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# Biotechnology of yogurt producing with specialized fermentation starters: safety indicators assessment

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**Abstract**

We are what we are eating. It is critically important to know you are eating a safe and quality meal. And the big companies have not to be excluded. Checking of the manufacturing technologies can show points which need more attention for getting a useful product. The aim of the research was to evaluate the yogurt production process according to biotechnology qualitative and safety. The authors have studied technical regiments of the yogurt "Dolce" and "Bios" ("Foodmaster" joint-stock company) preparation. The study results declare the milk-products by the "Foodmaster" joint-stock company meets the requirements for fermented milk products raw materials production. Specialized microorganisms for dairy products manufacturing from lactic acid bacteria and bifidobacteria strains were tested: "Bios" yogurt B. longum-B-5-M, "Dolce" yogurt lactic acid bacteria *Str. thermophilus-SV-M*, *L. acidophilus-L1-M*. Microorganisms starter cultures were meet the requirements for fermented milk products manufacturing with prophylactic and therapeutic purposes. We have checked the yogurt "Bios" and "Dolce" manufacture process in the "Foodmaster" joint-stock company. And can conclude the checked products can be getting a useful product in our meal list.

**Keywords:** healthy nutrition; milk; yogurt; microorganisms; dairy products.

**Practical Application:** We have made checking of the manufacturing process of the yogurt production for ensuring the safe meal will be getting.

## 1 Introduction

There are many factors that have their influence on normal bifidobacteria concentrations in the human digestive system. It can be diet, antibiotics, or stress (Shori, 2021). Yogurt is a fermented milk drink, mostly including bifidobacteria, Bulgarian bacillus (lactobacillus), and acidophilus bacillus. As a result of the yogurt's microflora vital activity, some substances are forming: lactic acid, alcohol, carbon dioxide, antibiotics, vitamins. All these compounds in small quantities have positive human body impact: normalize the activity of the gastrointestinal tract activity, inhibit the development of pathogenic microflora, and increase immunity level. Undoubtedly, nowadays, yogurt is one of the most commonly consumed fermented milk products. The assortment of yogurts produced by the international community is very wide and diverse (Gudkov et al., 1981; Russia, 1984, 1987; Sabdenov, 1989; Stepanenko, 1999).

Along with classic natural yogurt, which includes thermophilic lactic streptococcus and Bulgarian bacillus, there are many types of products of various compositions with numerous fillers and flavors that have a dense undisturbed or broken clot. Bio-yogurts containing probiotic cultures living cells, as well as various yogurt drinks containing fruit juices and enriched with vitamins, micro- and macroelements, and dietary fiber are getting more and more popular. Yogurts and desserts with an extended expiration date based on heat processing of the finished product are also known (Krasnikova et al., 1991; Russia, 1996; Phuyal, 2016).

In Kazakhstan, after thermophilic streptococcus thermophilic and Bulgarian bacillus implementation, fermented milk products such as yogurt, fermented baked milk, sour milk is in great demand for a long time. Domestic manufacturers, after achieving some success in this area, firmly settled in the Kazakhstan yogurt market. Commercial yogurts are divided into three main categories: natural, fruit, and flavored. These yogurts are available in various forms - thick, mixed, and drinkable, the last form is more popular (Krus et al., 2000; Shepelev & Kozhukhova, 2001; Bredikhin et al., 2003).

The current trend anticipates the commercial production of low-calorie yogurts. So there many ways of such goal-achieving: reducing milk-based fat content, replacing sugar with low-calorie synthetic sweeteners, replacing milk fat with fat substitutes, adding fiber, reducing dry skimmed milk residue, stabilizers adding (Kabir et al., 1997; Bibiloni et al., 2000; Shepelev et al., 2002). Nowadays, the dairy products market is widely represented around the globe with more than $ 125 billion in total income

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per year. Meanwhile, worldwide sugar consumption is growing markedly leading to a growing number of different diseases (Mc Cain et al., 2018).

