioxide and tetracarbon (diacetyl, acetoin) and other compounds (heterofermentative fermentation). In broad limits the process proceeds according to the following scheme:



The most important species of the g. Streptococcus of homofermentative fermentation is the Streptococcus lactis.

In milk St. lactis has oval shaped cells, which occur in pairs or in the form of short chains.

The optimum temperature of reproduction, likewise of acid production is 28 - 30°C, the minimum temp. being 10 - 12°C and the maximum temp. 42 - 45°C.

The acid produced by a small plantation rate of Str. lactis bacteria (0.1 - 0.5 %) causes milk to coagulate at the temperature of 28 - 30 °C in 10 - 12 hours.

The critical acidity of Str. lactis (maximum amount of acid) at the optimum temperature after 7 days is 110 - 120 °T (0.8 - 1.0 per cent of lactic acid).

Because of intensive reproduction but a moderate amount of lactic acid, the Str. lactis is used as a constituent of ferment in making of sour milk products, sour cream, butter and natural cheese.

Developing in milk, Str. cremoris has frequently very long chains of cells - consisting of 20 and more cells. The optimum temp. of cultivation of Str. cremoris is 25 - 30 °C, the minimum being 8 - 10 °C and the maximum 40 °C.

At optimum temperature, by a small plantation rate of Str. cremoris, milk coagulates in 10 - 12 hours. In milk the critical acidity of Str. cremoris is 110 - 115 °T. Together with Str. lactis it is an important ingredient of starter in the technology of sour milk products, sour cream butter and cheese.

At an optimum temperature, the Str. cremoris produces from fermented milk sugar 90 - 95 % of lactic acid. At a lower temperature (20 - 22 °C) Str. lactis produces less lactic acid - the fermentation process turns towards heterofermentation, which forms more volatile acids (mainly acetic acid-Bang).

The aroma-forming heterofermentative lactic acid bacteria are:

1. Str. citrovorus (Leuconostoc dextranicum, Betacoccus cremoris).
2. Str. paracitrovorus (Betacoccus bovis).
3. Str. diacetilactis.

Those Str-i have global cells, which grow single, in pairs or in short chains.

The optimum temperature of their reproduction is 25 - 30 °C, the minimum temperature being 8 - 10 °C, the maximum 43 - 45 °C.

In milk the activity of aroma forming Str. is moderate. Str. paracitrovorus coagulates milk in 2 - 3 days, but. Str. citrovorus does not turn milk sour.

In the fermentation process, 50 - 60 per cent of milk sugar is transformed into lactic acid, while CO_2, ethyl alcohol and volatile acids are formed of the rest of sugar. Hence the critical acidity of those aroma-forming streptococci is only 60 - 80 ^{o}T.

Of citrates, citrovorus bacteria form CO_2, volatile acids and tetracarbon compounds.

Str. diacetilactis, which was identified only in 1936, is of great practical importance, while its strains produce diacetyl more intensively than the above mentioned aroma forming streptococci. The central laboratories of dairying microbiology (The All-Union Research Institute of Milk Industry, The All-Union Cheese Research Institute in Uglich and the Microbiological Faculty of the Moscow University) have divided the strains of isolated Str. diacetilactis into three groups:

a) Intensive acid formers, turning milk sour in 15 - 18 hours. In addition to diacetyl they also produce acetoin and therefore they are called Pimenovas (MGU) Str. acetoinicus.

Their critical acidity is 110 - 115 ^{o}T.

b) Weak acid-, but strong diacetyl formers. They turn milk sour in 45 - 48 hours and their critical acidity is 90 - 100 ^{o}T.

c) Weak acid and diacetyl formers. They turn milk sour in 2 days. Their critical acidity is 90 - 100 ^{o}T. Str. diacetilactis as Str. citrovorus, ferment citric acid and its citrates, producing CO_2, volatile acids and tetracarbon compounds.

Aroma-forming bacteria of the type Str. diacetilactis, have cells in pairs or in short chains. The temperature of their optimum reproduction is 25 - 30 ^{o}C, the minimum being 10 ^{o}C and the maximum 45 ^{o}C.

Aroma-forming streptococci are used together with Str.lactis and Str. cremoris in preparing of starters. Because of the formation of aromatic substances, the use of starters in the production of milk products strongly influence their aroma.

The Str. Termophilus

Its oval cells occur in long chains of scores of cells.

Its optimum temperature of reproduction and the production of acid is more than 40 °C, reaching 45 - 48 °C. They turn milk sour in 12 - 14 hours and their critical acidity is 100 - 115 °T.

Thermophilic streptococci are comparatively more resistant than other streptococci, therefore a part of them is preserved even in the pasteurization of milk. Because of heat resistance, they are preserved in the above mentioned kinds of cheese even at the temperature of 54 - 56 °C for 30 - 50 min.

Rod-shaped lactic acid bacteria, which are called lactobacteria, are divided according to their properties as follows:

the thermophilic,

the streptobacteria (mesophilic),

the betabacteria (aroma-producing).

The thermophilic lactobacteria are all intensive acid producers. On milk technology Lactobacterium bulgaricum, L. acidophilum, L. helveticum and L. lactis are of importance.

The cells of the thermophilic lactobacteria are remarkably large (8 - 10 x 0.8 - 1 micrometers). Characteristic is the granular structure of cells. The cells occur singly, but also in short chains.

The optimum temperature of their reproduction is 40 - 45 °C, when even by a small plantation rate they turn milk sour in 12 hours. Their critical acidity reached 200 - 300 °T (2 - 2.8 % of lactic acid).

Of thermophilic lactobacteria, in the production of Bulgarian sour milk-yoghurt the L. bulgaricum are used and in the production of acidophilin and acidophilous milk the strains of L. acidophilum. In cheese technology L. helveticum and L. lactis, as heat resistant lactobacteria of high proteolytic activity are used in the production of cheeses of high secondary heating temperature.

The Streptobacterium-Lactobact.

Sb. casei, Sb. plantarum, L. lactis are less active acid-formers. The diameter of their cells is mostly smaller than that of thermophilic lactobacteria, which are active acid formers. The cells occur singly or in chains.

Streptobacteria take part in the ripening process of cheese, L. lactis is a component of the microflora of kefir starter.

As regards the physiological qualities, the species of Betabacterium are similar to the aroma-producing lactic acid streptococci. They produce aromatic substances, volatile acids and CO_2.

Betabacteria are of low activity, theydo not coagulate milk and they have low critical acidity. They occur in ripening cheese and in souring of kefir.

The Propionbacterium g.

Propionic acid bacteria have short rod-shaped, sometimes even globular cells. They are immovable and sporeless. The optimum temperature of development is 30 - 35 °C. In milk they develop slowly, coagulating it by a small plantation rate only in 7 - 10 days. Propionic acid bacteria produce by the fermentation of milk sugar, lactic acid and lactates, propionic and acetic acids and CO_2.

Although propionic acid bacteria are slow acid-formers, their critical acidity is fairly high, being 160 - 170 °T.

The propionic acid fermentation process appears in slowly ripening kinds of cheeses, as the Russian-Swiss and the Soviet cheese.

The species of Propionbacteria: the Propionic bact. shermanii and Pr. jensenii have the ability for producing vitamin B_{12} within the limits of pH 6 - 7.0.

The Yeasts

The cells of yeasts are considerably larger than those of bacteria. Their shape varies greatly, being round, oval or elongated-

-oval. Yeasts, fermenting milk sugar, produce from it ethyl-alcohol and CO_2.

$$C_{12}H_{22}O_{11} \xrightarrow{H_2O} 2C_6H_{12}O_6 \longrightarrow 4CH_3CH_2OH + 4CO_2$$

The optimum temperature of their reproduction is 20 - 25 °C.

Yeasts get into milk from the air, from dairy utensils. In the pasteurization of milk, yeasts are destroyed without exception.

Yeasts are used in the production of sour milk beverages containing alcohol and CO_2, as the kefir, the kumyss, the kurunga and the acidophilous-yeast milk. Attempts have been made to utilize yeasts, which do not ferment milk sugar, for rising the stability of butter.

The Bacteria of the Coli-Aerogenes Group

This group includes coli-type bacteria, which occur in the digestive tract of domestic animals and human beings. The cells are rod-shaped and do not form spores. The opt. temperature of their reproduction is 38 - 40 °C. When they develop in milk the bacteria of coli-group form of the hydrolysis products of milk sugar the following acids: lactic acid, acetic acid, succinic acid and formic acid and in large quantities of CO_2 and H_2.

Because of formation of gas, the coagulum, precipitated by acids, is now lifted - we see a swelled fermentation. The critical acidity of coli-group bacteria does not reach 80 °T.

A part of the coli-group bacteria decomposes protein and imparts milk and milk products an unpleasant taste and odor. In the technology of cheese making, the coli-group bacteria, due to intensive separation of CO_2 and H_2 causes premature swelling of cheese in the course of pressing, straining and salting in the brine, at a temperature exceeding 13 °C.

The coli-group bacteria, which most often occur in milk are the Bact. coli commune, which descend from faecal contamination. Occurrence of this bacteria in milk indicate, that also other coli-groups, even pathogenic strains may be found there. The physiological qualities of the coli-group bacteria vary greatly, depending of the conditions of the surrounding environment.

Other coli-group bacteria occuring in milk are Bact. aerogenes and Bact. cloaceae, which are harmless from the sanitary point of view.

In milk and milk products the coli-group bacteria are determined by two means:

a) The determination of coli-titre by Kessler substratum, which comprises the whole group of Bacterium coli - aerogenes.

b) The determination of typical Bact. coli as indicator of faecal contamination.

The determination of the occurrence of coli-group bacteria shows also the sanitary level of milk products technology (the sufficiency of thermal application, also by the extermination of all the pathogenic bacteria).

The Butyric Acid Bacilli in Milk and Milk Products

Their typical representative is Bac. amylobacter, whose cell has large dimensions and the diameter of spore, which is formed in the cell, is comparatively large. The optimum temperature of their reproduction is 30 - 35 °C, the minimum being 8 - 10 °C, the maximum 45 °C.

The butyric acid bacilli ferment milk sugar and lactate. producing butyric acid, CO_2 and H_2. Beside butyric acid a small quantity of propionic and formic acids and butyl, propyl and ethyl alcohols are formed under the action of Bac. amylobacter.

$$C_6H_{12}O_6 \longrightarrow CH_3CH_2CH_2COOH + CO_2 + H_2$$

Due to separation of CO_2 and H_2 the bacilli of butyric acid cause by the fermentation process a late swelling of cheeses, which becomes apparent only in the 4th to 6th week. This is conditioned by slow reproduction of butyric acid bacilli in cheese. The butyric acid bacilli have the ability to hydrolyse proteins of milk to a certain extent, therefore, fermentation of butyric acid imparts cheese a bitter, stale flavour.

The butyric acid bacilli find their way into milk mainly from the excrements of cattle. As the spores of butyric acid bacilli are not exterminated in the pasteurization of milk, cleanliness must be observed during milking. Also, dairy herd must get only high-grade forage. This applies especially to silage and vegetables.

The acetic acid bacteria produce in milk products acetic acid from ethyl alcohol, which was formed by the action of yeasts.

$$CH_3CH_2OH \xrightarrow{O_2} CH_3COOH + H_2O$$

The acetic acid bacteria most frequently occurring in milk products are the Acetobacterium aceti and the Acetobacterium pasteurianum. These cultures do not develop in milk, but they develop together with the lactic acid bacteria and the yeasts. Acetic acid bacteria occur in kefir, self-soured milk and curd.

The Protein Decomposing Bacilli and Bacteria

Protein decomposing bacilli and bacteria cause the hydrolysis and decomposition of milk proteins, which results in the formation of substances with a bitter taste and an unpleasant odour.

In milk and milk products, the most frequent protein decomposers are the species of Bacillus genera - Bac. subtilis, Bac. mesentericus, Bac. cereus; in milk occur also the Bac. megathericum and the Bac. mycoides. All these microbes are able to move and their spores are not exterminated in the pasteurization. They get into milk from the excrements of cows, from dust and the surfaces of carelessly cleaned dairy utensils. The protein decomposing bacilli produce rennetlike ferment, which causes the coagulation of casein. Later, by formation of peptons, it is hydrolysed and the coagulum dissolves.

Protein decomposing bacilli are sensitive to acidic medium, but many of them develop even at the temperature of 4 - 6 °C, in consequence of what they deteriorate the quality of raw and pasteurized milk and cream. By the action of these bacteria, toxic decomposition products are also formed in pasteurized milk, causing in babies and small children indigestion.

Also has been observed in Swiss cheese putrefaction, which was caused by the action of protein decomposing anaerobic Bac. putrificus, where also are formed gaseous products CO_2, H_2S and H_2.

The protein decomposing bacteria, occurring in raw milk most frequently are the Pseudomonas fluorescence. Proteus vulgaris, Str. liquefaciens, the species of Micrococcus. Also some yeasts and most of moulds are decomposers of protein,

Pseudomonas fluorescens (Bact. fluorescence) finds its way into milk as Proteus vulgaris and other protein decomposers, by contamination in milking, from dairy utensils, dust and water.

The cells of Ps. fluorescens are rod-shaped and movable. They produce yellowish-green pigment, which dissolves in water. This property is an essential characteristic of the culture.

Pseudomonas fluorescens develops at a low temperature of 2 - 5 °C, in consequence of what raw milk in winter can turn bitter due to formation of peptons. Ps. fluorescens causes (because of its ability of hydrolyzing milk fat) rancid taste of butter.

Proteus vulgaris (Bact. vulgare) has cells of varied length, which are able to move. The optimum temperature of development is 30 - 37 °C, but development can occur also below 10 °C. By developing in milk no coagulation of casein takes place there. Casein is peptonized and decomposed, resulting in the formation of toxic compounds with a sharp odour. It occurs in the excrements of cattle and on carelessly cleaned dairy utensils, also in water. Proteus vulgaris is exterminated in the pasteurization of milk.

The species of Micrococci find their way into milk om the udder, water, excrements and also from the air.

Micrococci produce rennet ferment and lactic acid, under whose joint action casein coagulates after 3-5 days at a low acidity of 63 - 65 °T.

Because of contraction of coagulum, some whey separates on the surface and as a result of hydrolysis of proteins causes milk and milk products to obtain a bitter taste. By thermal manipulation

- pasteurization at lower temperatures, not only the spores of bacilli but also micrococci are preserved.

