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ORIGINAL RESEARCH PAPER

OPTIMIZATION OF TECHNOLOGICAL PARAMETERS FOR BIOTECHNOLOGICAL PRODUCTION OF FEED ADDITIVE BASED ON MICROBIOTA OF *"TIBETAN KEFIR GRAINS"*

Olena Vichko[1*], Olga Shved[2], Mykola Kukhtyn[1], Romanna Petrina[2], Andriy Mylyanych[2], Mariia Babii[1], Volodymyr Novikov[2]

[1]Ternopil Ivan Puluj National Technical University, 56 Ruska str., 46001 Ternopil, Ukraine
[2]Lviv Polytechnic National University, 12 Bandera str., 79013 Lviv, Ukraine

*Corresponding author: o.vichko.te@gmail.com

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Abstract: Biochemical and microbiological changes in processes of fermentation and storage of fermented milk product prepared using *Tibetan kefir grains* were studied. Principal stages for the production of feed additive were depicted in biotechnological and technological schemes with equipment. Technological parameters, conditions of cultivation and fermentation were selected to manufacture product in industrial scale.

Keywords: *biotechnological and technological schemes, fermented milk product, kefir grains*

INTRODUCTION

Current trends in the domestic and global food markets include the use of probiotic supplements, bioactive ingredients and functional dairy drinks in nutrition, as well as the use of feed additives to feed domestic animals and birds. The active use of fermented milk beverages and the development of new industrial technologies for the production of classic dairy products indicate that the optimization of biotechnology of natural dairy products is relevant and commercially attractive [1, 2]. An analysis of the literature on microbial association beverage production methods, including the preparation of fermented beverages based on "*Tibetan kefir grains*" (starter leaven), showed that most industrial fermentation beverages are made using technologies of specially collectible pure cultures in monoculture based on one type of bacterium, or using a mixture of microorganisms (2 to 30) in the form of associated probiotics [3 – 5].
We investigated the basic microbiological and biological properties of microbiota "*Tibetan kefir grains*". *Tibetan kefir grains* are rubbery and look like a small, cleanly structured cauliflower consisting of lots of single straps [6 – 11]. Determination of cultural and morphological characteristics of microbiological composition of symbiotic culture of microbiota *Tibetan kefir grains* establishment of its physiological and biochemical properties, metabolism features (consumption of lactose, ethanol formation, presence of biologically active substances), optical characteristics and biochemical analysis fermented milk product made using *Tibetan kefir grains* [6 – 7] were a prerequisite for the development of biotechnology for the production of bioproducts of this class. The results of experimental studies (chemical, microbiological, chromatographic, optical) of laboratory samples of fermentation bioproducts based on the microbiota culture of *Tibetan kefir grains* made it possible to offer the obtained bioproducts as a food functional fermented milk drink and as an associated probiotic against infections of the gastrointestinal tract [8].
The study of the microbiological characteristics of fermented milk feed additives showed their effect on the microbiocenosis of the pig intestines. In particular, the biological activity of fermented bioproduct on endurance against the action of bile, gastric juice and NaCl is shown, and the ability to positively influence intestinal microbiocenos is in piglets [9]. According to the results of studies on the influence of microbiota *Tibetan kefir grains* on the composition of gut microbiota in piglets before and after the use of fermented milk feed additives, there was an increase of two orders of magnitude (in CFO from 10^7 to 10^9) of various probiotic *Bifidobacterium* and *Lactobacillus strains* and absence of conditionally pathogenic microorganisms. This allowed us to confirm some of the probiotic characteristics of the dairy product based on the microbiota *Tibetan kefir grains* grown in households [10 – 11]. The properties and the growth of mixed microbial cultures are of potential interest food production because of the sophisticated compositional and structural expectations of food products [12]. In work [12] was shown mixed culture fermentation to produce cereal-based foods with good organoleptic properties and also high numbers of probiotic bacteria [12].
The aim of the study was to determine the optimal growth conditions and producing technological parameters of the fermentation of the producer on the basis of the microbiota *Tibetan kefir grains* with the formation of biotechnological feed probiotic additives and to model the hardware and technological scheme of production with industrial technological modes and biotechnological parameters of processes.

MATERIALS AND METHODS

For laboratory and production research the microbiota biomass (natural association of yeast, lactobacilli and acetobacteria) was used:
Producer - microbiota "*Tibetan kefir grains*", for which a qualitative and quantitative microbial composition (in the amount of 6.60 ± 0.54 lg CFU/g biomass) was determined - in the lump: lactic acid bacteria *Lactobacillus fermentum* (6.55 ± 0.44 - 89 % of biomass), *Lactobacillus spp.* (6.55 ± 0.44 - 9 % biomass), *Lactococcus lactis*, *Leuconostoc lactis* (2.56 ± 0.17 and 4.43 ± 0.35 - 0.7% biomass); acetic acid bacteria of the genus *Gluconobacter oxydans* (4.65 ± 0.37 - more than 1.1% of biomass); the yeast pseudomycetes *Candida kefir* (2.59 ± 0.17) and the ascomycetes yeast *Sacchromyces spp.* (3.56 ± 0.21 and 2.55 ± 0.17 - averaging 0.1 % biomass).
Fixed microbiota preparation. Cell sizes: rod-shaped cells (length 2.9 - 3.6 µm; width ~ 0.5 - 0.8 µm); yeast cells (diameter 2.5 - 11 microns). The ratio of yeast cells - rods depends on the conditions and duration of cultivation.
The isolation of pure cultures of lactic acid bacteria was performed on MRS-Agar medium, incubated at 37 ± 1 °C for 48 h; isolation of the yeast was performed on Sabouraud Dextrose Agar medium at a temperature of 25 ± 1 °C for 3-5 days; beer wort was used to isolate the acetic acid bacteria. Uses of salt - KH_2PO_4 - 0.2 g; $(NH_4)_2SO_4$ - 0.3 g; $MgSO_4 \cdot 7H_2O$ - 0.2 g; C_2H_5OH - 2 mL. The agar medium was sterilized in an autoclave at 1 atm for 20 min (0.5 % $CaCO_3$ and 3 % alcohol were added immediately before sowing the culture) [11]. To isolate the pure cultures of microorganisms that are part of the microbiota "*Tibetan kefir grains",* the commercial environments of the manufacturer (HiMedia, India) were used.
The microscopy of isolated, fuchsine stained and Gram-pure cultures of microorganisms were performed using a MBL2100 trinocular microscope ("Krus", Germany) at eyepiece 20 and lens 100.
The identification of pure cultures was performed according to the morphological, tinctorial, cultural and biochemical properties described in the bacterial determinant [13] and the yeast according to Kreger-van Rij N.I.W. [14]. Test systems were used to identify ARI 50CN microorganisms (Bio Meuxux, Inc, France) according to the manufacturer's recommendations. Species belonging to isolated cultures of lactic acid microorganisms have been studied using PCR (polymerase chain reaction) method with genus-specific species-specific primers according to known data [15, 16].
Determination of the active acidity of the nutrient medium and the culture fluid (*p*H = 3.3 - 4.3) – the *p*H of the solutions was measured on an ionomer (I-160 M) (4.02 - 4.00; 4.01 - 3.95; 4.01 - 3.94).The determination of titrated acidity (80 - 120 °T) of the culture fluid obtained as a result of milk fermentation (fat content of 2.5 %) was determined by DSTU [17]. The determination of the acidity of the culture fluid was carried out analogously to the determination of the acidity of milk by titration with 0.1 N NaOH aqueous suspension solution (10 : 20) in the presence of an alcoholic 1 % solution of phenolphthalein to a slightly pink color, which does not disappear for one minute at the time of titration at within the range of 18-20 °C. Differences between parallel determinations were ± 1 °T.
The dynamics of the concentration of the carbohydrate component of the substrate (the main carbon source) in fermented milk products was determined by the iodometric method [18]. The amino acid composition of milk and milk drink was determined using