To increase the yogurt demanding (to provide the consumer of different yogurts tastes), the product is mixed with a wide variety of ingredients. Step by step, the yogurt's attitude has changed from a special healthy diet product; it has turned into a popular and not expensive food. The methods of yogurt obtaining have changed slightly. Despite some new improvements in the technological processes of the production of the yogurt (especially the lactic acid bacteria fermentation technologies improvements), the main stages of the process remained the same (Edwards et al., 2016).

One of the driving powers for further assortment enlargement, improving technology and the quality of the product is hard competition among manufacturers. Hence, such rivalry requires technology processes improving, dilatation of yogurt's features knowledge, finding reliable ways to ensure product safety and appearance of a closer connection with the consumer.

The aim of our research was to analyze the technical stages of the yogurt manufacture, find the critical for the manufacture safety points, and the produced yogurt safe analysis.

## 2 Materials and methods

We have used conventional microbiology, physicochemical and organoleptic methods during our research. The control of "Dolce" and "Bios" yogurt was carried out under the 1065-2002 regulatory documents "Yogurt. General specifications", "Milk and dairy products. Methods of microbiological analysis", "Milk and dairy products. Sampling rules, sample selection methods, and preparation of samples for analysis", "Food products. Lactic acid microorganisms determining methods".

### *2.1 The acidity determination method*

The titratable yogurt acidity is an evaluation criterion of its freshness. Acidity expressed in Turner degrees T° (the number of ml 0.1 NaOH or KOH, needed to neutralize 100 mL of milk). Yogurt acidity can be expressed as a lactic acid percentage.

In a conical 150-200 $cm^3$ flask we have measured 10 $cm^3$ of yogurt with a pipette, add 20 $cm^3$ of distilled water, and three drops of phenolphthalein. The mixture was thoroughly mixed and titrated with a solution of sodium hydroxide (NaOH) until a faint pink color appears which does not disappear for one minute. The acidity of milk in Turner degrees is equal to the NaOH volume spent on the neutralization of 10 $cm^3$ of yogurt, multiplied by 10.

### *2.2 The fat mass fraction determination method*

10 $cm^3$ of sulfuric acid (p = 1811-1820 $kg/cm^3$) we have poured into a pure milk butyrometer and, carefully, to avoid the liquids blending, add 10.77 $cm^3$ of a mixture (10 $cm^3$ of yogurt + 10 $cm^3$ distilled water); and 1 $cm^3$ of isoamyl alcohol was added. The butyrometer is corked, mixed, and centrifuged for 5 minutes. After laboratory water bath exposure and centrifugation, the fat is counted.

### *2.3 Microscopy method*

To detect the presented in the samples microorganisms, stained preparations were checking viewing under a microscope for the dairy products microflora determination. A small drop of the material was loaded onto a clean glass with further distribution over an area of about 1 $cm^2$. Then, the product was placed on the glass by inoculation loop, and thoroughly mixed. The preparation has been dried at room temperature, fixed on a burner flame, and stained.

### *2.4 The total bacteria number determination method*

The method is based on counting colonies of mesophilic aerobic and facultative aerobic microorganisms growing on nutrient agar at 30 ± 1 °C for 72 hours. The inoculation of the microbial suspension was made a selection of bacterial suspension seeding dilutions. The amount of inoculated samples has been determined with the consideration of the most probable microbial growth. Were acceptable only dilutions with growing at least 30 and no more than 300 colonies. Three Petri plates have been sowed for each sample. Each dilution has been seeded in an amount of 1 $cm^3$ in one pre-marked Petri plate and filled in 14 + 1 $cm^3$ of molten and cooled to a temperature of 40-50 °C temperature nutrient medium for bacteria number determination. Sowing of the test samples in a Petri plate from the same dilution in the amount of 1 and 0.1 $cm^3$ was possible. Immediately after the agar addition, the Petri dishes were thoroughly mixed by gentle rotary shaking to even sowing material distribution.

Cultivation was conducted in a thermostat with a temperature of 30 ± 1 °C for 72 hours. The count of the grown colonies was counted on each plate, using a magnifier with an increase of 4-10 times; and with marking of each counted colony. In a case of a large colonies number and their even distribution the bottom of the Petri plate was divided into four or more identical sectors, the colonies number into two or three sectors (but not less than 1/3 of the surface of the cup) were calculated. So, was finding an average arithmetic colonies number and multiplied by the total sectors number of the whole plate. This was a way to find total grown colonies number in one plate. The total bacteria number in 1 $cm^3$/1 g of the product (X) has been calculated by the Formula 1:

$$\tilde{O} = n \times 10^{m.} \quad (1)$$

Where **n** is the colonies number on a Petri plate; and **m** the tenfold dilution number.

