

ENGINEERING MANAGEMENT OF STARTER CULTURES IN STUDY OF TEMPERATURE OF FERMENTATION OF SOUR-MILK DRINK WITH APIPRODUCTS

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ABSTRACT

The article considers the solution of problematic issues of engineering management of poly fermentation in the study of fermentation temperature of sour-milk drink with apiproducs. In the development of fermented dairy products, the components that are part of them, changes in their composition, and properties in the interconnection are considered as a technological system. The authors took into account that food technologies based on the use of the pure culture of one microorganism are limited by the capabilities of its fermentation system systems, the ultimate goal may not be achieved even by changing the conditions and parameters of cultivation. To successfully carry out fermentation processes in the technological system, a combination of cultures, associations of microorganisms with a wide range of fermentation products in contrast to one culture is promising to use. All experimental samples on a set of indicators prevailed control ones. The leader was a sample fermented with yeast with an equal ratio of cultures at a temperature of 38 – 40 °C. The authors found that the set of indicators of finished products for the production of sour-milk drinks with a complex of apiproducs, it is necessary to choose a three-strain poly fermentation product with a congruent ratio of cultures and set optimal fermentation regimes 39 ±1°C for 5.0 ±0.3 hours.

Keywords: apiproducs; sour-milk drink; fermentation temperature; technological systems

INTRODUCTION

It is known that in the development of fermented dairy products, the components that are part of raw milk, changes in their composition, and properties in the connection are considered as a technological system (Parmjit, 2011; Palamarchuk et al., 2019). Its elements are independent and conditionally indivisible units that interact with each other and with the external environment (Balduzzi and Tononi, 2008; Kozelová et al., 2011). The set of elements and their connections form the structure of the system, and its spatio-temporal fragments – functional subsystems (Mda et al., 2019; Kim et al., 2015). The structure of the technological system for the manufacture of a sour-milk drink from apiproducs (Grujić et al., 2011) is presented in Table 1. The elements of the subsystems can function together and sequentially, in accordance with the tasks (Giachetti, 2006; Rogoskii et al., 2019b). The main element of subsystem A is natural cow's milk, which provides the nutritional and biological value of the developed product (Górska-Warsewicz et al., 2019). The nutritional value of milk is determined by its chemical composition, which is well studied and available in the literature (Barłowska et al., 2011), is relatively stable and does not require additional research (Rogoskii et al.,

2019a). Milk is a structural unit of the technological system “raw milk-apiproducs-starter cultures”, which is the environment of bioagents of fermentation – representatives of subsystem B (Vieites et al., 2008). The result of this process is the biotransformation of components of subsystems A and B, which ensures the quality and properties of the future product (Nikolic, 2018). The elements of subsystem B are lyophilized lactic acid microorganisms with known characteristics (Table 2) and therefore it did not require in-depth study (Azat et al., 2016). In contrast to the components of subsystem B (apiproducs), which required careful examination, their characteristics may change over time (Bishop et al., 2012) and botanical origin (Bober et al., 2020).

Scientific hypothesis

The scientific hypothesis assumes the existence of functional dependence in the fermentation process of three types of associations of lactic acid bacteria in the technological system with a complex of apiproducs and without them. The fermented milk process should be more active in the experimental media, as the hydrogen index should be lower than in the control ones.

Table 1 The structure of the technological system of sour-milk drink with apiproducs.

Subsystem	Elements of subsystems	Subsystem components
A	The components, that regulate the nutritional, biological and energy value of sour milk drink, are the living environment of biological objects	Raw milk
B	Components (growth factors) that regulate the activity and viability of biological objects	Apiproducs
C	Bioobjects that regulate fermentation	Starter cultures

Table 2 Characteristics of lactic acid bacteria.

Lactic acid bacteria	Temperature optimum, °C	Interfacial acidity, °T	Coagulation time, hour	Temperature range, °C
<i>Lactococcus lactis</i>	25 – 30	125*	4 – 6	10 – 40
<i>S. thermophilus</i>	40 – 45	110 – 115	3.5 – 4	20 – 50
<i>L. acidophilus</i>	37 – 38	260 – 280	5 – 8	5 – 53
<i>L. bulgaricus</i>	40 – 45	200 – 350	4 – 6	20 – 50
<i>B. longum</i>	36 – 40	120 – 130	10 – 12	20 – 50

Note: * – achieved in 5 – 7 days.