Another protein decomposer in raw milk is Str. liquefaciens, which is morphologically close to Str. lactis. The optimum temperature of its development is 37 - 38 ^{o}C, the critical acidity 110 - 115 ^{o}T. This species of streptococci also produces rennet ferment, causing coagulation of milk already at 35 - 40 ^{o}T. By developing in milk and milk products, because of formation of peptones, these products obtain a bitter or bitterish taste.

Of yeasts, the Mycoderma has a protein decomposing ability. It has longish-oval cells. Using lactic acid in the oxydation process, the acidity in the upper layers of curd and sour milk beverages decreases, resulting in the development of spores of the protein decomposing bacilli, which were preserved in the pasteurization. Caused by this, the protein decomposing process is intensified.

The Moulds

Conditionally, moulds can also be considered as decomposers of protein. Morphologically, moulds have a structure considerably more complicated than the bacteria and the yeasts. The cells of moulds are rod-shaped, with angular ends, forming a more or less conspicuous fibrous or felted growth upon the surface of substrata, forming from hypha mycelicum. Moulds develop by means of spores, which are formed in the shape of conits, on the tops of hyphas, on special cells-sterigmas by means of gemmation. Some moulds develop on the segmentation of the top cells of hyphas (Geotrichum candidum) or by the gemmation of cells in hyphas (Candida sp.). Few genera of moulds (Mucor) form spores in special sporangiums. The spores of moulds and the mycelicum are in most cases pigmentized.

Moulds are determined by the structure of their reproductive organs, colour and structure of the colonies, but also by physiological properties.

Moulds find their way into milk in production and handling after milking. Because of slow development they do not affect the quality of raw milk. The vegetative cells of moulds and also their spores are exterminated in the pasteurization of milk. They find their way into milk products from the air and from dairy utensils by means of contamination.

Moulds in milk products decompose protein, fat and lactic acid. Because of the above mentioned physiological qualities, only a few moulds can be used in milk technology. So Penicillum roqueforti is used in the production of roquefort cheese, Pen.album and Penicillum candidum in the production of camamber cheese. Moulds and yeasts have some importance also in the formation of surface--microflora of the cheeses of Limburg type.

Moulds, most frequently found in sour milk beverages, curd and butter are Geotrichium candidum - Oidium lactis. It does not form exogenous spores and its colonies are white in colour. The spores are formed on the segmentation of top cells, whereby by drying, the whole system of hyphas will be segmentized. On the surface of milk products Geotrichum candidum creates white islets, which after a few days cover the surface of sour milk or sour cream with a velvety film.

Geotrichum candidum intensively hydrolyzes also milk fat, causing its rancid taste. It can develop also deep in butter mass by a minimum amount of available oxygen.

Of other moulds in milk products we more frequently encounter the Penicillium glaucum and P. brevicaule, Aspergillus glaucus and Asp. niger, Cladosporium herbarum, Alternaria.

All moulds hydrolyze milk proteins and fats, deteriorating thus the quality and stability of butter.

Catenularia occurs in saccharified condensed milk in the shape of brownish clots. The acid reaction and the access of air favour the development of this mould.

The Physiology of Microbes

The physiological activities of microbes, like that of other living beings, consist in the reception of nutritive substances, in their transformation, in the process of obtaining energy and in reproduction. In carrying out these processes microbes cause considerable changes in the medium, where they develop. All changes, which take place because of the activities of microbes in the medium - in milk and milk products, are conditioned by the action of enzymes - of special biocatalyzers, which are produced by live cells of microbes. According to their field of activities, enzymes are divided into 1) ectoenzymes, which being produced by the protoplasm of the cell and excreted by the cell into surrounding medium and acting outside the cell and 2) the second group of enzymes acting within the living cell. We call them endoenzymes. Their activities do not cease with the death of cell, but those enzymes, causing the autolysis of cell, get free and penetrate into the medium, causing changes in it after the death of cell.

The Nutritional Process of Microbes

For the processes, common to all living matter, the microbes need nutritive substances. These substances are necessary for the synthesis of the components of cell, for growth, reproduction and obtaining of energy. The bacterium's cell receives the nutritive substances through the shell and the surface film of protoplasm - ectoplasm, through osmosis phenomenon. Through the same films and the same way products of metabolism are excreted. Only the nutritive substances of certain structure, which dissolve in water, such as salts, acids, alcohols, hexoses and free amino acids can penetrate into the cell without transformation.

The carbohydrates, beginning with disaccharides, milk proteins - casein, albumin and globulin, also milk fats in the form of glycerides must be split into compounds, which dissolve in water, after which they are assimilated by microbe cell. Thus the above mentioned nutritive substances are split by the action of enzymes-ectoenzymes by means of hydrolytical processes into compounds, dissolving in water. After that they can penetrate into the cell of microbe.

Further decomposition of organic matter and the process of synthesis of new compounds occurs inside the microbe's cell by the action of endoenzymes. Enzymes, active outside the cell, are splitting as biocatalysers perceivably more organic matter, than is necessary for the nourishment of cell.

The Processes by which Microbes Obtain Energy

Microbes obtain the necessary energy for the processes, which take place in the cell in two ways - the oxydation processes and the fermentation processes. By the first way energy is obtained through absorbation of air oxygen by aerobic oxydation. By the fermentation processes the energy is received without the presence of free oxygen. By the aerobic oxydation the organic-energetic material is acidified into CO_2 and into water, whereby energy reserves of the initial material are utilized in maximum. Such form of obtaining energy is typical for the protein decomposing bacilli and bacteria which occur in milk, for the most of moulds andforsome genera of yeasts (Mycoderma sp.).

Obtaining energy by the anaerobic process (fermentation), the energy is released as the result of oxydation-reduction reactions, by which organic compounds of complicated structure are simplified. So from hexoses through typical milk acid fermentation, lactic acid is formed. By the fermentation processes oxydation occurs, when the hydrogen ions are removed and thus some energy is released. By deoxidation of organic matter,into the compound are introduced hydrogen ions, followed by absorption of energy. By the anaerobic fermentation process only a small part of energy is released - only ca 1/20 from that, which is released by the aerobic oxydation process. Conditioned by the above mentioned, the energetic material for fermentation processes is used uneconomically - the end products of fermentation procésses, rich in energy, are used by other groups as energetic material.

In milk technology, the most important fermentation types are the lactic acid, propionic acid, butyric acid fermentation and the fermentation process of ethyl alcohol.

By oxydation and fermentation processes a relationship is perceivable between some of bacteria.So, for instance, the yeasts obtain energy through oxydation in such medium, where oxygen occurs, but in a medium, where there is no oxygen, they obtain energy through fermentation process, as a result of which large amount of ethyl alcohol is formed. In milk and milk products the fermentation processes take place on the basis of milk sugar and in some cases on the basis of lactates. But only a comparatively small number of microbes can use the milk fat.

Reproduction of Bacteria

Most bacteria reproduce by cell division, which in favourable conditions of external medium takes 20 - 30 minutes. The reproduction of bacteria in milk, as in other media does not take place on all stages of development at the same speed. By cultivating microbes in milk, we can observe, that at the optimum temperature they proceed through the following phases of reproduction:

1. The phase of adaptability is a period of ca 2 hours, during which bacteria adapt themselves to the conditions of the external medium and do not reproduce.

2. The accelerated (logarithmical phase), the phase of reproduction, which is characterized by the maximum intensity of reproduction (the cell division lasts only 2o - 3o minutes).

3. On the inactive phase of reproduction the number of bacteria reaches in the given conditions of medium the maximum and remains at this level till under the influence of metabolism products, reproduction is restrained and the dying off of cells begins.

4.The dying off of cells takes place as result of the exhaustion in the medium of nutritive substances and the accumulation of metabolism products. The general outline of the development of bacteria may be changed considerably by the effect of external condition. Usually after 10 - 20 generations,cells of bacteria die.

The Effect of External Conditions of Medium on the Development of Microbes

1. The effect of air oxygen

In conformity with need of air oxygen, micro-organisms are divided in two groups: aerobic, which need oxygen for their development; anaerobic, whose metabolism processes take place in oxygen-free medium. The anaerobic microbes are further divided as follows: the facultative anaerobic microbes (develop in the occurrence of oxygen and in its absence) and the obligatory anaerobic ones (they develop only by the absence of oxygen in the medium).

Accordingly, it is possible to arrange microbes, occurring in milk technology, into the following groups:

aerobic: moulds, protein decomposing bacteria and bacilli, acetic acid bacteria;

facultatively anaerobic (but they develop better by the occurrence of oxygen): yeasts, micrococci, coli-bacteria;

facultatively anaerobic: lactic acid streptococci;

facultatively anaerobic (develop better, when there is no air): lactobacterium, Propionic bacterium;

obligatorily anaerobic: the butyric acid bacilli.

2. The effect of temperature

We are subsequently going to discuss the temperature, which is one of the main factors determining the life processes of bacteria in following order:

a) the minimum temperature below which reproduction of microbes reduces and ceases;

b) the optimum temperature, which is most favourable for the reproduction and metabolism of microbes;

c) the maximum temperature, above which the reproduction of microbes ceases.

At a temperature below minimum, the bacteria do not die,- only their reproduction retards, but if the temperature is higher of maximum, the dying of cells begins. According to this, in milk technology high temperatures are used to exterminate harmful microflora and low temperatures, for restraining of reproduction of such microbes, which were preserved in the pasteurization, or descended from reinfection.

According to the optimum temperature, bacteria are arranged in 3 groups:

1) psychrophilic - the optimum temperature being 5 - 10 °C (Bac. subtilis, moulds, Pseudomonas ps.);

2) mesophilic - the optimum temperature being 25 - 40 °C (the majority of bacteria, including the lactic acid streptococci, lactobacteria, colibacteria, micrococci, the bacilli of butyric acid and the propionic acid bacteria);

3) thermophilic bacteria with the optimum temperature 45 - 60 °C (im milk and milk products the optimum temperature of thermophilic bacteria is 45 °C). By thermophilic bacteria we mean thermophilic streptococci and lactobacteria, like L. bulgaricum, L. acidophilum, L. helveticum.

3. The effect of moisture

Moulds and bacteria can develop by a 20 - 30 % moisture content in the medium. They can develop even by a 15 - 20 % moisture content in the medium, therefore, drying milk products, moisture content must be reduced below 10 %.

4. Osmotic pressure of the medium

Common salt and sugar have the ability by strengthening osmotic pressure to increase the stability of milk products. If the concentration of salt or sugar rises, the cells are dehydrated and their reproduction ceases, but they do not perish (anabiosis). With the help of common salt we can direct the microbiological processes on the surface of cheese. With the help of sugar we restrain development of the spores of bacilli and moulds in saccharified condensed milk.

5. Spores of bacteria and moulds are sensitive to the ultraviolet irradiation, so we can use it in order to exterminate microbes on the surface of cheese and on the packing material.

6. Applying reaction (pH) we can considerably influence the microbiological processes in the medium. A great majority of milk bacteria develop by neutral reaction, but moulds and yeasts can reproduce at a rather low value - 3 - 3.5 pH.

Of bacteria, which occur in milk and milk products, the most sensitive in respect to acidity are the protein decomposers and the Pseudomonas, also the butyric acid bacilli. The fermenters of milk sugar have different resistance to acidity. So the critical acidity of micrococci does not rise above 60 ^{o}T, that of colibacteria 80 ^{o}T and lactic acid bacteria up to 120 ^{o}T, propionic acid bacteria 160 - 170 ^{o}T, lactobacteria 200 - 300 ^{o}T. By adding substances rich in protein, which increase the buffering ability of medium (cheese curd, condensed milk), the critical acidity of bacteria is considerably higher, - the reproduction of bacteria is not so much restrained by titration acidity as by active acidity. But milk fat lacks this quality.

7. The effect of chemical substances

Many chemical substances, affecting cell's proroplasm, cause death of bacterial cell. So in conservation of milk samples we use the potassium bicromate, formalin and hydrogen peroxide. Calcium hypochlorite and chloramine are used for the desinfection of dairy utensils and the equipment and apparatus of milk industry. Copper sulphate restrains the reproduction of moulds on white-washed walls.

Interaction Between Microbes

Microbes developing in the same medium, affect each other with their metabolism products. Interaction between microbes becomes apparent in symbiosis, metabiosis and antagonism.

The Symbiosis

Symbiotic relations are those, in which metabolism products of one microbe favourably affect the reproduction of the other group. So yeasts favour the reproduction of lactic acid bacteria, as they produce vitamins. The latter in turn create an acid reaction of medium, favouring the reproduction of yeasts. Such symbiotic relations occur in kefir moulds.

Metabiotic relations are those, where the metabolism products of one group of microbes are a favourable energetic material for the other group, or by means of hydrolysis products favour the reproduction of other bacteria.

Antagonistic relations are those, where one group of microbes suppresses the reproduction of another group. Antagonism can be observed between the lactic acid bacteria and protein decomposers. If the reproduction of lactic acid bacteria takes place in milk, the reproduction of protein decomposers is suppressed. Especially clearly antagonism may be observed in such microbes which produce antibiotic substances. Antibiotic substances are products of normal metabolism of living cells of certain groups of microbes. Small amounts of such products suppress activity of other microbes (the bacteriostatic effect). But if antibiotics destroy bacteria of another group, bactericidal action becomes evident.

The antibiotics can influence the reproduction of bacterial cells or affect the system of ferments and consequently, metabolism and reproduction.

Although generally moulds, actinomycetes and bacilli are known as producers of antibiotics, we find also producers of antibiotic substances among the lactic acid bacteria. So are to be found isolated strains of Str. lactis, which producing antibiotic nisine, restrain the reproduction of staphylococci, of other streptococci, of butyric acid bacilli and even the bacteria of tuberculosis. The antibiotic diplococyn was isolated from one strain of Str. cremoris, which restrained the reproduction of colibacteria and Staphylococcus aureus.

Although among the strains of Str. lactis are races, which, because of their antibiotic qualities can demolish microflora, while harmful from the technological point of view, can those races at the same time restrain the reproduction of lactic acid bacteria in cultures, causing a drop in the intensity of development of those bacteria. The latter circumstance must be taken into account by starters of many components, in order to maintain balance between the species.