The final result of the analysis was the arithmetic average of all plates. Mathematical processing of the research results was carried by the Lakin biometric method (Saxelin et al., 1993).

## 3 Results

The study examined the parameters of milk used for fermented dairy products manufacturing, using the "Bios" and "Dolce" yogurts examples. The main physical and chemical parameters of milk were checking by us. Cow's raw milk grade 1, with a fat mass fraction of 3.5%, supplied by farms of the Almaty region as a fermented milk drink raw material, was taking into the

manufacture was detected as the first critical point of the safety manufacture. In checking was used the normative document GOST 1326488, and the raw milk was analyzed following the regulatory documentation.

Our study showed that the raw milk which was taken in the yogurt manufacture process was in the normal ranges in such parameters as the mass fraction of fat, density, acidity, purity, the temperature of the raw milk. And the important point was the microbiology safety of the milk after the pasteurization process: were no find coliforms in the tested samples. The manufacture stages from the milk collection to prepared product getting were represented on the Figure 1.

So the yogurt preparation manufacture consist such stages in the technical process: collection, cleaning, and separation of milk; collection and preparation of components; milk stabilization and mixture preparation; pasteurization; homogenization, mixture cooling; mixture fermentation; fillers addition; bottling, packaging, labeling, pre-cooling of the finished product; and the finish stage – storage, transportation.

The second critical point in safety yogurt preparation was detecting the phase of the milk fermenting. It should be noted a certain technology for the starter culture's preparation at domestic enterprises existing. Starter preparation was made by fermenting milk with pure cultures of lactic acid bacteria (strains). Strains of lactic acid bacteria pure cultures are isolated from milk, soured milk products, plants in special laboratories, and delivered to enterprises in the form of dry or liquid starter culture, dry or frozen bacterial concentrate, strains of lactic acid bacteria and yeast, kefir fungi. Liquid starter cultures are strains of lactic acid bacteria grown in sterile milk, used in dry form after desiccation.

The dry bacterial concentrate was obtained. The expiration date of dry starter cultures and the bacterial concentrate is not more than 3 months, and liquid starter cultures not more than 2 weeks at a temperature of 4 ± 2 °C. Has been formulated such basic requirements starter cultures production (Russia, 1996).

- 1. The fermentation department should be isolated from production and be as close as possible to the consumers of starter cultures.
- 2. At the department entrance there should be a vestibule for sanitary clothing changing.
- 3. The fermentation department must have separate rooms: for preparing starter cultures on pure seedings; and for kefir, yeast obtaining; for washing and disinfecting of the equipment.
- 4. It is allowed to prepare starter cultures and kefir in one room for small-capacity enterprises. However, containers for the various starter cultures preparation and pipelines for their supply must be separated.
- 5. The bactericidal lamps must be used for air sterilization in the fermentation departments.
- 6. It is taboo to pass communications for steam, water, and cold supply, as well as ventilation and sewage in fermentation departments.

All these actions can provide pure in the yogurt starters process preparation and allow getting a quality product for food and treatment. Have to be noted, yogurt manufacture has declared instructions for the starter culture preparation for the fermented milk product preparations. First of all were detected bacterial agents for the milk product preparation and was formed its way using.