MATERIAL AND METHODOLOGY

Given that food technology, based on the use of the pure culture of one microorganism, is limited by the possibilities of its enzyme systems, the ultimate goal may not be achieved even by changing the conditions and parameters of cultivation for ISO 27205:2010 (ISO 27205:2010). Biological systems with a wider internal variety of signs, properties, and qualities have higher viability. For the success of fermentation processes in the technological system, it is promising to use a combination of cultures, associations of microorganisms with a wide range of enzymes in contrast to one culture.

The next series of experiments were conducted for a comparative study of technological processes in normalized milk (control technological system) and the milk environment with the complex of apiproducs (experimental technological system) with the participation of poly ferments of different bacterial composition.

First of all, two variants of associations were studied: the first consisted of three cultures – *S. thermophilus*, *L. acidophilus*, *L. bulgaricus*. The second, in addition to these, additionally contained a fourth species of microorganisms – *Lactococcus lactis* (Table 3).

Table 3 Scheme of the presence of cultures in associations.

Component of the technological system	Samples			
	K1	K2	D1	D2
Prepared cow's milk	+	+	+	+
Complex of apiproducs	–	–	+	+
<i>S. thermophilus</i>	+	+	+	+
<i>L. acidophilum</i>	+	+	+	+
<i>L. bulgaricus</i>	+	+	+	+
<i>Lactococcus lactis</i>	+	–	+	–

Statistic analysis

Statistical analysis of the results of experimental studies was performed in five replicates using standard methods of research of organoleptic, physical, physicochemical, microbiological, and other indicators. The obtained results of experimental research are processed using modern analytical integrated systems Microsoft Excel 2016 and Statistica 13.3. Adequacy of decision-making was carried out according to the criteria of Fisher, Cochran, and Student.

RESULTS AND DISCUSSION

Four samples took part in the experiment: two control samples – K1 and K2, which did not contain apiproducs and two experimental samples – D1 and D2, obtained by fermentation of the technological system “raw milk-apiproducs-starter cultures”. Similar studies were conducted by the authors of the following scientific papers (Banik et al., 2003; Abdul Manan and Webb, 2017; Montemurro et al., 2019). The ratio of cultures in fermentation products was congruent within each, incubation was performed at a temperature of 39 – 40 °C. In scientific works (Ospina, Merrill and Benson, 2018; Smith et al., 2018) incubation was performed at a temperature of 28 – 34 °C. Titrated and active acidity was determined within 6 to 12 hours every 60 minutes. In scientific works (Shanina et al., 2019; Wijnen et al., 2020) titrated and active acidity were determined for seven hours with an interval of 30 to 120 minutes. Samples D1 and D2 were compared with control ones (Figure 1).

Studies of acid-forming activity in control and experimental environments have shown that apiproducs has a positive effect on the fermentation process, accelerating it (Todorov et al., 2017). In particular, cultures grown in experimental technological systems with apiproducs overcame the stage of preparation of microorganisms for reproduction (lag phase) 2 times faster. This was more noticeable in the example of sample K2 (Furtado et al., 2014). This may be due to the lower acid-forming capacity