It must also be mentioned, that intensive producers of antibiotic substances of a wide spectrum are L. acidophilum and some yeasts, fermenting milk sugar. Also are to be found among lactic acid bacteria of plants producers of antibiotic substances, like some strains of L. plantarum.

III. THE MICROBIOLOGY OF MILK

Already in the udder, despite the bactericidal effect of milk, it contains many bacteria, mainly globular ones, which are called mammococci. Milk, which contains only the microflora of the udder, is called aseptic milk, but even in such milk the number of bacteria in 1 ml can reach to a few thousands. Especially great amount of microbes are to be found in the opening of teat, therefore the first streams must be milked into a special vessel.

Many microbes occur on the surface of teats and the udder. Therefore, careful cleaning of udder is of great importance, if we want to get milk with small number of spores.

Another important source of microbes in milk is cow's hair. If cows are carelessly cleaned, milk can get contaminated by dung. If animals are not cleaned of excrements sticking to their hair, there will occur bacteria of coli-aerogenes group, butyric acid bacilli and protein decomposers, also spores of yeasts.

The Air in Cow-Shed

Although microbes cannot develop in the air, the latter is one of the media, where they spread and from where they get into milk. The air in cow-shed is especially then a good spreader, if it contains much dust of fodder. Cleanliness of milker's hands and clothes has also great effect to the number of spores in milk.

From the above mentioned sources mainly the coli-group bacteria get into milk, but also some patogenic bacteria

Milking Technique

The number of bacteria in milk and milk products greatly depends from the milking technique. In order to get clean milk with a small number of spores by hand-milking, but also milking by machine, cows and especially their udders must be clean. Machine-milking, especially when pipe-line appliances of milking machines are used, enables to get milk with a small number of spores, because such sources of contamination as animal's hair, air, milker's hands and clothes are excluded. By mechanical milking special care must be taken that the dairy utensils and milk tanks were well cleaned and desinfected. By the utilization of well cleaned and desinfected milking equipment, the number of spores in milk can approach the number of spores in aseptically produced milk.

Beside dairy utensils, great importance has the orderly cleaning and disinfection of vessels, which are used by keeping and transportation of milk. If milk cans and cisterns, used by transportation, are not carefully cleaned, in milk begin to develop mainly the lactic acid bacteria as a result of which milk's acidity rises.

If by rinsing inside the reservoirs of milk remains some water, a development of micrococci, bacilli and protein decomposing bacteria begins on their surface.

In order to diminish the number of microbes in milk, it is necessary to wash carefully all dairy utensils, using alkaline solutions together with surface-active substances and disinfect. After that to rinse with hot, clean water and maintain the reservoirs so, that no microbe-polluted dust will get into them from the air.

The Microflora of Raw Milk

The number of microbes and the composition of their species depends from conditions, in which milk is produced. Especially high is the number of microbes in milk, when milking was carried out in insanitary conditions and when carelessly cleaned dairy utensils were used.

While a great part of microorganisms, which have found their way into milk, descend from the mechanical particles of dirt, it is necessary to strain milk immediately after milking. The most adequate straining material is cotton wool, but in case of carefully washed straining towels, if they are changed after each straining of 50 - 60 liters of milk, clean milk can also be obtained.

Cooling of Milk

The quality of milk depends beside clean milking and cleanliness of dairy utensils, yet from the temperature of keeping. In sweet milk, reproduction of microbes does not begin immediately; it even decreases by some extent, which condition is caused through bactericidal substances in milk - the lactenine I and the lactenine II.

The period, during which microbes in newly milked milk do not reproduce, is called the bactericidal phase. Duration of this phase depends from purity of milk (the smaller the number of microbes in milk, the longer is the bactericidal phase) and also from keeping temperature of milk (by lowering of temperature, the duration of phase lengthens, milk is kept fresh longer).

By uncooled milk the duration of bactericidal phase is 2 - 3 h, but in cooled state, below 10 °C, it continues up to 24 hours, being in case of clean milking at the temperature of 0 °C nearly 48 hours. That shows that, the quicker we cool milk to a low temperature, the longer is the continuation of the bactericidal phase,- the longer milk keeps fresh.

If milk is cooled after milking and drying below 10° C (the minimum temperature of reproduction of lactic acid bacteria), it can be kept at the farm one day. But if milk of clean milking is cooled down to the temperature of 0 - 5 °C, it can be kept at the farm, before delivering, up to 2 days. If milk is contaminated with microbes by milking, in it begins, when kept over one day, the reproduction of psychrophilic bacteria, causing a bitterish flavour of milk. At a temperature above 10 °C, the lactic acid streptococci begin to develop. Thus, dependent of keeping temperature, the acidity of milk rises, what condition restrains the reproduction of protein decomposing bacteria.

Therefore, after milking, milk is to be cooled at all events to below 10 °C or even to a temperature of 6 - 8 °C, while care must be taken, that during transportation temperature of milk does not rise.

Manipulation of Milk at the Industry

Manipulation of milk at the industry consists in diminishing the number of microbes and in creating conditions, which restrain their reproduction. Handling of milk begins by centrifugation with cleaning centrifuge. With cleaning centrifuge it is easily possible to isolate the protein decomposing bacilli, but due to diminutive dimensions of the cell, the coli-group bacteria are isolated unsuccessfully. While by the use of milk-cleaning centrifuge particles of mechanical impurities are isolated almost completely, is the effect of destroying of microbes in the pasteurization of previously cleaned milk the stronger.

A general and principal method in the contemporary milk industry for diminishing microflora in milk, is the moderate thermal application - pasteurization, where beside destroying of saprophytic microbes are exterminated also the pathogenic microbes.

In pasteurization are also decomposed the enzymes of milk.

In milk technology the short-time (68 - 72 °C) pasteurization is mainly applied with a duration of 15 - 20 sec. Also is practised short-time pasteurization at the temperature of 80 - 85°C together with prompt cooling. Of the pasteurizing installations plate-pasteurizers are mostly used in the technology of drinking milk. The purpose for pasteurization is the destroying of patogenic bacteria (which do not form spores). To check this was employed the phosphatase test. The research work has shown that milk enzyme phosphatase can be decomposed only by employing high temperature for a longer period, than is necessary for destroying of pathogenic bacteria. So the bacterium of typhoid fever dies at a temperature of 75 °C during 2 sec, the tuberculosis bacterium during 7 - 10 sec and the brucellosis bacterium after 12 sec. But the phosphatase is decomposed by this temperature only after 23 sec. Nevertheless, by heating milk until the phosphatase test will prove negative, it is not warranted, that the bacterial toxines are destroyed. Although in the industry pasteurization is being controlled, is strictly prohibited the reproduction of pathogenic bacteria in raw milk.

The non-patogenic microflora diminishes by pasteurization up to 99.5 per cent. The remaining microflora in the amount of 0.5 per cent, dependent of the number of spores in raw milk and the species of microlora, consists of a few thousand bacteria per 1 ml of raw milk.

Resistant to pasteurization are beside the spores of bacilli the micrococci, the microbacteria (sporeless diminutive rod--shaped bacteria, among which are weak acid formers, that will not turn milk sour), thermophilic streptococci and streptococci, which descend from the intestines (Str. faecium, Str. bovis, Str. durans). The latter coagulate milk at an optimum temperature (37 - 38 °C) during 16 - 20 hours.

Beside microbes, which were preserved by thermal working, there always occur in pasteurized milk microbes, which descend from reinfection. They descend from pipe-lines, pumps, pipe connections and surfaces of milk preservation tanks and their amount depends from the cleanliness of installation and from the thoroughness of desinfection.

Dependent from different microbe groups, the microbiological processes of pasteurized milk differ from that of raw milk (table 1).

Table 1

The changes of microflora of raw- and pasteurized milk by keeping at a temperature of 30 °C (Krause)

Kind of milk		Number of bacteria		
		Total number of spores	Lactic acid bacteria (%)	Protein decomposers (%)
Fresh		14 800	29.4	63.6
Raw	After 24 h	241 000 000	92.1	7.4
	After 48 h	1 500 000 000	96.0	4.0
Fresh		2 070	18.8	76.2
Pasteurized	After 24 h	7 300 000	12.4	86.4
	After 48 h	960 000 000	76.1	23.7

Thus, at a temperature of 30 °C, bacteria in raw milk develop much more intensively than in pasteurized milk, whereby in pasteurized milk the number of bacteria reached $7.3.10^9$/ml, while in raw milk it was after the same period $2.41.10^8$/ml. There are differences also in the qualitative composition - in raw milk are predominating the lactic acid bacteria, while in pasteurized milk during 24 hours the protein decomposers greatly predominated. It is possible to keep pasteurized milk because of protein decomposing bacteria at a temperature of +5°C during two days, but after three days the reproduction of protein decomposing bacteria intensifies and toxic compounds are formed.

Irradiation of milk with ultraviolet rays can diminish (without rise of temperature) the amount of microbes, but this is possibl only in a thin layer, whereby milk may obtain an upleasant flavour because of separation of ozone. Irradiation with ultraviolet rays can successfully be utilized for disinfection of the air in storehouses, also for defence against mould in cheese vaults and by destroying of the microflora on the surface of meat.

By irradiation of milk with ultraviolet rays, the content of vitamin D in it also rises.

Beside pasteurization has been utilized in recent years sterilization of drinking milk, which is carried out at a temperature of 110 - 112 °C, but in such case the number of bacilli in raw milk must be low, because the spores of bacilli are not completely destroyed at the said temperatures, and milk may become spoilt.

Requirements for pasteurized milk, based on MRTU 18/91-65 are the following:

The categories of pasteurized milk	Total number of spores per 1 ml, not exceeding	The occurrence of coli-bacteria, per ml
Bottle milk, categ. A	75,000	3
B	150,000	0.3
C	300,000	0.3
The can milk (and milk in cisterns)	400,000	0.3

The pasteurized milk must not be preserved over 36 hours. In pasteurized milk the occurrence of pathogenic microbes is not allowed. The repeated pasteurization of milk is prohibited.

In the pasteurized fresh cream the number of bacteria must not exceed 200,000 per 1 ml, the occurrence of coli-bacteria in cream is permitted, but not exceeding 0.3 per 1 ml of cream.

Defects of Milk of Microbal Origin

Bitter flavour of raw milk, especially of milk, which was kept for some time in cooled state, is caused by micrococci and by protein decomposing bacteria. Bitter flavour of pasteurized milk cause the protein decomposing bacteria. The above mentioned bacteria are able to decompose protein into peptons, that lend milk a bitter taste.

Rancid flavour appears in raw milk, which has been kept for a longer period in a cool place, where the reproduction of species of Pseudomonas g., especially Ps. fluorescens, which produce the enzyme lipase takes place. Under the effect of lipase, milk fat is hydrolysed, whereby butyric acid, aldehydes, ketons and other compounds are formed, which cause rancid flavour of milk.

A symptom for fermenting milk is the formation of gas. In raw milk it is caused by yeasts and coli-bacteria, in pasteurized milk by butyric acid bacilli.

Coagulation of milk by low acidity is caused byreproduction of micrococci and mammococci, but also Bac. Cereus in milk. They produce the rennet-like enzyme.

Milk with by-flavour and -aroma is the result of reproduction of coli-group bacteria and the species of Pseudomonas in milk. The defect is caused by unclean dairy utensils, unclean milking and prolonged cooling down of milk.

Viscous milk is caused by Bac. lactis viscosum, which descends from water and by the action of which mucous substance is produced from milk sugar. Viscosity of milk cause also some mucus producing streptococci.

Pathogenic Bacteria in Milk

Pathogenic microbes, which are spread through milk, can be divided into two groups:

1. Microbes, that cause diseases by animals, which are also pathogenic for human beings (tuberculosis, brucellosis, mastitis, foot- and mouth disease).

2. Microbes, that call forth diseases by human beings (typhoid fever, paratyphoid, dysentery, scarlet fever, cholera etc.).

The tuberculosis bacterium has an immovable cell of diminutive diameter. As the shell of the cell is covered with a wax- and fat-like substance, it is not stained by usual procedure. There occurRs in milk two types of tuberculosis bacteria - the human- and the cattle type. They are similar and cause tuberculosis by man. Children are especially susceptible to the cattle type of tuberculosis. Tuberculosis is the most frequent disease in cattle, which through milk infects man.

By thermal working of milk, the tuberculosis bacterium dies as follows:

82.2°	20 sec
76.7°	20 sec
71.1°	20 sec
68.3°	30 sec
65.6°	120 sec
62.8°	360 sek

In milk products, the tuberculosis bacteria are preserved comparatively long - in various sour milk kinds, up to 20 days, in butter 100 days, in Swiss cheese 5 - 30 days, in semi-hard cheeses 305 days and in ice cream up to 6.5 years.

The brucellosis is a disease of cattle, sheep and goats, which is caused in case of cattle by a diminutive-dimensioned sporeless immovable Bact. abortus. Brucellosis is a widely spread disease, by which were infected in a number of countries 10 - 20 per cent of cattle. Milk, yielded by brucellosis-infected cattle, must be pasteurized at a temperature of 70 °C during 30 minutes. Milk, which was milked by a cow with clinical symptoms of brucellosis, must be boiled during 5 minutes. After that, in the industry, milk is to be pasteurized in the usual way. Of brucellosis the composition and quality of milk does not change noticeably.

The Mastitis (the acute inflammation of udder) is caused by streptococci (Str. mastiditis), micrococci (Staphylococcus sp.), coli-bacteria (Bact. coli) etc.

By the acute inflammation of udder, the composition of milk changes vitally; changes the reaction, milk obtains a saltish flavour, in milk occur flakes, diminishes milk sugar and casein content, but increases the content of albumin. In case of mastitis in milk sharply increases the number of leucocytes. If by a cow with a healthy udder, the number of leucocytes per 1 ml of milk does not exceed 500,000, then in case of mastitis it rises to a few millions. At the same time in milk are to be detected leucocytes, which are positive symptoms of the inflammation of udder. Milk from mastitis cows cannot be used as food, as it may cause inflammation of the throat.

Of microbes, which cause diseases by man, through milk are spread more frequently the typhoid fever, the paratyphoid and the dysentery. Although milk is not a proper medium for the reproduction of those pathogenic bacteria, they remain in it for a sufficiently long period. By consumption of raw milk, there has occurred in summer months infections from coli-bacillosis by children. It was spread by a bacilli-carrier - a farm worker.