an automatic amino acid analyzer type T339, firm "Mikrotechna" (Prague, Czech Republic) by ion-exchange chromatography. The content of the ethanol enzyme produced by the yeast of the microbiota did not exceed 0.0057±0.0001 % during the 96 hour shelf life, which indicates its utilization by acetic acid bacteria, which makes it possible to use bioproduct for dietetic nutrition as for people, as well for animals, in particular pigs during their weaning period, which positively influenced the formation of stable microbiocenos is in experimental animals.

The culture liquid formed in the process of hetero-enzymatic lactic acid fermentation of the microbiota is characterized by a peculiar taste bouquet of the drink due to the isolated organic acids (lactic and pyruvic, acetic, glucuronic, malic, citric and phosphoric), as well as the number of sugars.

Organoleptic evaluation of the bioproduct was made according to standard indicators: taste and smell, consistency of microbial biomass in the form of kefir grains with a tubular surface, color of the culture fluid, appearance of the concentrate at (18 ± 2) °C, by the described methods [19].

RESULTS AND DISCUSSION

The obtained experimental data formed the basis for laboratory scaling, modeling of technological process and study of technological parameters of biotechnology of fermented bioproducts. The rationalization of the biotechnological process for the production of these bioproducts was carried out on the basis of the developed biotechnology of cultivation of biomass of microbiota *Tibetan kefir grains* and fermented milk fermentation according to certain rational technological parameters. The ways of implementation of the results in the fields of possible application of these bioproducts as fermented functional fermented beverage and concentrate of probiotic feed additive for feeding of domestic animals are offered.

The main important parameters for the design of the production process are: concentration of solids in a nutrient medium with a carbohydrate substrate, pasteurization temperature of milk, microbial quality of starter-inoculum, temperature of inoculation and fermentation, final acidity of the bunch, modes of mixing [10].

Compliance with these parameters ensures the passage of the desired metabolic processes and the production of bioproduct. The composition of the milk-containing nutrient medium (the main carbon source is lactose) to perform the process and obtain the fermented milk product and concentrate: lactose content - 4.6 %, protein - 3.0 %, fat - 2.5 %.

The primary substrate that initiates enzymatic processes in milk is lactose, which is cleaved by microorganisms inoculum under the action of the amylolytic enzyme *β*-galactosidase to monosaccharides, and organic acids are formed, which change the active acidity of the culture fluid. The lactose content of the final fermented milk products may be different (3.5 % - 4.1 %). In our case, for technological readiness of the bioproduct - fermentolysate at different temperatures of milk fermentation by the microbiota *Tibetan kefir grains* within 48 hours for the growth of microbial biomass and the formation of fermentation products, an average of 25 to 35 % of milk lactose is consumed, resulting in its composition and content in the product 3.2 ± 0.1 %. Subsequent exposure under the experimental conditions does not reduce the content of lactose in the substrate, and does not increase the acidity of the culture medium, indicating a stationary phase of development of mixed microorganisms that exhibit significant β-galactosidase activity during fermentation.

The protein value of bioproducts was determined by the content of amino acids. In the initial milk substrate, the average content of casein, albumin and globulin is about 3.3 %. *Tibetan kefir grains* in culture fluid decreases protein composition to 2.2 ± 0.1 % due to peptidase activity of microbiota with free amino acids formation (12 amino acids determined). Analysis of the amino acid composition showed that during cultivation for 24 h, content essential acids - decreases on average 2.0 times from 180.3 ± 7.7 $mg\cdot cm^{-3}$ to 90.5 ± 4.10 $mg\cdot cm^{-3}$ ($P \leq 0.05$), as the use of amino acids in the life of microorganisms, but their subsequent formation at 48 hours of cultivation does not indicate that the microorganisms in the fermented milk product virtually stop their development (total mass of aromatic amino acids in medium is 236.8 ± 12.5 $mg\cdot cm^{-3}$), while continuing the biosynthetic peptidase activity, which increases the biological value of the target bioproduct.

The preparation of the seed was carried out by incubation of selected laboratory samples of the microbiota *Tibetan kefir grains* by standard, but partially modified methods to the state of symbiotic starter culture for inoculation.

Homogenized cell mass, formed by trituration of 1 g of the body of the microbiota *Tibetan kefir grains* in a mortar with glass powder, followed by ten-fold dilution in tubes from 9 mL of sterile tap water to 10-3 $mg\cdot mL^{-1}$, sown on Petri dishes in an amount of 0.1 mL, placed in a thermostat and maintained at a temperature of 28 and 35 °C for 1 day on medium with 0.5 % $CaCO_3$ and after 10-14 days observed the appearance of lightening zones due to the action of the acids formed.

The dynamics of microbial association biomass growth was determined under different conditions of cultivation due to the establishment of mass gain, depending on the degree of fermentation: by 24 h by 5.4 ± 0.34 %; for 48 h - by 7.6 ± 0.51 %; for 72 h - by 10.3 ± 0.62 %; for 96 h - by 10.7 ± 0.64 %. Taking into account the experimental data, we believe that an increase of 72 h at 28 °C at an average of 10 % gives the opportunity to carry out several cycles of product production without changing the seed material (symbiotic leaven).

Analysis of the dynamics of the process of cultivation of the seed material shows that at temperatures from 17 to 25 °C yeast cells predominate, rod-shaped are very rare; and from 25 to 30 °C, the number of sticks increases sharply, but remains in the minority; in the temperature range of 12 - 17 °C, there is a slow accumulation of biomass, which does not form a film on the surface. At 18 - 25 °C more active accumulation of biomass with the formation of film on the surface is observed. At 26 - 32 °C there is rapid growth of biomass with the active release of CO_2.