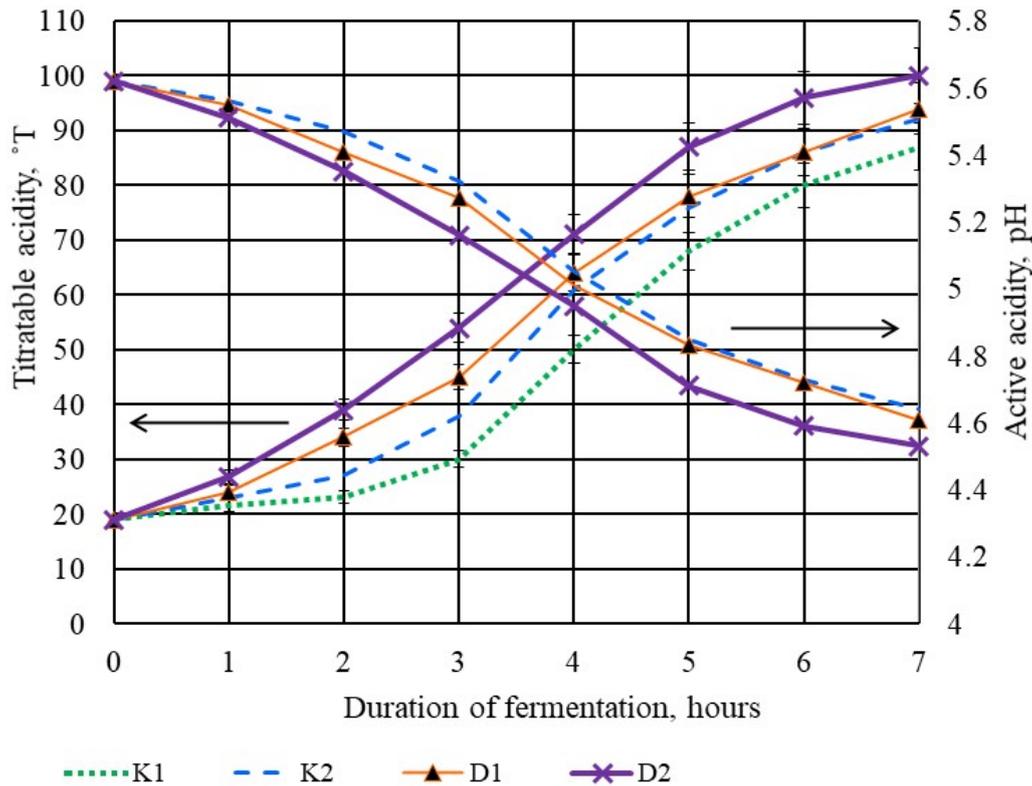


Figure 1 Dynamics of acidity of technological systems.

Table 4 Organoleptic parameters of sour-milk clots under the action of various associations of microorganisms.

Sample	Organoleptic parameters	
	consistency and color	taste and smell
K1	Moderately dense, slightly brittle and viscous	Pure, sour-milk, fresh taste
K2	Viscous, dense, homogeneous	Sour milk, moderately sour
K3	Homogeneous, viscous	Taste and smell are satisfactory
K4	The texture is viscous, slightly heterogeneous	Taste and smell are satisfactory
D1	The yellow clot is homogeneous, moderately dense	Sweet, not sour enough with a pleasant taste and smell of honey and pollen
D2	The clot of pleasant yellow color, homogeneous, dense, viscous	Harmonious sweet and sour, with a pleasant taste and smell of apiproducts
D3	Viscous sour milk gel with a yellow tint	Taste and smell are satisfactory
D4	Texture and color are satisfactory	Taste and smell are satisfactory

of *Lactococcus lactis* compared to *L. acidophilus* and *L. bulgaricus*. The reduction of the lag phase is a positive phenomenon, as it will reduce the likelihood of the development of unwanted microflora in the initial stages of fermentation of the technological system (Sahu and Panda, 2018), when the acidity has not risen to a safe level yet (Adebo et al., 2017).

The phase of smooth growth of biomass (log-phase) was also more productive in the technological system with the apiproduct complex for both starter cultures complex, but the association, which contained *Lactococcus lactis*, lagged behind the speed and efficiency of fermentation at all stages of microflora development (Singh et al., 2015). So, the starter cultures complex, which includes *S. thermophilus*, *L. acidophilus*, *L. bulgaricus*, proved to be better in both control and experimental environments (Glušac et al., 2015). To justify the choice of one of the two options for combinations of poly fermentation products (Dafeo and Daugulis, 2013), an additional organoleptic evaluation was

performed (Table 4) and the ability of the sour-milk drink to emit the moisture (syneresis) was compared.

It was found that the use of different ferments in the presence of apiproduct in milk provides products with different flavors (Widyastuti et al., 2014). Thus, sour-milk drinks obtained by fermentation of the technological system “raw milk-apiproduct-starter cultures” had an appetizing appearance, pleasant color, pure sour-milk-honey smell, but the taste was not harmonious enough due to insufficient acidity. Sample D2, obtained by using three cultures (*S. thermophilus*, *L. acidophilus*, *L. bulgaricus*) had an appetizing appearance, pleasant sour-milk-honey aroma, and harmonious taste (Spence et al., 2017).