Also has spread through milk the dysentery, the paratyphoid and the scarlet fever. By the centralized production of drinking milk, spread of said diseaseshas sharply decreased, while by single very rare occasions have the originators of the above-mentioned diseases got into milk through the mediation of bacilli-carriers, who work in milk industry and in retail trade.

Certain milk products may, because of reproduction of some microbes in summer, cause toxication. One of such milk products is the whipped cream. So the Staphylococcus aureus, which produces toxines, has caused toxications by consumption of layer-caces and whipped cream, which were kept for a longer period at a high temperature.

Thus, pasteurization of milk and the utilization of pasteurized milk by manufacturing of milk products and the refrigerating and keeping those products at low temperatures has completely justified itself also from the hygienic point of view, since it restrains the spread of pathogenic bacteria.

IV. THE MICROBIOLOGY OF SOUR MILK PRODUCTS

The technology of sour milk products is based mainly on the lactic acid fermentation, but partially also on the fermentation of alcohol. In comparison with fresh milk, sour milk products have dietetic and also curing qualities. In the contemporary milk technology, sour milk products are mainly produced with the help of special starters and rapidly is decreasing the role of naturally soured products in the menue of the population.

Sour milk products are assimilated by organism easier and quicker, as can be seen from table 2.

Table 2

The percentage of assimilation of milk and sour milk

Time (hours)	Milk	Sour milk
1	32	91
2	36	92
3	44	95.5

This difference between assimilation of milk and sour milk is due to the fact that, during the souring process, proteins undergo chemical transformation. Under the effect of gastric juice from fresh milk a coarse-structured coagulum is formed, but in case of sour milk, the coagulum is softer and the assimilation of phosphorus and calcium better.

Sour milk products are divided into 2 groups: - the products, which are produced with starters of mesophilic lactic acid bacteria (the optimum temperature 30 °C); secondly - the products, which are produced with the help of starters of thermophilic lactic acid bacteria (the optimum temperature of souring 40 °C).

Sour Milk Products of Mesophilic Starter,

Cultured Milk, Sour Cream and Curd

For the production of cultured milk, pasteurized milk is used, to which 2 - 5 % starters of cream souring at a temperature of 25 - 30 °C is added. The microflora of this starter the Str.lactis and Str. cremoris give a thick homogeneous coagulum. But Str. diacetilactis and Str. paracitrovorus, forming the aromatic substance diacetyl, improve flavour and aroma of cultured milk.

The Microflora of Kefir

The microflora of this sour milk beverage, in the shape of the so called kefir grain, comes from the North-Ossetia. The microflora of kefir grain, consisting of galactane and mucine (formed from milk sugar) is variable, but the mesophilic streptococci (Str. lactis, Str. cremoris and aroma-forming bacteria) must be regarded as its main components.

2. Mesophilic streptobacteria and betabacteria.

3. Yeasts, which do not and do ferment milk sugar.

4. The acetic acid bacteria.

In the microflora of kefir grain we find the Betabacterium caucasicum, which very badly develops in usual media. But if we add yeast extract to the agar medium or use the hydrolysed milk medium, it is possible to see small colonies of this lactic acid bacterium.

If the microflora of kefir grain is freed from yeasts, then in replanting, such starter loses the typical qualities of kefir, being a product, which in flavour and odour is similar to the cultured milk, with streptococci predominating. It proves the fact, that the lactic acid streptococci are the most active components in the microflora of kefir grain. It also shows, that the rest of the microflora develops considerably slower, being thus of secondary importance.

In kefir ethyl alcohol and carbon dioxide are formed mainly by the action of Torula kefiri, which is a weak alcohol fermenter.

Therefore, in order to keep balance of kefir's symbiotic microflora, it is necessary to choose the temperature of souring possible low -20 - 22 °C, while by souring of kefir at higher temperature, the mesophilic streptococci and yeasts are inevitably driven out by other lactic acid bacteria. The temperature of 10 - 12 °C restrains the fermentation process of lactic acid, enabling development of yeasts, which saturate milk with carbon dioxide.

By the data of the All-Union Dairying Institute, the most characteristic flavour and aroma of kefir is obtained, when milk is soured with mother starter, which has been cultivated on kefir-grain. If kefir is produced in tanks, the reproduction of yeasts can be intensified, if milk is stirred during the fermentation process and after formation of coagulum. The homogenization of milk also helps to improve the consistency of kefir.

To some degree the microflora of kefir hydrolyses also casein, which results in the increase of the amount of peptons and the acid albumin. So kefir is a typical curing-dietetic sour milk beverage, which can be used in the treatment of stomach and intestine diseases.

While by the multi-component microflora of kefir the balance of some physiological groups changes, which gives a product of unstable qualities, attempts have been made to produce kefir with the help of pure cultures.

By the data of the All-Union Dairying Research Institute in Moscow, the making of kefir with the help of streptococci, lactobacteria, acetic acid bacteria and yeasts has become possible. The results were the same as achieved by using kefir grain.

The Microflora of Koumyss

On the basis of multi-component microflora the southern districts of the Soviet-Union - Turkmenia and South-Kazakhstan, produce koumyss from mare's and camel's milk. In this sour milk beverage, which is still produced mostly in home conditions in sufficiently large scope, the fermenters of lactic acid are the local strains of lactobacteria (similar to L. bulgaricum).

Beside the lactobacteria and other lactic acid bacteria koumyss always contains yeasts, which fermenting the lactose produce up to 1 % ethyl alcohol and saturate the sour milk beverage with CO_2.

Beside the yeasts, which ferment milk sugar, koumyss also contains such alcohol fermenters, which can hydrolyse only the hydrolysis products of lactose - the glucose and the galactose.

Since the intensive reproduction of lactobacteria brings about a quick acidity rise in koumyss, the Str. lactis cannot develop because of weak buffering ability of mare's milk. In the horse-breeding districts of the Soviet Union, koumyss has been used for centuries as a remedy, which favours the treatment of tuberculosis and stomach-intestine diseases.

Earlier, the curing effect of koumyss was not clear, but now it has become evident, that by the action of yeasts and lactic acid bacteria, which ferment milk sugar, antibiotic substances are formed, which do not restrain only the reproduction of staphylococci, but also the reproduction of the bacilli of stomach- and intestine diseases and the bacteria of tuberculosis.

In order to standardize the qualities of koumyss as a curing-dietetic sour milk beverage, its production was begun with the help of yeasts cultures, which ferment L. bulgaricum and milk sugar.

The Kurunga

The Kurunga is a fermented sour milk beverage, which was produced in the north-east regions of the Soviet Union in ancient times already. This is a sour milk beverage, in which simultaneously lactic acid and alcohol fermentation processes take place. The lactic acid fermentation process in the production of kurunga is mainly effected by L. bulgaricum, but also by Str. lactis. The alcohol fermentation, which is formed in the amount of 1 %, proceeds under the effect of Torula curunga's. The Kurunga is a curing-dietetic milk beverage for treating the diseases of stomach and intestines and also the lung tuberculosis.

Sour Cream

The starter of mesophilic streptococci is used in the technology of sour cream production, where the lactic acid fermentation takes place under the action of Str. lactis and Str. cremoris. Using cream, which was pasteurized at a temp. of 80 - 90 °C and adding 3 - 5 % of starter for cream fermentation, cream coagulates at a temperature of 12 - 16 °C in 10 - 12 hours already. Then it is kept at a low temperature (4 - 6 °C), in order to swell the proteins. During the fermentation process, beside the streptococci also the yeasts Saccharomyces lactis, descending from contamination, the species of Torula and moulds - mainly Oidium lactis begin to develop. By keeping of sour cream the microflora, descending from contamination, appears on the surface in the form of blotches. By the reproduction of yeasts, in sour cream the aroma of yeast is formed, but by the reproduction of moulds the surface layers acquire a rancid taste and odour.

The Curd

In the production of curd, the starter of cream fermentation is used, in which the streptococci Str. lactis, Str. cremoris give a strong coagulum of casein and to which aroma-formers (Str. citrovorus and Str. diacetilactis) impart aroma. In the production of curd, pasteurized milk and 3 - 5 % of starter are used. In the souring process, contamination with yeasts and moulds and also the reproduction of bacilli (spores), which were preserved in the pasteurization take place.

If curd is not tightly packed, begin to develop in the surface layers Oidium lactis, the species of Mycoderma, mainly the Mycoderma casei. The latter using lactic acid, brings curd within the limits of 5.0 pH, where the development of spores of bacilli, which were preserved in pasteurization begins. That, together with yeasts and moulds calls forth putrefaction of curd. So, in order to preserve the quality of curd, beside the utilization of pasteurized milk and starters, is of greatest importance tight packing and keeping of curd at a low temperature.

Sour Milk Products of Thermophilic Starter

With the help of starters of thermophilic lactic acid bacteria of the g. Lactobacterium we produce Bulgarian and acidophilous milk, acidophilin, acidophilous paste, acidophilous-yeast milk and dried acidophilin starter.

The Bulgarian sour milk is produced from pasteurized milk at a temp. of 85 - 90°C, with the starter Str. thermophilus and L. bulgaricum (1:1).

The thermophilic streptococcus intensifies the reproduction of L. bulgaricum and rises the aroma of starter. If fermentation takes place at a temperature of 40 - 42 °C and in all 4 - 5 % of starters are used, milk coagulates already in 2.5 - 3 hours. L. bulgaricum has the ability to restrain the reproduction of protein decomposing bacteria. It has been observed, that continuous use of Bulgarian sour milk restrains the reproduction of protein decomposing bacteria in the digestive tract and the formation of toxic products.

The Curing-Dietetic Sour Milk Beverages, Produced on the Basis of L. Acidophilum

L. acidophilum is a typical fermenter of lactic acid in the digestive tract of human beings and animals and if sour milk products, made with the help of this bacteria are used, it is possible to direct the microflora of the digestive tract and to increase the number of lactic acid bacteria there. L. acidophilum, as an intensive lactic acid fermenter forms specific substances of the type antibiotics. These substances are heat-resistant and do not decompose by boiling. These substances also penetrate the bacteriologic filters. Their characteristic feature is the highest activity at pH 5.0 - 5.6. The antibiotic substances, produced by L. acidophilum, restrain the reproduction of protein decomposing bacteria (Bac. subtilis, Bac. mycoides, Bac. mesentericus etc.), as well as the pathogenic bacteria (typhoid fever, paratyphoid, dysentery and colibacteria). L. acidophilum is resistant to the external medium, it is resistant to biles (20 %), common salt (2 %), phenol (0.4 %). It is resistant to the antagonistic substances, produced by coli--bacteria. L. acidophilum is also resistant to a medium with an alkaline reaction. L. acidophilum can obtain energy from the carbo--hydrates, maltose, dextrine and saccharose etc., which always occur in the digestive tract. So L. acidophilum is more resistant to conditions of external medium than L. bulgaricum, bearing also such strong antibiotics as tetracyclin, oxytetracyclin and penicillin.

In a prolonged cultivation in laboratories L. acidophilum loses certain physiological qualities, such as adaptation to the medium of the alimentary canal, resistance to phenol, bile, alkaline reaction etc. Therefore it is useful to check periodically the physiological qualities of L. acidophilum and to isolate the new strains. It has been observed, that active strains of L. acidophilum occur only in the alimentary canal of those people, who continuously drink milk. Those strains have also the highest active acidity and they are able to live in the alimentary canal.

Of the curing-dietetic sour milk beverages, which are produced with the help of L. acidophilum, are to be mentioned acidophilous milk, the acidophilin, the acidophilin-yeast milk, the acidophilin paste and the biomass of the acidophilic bacteria.

The acidophilous milk

The acidophilous milk is produced with the help of cultures of the same bacterium, using mucus forming and mucus not forming strains (1:4), which are cultivated separately. By using two starters, sour milk is obtained, which is not strongly viscous, but has a consistency similar that of sour cream. Acidophilous milk is used as a curing-dietetic sour milk beverage for treating intestinal ailments. It is also used for breeding young animals - calves, piglets, lambs and chickens. A shortcoming of the acidophilous milk is, that it is apt to become over-acidified at the milk trader and in the hands of the consumer. Therefore, after coagulation, it should be kept at the lowest possible temperature.

The acidophilin

In order to obtain an acidophilic b. sour milk beverage of low acidity, beside the starter L.acidophilum the starter for cream souring and kefir starter (1:1:1) are used. Milk is soured at a temperature of 30 °C.

The acidophilous yeast milk

The acidophilous yeast milk is produced with the help of L. acidophilum culture and yeasts, fermenting milk sugar. They are selected on the basis of their antibiotic qualities.

The acidophilous yeast milk is used as a curative-dietetic sour milk beverage in the treatment of alimentary canal and some skin diseases. Good results have been obtained in the treatment of tuberculosis by using acidophilous yeast milk. Especially great effect was obtained in the restauration of microflora of alimentary canal by patients, who had used antibiotics. The use of antibiotics causes beside the destruction of protein decomposing bacteria in the digestive tract also destruction of other bacteria occurring in the microflora of intestines. The normal microflora of alimentary canal plays an important role in humanslife: it increases resistance of organism to toxic, pathogenic microorganisms, produces many vitamins (biotin, nicotine acid, lactoflavine, fole-acid, vitamines B_1, B_{12} and K). The action of normal microflora of the alimentary canal produces also some amino acids and unidentified factors of growth. The active life processes of the microflora of intestines suppress the harmful microflora (included the pathogenic) and makes harmless the toxic products of metabolism.

Sudden restraining of reproduction of useful microflora by the use of antibiotics can cause serious illnesses, caused by pathogenic microbes, which are resistant to antibiotics. Milk products, which are soured with the help of acidophilic bacteria, together with a large amount of its metabolism products and with the acidophilic bacteria, to be found in the alimentary canal, create conditions for the formation of normal microflora in digestive tract.

It has been also observed, that to the restauration of alimentary canal's microflora after a use of antibiotics, beside the acidophilic bacteria contribute also other lactic acid bacteria.

The acidophilous paste

Acidophilous paste represents acidophilous milk, from which part of whey has been removed through draining. The paste obtained, has a homogeneous consistency, similar to sour cream, with a pleasant sour aroma and a strong sour flavour. The acidophilous paste is used in the treatment of stomach-intestinal diseases as a dietetic food, but it is also used by badly healing wounds.