The quantitative ratio of yeast and lactobacilli in the culture fluid of fermented milk enzyme is optimally achieved (1 : 99.9 $x10^4$) for 24 h of cultivation: at 28 °C the number of yeast cells in 1 cm^3 is (3.7 ± 0.27) $x10^4$, and the number of cells of lactobacilli in 1 cm^3 is (2.9 ± 0.22) $x10^8$.

The most optimal initial acidity of the milk, as a culture medium, is 17.0 ± 0.5 °T, and the most intense dynamics of acidification takes place in the temperature range from 15 to 37 °C reaching a titrated acidity of 101.5 ± 8.7 °C for 24 h at 28 °C.

Experimental laboratory studies have determined the optimal growth of producer biomass under conditions of growth of the microbiota *Tibetan kefir grains* (when sowing 2.5 % in the amount of 10^9 CFU·cm^{-3} mixed symbiotic microbiota) and determined the highest intensity of the acid formation process at temperatures from 15 to 37 °C hours with the introduction of the seed for fermentation - up to 5.0 % by weight of the nutrient medium. Therefore, the optimal fermentation temperature is 27-29 °C, which causes the development of all associates of microbial ferment *Tibetan kefir grains.*

The most optimal initial acidity of the milk, as a culture medium, is 17.0 ± 0.5 ºT, and the most intense dynamics of acidification takes place in the temperature range from 15 to 37 ºC, reaching a titrated acidity of 101.5 ± 8.7 °T for 24 h at 28 °C. The qualitative characteristic of the process of obtaining the additive was the active acidity, which during the fermentation period fluctuated within the *p*H of 3.9 ± 0.3 ÷ 0.4, and with a change in the concentration of sugars for 48 h the value of the final titrated acidity of the bioproduct ranged from 80 ºT up to 120 ºT .

Optimal acidity parameters were established in studying the dynamics of changes in the quality indices of the test samples of bioproducts during storage of the biomass of microbiote and bioproducts by the mass and acidity of the samples. An increase of 1.2 times the titrated acidity of the fermented milk beverage made on the leaven of *Tibetan kefir grains* to 108.4 ± 8.3 °T at a constant level of microbial population was observed at a temperature of 5 ± 1 ºC for 10 days (89.3 ± 7, 7 ºT for 1 day; 94.5 ± 8.2 ºT for 3 days; 97.9 ± 7.4 ºT for 5 days; 101.8 ± 6.8 ºT for 7 days; 108.4 ± 8.3 ºT for 10 days)

Studies of the effect of temperature on the organoleptic and probiotic properties of bioproducts were carried out in the temperature ranges from 12 - 17 °C; from 17 to 25 ºC; from 25 to 32 ºC on the control of film formation on the surface of biomass at its growth for 10 days. The conditions of storage of biomass are provided that provide high rates of microbiotic producer of bioproducts at the storage temperature within + 4 ÷ +8 ºC. Producer *Tibetan kefir grains* can be stored in the biomass of the association in the frozen state (temperature - 18 °C) and for ten days at a temperature of 5 °C, which practically did not affect its organoleptic properties, microbiota composition and probiotic properties. The shelf life of the bioconcentrate at 8 ± 1 °C is up to 7 days and at 4 ± 1 °C for 10 days and meets the requirements of ISO 4417.

<u>The fermented beverage</u> contains 0.5 % biomass, the lactic acid concentration is 0.15 %, the protein concentration is 16 mg·mL^{-1}, the sugar concentration is 10 mg·mL^{-1}, the titrated acidity is 80-100 ºT.

<u>Bioconcentrate (feed additive)</u> contains 14 % biomass and has a moisture content of 86 - 89 % with good organoleptic properties.

On the basis of scientifically substantiated results of scaling of the laboratory process, the basic schemes of technical realization of industrial production of functional culture fluid and on its basis the line of fermented products - fermented beverage and feed additive in the form of concentrate were developed, and a design batch of feed additive was approved, which was confirmed.

The production uses two methods of industrial fermentation of milk - thermostatic (fermentation to the intact bunch in packaged small containers in thermostatic chambers) and tank (fermentation of the mixture in large containers with careful mixing of the bunch before packaging) [20]. To obtain feed additives on microbial grain biomass, the tank method used in this technology is more economical.

Description of biotechnological process of bioproduct production with modes of technological stages and appliance design

I. Biotechnological flowchart

The biotechnological process for the production of fermented milk products includes the standard stages of a typical technological scheme of cultivation of a microbial bio-agent on a milk substrate. The main biotechnological stages are incubation, inoculation, fermentation, separation of biomass from the culture fluid (Figure 1).
The use in the process of biomass inoculation of microbiota and the introduction of this seed material in the nutrient medium has significant advantages over the separate accumulation of cultures of components of biomass and subsequent mixing, similar to the technologies of simultaneous introduction of seed material multivariate bacterial preparations containing all compositions the quality of bioproducts and the stability of all determinants to reach the required level of acidification taste and aromatic substances during fermentation.
The technological parameters of industrial fermentation of milk, which is carried out by cultivation of the inoculum of the microbiota *Tibetan kefir grains*, are determined at a temperature 27 - 29 °C for 24 h with subsequent maturation at temperatures 14 - 15 °C for 4 - 6 h for accumulation aroma, and then it keeping the bio-product as cooled to a temperature of (8 ± 1) °C for 6-12 hours. Nutrient medium - sterile cow's milk with an acidity of 17 - 18 ºT - with a fat content by weight of 2.5 %; lactose content - 4.6 %, protein - 3.0%. Number of cells in the microbiota - in the amount of 1 $g \cdot dm^{-3}$: lactic acid bacteria - (2.9 ± 0.22) $x10^8$ $CFU \cdot cm^{-3}$; fungi and yeast - (3.7 ± 0.27) $x10^4$ $CFU \cdot cm^{-3}$. Incubation biomass fraction - 2.5 %; (seed – starter: 4 ± 1 mm in diameter of the grains up to 9 - 10 days of cultivation). Inoculation biomass fraction - 3.5 - 5.0 % (seed – starter: 4 ± 1 mm in diameter of the grains up to 17 - 18 days of cultivation). Cultivation of biomass at a fermentation - temperature of 28 ± 1 °C for 24 h. Active acidity of the culture fluid in the inoculation - *p*H = 4.02 - 4.00; 4.01- 3.95. Fermentation time was 24 hours; fermentation temperature was 28 ± 1 ºC. The active acidity of the culture fluid in the fermentation period was *p*H 3.9 ± 0.3. Titrated acidity of fermented beverage was from 85 to 120 °T. Titrated acidity of the bioconcentrate was from 90 to 118 °T. The amount of moisture in the lump of the *Tibetan kefir grains* was from 89.3 ± 0.84 % to 86.1 ± 0.77 %.
The sample obtained corresponded to the values prescribed by ISO 4417.