- 1. Bulgarian bacillus starter culture (*Lactobacillus bulgaricus*) preparation rules: one portion of the dry fermented

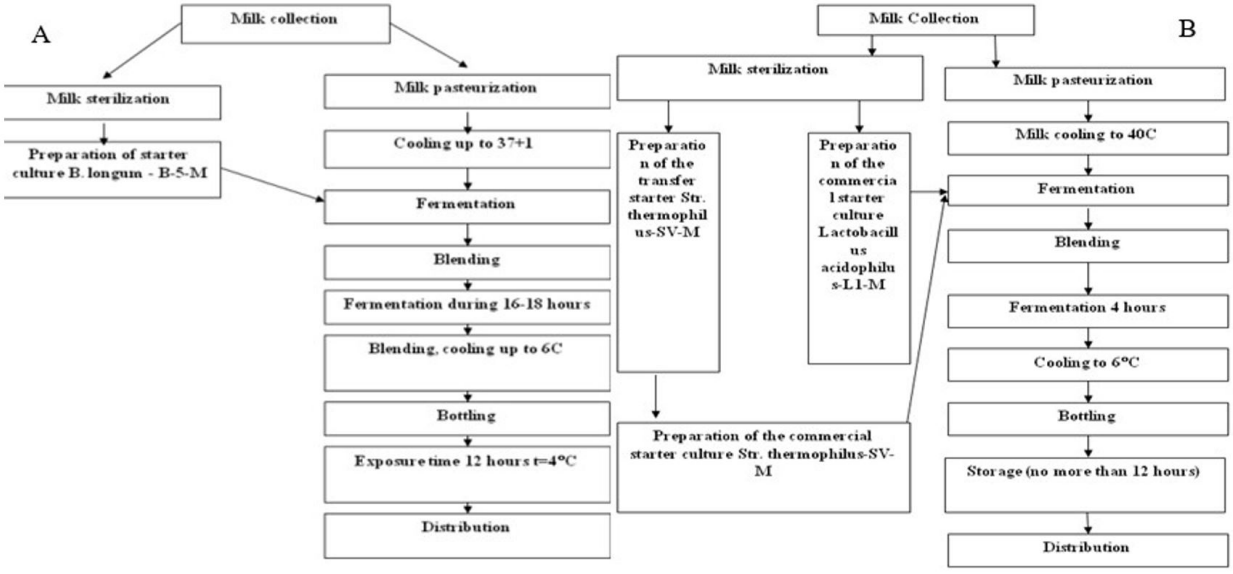


**Figure 1**. Technological scheme of the yogurt manufacturing: (A) "Bios" yogurt manufacture; (B) "Dolce" yogurt manufacture.

*Lactobacillus bulgaricus* was diluted with 100 $cm^3$ of sterilized milk and cooled to 40 ± 1 °C. Before dilution, dry starter culture was previously grounded in a sterile mortar with 10-15 $cm^3$ of sterilized milk addition. After that starter culture placed in a thermostat at 40 ± 1 °C for 12-18 hours for a clot formed. Prepared primary laboratory starter culture was cooled to 4-8 °C.

Secondary laboratory starter culture was prepared in sterilized and cooled to 40 ± 1 °C milk: 0.5-1 or 2-3% of the primary laboratory starter culture was added to this milk and kept at the previous temperature for 10-12 or 4-6 hours until a clot has been formed. Then, the prepared starter culture was cooled to 4-8 °C and stored to the using-time. The production ferment was obtained similarly to the secondary laboratory ferment.

- 2. *Lactobacillus acidophilus* starter has been prepared from a dry starter culture and bacterial concentrate. In preparation-time of the starter culture by an initial laboratory single portion of dry *Lactobacillus acidophilus* starter culture was diluted into 100 $cm^3$ of sterilized and cooled to 38 ± 1 °C milk. Dry starter culture was previously ground in a sterile mortar with 10-15 ml of sterilized milk. Fermented milk stood in a thermostat (t = 38 ± 1 °C) for 12-14 hours for a clot formed. The prepared starter has been cooled to 4-8 °C.

To prepare a secondary laboratory starter culture, the primary laboratory starter culture was added in an amount of 0.5-1 or 2-3% to sterilized milk (t = 38 ± 1 °C). Then, the milk was kept in thermostat for 10-12 and 4-6 h at the same temperature until a clot formed.

Before preparing the starter culture from the bacterial concentrate, it was activated: a single concentrate portion was added to 1-2 L sterilized or pasteurized milk at 95 °C and exposed for 45 min. After that time, milk was stirred, and 1 hour after the concentrate has been added the fermented milk had let at 38 ± 1 °C for 1.5-2 hours. The acidity of the activated concentrate should be of 40-45 °T (Lakin, 1990; Saxelin et al., 1993; Karagül-Yüceer et al., 2001).