The synergistic properties of sour-milk gels obtained by fermentation of the technological system “raw milk-apiproduct-starter cultures” indicate in favor of a three-strain combination of microorganisms, as sour-milk drinks, obtained by its use, better retain moisture in their structure (Figure 2).

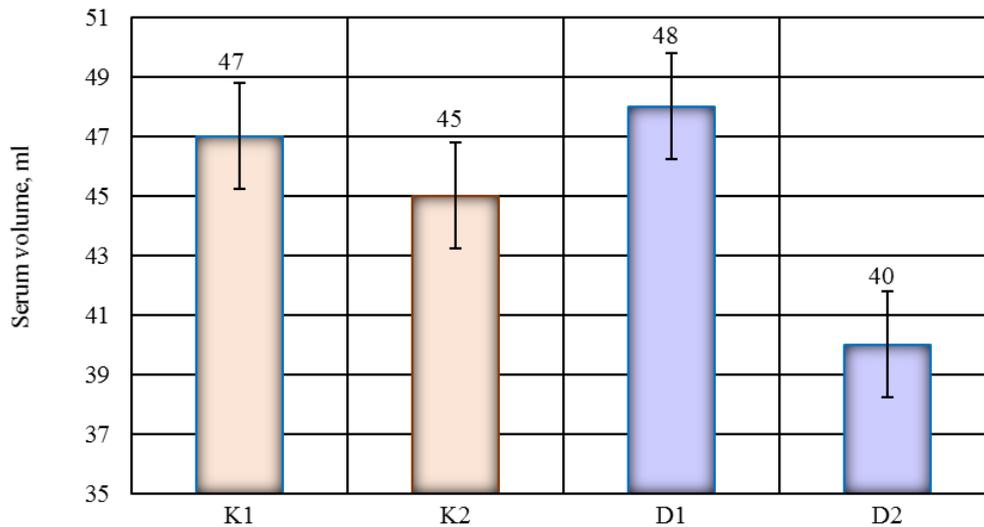


Figure 2 Synergetic properties of fermented technological systems.

Table 5 The structure of technological systems for studying the properties of associations of microorganisms.

Component of the technological system	Samples							
	control				experimental			
	K1	K2	K3	K4	D1	D2	D3	D4
Prepared cow's milk	+	+	+	+	+	+	+	+
Complex of apiproducs	-	-	-	-	+	+	+	+
2/4 <i>S. thermophilus</i> , 1/4 <i>L. acidophilus</i> , 1/4 <i>L. bulgaricus</i>	+	-	-	-	+	-	-	-
1/3 <i>S. thermophilus</i> , 1/3 <i>L. acidophilus</i> , 1/3 <i>L. bulgaricus</i>	-	+	-	-	-	+	-	-
1/4 <i>S. thermophilus</i> , 1/4 <i>L. acidophilus</i> , 2/4 <i>L. bulgaricus</i>	-	-	+	-	-	-	+	-
1/4 <i>S. thermophilus</i> , 2/4 <i>L. acidophilus</i> , 1/4 <i>L. bulgaricus</i>	-	-	-	+	-	-	-	+

After 3 hours of filtration of the 100 ml sample, less serum was released in K1 samples than in D1 by 1 ml and in K2 more than in D2 by 5 ml. The difference between samples K1 and K2 was 2 ml (4 %), and between D1 and D2 – 8 ml (16.7 %). Incubation of four-strain starter cultures complex in the medium with apiproducs led to a deterioration in the ability of the gel, formed by it, to retain moisture in the structure (Mushtruk et al., 2020).