1. Preparation of nutrient medium
Tank fermentation method for receiving milk with an acidity not exceeding 18 ºT, with microbial contamination of not more than 100 thousand $CFU \cdot mL^{-1}$, with mechanical contamination not lower than group I, provides for the milk to be heated to 40 - 50 °C, normalization to 2.5 % fat; homogenization at 55 - 60 °C for 30 minutes, at a temperature not lower than 55 °C and a pressure of 17.5 MPa, followed by pasteurization of milk within 92 - 95 °C for 10 - 20 min and immediately cooled to a fermentation temperature of 26-28 °C in the fermentation tank.
Conditions for heat treatment of raw materials for bioproducts are defined in units of pasteurization. The most optimal mode of pasteurization of milk for dairy products -

temperature 85 - 87 °C with keeping for 5 - 10 minutes or 90 - 92 °C with holding for 2 - 3 minutes.

2. Incubation of primary (maternal) starter culture

Producer as working laboratory starter leaven culture for fermentation is prepared from wet synergistic microbial cultures of *Tibetan kefir grains* microbiota (or from dry or frozen lumps microbiota): 1 g the body microbiota is applied into 1 L of cooled milk substrate, closed with a stopper, closed with a stopper temperature of 26 - 28 °C to formation after 10 h of clot grains and another 2 h to acidity of 75 - 85 °T with sufficiently active microbiota (1 g contains 10^7 - 10^8 CFU, 99 % of them are acid - forming lactic acid, about 0.45 % of yeast) in microaerophilic conditions.

3. Inoculation of secondary (transplant) starter culture

Fermenting culture - secondary starter culture, made by inoculation of 2.5 % fresh liquid leaven similar to that of the maternal culture (25 g per 1 liter of milk), may be the main for obtaining the seed - production (working) leaven (when using dry leaven can prepare an additional third of the leaven), stirring gently so as not to damage the body of the upper layer microbiota (2 - 3 cm) of the leaven.

The milk is fermented at the same temperature of 28 °C as in the production of primary fermentation for 8-12 h to an acidity of 80 - 90 °T. The finished leaven inoculate (transplant fermentation) is cooled to 8 °C and stored at the same temperature.

4. Growing-cultivation of industrial (working) leaven

The working fermentation is prepared by adding 3.5 to 5% of the transplanting leaven into the substrate culture medium, at a cultivation temperature of 28 ± 1 °C for 20 to 24 hours to an acidity of 90 - 100 °T in a tank for fermenting milk (500 g per 10 l). The starter fermentation must meet the organoleptic requirements and have a pure, sour milk taste and odor, homogeneous, serum-free consistency; the body of grain should be stable. After 6 - 12 hours of storage in the inoculator at a temperature not exceeding 6 °C, symbiotic microbiota restores their properties and begins to give weight gain, which indicates its viability in the first cycle of fermentation.

The starter culture can also be stored after filtration and washing with fresh substrate water at 6 - 8 °C until use as leaven. It can be renewed every 10 - 12 days for use in subsequent cycles of fermentation, using a new packaging of laboratory culture, carrying out cultivation in specially designated areas, conducting cultivation in specially designated areas, in which equipment should be kept clean and periodically disinfected.

Determination of biomass growth of the microbiota body placed in a nutrient medium for 12 hours, repeatedly use to obtain the growth of biomass (every 12 hours), depending up to the degree of fermentation of the medium as to the depletion of the nutrient medium by lactose. The moisture content of the body of the microbiota ranges from 84 to 89 %, with an average of 86 % (Figure 2).

5. Working fermentation of fermented functional beverage

For the formation of the target bioproduct, in the form of a metabolic bouquet of the culture fluid, the inoculum (working starter) is fed within 5 % to the fermentation medium (200 L), with the starter being introduced immediately into the prepared milk to prevent any contamination in the tank.