The biomass of acidophilic bacteria

In the Estonian S.S.R. a concentrate of acidophilic bacteria - the biomass is produced, which is obtained through the cultivation of acidophilic bacteria in the medium of cheese whey, to which hydrolyzate of casein or special biological preparation is added for the intensification of growth. In the periodical neutralization, already in 24 hours the number of spores of L. acidophilum rises up to 3-4 milliard per 1 ml, after which the cells are separated by means of milk cleaning centrifuge. Thus the biomass is obtained, in which the number of cells is provisionally 30 - 40 times higher than in sour milk products, made with the help of L. acidiphilum.

V. THE MICROBIOLOGY OF BUTTER

Despite the employing of contemporary technological equipment, their careful cleaning and disinfection and despite pasteurization of cream, microbes are always to be found in butter. Microbes, which get into butter in the productional process and during packing, begin to develop, because in butter they find all the necessary nutritive substances needed for reproduction. Therefore, beside the oxydation processes that take place in storing of butter, changes are caused mainly through microbiological processes

From the microbiological point of view, the large assortment of up-to-date butter products can be classified into two groups:

1. The kinds of fresh cream butter (from pasteurized cream).

2. The kinds of sour cream butter, in the production of which special starters of streptococci are used, for souring pasteurized cream.

Contamination Sources of Butter

As the destroying of microbes in cream is more difficult than in milk, cream must be pesteurized at least at a temperature of 85 °C. Pasteurization must also inactivate the fat splitting enzyme lipase, which is to be found in cream. If cream, which is to be pasteurized, is not strongly contaminated by spores of bacilli, is the number of bacteria comparatively low, - a few hundred or thousand per 1 ml. The microflora, which is preserved in the pasteurization of cream, consists mainly of spores of bacilli. Lactic acid bacteria, micrococci and microbacteria occur in pasteurized cream in a comparatively small amount.

Contamination of Cream by Cooling

In further manipulation, cream gets contaminated with various microbes in cooling and physical ripening processes. They find their way into cream from piping, from the inner surface of its connecting pieces, from pumps, coolers, from the walls of cream tanks, from rinsing water and air. The number and make-up of species of microbes, that are to be found on the surfaces of equipment, used in working of cream, dependson the thoroughness of cleaning and disinfection of such equipment. If properly cared, on their surfaces remain only bacteria, which are to be found in rinsing water. If the equipment, used by working of cream is not carefully cleaned, in the residue of organic matter begin to develop during the intervals of work, the protein decomposing bacteria and bacilli and also the lactic acid bacteria, with which cream is later strongly contaminated. During the physical ripening of cream, due to low temperature, no notable reproduction (except the cold psyhrophilic) of bacteria takes place.

The Butter Churn

The butter churn is an important infection source in the technology of butter production, therefore, it must be especially carefully washed and disinfected. Since in place of wooden churns the utilization of metal churns of different construction is widely practiced, contamination from said source has diminished.

Water

The quality of water is of great importance in the technology of butter. We use water for washing butter granule. In this way it gets into the composition of butter. Water of poor quality contains fluorescent bacteria, protein decomposers and coli-group bacteria. Water, used in butter making, must correspond to all requirements of quality: 1 ml of water must not contain over 300 million bacteria and the occurrence of coli-bacteria must not exceed 3. Water with greater bacterial count must be pasteurized or chlorized.

Salt

In salt of butter only spores of bacilli, micrococci and some yeasts occur, whose number does not exceed 100 per 1 g. As these microbe groups are protein decomposers, they affect the quality of butter negatively.

Since in pasteurization of cream, mainly the spores of protein decomposing bacilli and micrococci are preserved and by cooling and physical ripening of cream, contamination also takes place mainly by the action of protein decomposing microbes, it is very important in the technology of fresh cream butter, to have a low number of microbes, while here the said microbes are especially perilous, because of absence of the effect of acidity, which restrains the protein decomposing process.

Although by sour cream butter the protein decomposing microbes are also harmful, they do not determine to such extent the formation of butter quality and the stability, as by fresh cream butter. The microflora of sour cream butter descends mainly from starters, consisting of lactic acid streptococci, which restrain the reproduction of harmful microflora in butter.

Starters for Sour Cream Butter

The purpose for the use of starters in butter technology is to obtain high-quality butter of stability. It must have a specific flavour and aroma, which is caused in case of sour cream butter by lactic acid and aromatic substances of diacetyl, the volatile acids, alcohols and esters.

First starters for souring cream were used in Denmark in 1888.

The Composition of Starters on Cream Souring

At first, attempts were made to produce starters on cream with the cultures of one strain of lactic acid streptococci. But very soon it became evident, that the starters, which were made from these cultures, do not give butter such a flavour and aroma, as has butter, which is produced with natural starters, whose lactic acid microflora is more variegated

Due to this circumstance scientists began to isolate aroma-producing lactic acid bacteria for starters.

In 1919 Str. citrovorus and Str. paracitrovorus were isolated and determined as aroma-formers. These lactic acid streptococci are able to ferment milk sugar and citric acid, forming aromatic substances. The cultures of these lactic acid streptococci are quite weak in milk and do not affect the quality of starters. But if these bacteria are cultivated together with Str. lactis, the flavour and aroma of starter improves noticeably.

Simultaneously with the aroma-producing bacteria, Str. cremoris, an intensive milk sugar fermenter was isolated.

Already in the 1920-es the four-component starters were introduced into the technology of sour cream butter. In those starters the aroma-producing streptococci Str. citrovorus and Str.

paracitrovorus were cultivated together with active acid formers Str. lactis and Str. cremoris.

The composition of the starter on cream was improved in 1935 - 1936 with new aroma-producing streptococci. From the aspect of butter technology, they display better qualities than Str. citrovorus and Str. paracitrovorus. These are weak acid producers, where certain strains produce diacetyl, which is the main component of the aroma of sour cream butter.

Three-component starters are used in the Soviet Union at the present, where the active acid producers are Str. lactis and Str. cremoris and the producer of aromatic substance - diacetyl, is the Str. diacetylactis.

The ability of producing of diacetyl is now one of the main qualities, on the basis of which, beside the ability of producing acid, is estimated the starter on cream souring.

The formation of diacetyl in cream souring culture has been thoroughly studied in the All-Union Dairying Institute. It has become evident, that one of the most important factors, which determine the intensity of the formation of diacetyl is the oxydation-reduction potentiality of the medium. Its rise activizes oxydation processes, giving oxydized products, including diacetyl. The decrease of oxydation-reduction potentiality brings forth deoxidation of diacetyl - that is, the formation of a substance lacking aroma.

Of the bacteria, belonging to the components of cream souring starters, of the aroma formers are the Str. diacetilactis of highest oxydation - reduction potentiality. The extent of reduction of other components of cream souring starter is greater, so Str. diacetilactis forms the greatest amount of diacetyl.

Thus, selecting components for cream souring culture, it is necessary beside other factors to take into account the reduction ability. Only strains of weak reduction ability can be used as components of cream souring culture.

Of the acid formers of cream souring starter, the Str. cremoris has the lowest reduction capacity.

If the cultures of Str. diacetilactis and Str. cremoris are combined, more diacetyl is formed in the culture, as when Str. diacetilactis and Str. lactis were combined. Therefore Str. cremoris must be a component of cream souring starter.

The aroma producing bacteria Str. citrovorus and Str. paracitrovorus are not used at the present time as components of cream souring starter. Some of the strains of the above mentioned aroma-forming bacteria have strong reducing qualities, forming only acetoin in milk, reducing thus the amount of diacetyl in the culture. The relative importance of Str. citrovorus and Str. paracitrovorus is considerably diminished, if cream souring culture contains Str. diacetilactis.

The cultivation of cultures at the temperature of 25 °C and the stirring of culture, when its acidity had risen up to 40 - 45°T and after the formation of coagulum, the formation of diacetyl is favouredin the cream-souring culture, which has Str. diacetilactis as its component part. The stirring-aerating has a positive effect, because it increases the oxydation-reduction potentiality.

The Preparation of Laboratory Cultures

The isolation of cream souring cultures, the determination of their qualities and the making of laboratory cultures take place in the laboratories of dairy microbiology in the Soviet Union, in Moscow, Uglich, Kiev, Novo-Sibirsk etc.

The lactic acid bacteria of cream souring cultures, both, the acid-producers and aroma-formers, are isolated in a natural way from soured milk, cream, milk products, from green plants and the soil of their risosphere. The strains of single lactic acid bacteria-the streptococci,which are used for the making of cream souring culture, must have certain biochemical qualities and must not influence each-other antagonistically.

Cream-souring cultures are prepared either as liquid or as dry cultures. It is advisable to make liquid cultures for short-time keeping, because drying exterminates the greatest part of microbes and by making of mother starter, not all the components of microflora of dry culture are present in active state. The activity of liquid cultures decreases according to the keeping temperature, so they can be used within 2 - 3 weeks, when the relations of different species have not yet undergone considerable changes. The duration of cream souring cultures rises, if we use skim milk in their making. The total solids consistency of skim milk is increased up to 16 %, if skim milk powder is added.

To increase durability of cream souring cultures, yeasts can be added, which are used in increasing the stability of butter. The addition of yeasts increases the duration of cultures up to 3 months. The importance and effectivity of yeasts to the activity of lactic acid bacteria lies in the fact, that they enrich the medium with vitamins, partly hydrolyse proteins and decrease the acidity.

Liquid cultures on cream souring can be preserved in active state up to 6 months if they are frozen down to -28 °C, and kept afterwards at the same low temperature.

Although in dry cultures the streptococci, which we use for cream souring, cannot be so well preserved, is their use inevitable in such enterprises, which are far away from laboratories. Dry cultures for cream souring are produced in the Soviet Union by the method of sublimation-lyophilization and spraying-by-drying procedure. Dry cultures, produced in this way, contain scores of milliards of cells per 1 g. They dissolve in milk well and can be used during 3 months, if they are packed into hermetic tare. These dry cultures can be successfully used in the course of 3 months for the making of mother starter.

Making Mother- and Commercial Starters in Butter Industry

In order to restore the activity of liquid-, as well as of dry cultures of acid producers and aroma formers, it is necessary to inoculate the cultures, obtained from the laboratory, 2-3 times. Thus mother starter is obtained.

Restoration of activity of starter can be tested on the ground of coagulation speed and on the intensity of production of acid (the acidity ^{o}T after a certain period). After that it can be used in the production of commercial starter, - which we use for cream souring. The quality of mother- and commercial starter must be checked every day. Their quality, as well as the quality and activity of cultures depends greatly from milk, which is used for their making. In making cultures and starters, pure, fresh milk must be used, which has normal organoleptic qualities and which was obtained from healthy cows in their 2-nd to 5-th lactation month.

For the making of cream souring cultures and starters, milk must be biologically of full value - must contain all substances, which are necessary for the reproduction of lactic acid bacteria and it must not contain substances, restraining the development of bacteria. By making of cultures and starters, the use of milk of udder-diseased cows is improper. If starter's milk 10 % of milk from cows, suffering from mastitis is added, coagulation of starter's milk slows down and the coagulum has an insufficient consistency. In order to prevent this, the occurrence of leucocytes must be checked with the help of a microscope in the preparate of raw milk. Also, by making of cultures and starters, the use of milk from cows, which were lately treated with antibiotics, is not allowed. In milk, which contains antibiotics, the lactic acid bacteria develop badly and their physiological qualities are changed. The making and evaluation of mother- and commercial starters is treated in the cycle of lectures: " The technology of butter manufacturing".

The Changes of Microflora by Keeping of Butter

The microflora of sweet cream butter consists mainly of microbes, which remained in cream in the pasteurization, or found their way there by cooling, physical ripening and churning. One gram of fresh sweet cream butter, which was made in the butter machine with wooden churn, contains from a few hundred up to a few thousand cells of bacteria. If butter was made in the butter machine with metal churn, or was made by the line production method, the number of bacteria in sweet cream butter is noticeably lower, being only a few hundred per 1 gram.

In sour cream butter the number of bacteria after churning is noticeably higher, reaching some tens of millions per 1 gram. But the microflora of sour cream butter consists mainly of lactic acid streptococci, which restrain the reproduction of protein decomposing bacteria. Whereas sour cream butter of long-term souring contains more bacteria than butter, which was produced by short-term souring of cream.

The reproduction of bacteria in butter mass takes place mainly in butter plasma, i.e. in water, in which proteins, salts, milk sugar, lactic acid and other substances were dissolved. The main substance of butter, i.e. butter fat, is not easily accessible to microbes, as by fat globules the protein membrane is partly preserved. The butter plasma occurs in the shape of droplets of various size, which are partly connected by very thin capillaries. The number of plasma-droplets in butter mass is considerably higher than the number of bacteria. Thus, the finer the dispersion of plasma, the more droplets are free from bacteria.

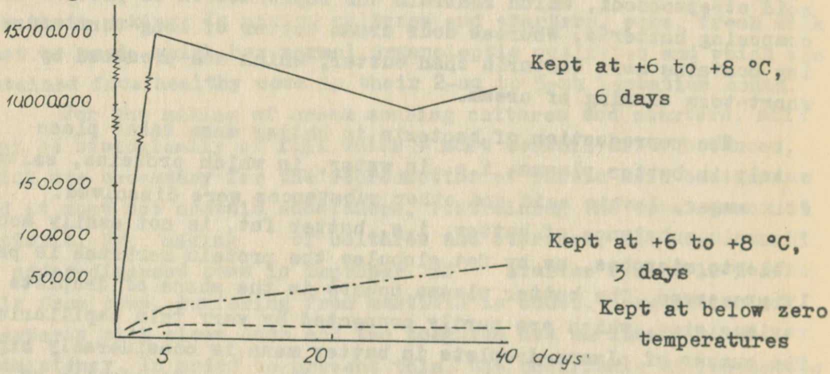
Thus the preliminary small number of bacteria and the dispersion stage of liquid phase restrain the development of microbiological processes in butter mass.

The intensity of reproduction of microbes in butter mass, after making of butter, depends greatly from the temperature of keeping.

In order to restrain reproduction of microbes, butter, especially sweet cream butter must be cooled down immediately after packing. Stability of butter is the better, the quicker and deeper it is cooled. The All-Union Scientific Research Institute of Milk Industry and the All-Union Institute of Refrigeration have ascertained, that the temperature, to which butter was cooled after packing is one of the main factors, determining its stability.