For commercial starter culture, the activated bacterial concentrate was added to 200 L of milk pasteurized at 95 °C for 45 min. Then the milk was stirred and kept for 8-10 hours at 38 ± 1 °C until a clot formed. The prepared starter culture was cooled to 4-8 °C and stored (International Standard Organization, 2003).

- 3. *Bacillus longum* starter has been prepared from the dry material analogy to the represented starter samples, and the prepared starter culture has such characteristics: the acid formation energy was on the point 9,0-11,0 (per hour); the number of colonies formed units was 1 x $10^{12}$ for a $cm^3$ of the finished product. The preparing of the bacterial concentrate was done in the volume 210 milk L and the temperature for the bacterial concentrate preparing was in the rage 37 ± 2 °C.

- 4. *Str. thermophilus* starter culture has been prepared for the 22 hours and had the acid formation energy of the secondary starter culture at the rage 7 hours. The quantity of colony-forming units in 1g of the bacterial sample was 1,0 x $10^{10}$.

Freshly prepared fermentation starter has the highest activity. The finished fermentation starter should immediately be delivered to production, otherwise, it should be cooled to 3-10 °C range. The permissible storage time of starter cultures made on pasteurized milk is 24 hours, on sterilized milk 72 hours. During the fermented milk products, arrangement laboratory or commercial starter is added to milk or cream in 1.0-5.0% amount, depending on the starter activity. No more than 10% of starter culture is allowed to introduce. The introduced starter culture should be carried out according to "Instructions for the preparation and use of starter culture for the ripening of each type of product."

### *3.1 Yogurt starter culture testing*

The main agent in yogurt "Bios" manufacture is *Bacillus longum* strain *B-5-M* (*B. longum-B-5-M)*, and the microorganisms *Streptococcus thermophilus* strain *SV-M* (*Str. thermophilus-SV-M)* and *Lactobacillus acidophilus* strain *L1-M* (*L. acidophilus-L1-M)* are used in yogurt "Dolce" manufacture. And the accordance to the responds to the requirements of the relevant instructions for the preparation and use of starter cultures was checking as the third critical safety point.

Thirst of all, we had provided a microscopic analyze of the starter cultures to proof there purellity and microbiological safety for the food-products preparation. Microscopy of the starter culture samples showed there fully responds to the requirements of the relevant instructions for the preparation and use of starter cultures. The microscopy results declare pure microbial cultures in the tested samples (Figure 2).


**Figure 2**. Gram stain, x1000, immersion, cedar oil: (A)-(B) *longum* strain *B-5-M*; (B) *L. acidophilus* strain *L1-M*; (C) *Str. thermophilus* strain *SV-M*.

So we have seen pure culture in each tested sample of starter cultures. In the case of the B. longum-B-5M we have seen Gram-positive (Gr+) rods often branched and connected in short chains; there were some cells with bad dye perceive – spore cell. In the samples from the second tested starter culture (*L. acidophilus-L1-M*) we have constant present of the two cell-types – vegetative (long, slightly curved and straight thin, connected in the short chains Gr+ rods) and tranquility cells – spores (fatty single rods which have bad dye perceive; they were single or connected in groups). The presence of two cell-types in such starter culture is normal due to the nature of the represented microorganisms. The third starter culture was more different. *Str. thermophilus-SV-M* was detected like round big cells, Gr+, connected in different length chains.

The next checking safety point was testing milk after pasteurization and adding the starter culture. Microscopy of commercial starter culture obtained from *B. longum-B-5-M*, *Str. thermophilus-SV-M* and *Lactobacillus acidophilus-L1-M* bacterial concentrate in sterilized milk showed the same microscopic picture as prepared from the dry starter cultures. More other, we have checking pasteurized milk samples with added starter and prepared for the bottled to the presence of the coliforms and accordance with the organoleptic indicators (Table 1).