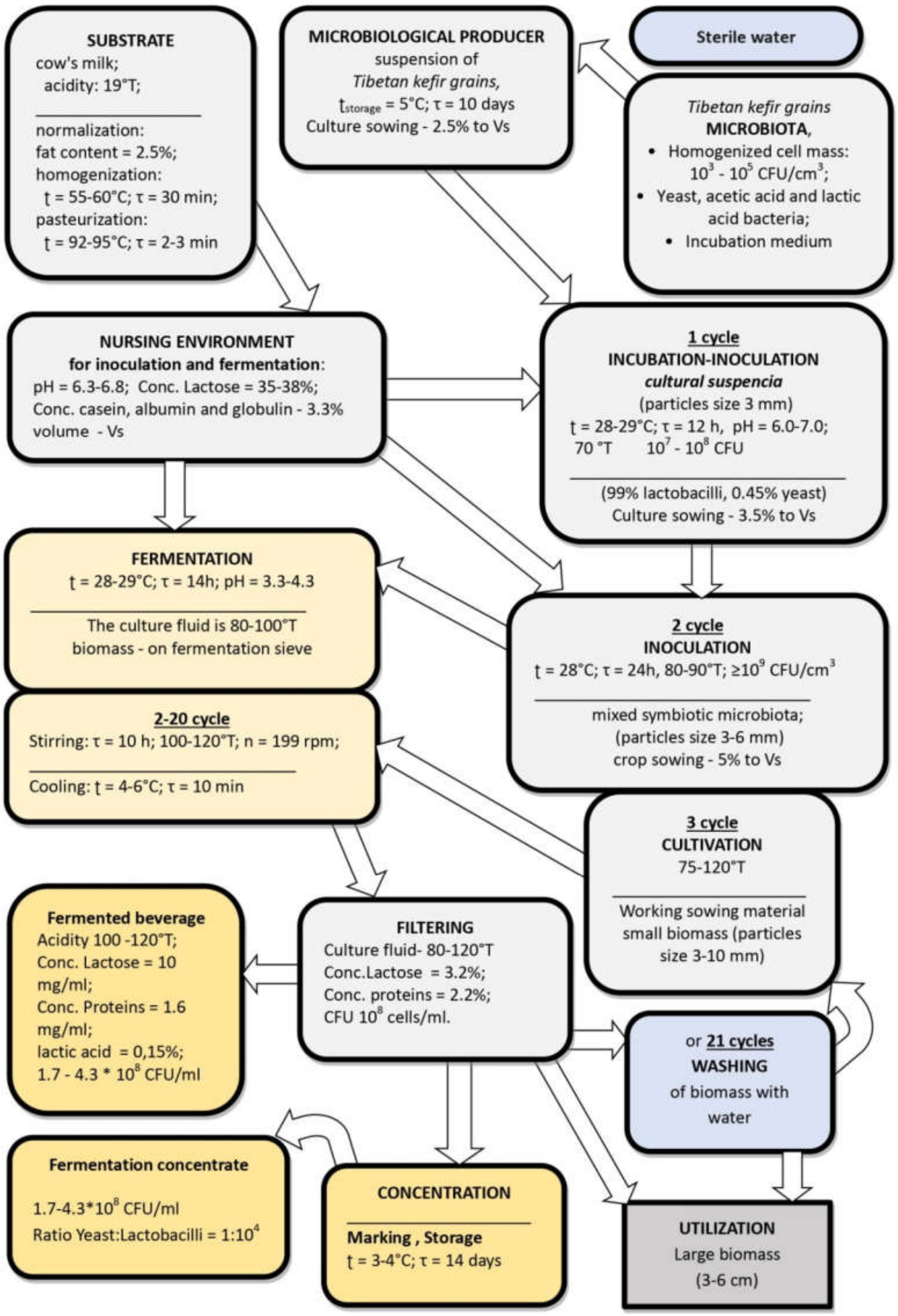

Figure 1. *Flowchart of fermentation beverage and concentrate based on microbiota Tibetan kefir grains*

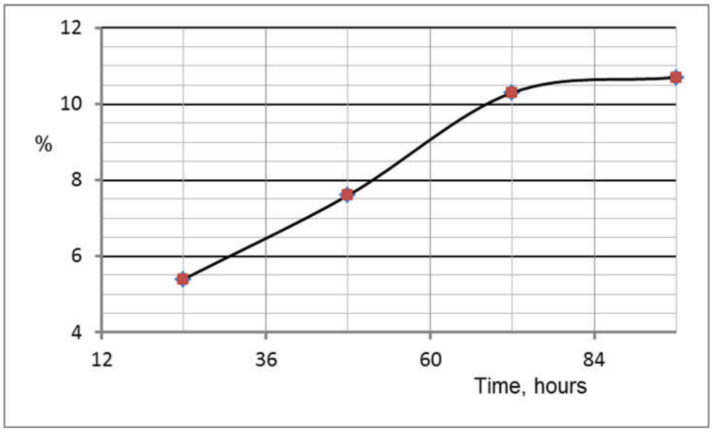

Figure 2. *The dynamics of biomass growth of the microbiota was determined*

To accelerate the fermentation process, it is also recommended to make about 10 % of the fermented wort from the previous cycle in subsequent cycles. The technological measures in the reservoir method of production of the target bioproducts are somewhat different from the way of production of fermented milk products.

The fermentation of the first cycle is carried out for 14 h without stirring at a temperature of 28 ± 1°C, and then the culture medium with a consortium of microorganisms matures for another 10 h with stirring 199 rpm to acidity of 100 - 120 °T, enriched with amino acids and vitamins in the process of biosynthesis and acquires taste and physic-chemical characteristics (reducing sugars - 10 ± 0.2 $mg{\cdot}mL^{-1}$, total protein 1.6 ± 0.1 $mg{\cdot}mL^{-1}$, lactic acid (0.15 ± 0.02 %). In the first cycle, when the reservoir production method of feed additives based on microbiota *Tibetan kefir grains*, the inoculation and fermentation process is carried out at temperatures of 24 - 28 °C for 24 - 48 h, cooling and maturing of the culture fluid occur in the same container. After partial filtration, the bioproducts are kept for 6 - 12 h for maturation at a temperature not higher than 8 °C, which helps to slow down the process of lactic acid fermentation and does not release serum. After 2.5 days of maturation, the *p*H of the lactic acid beverage reaches 3.4 ± 0.1, the optimal biomass of the body of the microbiota increases almost twice (40 - 50 grams per liter), and the number of viable cells in the culture fluid is just over 10^8 $cells{\cdot}mL^{-1}$.

The finished bioproduct is packed into appropriate containers (bottles, packages). In all subsequent cycles, the inoculum (working biomass) of the microbiota is used for seeding, which can be separated through a special window of the manual unloading hatch (Figure 3).

II Principal technological diagram of the process with the modes of carrying out the corresponding stage of production fermented milk product

The fine culture liquid is filtered off for cooling and packaging in the form of a bioproduct with the organoleptic properties of the fermented milk beverage at a total acidity of 85 - 120 °C. The fermented beverage is stored in the container under cooling in the form of a fine culture fluid with a fat content by weight of 2.5 %, with a solids content of 4.1 - 5.2 $g{\cdot}dm^{-3}$ and retains probiotic properties (high concentration of the live probiotic microorganisms) at 8 °C for 7 days. The amount of lactic acid-forming microorganisms in the beverage is 1.7 - 4.3 × 10^8 CFU·cm^{-3}, the amount of yeast is 4 × 10^4 CFU·cm^{-3}. The wet biomass concentrate is also passed on to cooling and packing as a ready-made probiotic feed additive by the nature of the bunch and the acidity of 90 - 120 °C.

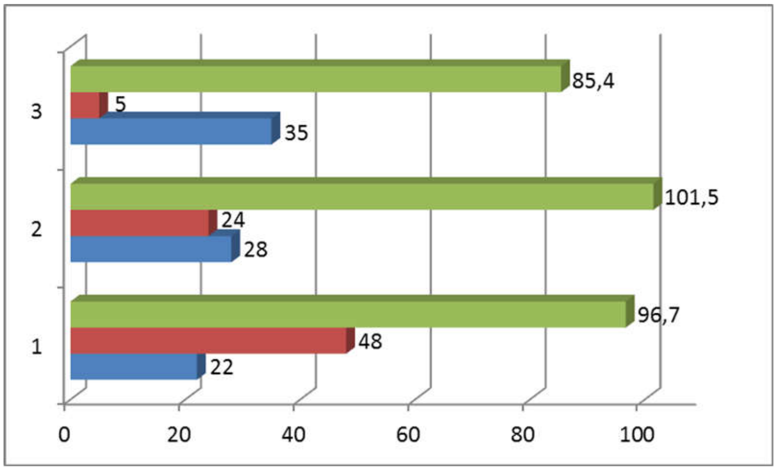

Figure 3. *Dynamics of acidification of the culture fluid of the microbiota in the time interval of temperatures.* - t, °C, - time, hours, - °T

Concentrated feed bioproduct is stored hermetically closed when cooled, with fat solidified, casein binds water, bubbling, resulting in a consistency of products, becomes dense with humidity 86 % and retains probiotic properties at 4 °C for 10 days. Quantity of lactic acid bacteria - (2.9 ± 0.22) × 10^8 CFU·cm^{-3}, yeast - (3.7 ± 0.27) ×10^4 CFU·cm^{-3}, which most meets the regulatory requirements DSTU [19].
Production process (PP) fermented milk bioproduct is presented in Figure 4, process technology includes the main stages - cultivation of biomass (inoculation) and fermentation (fermentation) of milk and packaging and storage of the target product, as well as ancillary stages (AS) - preparation of milk (nutrient medium) and preparation of seed.