The All-Union Scientific Research Institute of Milk Industry has established, that in butter of small number of spores, which was placed after making and packing at a low temperature, no noticeable reproduction of bacteria takes place.

But if butter was preliminarily kept at a temp. of 6 to 8 °C, the number of microbes increased hundreds of times during 3 days. After the 6th day, the number of microbes did not increase any more (drawing 1).



The effect of preliminary keeping temperature and its duration on the preservation of butter's microflora, at the preservation temperatures.

In further keeping at low temperatures, the number of microbes in butter decreases, but the accumulation of great numbers of bacteria during the first days and the releasing of enzymes during their autolysis harms the quality of butter.

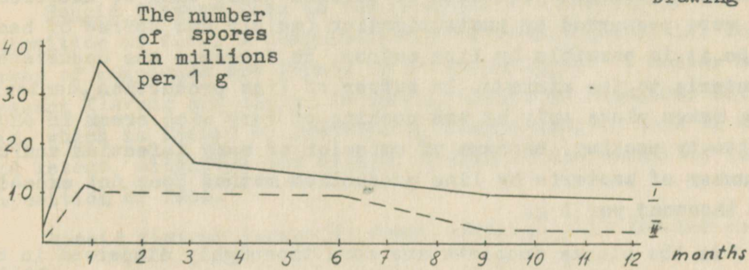
Keeping butter at below 0 °C temperatures, -5 to -7 °C and even at -10 to -12 °C, does not stop the action of microbes entirely. The action of microbes stops by unsalted butter at the temperature of -18 °C, but by salted butter at the temp. of -23 °C only.

But in the creamery conditions it is practically impossible to cool butter promptly to the above mentioned temperatures. In production and during transport, butter is kept at a temperature above 0 °C, for shorter or longer periods. In these conditions the reproduction of microbes in the mass of butter continues. It becomes evident especially in case of sweet cream butter, where the increase in number of microbes is obvious.

Thus, the number of microbes in butter depends on the temperature, at which butter was kept before storing in cold storage (drawing 2).

The number of lactic acid bacteria in sour cream butter diminishes, especially in short-term souring of cream.

Drawing 2



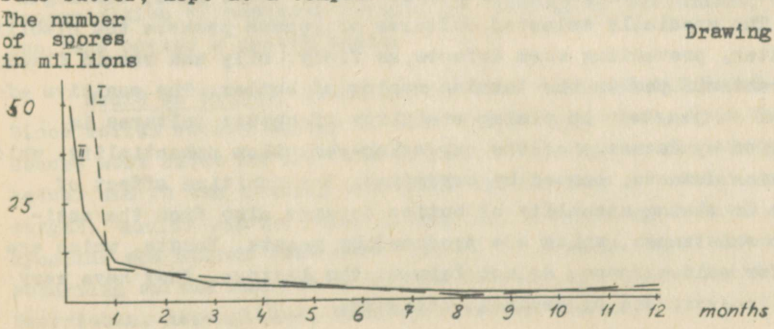
I. Sweet cream butter, kept preliminarily at +12 to +14 °C temperatures.

II. Sweet cream butter, kept preliminarily at -2 to 0 °C temperatures.

The amount of alien microflora, being more resistant to cold, can, despite the acidious medium, increase to some extent.

If sour cream butter was preliminarily kept at a temperature above 10 °C, the number of spores decreased quicker than by the same butter, kept at a temperature of -2 to 0 °C.

Drawing 3



I. Sour cream butter, kept at a temperature of +12 to +14 °C.
II. The same sour cream butter, kept at a temperature of -2 to 0 °C.

The Microbiological Processes in Butter, Produced by Line-Production Method

The microflora of butter, which was produced by line production method, consists (because of covered line) only of microbes, which were preserved by pasteurization (mainly the spores of bacilli). So it is possible by line method, to decrease the occurrence of bacteria to the minimum. In butter of line production ,contamination takes place only by the cooling of very rich cream in churn and also by packing. Because of omission of many infection sources, the number of bacteria by line production method does not exceed a few thousand per 1 g.

As the plasma droplets are more thoroughly dispersed in butter, produced by line production method, the microbiological processes in it take place slower than by usual sour cream butter, by the same conditions.

The Use of Cultures of Yeast-Fungi in Rising Stability of Butter

Beside cream souring starters, yeasts have been taken into use for rising the stability of butter (G.G. Blok, V.M.Bogdanov). The specially selected cultures of yeasts promote the stability of butter, preventing such defects, as fishy, oily and rancid flavour. Also restrain yeasts the turning mouldy of butter. The positive effect of yeasts in rising stability of butter cultures is explained by decrease of the oxydation-reduction potentiality, which restrains defects, caused by oxydation. The positive effect of yeasts in rising stability of butter depends also from the antibiotic substances, which are produced by yeasts. Yeasts, which are used for said purpose, do not ferment the lactose. They have very weak lipolytic and proteolytic activity.

Butter Defects of Microbal Origin

Sour flavour. Butter acquires a sour flavour, if much lactic acid is accumulated in it. Sweet cream butter can acquire a sour flavour, if it is kept at a temperature of +10 °C. Butter acquires a strong sour flavour also then, when it is made of over-acidified cream.

Rancid flavour. This is the result of the hydrolysis and decomposition of fats. A characteristic of rancid butter is, that a number of compounds are formed in it which impart butter an unpleasant flavour and odour. In rancid butter we encounter butyric acid, which is formed by hydrolysis. Beside butyric acid we also find ketons, aldehydes and esters, formed as the result of decomposition of fats.

Rancid flavour occurs in sweet cream, as well as sour cream butter.

Rancid flavour in butter is caused by fluorescent bacteria, some proteolytic bacteria and by moulds and yeasts.

Bitter taste. This defect has been observed more often in case of sweet cream butter. It is the result of the activity of the proteolytic bacteria (bacilli and fluorescent bacteria), which are able to develop at low temperatures. By the action of proteolytic bacteria, peptones are formed in the hydrolysis of proteins. The decomposition of peptones results in forming of substances, which can lend butter a cheesy taste.

Mould on butter. Mould is the most frequent defect of butter. Since moulds obtain energy in the oxydation process, this defect occurs most often in the form of mould blots on the surface of butter and on the packing material. As the mass of butter contains oxygen, moulds can be found inside it, where species of coloured mycelium and spores form dark blots. Moulds most frequently occurring on the surface of butter are the species of Penicillium, Geotrichum, Aspergillus, Cladosporium and Mucor.

Inside the mass of butter moulds most frequently occur in hollows, caused by packing. Even very small hollows may have moulds. Since the Geotrichum can develop at a low concentration of oxygen, it occurs in the mass of butter more frequently than the other moulds. Moulds are proteolythically and lipolythically active, imparting butter a rancid and mouldy flavour and smell, which is one of the greatest defects of butter.

In order to prevent the occurrence of moulds in butter, it is necessary to create sanitary-hygienic conditions in the process of butter making, that enables us to prevent spores of moulds to find their way into butter. But it is also necessary to create conditions, which prevent developing of moulds in butter mass.

Since air humidity favours the reproduction of moulds, they can begin to develop even on the walls and ceilings of the premises of creamery and in storage rooms. In order to restrain development of moulds, the per cent of relative humidity in storage room must be 80 to 85. Moulds can develop even at rather low below zero temperatures, but at the temperature of -10 to -12 ^{o}C, their reproduction ceases. High dispersion stage of water droplets restrains the development of moulds, while it is associated with more intensive dispersion of droplets. This condition makes harder the separation of moisture on the surface of butter. So are created unfavourable conditions for the reproduction of moulds.

The per cent of salt in butter by the limits of standard is 1.5 to 2. This only slows down the reproduction of moulds. The reproduction ceases at a 4 per cent concentration of common salt.

Untight packing is often the cause of reproduction of moulds. The hollows inside butter or under packing material make the reproduction of moulds possible.

<u>Fishy flavour</u>. Although fishy flavour of butter is most frequently caused by high acidity of cream and metallic salts (copper, iron), which have got into butter and also by the oxidation of butter fat, it can also be caused (after Djatchenko) by the reproduction of protheolytic bacteria in butter plasma. These bacteria hydrolyse the leci-tinic complex of proteins of fat globules.

VI. THE MICROBIOLOGY OF CHEESE

Beginning with the coagulation of milk till the ripening of cheese, the microbiology of cheese is based on the microbiological processes, among which of greatest importance is the lactic acid fermentation process. If cheese is produced of raw milk, milk itself is a partial source of microflora. In the production of cheese from pasteurized milk,starters of lactic acid bacteria must be used for the direction of the microbiological processes.

Since 1950, in the Soviet cheese technology, pasteurization of milk is being introduced for cheese making. In connection with this, special starters are used. In the production of mould cheeses, Penicillium roquefort, Penicillium album and Penicillium candidum are used beside the lactic acid bacteria.

By soft cheeses of slimy surface, ripening process takes place under the joint action of Bact. linens and micrococci, which occur in the slime of cheese and form a protective slimy layer on the surface of Latvian and Yartsev cheeses.

By cheeses of high secondary heating temperature, the propionic acid bacteria take part in the ripening process. All the above mentioned microbes, together with lactic acid bacteria, take part in the ripening process of respective kind of cheese.

The supply of cheese industry with cultures, necessary for the making of cheese starters and moulds, is centralized in the Soviet Union.

Research work on the microbiology of cheese and the working out of cultures, is chiefly concentrated at the Uglich Institute. The microbiological laboratory of the Uglich Institute delivers dry cultures for the making of cheese. The laboratories of the republics and the territories deliver cheese cultures in liquid state.

Starters on Cheese

The run of lactic acid fermentation and the quality of cheese depend greatly of starters and from the qualities of lactic acid bacteria, which occur in the starter. Therefore lactic acid microflora, that causes and of what depends the ripening process, must occur in the microflora of starter.

In conformity with the characteristics of microflora, which collaborates in the ripening process of cheese, the cheeses are divided into two groups:

1. Cheeses, the ripening processes of which takes place mainly under the action of mesophilic streptococci;

2. Cheeses, the ripening process of which takes place mainly under the action of thermophilic lactic acid bacteria, and only partly under the action of mesophilic bacteria.

In conformity with this, in cheese making are used:

1. the cultures, which consist of mesophilic lactic acid streptococci;

2. the cultures, which consist of mesophilic and thermophilic lactic acid bacteria.

Starters of the first group are used in the making of semi-hard cheeses of low secondary heating temperature - mainly of the Dutch type - the round and angular Dutch cheese, the Kostroma, the Yaroslav and the Steppe cheeses, which are produced in the Soviet Union the most.

Starters of mesophilic lactic acid bacteria are used also in making of soft cheeses: those, ripened with the help of molds and those of slimy surface layer.

In the production of hard rennet cheeses of high secondary heating temperature (by the technology of Russian-Swiss, Soviet, Moscow and Altai cheeses), 2 types of starters are used: those containing lactic acid streptococci and those containing lactobacteria.

The starters of streptococci, which are used in cheese making, must have a high acid-forming intensity. They must also produce CO_2 and have an intensive ability of proteolysis.

<u>Starters for Cheeses of Low Secondary Heating Temperature</u>

To the composition of starters for cheeses of low secondary heating temperature belong the active acid- and aromatic substance producers. The lactic acid bacteria, which are to be found in the starter of cheese, must be active in order to ferment the whole of milk sugar, dependent of the kind of cheese and they must have a higher ability of proteolysis. The streptococci, producing aromatic substances, must beside lactic acid produce a rather large amount of other subsidiary products, which are important components in the flavour and aroma of cheese. They play an important role in the formation of eyes in the texture of cheese.

Concerning the aroma-producing streptococci, there are other requirements in the making of cheese, than in the making of butter. Regarding cheese starters, it is not essential, that a large amount of diacetyl were produced. Therefore, the reductive qualities of cultures are not of basic importance. But it is required, that in the lactic acid fermentation process they would produce CO_2, which is necessary in the formation of texture of this cheese.

Research on the composition of starters for cheeses of low secondary heating temperature has been carried out in the Central Research Institute for Butter and Cheese Industry. The effect of 4 starters of different composition on the microbiological processes of Kostroma cheese were investigated.

The following experimental samples of starters were used:

1. The culture of Str. lactis;

2. The cultures of Str. lactis + Str. paracitrovorus;

3. The cultures of Str. lactis + Str. diacetilactis;

4. The cultures of Str. lactis + Str. diacetilactis + Str. paracitrovorus.

The one-component homofermentative culture of Str. lactis had a negative influence on the quality of cheese. The active acidity rose quickly and towards the end of ripening, the acidity remained high (pH 5.14 to 5.29). Therefore the hydrolysis of proteins was restrained and cheese had a sourish and unclean flavour. The eyes in cheese were missing, the texture was hard and fissures occurred.

In the second experiment, where to Str. lactis Str. paracitrovorus was added, the latter developed very slowly and in a month constituted only 6 per cent of the microflora of cheese.

Although the active acidity of cheese at the beginning was 5.18, it dropped during the ripening process down to 5.41, which made possible the normal course of the biological processes in it As a result, typical flavour and the formation of eyes took place, but the texture was unsatisfactory.

During the third series of experiments, were to the culture of Str. lactis, Str. diacetilactis was added, the microbiological processes became notably intensified, the result of which was a considerably larger amount of aroma-producing streptococci. So Str. diacetilactis constituted ca 40 % of the microflora of one month old cheese. Those cheeses had a lower active acidity, which caused an acceleration of autolysis of cells and in connection with this the hydrolysis of proteins. By the time the conditional ripening was over, those cheeses contained 1.5 times more amino acids, than the cheeses, which were produced only with the help of Str. lactis culture.

The fourth experiment, in which beside the Str. lactis were the cultures of both aroma producers, the quality of cheese was the best. Cheese had a fine aroma, flavour, formation of eyes and texture.

Hence the microflora of cheeses of low secondary heating temperature must include beside the Str. lactis also both the aroma producers - Str. diacetilactis and Str. paracitrovorus. The starter of such composition and of low secondary heating temperature is the so called Uglich starter, which is delivered in dry form.

In the Baltic republics and in the Byelorussia, the Estonian cheese starter is used. It is used in parallel with the Uglich starter. The Estonian cheese starter contains local lactic acid streptococci, where the basic lactic acid fermenter is the Str. cremoris beside the aroma producers and also the Str. lactis.