**Table 1**. The results of the starter cultures checking.

| Starter microbe name and tests counter | Coliform bacteria presence | Clot condition | Taste indicators |
|---|---|---|---|
| *B. longum-B-5-M* (10) | - | Tender, prickly. | Sour taste |
| *Lactobacillus acidophilus-L1-M* (10) | - | Dense, prickly, uniform (sometimes a small serum separation) | Sharp taste |
| *Str. thermophilus-SV-M* (10) | - | Dense, viscous. | Sour and soft taste. |

And the last checking point we have studied the microbiological and physicochemical parameters of "Bios" and "Dolce" yogurts according to the following scheme (Figure 3).

The acidity of "Bios" yogurt is below standards 75-90 °T. However, the taste is not impaired. Fat fraction is above the established standard (3.3%), determined by the fact, the product is prepared based on not normalized whole milk. The prepared product meets the requirements of microbiological control (bifidobacteria count, and absence of the coliforms). Microscopy of the "Bios" product showed the availability of Gr +, thin, straight and slightly curved rods (typical for *Bifidobacteria spp*.). In this case, the shape changes depending on the quality of the milk. The "Bios" yogurt has a delicate, sour taste, and a delicate texture.

"Dolce" yogurt, as a finished product, has lower acidity compare to recommended (65-70 °T), and the fat fraction is above the established standard. The count of the lactic-acid bacteria into a 1 $cm^3$ corresponds to standards, and external microflora has no present. The microscopy of the "Dolce"-samples showed the availability of Gr + straight immovable roads and cocci chains. At the same time, organoleptic testing declare the taste of the product is soft sour, a consistency is viscous, and the clot is broken.

## 4 Discussion

Nutrition is one of the most important factors determining the human's health and wellness, and we can observe some new nutrient-depending directions, as nutrigenomics, nutrigenetics, nutrimetabolomics, and proteomics (Rolfe, 2000). Creation and production of functional nutrition supplements (FNS) are one of the UN human nutrition program's main directions; and 65% of the FNS market consists of dairy products. Dairy products include bifidobacteria and lactobacilli or their consortiums, as well as stimulators of their growth, biologically active proteins,

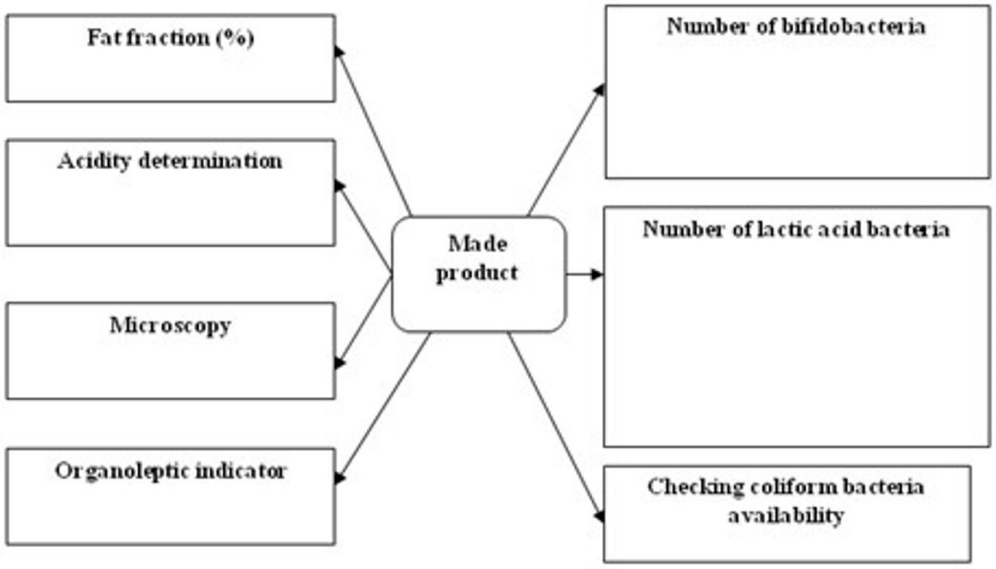


**Figure 3**. A sequence of the "Dolce" and "Bios" yogurts testing.

peptides, amino acids, oligosaccharides, vitamins and minerals, dietary fiber and other nutrients. For example, over the past ten years, the FNS production and biologically active food supplements have increased by more than 350 times in France (Doronin and Shenderov 2002).