DS 1. Milk acceptance and reservation. Milk from the tank truck, passing through the milk filter F-1, pump H-1 into the intermediate tank cooler CX-1, in which it is cooled to 4 °C and stored for further processing.

DS 2. Preparation of milk for fermentation
DS 2.1. Normalization. Normalization is carried out in a stream in the separator-normalizer SN-1. Prior to normalization, the milk is heated to 40 - 50 °C in the To-1 heat exchanger, into which it is fed from the CX-1 tank by means of the H-2 metering pump. The cream, which is separated in the separator from the milk, comes to the tank E-1, from where the pump H-3 is transferred for further processing. The skim milk is collected in a container E-2 and pumped to the H-4 for homogenization.
DS 2.2. Homogenization of milk. To improve the consistency of dairy products and prevent the separation of whey milk is heated to a temperature of 55 - 60 °C in the To-2

heat exchanger and homogenized at a pressure of 17.5 MPa in a plunger homogenizer valve type G-1.

DS 2.3. Pasteurization. Pasteurization is carried out at a temperature of 92 - 95 °C for 10-20 minutes. In this case, homogenized milk is first fed into a tube-to-tube heat exchanger To-3, where it is heated to a pasteurization temperature using saturated water vapor. The heated milk enters the pasteurizer P-1, where it is kept at pasteurization temperature for 10 minutes. The heating is carried out through a water vapor shell. After holding, using the dosing pump H-5, the pasteurized milk is fed into a tube-tube heat exchanger To-4, in which it is cooled to the fermentation temperature, *i.e.* to 26 - 28 °C, and fed to the fermenter F-1. The pasteurizer P-1 released from milk is purified first with water vapor and then with drinking water.

DS 2.4. Cultivation of seed biomaterial in the inoculators. The fermenting culture grown in the laboratory under sterile conditions is placed in a wide-necked flask of 5 l capacity and transferred to a steam sterilized I-1 inoculator with pasteurized milk. The duration of cultivation of the starter seed in the inoculator is about 24 h at a temperature of 28 ± 1 °C. The obtained seed was checked for the absence of contaminating microbiota (seeded for nutrient agar and microscopy). It is permissible, but not recommended, to store the seed biomaterial for 6 - 8 h in an inoculator in a cooled state (6 - 10 °C), then transfer it to the seed in the fermenter.

TP 3. Working fermentation (fermentation). First cycle: 200 liters of pasteurized milk, cooled to fermentation temperature, are fed to the sterile Fer-1 fermenter from the pasteurizer P-1 and biomass of the *Tibetan kefir grains* from the I-1 inoculator is introduced. In the process of fermentation there is an increase in the microbiota, increase in acidity, coagulation of casein and the formation of a clot. The end of fermentation is determined by the formation of a sufficiently dense clot and the acidity of 80 - 120 °T.

The first 14 hours of fermentation process is carried out without stirring, and the last hour includes a stirrer. The process is conducted at a constant temperature of 28 ± 1 °C, which is achieved by the supply of hot water into the interstitial space of the fermenter. After fermentation, the product is immediately cooled to 4 - 6 °C by supplying brine from the XM-2 refrigerating machine to the interstitial space of the fermenter.

The cooled fermented milk product is pumped from the fermenter by pump H-6 into the intermediate tank E-3, and the fungus remains on the filter partition in the fermenter. After pumping the fermented milk product into the fermenter pour the next portion of pasteurized milk for fermentation. In all other cycles, the milk is loaded into a fermenter that already contains the biomass of the fungus. The fermenter must be equipped with a filter mesh to accommodate the biomass and a window for its manual unloading. After the 20th cycle of fermentation, the microbial fungus on the F-1 fermenter partition is washed with purified water, and then discharged through a hatch into a vessel E-4, into which two filter partitions are mounted: the upper and the lower (upper with larger openings). Thus, a large fraction of the fungus that is disposed of is retained on the upper partition, and a smaller fraction of the fungus is retained on the lower partition, which is returned back to the F-1 fermenter for further fermentation of milk.

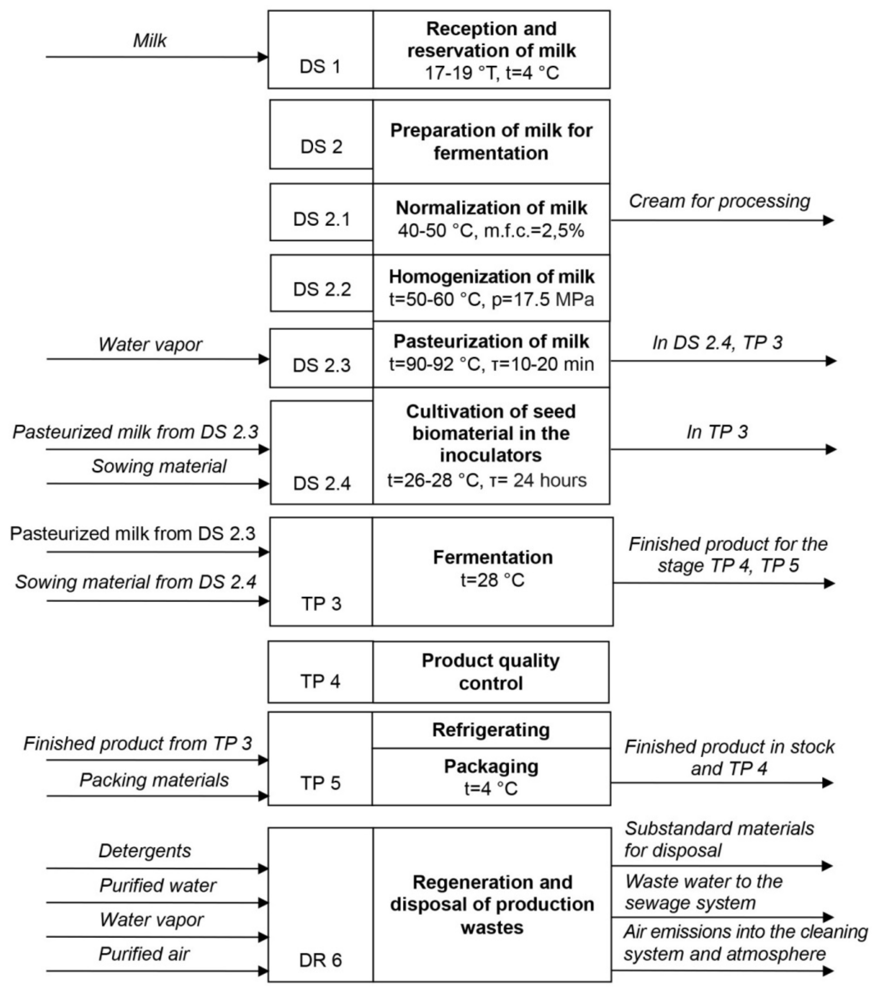

Figure 4. *Flowchart of the fermentation production process sour milk drink and probiotic feed concentrate additive*