Starters for Cheeses of High Secondary Heating Temperature

Cheeses of high secondary heating temperature are the Swiss, the Soviet, the Altai and the Moscow cheese. These, the so-called great cheeses are produced by the action of lactic acid streptococci and the lactobacteria, i.e. under the action of mesophilic and thermophilic microflora. During the ripening process, beside the intensive hydrolysis of the cheese mass, the fermentation of the propionic acid, typical to these cheeses, takes place. These bacteria lend cheeses a characteristic, slightly sweetish flavour and favour the formation of typical eyes. For the direction of fermentation of lactic acid, which takes place in the first stage of the process of making of cheese, as the mesophilic starter on great cheeses, the multi-component starter of streptococci of small cheeses together with the starter of lactobacteria is being used at the present time. The latter is cultivated separately, otherwise balance between the species is not guaranteed.

As thermophilic starters we use the Lactobacterium helveticum, the L. lactis and beside these also the Str. thermophilus. The cultures of propionic acid bacteria are delivered by the laboratories and they are used in such cases, when it is necessary to intensify the formation of eyes in cheese. Beside L. helveticum and L. lactis, in the ripening process of cheeses of high secondary heating temperature, especially during the later stages of this process is also of importance L. casei. However, when L. casei was used as cheese starter together with L. helveticum, the first quickly disappeared from the starter. The cultivation of said starters separately is troublesome in practice, because L. casei prefers a lower temperature. At the same time it became evident, that L. casei appears in great cheeses at a certain ripening stage also then, when it was not added to cheese as starter. Thus, the source of L. casei is milk, in which these microbes are preserved even in case of pasteurization. So it is probably not necessary to use the starter of L. casei separately in the production of great cheeses.

The mesophilic lactic acid bacteria, which are introduced into the pasteurized milk as starter on cheese, also the streptococci descending from raw milk (the Swiss cheese) are exterminated at a high (52 - 56 °C) secondary heating temperature. Here the question is rised: must the mesophilic streptococci not be substituted in the production of great cheeses by the thermophilic ones?

The thermophilic streptococci favour the formation of acids during the first stage of the productional process and so stimulate the reproduction of lactobacteria. Therefore the utilization of Str. thermophilus is justified, but it must constitute only 0.05 - 0.1%, otherwise the acidity of cheese is too high.

Selection of Lactic Acid Bacteria, which are Used as Starters on Cheese

By selection of cultures must be taken into consideration the influence of separate species to each other and to prevent the introduction of antagonistic strains. By cultivation in practice, the qualities of starters can change, therefore they cannot be used over 6 - 7 days. Changes in the qualities of the cultures can be caused also by the bacteriophage. Investigations of latest years have shown, that in the technology of those milk products, in which lactic acid fermentation takes place, the bacteriophage occurs more extensively as was thought up till now.

Especially harmful is the bacteriophage in cheese making, as the lactic acid bacteria are important factors in the ripening process of cheese. The problem of bacteriophage in the technology of cheese cropped up especially then, when the utilization of pasteurized milk and in connection with this, the use of starters of lactic acid bacteria began. All cultures, which are used in the production during a prolonged period, inevitably contaminate sooner or later by bacteriophage. Among the lactic acid bacteria the most susceptible to bacteriophage are the lactic acid streptococci. The Lactobacteria are less susceptible to the phage. Thus the bacteriophage is most perilous on small cheeses - on cheeses of low secondary heating temperature. If the culture or starter has been harmed by bacteriophage, the reproduction of lactic acid bacteria takes place normally during 3 - 5 hours only. Further on, the rise of acidity decelerates and the fermentation process of lactic acid is restrained, or totally stopped. On the microscopic preparate is observed at firt the agglutination of bacterial cells, after which follows their lysis.

By making of cheeses, where the bacteriophage occurs in milk, the rise of acidity in whey is slow and there can begin to develop microbes, which are harmful to the quality of cheese: the bacteria of Coli aerogenes group and the mammococci. They cause such defects as early swelling, unclean and stale flavour, which lower the quality of cheese. Many bacteriophages are of a specific action, damaging only one species or strain (the monovalent phage) But beside the monovalent phage, such bacteriophages occur, which harm many species of lactic acid bacteria.

In the bacterial culture, which has been harmed by bacteriophage are to be found cells, which are resistant to phage. In respect to this may occur in practice a partial lysis of cells and the strains, resistant to phage are selected, causing alteration in the quality of starter.

It has been observed, that the bacteriophage, which is active in regard to Str. lactis, is of widespread activity - it can harm also other species of streptococci. The bacteriophages of other streptococci are of more specific action.

The bacteriophages of lactic acid streptococci are rather extensively spread in the nature. Investigations have shown, that bacteriophage occurs in milk of some farms. The bacteriophage of streptococci occurred quite seldom in milk of healthy cows, but it occurred in milk, obtained from udder-diseased cows.

In the industry the bacteriophage occurred in milk of certain farms, in milk of cheese (mixture), in whey, brine, on the utensils used by cheese making, but also on the walls and floors of the premises. Most likely the sources of phage contamination in the farm are the improperly sterilized dairy utensils, but there are also bacteriophages occurring in cheese making room. in the air and on the equipment.

In starters the bacteriophage occurs more seldom and its getting there is conditioned through contamination, which took place by the inoculation of starter. Reproduction of bacteriophage in conditions of cheese making is favoured by low secondary heating temperature. Also the use of $CaCl_2$ promotes the reproduction of bacteriophage, because $Ca^{\cdot\cdot}$ activizes the bacteriophage (without Ca°° the bacteriophage develops slowly).

In order to avoid of bacteriophage in cheese making, it is necessary to use the multi-component starter, while the lactic acid bacteria, used for its making must be resistant to phage. Starters must be often changed, at least after 6-7 days. By taking into use a new culture, rooms and utensils must be desinfected with special care, using for that purpose calcium hypochloriti or chloramine. By making of cheese, all utensils and equipment must, before use, be thoroughly disinfected. The making of mother-, as well as of commercial starter must be effected in a separate room, irradiated with a quartz lamp.

Making of Cheese Starters in Practice

In making of starters in practice, it is very essential to favour the reproduction of aroma-forming streptococci. If starter has been used for a long period, from its composition disappear fist of all the aroma producing bacteria. To prevent this, must be observed the regime of temperature during cultivation, and taken into consideration the seasonal instability of milk. Milk, containing antibiotics, is not allowed to be used in the making of starters.

Microbiological Processes in Making of Raw Cheese

In making of cheese, starter is being added to cheese milk at a temperature of 30 °C, i.e. by the optimum temperature for the reproduction of lactic acid streptococci. In working of cheese curd, noticeably more bacteria are being detained by the coagulum, for which reason microbiological processes in curd take place more intensively than in whey. The reproduction of lactic acid bacteria in cheese curd is favoured through higher buffering ability, than by whey.

The secondary heating temperature affects the composition of microflora, which later affects the ripening process.

The workers of the All-Union Cheese Research Institute have shown, that in the technology of Dutch cheeses (the Kostroma and the Steppe), the rising of secondary heating temperature over 40°C greatly affected the reproduction of more sensitive lactic acid bacteria. When the secondary heating temperature was raised from 40° up to 43 °C, the number of bacteria after pressing was perceivably lower, being at the temperature of 46 °C only 1/5 of the number of that of 40 °C.

By rising the temperature from 40° to 43 °C, took place an increase in the number of aroma-producing streptococci of the Str. diacetilactis and L. casei. But when by secondary heating, the temperature was raised up to 46 °C, the reproduction of aroma producing streptococci was restrained, whereby the relative importance of thermophilic streptococci increased.

By cheeses of high secondary heating temperature (the Swiss and the Soviet cheese), at the temperature of 55 - 59 °C takes place, during 30 - 50 minutes a prompt restriction of the reproduction of mesophilic streptococci and mesophilic lactobacteria (L. casei), but at the temperature of over 48 - 50 °C, the reproduction of the thermophilic lactobacteria (L. helveticum, L. lactis) is also restricted. Thus, high secondary heating temperature has a selective effect to the microflora of the above mentioned cheeses, emphasizing the thermophilic lactobacteria and Str. thermophilus.

Microbiological Processes by Pressing, Straining and Salting of Cheeses

Since by forming, pressing and straining of cheeses the temperature slowly decreases, the microbiological processes in cheese mass proceed very intensively. Toward the end of pressing, the number of bacteria in cheese rises to many milliards per 1 g, whereas the streptococci entirely predominate.

By salting, which takes place at the temperature of 10 - 12°C, the fermentation of lactic acid is resticted, but also is restricted the reproduction of harmful microbe groups (the coli-group bacteria).

Microbiological Processes by Making of Cheeses of Low Secondary Heating Temperature

The ripening process of cheese takes place by the action of enzymes of lactic acid bacteria and also, to a certain extent, under the action of the rennet enzyme. The intensive lactic acid fermentation restricts the reproduction of coli-group bacteria: the micrococci, the aerobic and anaerobic decomposers of protein and the butyric acid bacilli. From harmful microbes the coli-group bacteria become apparent because of their intensive gas forming already by pressing, straining and salting, if the temperature of brine exceeds 13 - 14 °C, The fermentation process of butyric acid bacilli takes place in the 3rd to 5th week. By different cheese groups, the microbiological processes run according to the technological regime and the used starters.

In cheeses of low secondary heating temperature, the conditions for reproduction of mesophilic lactic acid bacteria, whose optimum temperature is 30 - 35 °C exist, but their reproduction takes place also at a temperature of 20 °C and even at a lower temperature, although slower.

The ripening process of small cheeses, which are made, without exception, from pasteurized milk, takes place by the action of enzymes of streptococci of starters, the Str. lactis, the Str. diacetilactis and the Str. paracitrovorus. Of the lactobacteria in the microflora of small cheeses, there occur only b. casei and L. plantarum, but the latter occur in smaller number than the streptococci.

In the Dutch-type cheeses (the round Dutch cheese, the angular Dutch cheese, the Kostroma, the Yaroslav, the Steppe, the Uglich cheese) the number of lactic acid bacteria rises after pressing up to 2 - 4 milliards per 1 g. The reproduction of lactic acid bacteria takes place also after salting, in consequence of what after 5 to 7 days of salting, milk sugar in cheese mass is completely fermented. By the intensive reproduction of lactic acid streptococci, plenty lactic acid is formed in cheese mass, but because of the buffering ability of cheese mass the pH remains within the limits of 5.4 to 5.6. The high content of lactic acid restrains the reproduction of lactic acid bacteria and the amount of streptococci begins to decrease. The number of lactobacteria in the ripening cheeses during the first two weeks is low, but being resistant to acidity, they later begin to reproduce in conditions, where the streptococci die out. The reproduction of lactobacteria takes place in ripening cheese because of absence of milk sugar on the basis of hydrolysis products of protein. The number of lactobacteria in the ripening cheese reaches after 6 to 8 weeks 400 to 500 millions, after that their number begins to decrease.

The Microbiological Processes in the Cheddarized Cheeses

Through cheddarization are produced in the Soviet Union the cheddarized cheese, the Pioneer cheese and the Altai-mountain cheese. By cheddarization, the reproduction of lactic acid bacteria is stimulated, by the help of which in the course of 24 hours a perceivably higher number of strains is obtained, than witout it. This favours a quick fermentation of milk sugar (during the first 2 - 4 days) and the formation of a large amount of lactic acid.

During the first days in the cheddarized cheeses predominate the lactic acid streptococci. Their number reaching 1 to 2 milliards per 1 g, after which it begins to decrease.

The number of lactobacteria in fresh cheese is very low, reaching only a few thousands per 1 g, but in ripening of cheese, their number rises step by step. In 1.5 to 2 months old cheese, the number of lactobacteria makes out 50 % of the total number of microbes, reaching 100 millions per 1 g of cheese mass.

The surface microflora, especially the moulds play no role in the ripening process of the cheddarized cheese, therefore the surfaces must be kept clean.

The Microbiological Processes in Strained and Slightly Pressed Cheeses (Latvian, Yartsev, Volga cheeses)

The strained cheeses contain more whey and consequently more milk sugar. That is the reason, why the microbiological processes of these cheeses are more extensive, compared with Dutch-type and cheddarized cheeses. During the first days of ripening process, the number of lactic acid bacteria reaches 8 to 10 milliards, from which more than 90 % are the lactic acid streptococci.

The Lactobacteria (L.casei) appear in the microflora of cheese in the 5th to 10th day and their number reaches 50 - 100 millions per 1 g of cheese mass.

In order to prevent the surface of cheese from drying and to lend it a specific flavour and aroma, the surfaces are kept moist. This circumstance causes the reproduction of many species ofprotein decomposers, under whose action on the surface a slimy cover - the cheese slime is formed. On the surface of cheese begin to reproduce, at first, the micrococci and yeasts, but by penetration of salt into the mass of cheese , also the proteolytically active Bact. linens and Bact. bruneum. The number of bacteria in slime of cheese reaches tens of milliards per 1 g and even more. By the process of hydrolysis of protein in slime of cheese ammonia is formed, which lends cheeses a specific sharp flavour and aroma, slightly reminding of ammonia.

The Microbiological Processes in Cheeses of High Secondary Heating Temperature

The high secondary heating temperature, which is utilized in the production of cheeses has a selective effect to the mesophilic streptococci, bringing forth a great predominance of lactobacteria, which are used as starter and also the thermophilic streptococci in cheese. As the L. helveticum attains predominance already in the beginning of ripening process, deeper hydrolysis of protein is brought about as by lactic acid streptococci. So, by ripening process, the hydrolysis of protein turns out deeper, what lends cheeses of high secondary heating temperature a sweetish flavour and aroma. Sweet flavour of these cheeses is brought about not only by protein decomposing products but is conditioned also by milk sugar.

Because of difference of microflora and of drier working, the consistency and texture of cheeses of high secondary heating temperature are perceivably different from strained cheeses, also from the Dutch cheeses. Because of dryer working of cheese curd, the number of lactobacteria reaches but one milliard per 1 g. The propionic acid bacteria develop in cheese during the period of intensive hydrolysis process, i.e. beginning from the 4th to 5th week, until the 6th to 8th week.

That the intensity of proteolytic processes are determined by the moisture content in cheese becomes evident from the fact, that in Soviet cheese of higher moisture content, the microbiological processes run quicker. Cheese ripens in four months, while the ripening process of Swiss cheese lasts almost six months.