There are data about the increase the bacterial density in the large intestine in case of lactic acid bacteria ingestion. So, the bacterial activity enhances and an elevation of deconjugation of bile acid, and the cholesterin rate becomes lower due to this. This can greatly help to person with hypercholesterolemic subjects (Ahmad et al., 2021). I. Mechnikov has noted life expectancy can be significantly increased by the intestine putrefactive microflora. Its suppression and elimination can be reached with lactic microorganisms help. Has to be remembered, fermented milk products contain all the constituent milk parts in a more digestible form (Kurman & Rasic, 1984; Klebanoff & Coombs, 1991).

Japanese researchers have found that yogurts help to eliminate harmful substances from the body and normalize digestion. Epidemiological studies indicate a reduced bowel cancer risk with regular yogurts consumption. The antimutagenic properties of yogurt are associated with its fermentation. Yogurts are effective in treating mild cases of radiation exposure (Food Agriculture Organization, 2005).

### *4.1 Kazakh "yogurt" experience*

The Kazakhstan yogurt market is constantly evolving. The yogurts assortment enlargement is possible due to a new generation of enzymatic food fortifiers introduction, having antioxidant, immunomodulating, and neuroprotective properties. Fermented dairy products have to be compliant with established standards for organoleptic indicators, fat content, acidity, microbiological indicators. Also, should not be contained pathogenic microorganisms and an *E. coli* titer should be not lower than 0.3 mL (Kurmangaliev, 2009).

Organoleptic assessment is a simple but technologically important point. It makes it possible to determine the sensory parameters and to make a scorecard described. For this goal, have to be assessment such yogurt samples' sensory properties like appearance in the first viewing, consistency, smell, and taste (Arslaner, 2020).

Our researches have shown full compliance with starter culture production requirements for therapeutic and prophylactic nutrition. The quality factors affecting to fermented dairy products were analyzed by us by monitoring each process operation. By analyzing the JSC "Food Master Company" economic activity, a scientifically based conclusion was made – the production of fermented milk products like "Bios" or "Dolce" is economically feasible. For the first time, were examined the morphological, tinctorial, and cultural microorganisms features of specialized starter cultures: *B.longum-B-5-M*, *Str. thermophilus-SV-M* with *L. acidophilus-L1-M*. Was revealed full compliance with starter cultures production requirements for therapeutic and prophylactic nutrition.

What is yogurt? It is a dairy product after fermentation, the result of lactic acid fermentation under the effect of Streptococcus salivarius ssp. thermophilus and Lactobacillus delbrueckii ssp. bulgaricus. And it is extremely important for healthy feeding to contain live yogurt cultures in it. Must be noted, that including bifidobacteria enhances the therapeutic value of foods (in particular yogurt). But the survival of bifidobacteria in foods is dependent on varying food matrices, like the time, pH and temperature of incubation and storage, ratio and strains in the inoculum, availability of nutrients, presence of undesirable microflora and their enzymes, the presence of hydrogen peroxide and dissolved oxygen, the concentration of metabolites (Shori et al., 2020; Shori, 2021). So, the control of the manufacture yogurt process is very important to get a quality product.

We sustained a technology of the fermented milk products production with starter cultures from lactic acid and bifidobacteria strains using has been presented. The obtained fermented dairy products have a dense clot, a delicate taste, are characterized by a high content of lactic acid, have sufficiently developed useful microflora. They actively affect the gastrointestinal tract condition, improve its activity, increase enzyme activity, accelerate digestion processes, stimulate appetite, and have a positive effect on the immune system (Kurman & Rasic, 1984; Food Agriculture Organization, 2005; Kurmangaliev, 2009). JSC "Food Master Company" has the necessary conditions for the fermented dairy products manufacturing safe for human life and health.

## 5 Conclusions

Milk supplied to JSC "Food Master Company" meets the requirements for fermented dairy products raw material production, and our study results showed full compliance of the examined bacterial starter cultures with the requirements for the fermented milk products production of therapeutic and prophylactic nutrition. After described and studied the requirements for technological safety we could declare that yogurt "Bios" and "Dolce" manufacture take care of all safety needs for production the therapeutic and prophylactic food production.

## Conflict of interest

The authors declare no conflict of interest.

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