TP 4. Product quality control

TP 5. Packing. Dairy product from the intermediate tank E-3, the dosing pump H-7 is pumped into the packing machine D-1, by which the finished product is packed in packages of 1 kg. Package marking shall be in the official language and language specified in the supply contract. Each packing unit shall be labeled or labeled with the following information: the name of the manufacturer, its address, trademark, batch number, net weight, composition, date of manufacture, shelf life. The packages are packed in boxes and sent to the warehouse of finished goods, where it is stored at a temperature of +(3 - 4) ^{o}C. Duration of storage is 14 days (Figure 5).

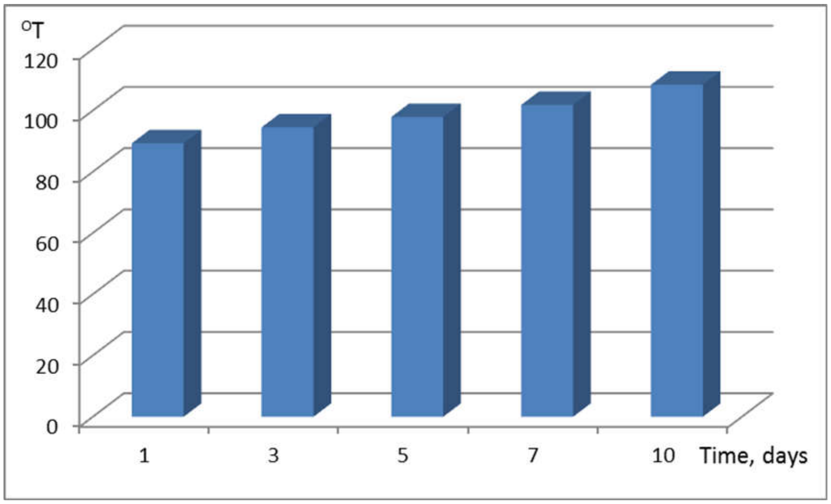

Figure 5. *Dynamics of changes in the acidity and quality of the studied samples of bioproducts during storage of biomass of microbiota and bioproducts*

The shelf life of the bioconcentrate at +8 ± 1 °C is up to 7 days, and at 4 ± 1 °C for 10 days and meets the requirements of ISO 4417 (Figure 6).

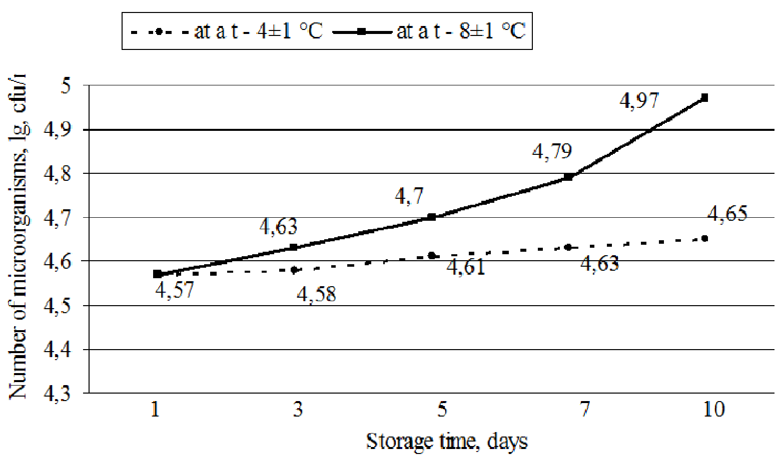

Figure 6. *Dynamics of storage of bioconcentrate based on microbiota "Tibetan kefir grains"*

DR 6. Regeneration and disposal of production waste. The last stage of the technological process is the utilization, regeneration and disposal of waste, namely the substandard large fraction of the body of the "*Tibetan kefir grains*", washing water, ventilation and process air, batches of defective product, residues of packaging materials and unused raw materials (milk), cleaning of pipelines. This stage ensures the ecological purity of the production of fermented milk beverage and the compliance of its production and storage in accordance with the requirements of GMP and HACCP.

After each stage, control of the environment for the content of amino acids, organic acids, organoleptic by the specified methods [21]. According to the requirements of the HACCP, the company should adhere to the sanitary-hygienic regime (SHR): in particular, after the end of the technological cycle, wash the equipment (at atmospheric pressure) with a detergent solution, and then purified water. The main equipment (inoculator, fermenter, storage tanks for raw materials, semi-finished products and finished beverage), provided by production technology, was sterilized of hot steam at a pressure of 0.18 - 0.20 MPa at a temperature of 125 - 130 ºC for an hour, so if necessary, equipment purged with purified air to remove moisture.

III. Principle equipment and technological scheme of biotechnological process

Appropriate apparatus and equipment are required for each stage of the process, namely: acceptance of raw materials (milk filter, pumps, cooler, separator-normalizer, pasteurizer homogenizer); preparation of nutrient medium and its heat treatment to eliminate contamination by foreign microbiota (heat exchangers, tanks); preparation of the inoculum (incubator); inoculum cultivation to obtain inoculum - fermentation (inoculator); fermentation - fermentation with acidification resulting from lactic acid fermentation with lactic acid to form a culture fluid containing the biomass of the original fermentation culture (fermenter); partial filtration and concentration of the native culture solution (filters); final packaging of fermented bioproducts (fermented beverage and concentrated feed additive) (Figures 7, 8).

Modified fermenter with a mesh partition meets the task of technology, as in the usual fermenter with a stirring means will lead to the destruction of lumps of the fungus, i.e. its cellular associations, which will affect the ratio of yeast and lactobacilli, and therefore will have a negative impact on the fermentation process.