Microbiological Processes in Soft Cheeses

The ripening process of one group of soft cheeses takes place under the action of enzymes of lactic acid bacteria and by the action of yeasts and bacteria, reproducing on the surface of cheese (the Dorogobush, the Medyn, the Smolensk cheese). The second group of soft cheeses ripen under joint action of the enzymes of lactic acid bacteria and the enzymes of special moulds (the Camambert cheese, the Roquefort cheese).

The cheeses of Dorogobush type contain perceivably more whey and consequently, more milk sugar, owing to what in the first days of ripening the number of bacteria reaches 7 - 8 milliards per 1 g. Due to this, cheeses have high acidity (pH 4.7 to 4.8). High acidity slows down ripening of cheeses, but in the surface layer begins already after salting, the reproduction of microflora, resistant to salt - the yeasts, moulds and micrococci. Yeasts and moulds, using lactic acid, are to be found in the surface layer of cheese, creating conditions for the reproduction of Bact. linens, Bact. bruneum and other protein decomposers. Metabolism products of these bacteria penetrate into cheese mass and cause neutralization of acid, favouring decomposition of proteins. In consequence of that ammonia is formed.

As the Smolensk cheese after salting is being dryed for 5 days, on its surface begin to develop moulds, which lend cheese a specific aroma, of which reason this cheese differs from the Dorogobush cheese.

In the Camambert cheese the reproduction of lactic acid bacteria, because of high content of whey takes place very intensively. High content of milk sugar causes a rise in the number of mesophilic lactic acid streptococci, bringing it up to 6.5 milliards per 1 g. After the 10th day their number decreases to a few hundred million. From lactobacteria the L. casei and L. plantarum begin to develop. Their reproduction in said cheeses is perceivably more activ than in the Dutch and Latvian cheese, so their number on the 15th day reaches a few milliards. On the surface of the Camambert cheese begin to reproduce the cultured moulds Penicillium candidum and Pen. camamberti, which were planted there. These, using lactic acid, create conditions for the activation of endoenzymes, released by the autolysis of lactic acid bacteria. Thus, ripening process of Camambert cheese takes places from the surface inward.

By the ripening process of Roquefort cheese, beside the lactic acid bacteria also the Penicillium roqueforti collaborate. Spores of this cultured mould are brought into cheese milk or cheese curd. As the moulds need oxygen for the oxidation processes, longitudinal canals are beaten into cheese with the help of a mechanical device.

In Roquefort cheese, in the first days, the lactic acid streptococci develop, reaching a spore number from 5 to 5.5 milliards per 1 g. After the tenth day, the mesophilic lactobacteria begin to reproduce and their number surpasses later the number of streptococci, because of continuous decreasing of the number of the latter.

The reproduction of lactobacteria is connected with the circumstance, that Pen. roqueforti, reproducing in cheese mass, uses lactic acid. Beside the hydrolysis of proteins, moulds cause also the hydrolysis of fats, which lend cheese the specific sharp taste. The development of moulds in cheese mass is comparatively rapid, as they use lactic acid, which allows to employ the system of ferments, released by the autolysis of lactic acid bateria.

On the surface of Roquefort cheese develop also yeasts, micrococci and many other protein decomposers, but they have importance only by processes taking place in the surface layer.

Microbiological Processes in the Brynza Cheeses

The Brynza cheeses are sticked, after making into a 22 to 23 % brine, where they remain until delivery. Thus, the microbiological processes can take place only by the making, forming and working of raw cheese. Lactic acid fermentation process runs in curd of the Brynza cheese very slowly, owing to that , even later, milk sugar is not completely fermented.

The Formation of Cheese's Texture

Texture of cheese mass is formed by the activity of bacteria, which are able to produce gas. Moderate gas producers in small cheeses are the streptococci, which produce aroma-forming substances. In great cheeses the texture is formed mainly by the action of propionic acid bacteria. In case curd is being formed into plate under whey, in cheese are formed by the action of gas round, sparsely scattered eyes (closed texture - by Dutch, Swiss and Soviet cheeses). But if cheese is shaped by pouring (Latvian, Yartsev and soft cheeses), between cheese curd penetrates some air, in consequence of what an irregular, slotty, the so-called open structure is formed.

By early swelling of cheeses, the bacteria of coli-aerogenes group damage its texture. By late swelling, which becomes apparent after 3-rd to 5-th week, are the causers the butyric acid bacilli (Bac. amylobacter).

Defects of Cheese of Microbiological Origin

Swelling of cheeses take place, as just mentioned, by the action of coli-group bacteria and butyric acid bacilli. The so-called early swelling, which takes place by the action of coli-bacteria, becomes evident by working and straining in brine and is effected on ~~the basis~~ of milk sugar. The formation of eyes is irregular, dense and cheeses have because of hydrolysis of proteins an unclean, a bit insipid flavour.

The late swelling is conditioned by the butyric acid bacilli and becomes evident, when the hydrolytic processes have started in cheeses by the action of lactic acid bacteria. In such case the mass of cheese is elastic, which makes possible the forming (by the action of CO_2 and H_2, which separate by the fermentation process of butyric acid) of especially big eyes, and often a honeycomb-like texture. The butyric acid-fermented cheese has a sweetish-insipid flavour and because of butyric acid, a sharp aroma. By an intensive butyric acid fermentation the eyes in cheese are irregular, dense and cheeses have because of decomposition of proteins an unclean, slightly insipid flavour.

Bitter taste of cheese is conditioned by different protein-hydrolysing bacteria. They form from casein products of bitter taste - the peptons. Producers of bitter taste are often the micrococci.

Purulent-spotted surface is conditioned by races of Oidium, which on the surface of cheese decompose proteins, making this surface "pock-marked". Because of that it is not possible to preserve cheeses for long, as in harmed places begin to develop protein decomposing bacteria.

An effective method against purulent-spotted cheese is the manipulating (by 2 to 2.5 weeks old cheeses) with hot whey (60 to 70 °C), in which cheeses are kept 3 to 5 minutes. After that cheese is to be rinsed in water at a temperature of 70 to 75 °C for 2 - 3 seconds.

Brown spots occur most often on the surface of Dutch cheeses, which were carelessly handled. In this case beside the micrococci, on cheese develop also the Bact. vulgare. As both have a strong proteolytic action, the brown colour is caused by the amino acid tyrosine. In order to prevent this defect, equipment, used by cheese making, must be carefully cleaned and disinfected with calcium hypochloriti, chloramine or steam. If necessary, even water must be manipulated with calcium hypochloriti.

Under-cortex mould is a frequent defect of worked cheeses, which is caused by reproduction of Penicillium and Aspergillus on the surface of cheese, the pores of which are, because of surface manipulation, not yet closed. The under-cortex mould appears also then, when the worked cheese has an uneven surface, or as a result of fissures, caused by drying.

In order to prevent this defect, the manipulation of cheese's surface must begin already a few days after salting and the surfaces of cheese must be kept clean during the cellar-manipulation.

Fissures on the surface of cheese and in its mass are conditioned by the circumstance, that the mass of cheese has not joined homogeneously; also of tears in consequence of later-constituted tensions. On the fissures of surface begin to develop moulds and yeasts, causing processes of protein decomposition.

The hydrogen sulphide is formed during the ripening process of cheese because of contamination or the Str.faecalis, which descends from milk, the reproduction of Varietas malodoratus in cheese. The reproduction of said bacteria occurs in cheese by low content of salt. In cheese are formed decomposition products of unpleasant odour (H_2S), which become apparent 3 to 4 weeks after commencement of cellar-manipulation.

In order to prevent this defect, cheese milk must be pasteurized at a higher temperature (72 to 74 ^{o}C) and all the technological requirements for the production of certain kind of cheese must be observed.

VII. THE MICROBIOLOGY OF CANNED MILK

In the Soviet Union the following kinds of canned milk are produced: the saccharified condensed milk, the condensed milk with coffee and sugar, the condensed milk with cocoa and sugar, the condensed sterile milk, the powder of full- and skim milk and dryed cream.

In preservation of canned milk the microbiological processes play a negative role, therefore, by their production, it is necessary either to reach a total destroying of micro-organisms (condensed sterilized milk), or to restrain their reproduction for the conservation (the saccharified condensed milk, the milk powder).

As condensed milk is foreseen for a long-period preservation, the reproduction of microbes, even if this takes place on a small scale, can cause defects and even taint of product.

Condensed, Sterilized Milk

In the technology of this product, milk is condensed 1:3, provided with stabilizators, is packed into hermetic tare and sterilized at a temperature of 115 - 118 °C during 15 minutes. If the microbes (mainly the spores of bacilli) are not completely destroyed, various defects may occur in condensed milk.

Rise of acidity of condensed, sterilized milk is caused by spore producing bacilli - the Bac.cereus and the Bac.coagulans. Precipitation of milk casein in condensed sterilized milk is caused by the anaerobic bacilli, which ferment milk sugar and link the decomposition of proteins. Of those the butyric acid bacilli, the Bac. amylobacter and the anaerobic protein decomposer Bac. putrificus is to be mentioned. By decomposition of proteins of condensed milk, casein deposits on the bottom, causing whey to raise to the surface. When coagulum is formed, it rises to the surface. In some cases milk smells of putrification, while coagulum, formed at the beginning, has dissolved. According to the State standard, the sample of condensed, sterilized milk, which has been packed into hermetic tare, must show no alteration after 10 days, at a temperature of 37 °C - the product must remain sterile.

Saccharified Condensed Milk

By the technological process of saccharified condensed milk, there are always preserved a certain amount of microbes, which descend from raw milk and are preserved in pasteurization and condensation. In condensed milk micrococci and spores of bacilli occur. Besides, there occur yeasts, lactic acid bacteria and also moulds. Condensed milk must be kept at a temperature not exceeding 10 °C. At higher temperatures there begins, despite high concentration of sugar, the reproduction of certain micrococci and yeasts.

According to standard, condensed milk must (with respect to microbes) correspond to the following requirements:

In saccharified condensed full milk, the number of bacteria per 1 g is not allowed to exceed 50 thousand and per three parallel samples of milk of 1 g each, the Bact.coli can occur only in one sample.

The condensed milk with coffee, cocoa and sugar is not allowed to contain over 35 thousand bacteria per 1 g.

In three parallel samples of the medium of Buliera, in each 0.1 g of the investigated milk, the coli can occur only in one sample. By saccharified condensed milk or condensed milk with other admixtures, no pathogenic microbes are permitted.

Defects of Saccharified Condensed Milk

The viscous milk. This defect is caused by acid producing microbes, by the action of which condensed milk obtains a viscous paste-like consistency. Most often this defect is caused by micrococci, which produce lactic acid, but affect at the same time the proteins, coagulating and also decomposing them.

Formation of Small Clots

Defect lies in this, that on the surface of saccharified condensed milk small clots are formed, consisting of coagulated casein, but having thereby a white to yellow and yellowish-redish colour. The inducers of this defect are mainy moulds, more frequently certain species of Aspergillus and Catenularia. In saccharified condensed milk sometimes chocolate-brown small clots are formed, which are caused by Catenolaria fulicinea, by reproduction of which viscous small clots are at first formed. Later these clots get firmer and obtain a bit stronger consistency, while later they obtain a redish-brown colour. The colonies on the surface of condensed milk are dense and of radial shape. These colonies are white at first, but after 10 to 15 days they get pigmented and have a chocolate-brown colour. The source for yeasts in saccharified condensed milk is the syrup, in which, because of high concentration of carbohydrates, the spores of moulds are not killed in thermal handling.

The Bombage

This defect becomes apparent in the formation of gas, as a result of which the ends of cans bulge out and can sometimes even burst. The formation of gas is caused by the reproduction of yeasts, butyric acid bacilli and coli bacteria. But the most frequent causers of bombage are the resistant yeasts, whose reproduction is not restrained by high osmotic pressure. The source for these yeasts in the technology of condensed milk are the bags of granulated sugar. The reproduction of yeasts in granulated sugar takes place, when sugar bags are kept in a damp room. Therefore sugar must be preserved in clean, well-aired rooms. Saccharified condensed milk must be packed into carefully cleaned tare.

The Mould

By this defect on the surface of condensed milk, or on the inner surface of barrel's lid, colonies of yeasts appear by storing. Most frequently occur in such case the species of Penicillium, headed by Penicillium glaucum, but sometimes through slight access of air, also the species of Cladosporium develop. The causer for the development of moulds on the surface of condensed milk or on the tare, is the air, which remains between milk and lid. The greater the gap between lid and condensed milk, the greater is the peril of mould. In the practical conditions this defect occurs more often, when condensed milk is packed into large tare.

In order to prevent the reproduction of yeasts and moulds, the saccharified condensed milk must be preserved at a temperature as low as possible.

Defects of Saccharified Condensed Milk, which are Caused by the Pigmentized Micrococci

Micrococci, being resistant to thermal handling are resistant also to high concentration of sugar. In storing of condensed milk, micrococci cause splitting and decomposition of protein, unclean taste and even the taste of cheese. Micrococci are able to develop at a comparatively low temperature, but in this case their reproduction is slower. The source of micrococci is raw milk, technological equipment, sugar-bags and the tare.

VIII. THE MICROBIOLOGY OF MILK POWDER

Milk powder is produced by two methods: by film-drying and by spray-drying. By film procedure a temperature, exceeding 100 °C is used by drying. Thus the microbes are destroyed more completely than by spray procedure. By film-drying mainly the spores of bacilli are preserved, but by spray-drying, at a spray-zone temperature of 65 to 70 °C, beside spores of bacilli yet the micrococci, microbacteria, lactic acid bacteria and yeasts occur.

As the per cent of moisture in milk powder is 4 to 6, there are no pre-requisites for the reproduction of microbes. The reproduction of microbes can take place only then, when milk powder is in non-hermetical tare and becomes moist through the air. In such case moulds sometimes begin to develop, but the reproduction of bacteria was usually not denoted. By the non-hermetical tare (the plywood barrels and drums), the moisture content rises, as a result of which the dissolubility of milk powder reduces because of reaction between milk sugar and protein.

According to state standard, the number of microbes in weak milk powder is not allowed to exceed 50 thousand per 1 g. By the semi-rich milk powder, used as childrens nourishment, the number of microbe content must not exceed 30 thousand per 1 g.

In milk powder no bacteria of coli-aerogenes group and no pathogenic microbes are permitted.

Ю. Клаар

Микробиология молока и молочных продуктов

На английском языке

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