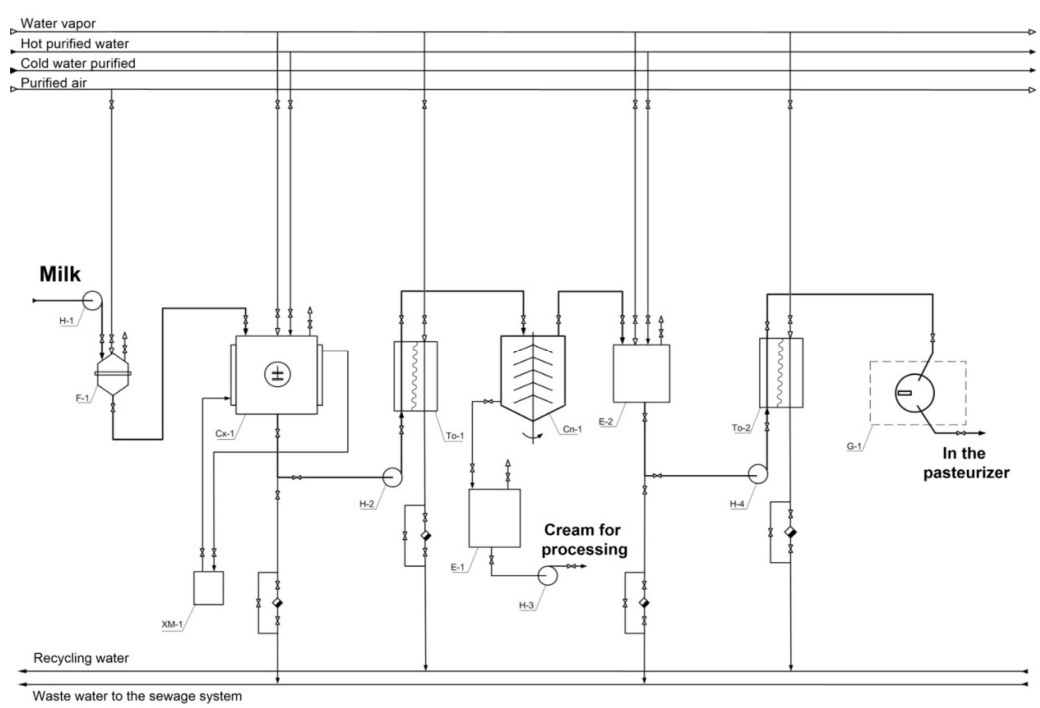

Figure 7. *Principal apparatus and technological scheme of the process of production of sour milk bioproduct*

The proposed equipment and technological scheme for the production of fermented milk feed additives with material and energy flows is shown in Figures 7 and 8, where the apparatus is accordingly indicated as follows: H-1, H-2, H-3, H-4, H-5, H-6, H-7 - pumps; F-1 - milk filter; Cx-1 - reservoir - cooler; CH-1- separator-normalizer; E-1, E-2, E-3, E-4 - intermediate tanks; To-1, To-2, To-3, To-4 - heat exchangers; G-1 - homogenizer; P-1 - pasteurizer; Fr-1 is a fermenter; I-1 - inoculator; Хм-1, Хм-2 - refrigerators; D-1 - packing machine.

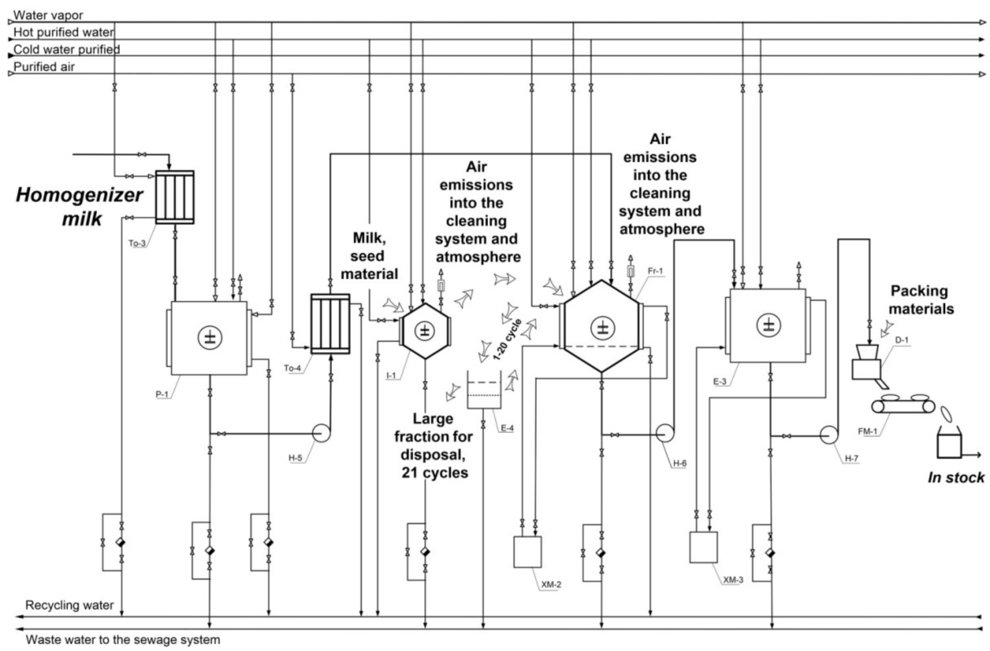

Figure 8. *Principal hardware and technology scheme of the production process fermented milk product*

It is advisable to use standard and modified equipment with selected optimal fermentation parameters for this type of production from available catalogs of apparatus and equipment, where the apparatus is accordingly indicated as follows: H-1, H-2, H-3, H-4, H-5, H-6, H-7 - pumps; F-1 - milk filter; Cx-1 - reservoir - cooler; Cn-1-separator-normalizer; E-1, E-2, E-3, E-4 - intermediate tanks; To-1, To-2, To-3, To-4 - heat exchangers; G-1 - homogenizer; P-1 - pasteurizer; Fr-1 is a fermenter; I-1 - inoculator; CM-1, CM-2 - refrigerators; D-1 - packing machine.

CONCLUSIONS

The implementation of the developed biotechnology makes it possible to increase the ecological compatibility of the product, to ensure the stability of physic-chemical and microbiological parameters during storage. Methods of preparation for quality assurance are determined, the composition of nutrient media for biomass accumulation and the parameters of cultivation are selected, which will allow to preserve the necessary ratio between strains in the dry bacterial preparation, which is one of the factors regulating the growth and consistency of metabolic processes. The optimal technological parameters of fermentation of milk by the microbiota "*Tibetan kefir grains*" in quantities sufficient to convert milk raw materials into quality products, the compliance of which ensures the passage of the desired metabolic processes and obtain a fermented milk product with good organoleptic properties.

The optimal growth conditions and producing technological parameters of the fermentation of the producer on the basis of the microbiota *Tibetan kefir grains* was determined. Biochemical and microbiological changes in processes of fermentation and storage of fermented milk product prepared using *Tibetan kefir grains* were studied in details. Principal stages for the production of feed additive were depicted in biotechnological and technological schemes with equipment. Technological parameters, conditions of cultivation and fermentation were selected to manufacture product in industrial scale.

CONFLICT OF INTEREST

All authors have no conflict of interest.

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