This is evidenced by a 2 % increase in the compression of the dispersion medium of sample D1 compared with K1. Thus, sour-milk gels formed by the starter cultures consisting of *S. thermophilus*, *L. acidophilus*, *L. bulgaricus*, retain moisture better than those formed by *S. thermophilus*, *L. acidophilus*, *L. bulgaricus*, *Lactococcus lactis*, the difference becomes more noticeable during the fermentation of the technological system “raw milk-apiproducs-starter cultures”. This may be due to the ability of the *L. acidophilus*, *L. bulgaricus*, *Lactococcus lactis* to form a sour-milk gel with a brittle structure that displaces the dispersion medium. In contrast to lactic acid mucus-forming rods (*L. acidophilus*, *L. bulgaricus*) and thermophilic streptococcus, which form clots of viscous and creamy consistency (Sfakianakis and Tzia, 2014), which provides low self-expression of serum.

A comparative evaluation of the action of two starter cultures by the set of signs of leaven, in dairy environments with a complex of apiproducs and without it, suggests that in further studies we should use the first of them. Because the combination of *S. thermophilus*, *L. acidophilus*, and *L. bulgaricus* provides the best quality in organoleptic,

biochemical, technological, and others. properties of the product than poly fermentation product with the composition: *S. thermophilus*, *L. acidophilus*, *L. bulgaricus*, *Lactococcus lactis*.

Substantiation of fermentation regimes and composition of poly cultural leaven (Kennedy and Krouse, 1999). The biochemical activity of microorganisms should be studied under the conditions of a set of influencing factors: the composition of the nutrient medium, the temperature of cultivation, the composition of the leaven (Beltran et al., 2008; Bober et al., 2020). The influence of the first factor was investigated using two variants of media: the first – normalized milk with a mass fraction of fat of 3.2 %, pasteurized at a temperature of 87 ± 2 °C, and cooled to fermentation temperature, the second – the same milk with a complex additive. The effect of the second factor was investigated by varying the cultivation temperature (Table 2) from 38 °C to 42 °C in increments of 2 °C. Temperatures above 42 °C are undesirable for the apiproducs. The influence of the third factor was studied by changing the ratio of cultures in the starter cultures. As a result of the technological system of design, eight samples of sour-milk clots were obtained (Table 5): control – K1-K4 and experimental – D1-D4.

As a result of designing the technological system, eight samples of sour-milk clots were obtained, which differed in the presence of apiproducs in their composition and were fermented by different. Lactic acid bacteria (LAB) associations (Fahrurrozi et al., 2019). Control and experimental samples were evaluated by coagulation time

and titrated acidity (Marchi et al., 2009; Zheplinska et al., 2020). The research results are presented in Table 6.

Analyzing the biochemical activity of the association of lactic acid cultures in control and experimental of the technological system at a cultivation temperature of 38 – 42 °C, we can conclude that, under these conditions, environments with apiproduct complex performed better than control ones.

The results of the study of culture activity under different cultivation conditions showed that an increase in biomass was obtained in environments with apiproducts, which is higher than in the control ones by 7.4 %. In scientific works (Barãoa et al., 2019; Abdulgader et al., 2019) descriptive experiments where the increase in biomass obtained in environments with a variety of products ranged only at the level of 3.5 - 4.6%.

Increasing the fermentation temperature by 2 °C causes an increase in acidity by 1 °T, a similar effect on the content of Log CFU ml⁻¹ is not observed.

The number of lactic acid bacteria grown at a temperature of 3 °C was higher than the number grown at a temperature of 40 °C by 1.0% in the control group and 2.0% in the experimental group.

A further increase in the fermentation temperature to 42 °C caused an inverse reaction, which resulted in a decrease in the number of viable cells, both in the control and in the experiment, by an average of 3.4 %, compared with samples cultured at 40 °C by 2 % compared to those samples that were fermented at a temperature of 38 °C.

As the fermentation temperature increases, the acidity of the clots increases – protein coagulation is faster by almost one hour, but under such conditions, there is a tendency to reduce the synthesis of biomass.

The time of clot formation has a directly proportional effect on the duration of the production process, and acid coagulation of milk correlates with the level of organic acids in the environment.

However, with the acceleration of coagulation, as can be seen from Table 6, the ability of microorganisms to

Table 6 Activity of cultures under different conditions of cultivation, *n* = 3, *p* ≤ 0.05

Sample	Titrated acidity, °T	Time of Gel formation, hour	Cell numbers log LAB/g
Cultivation temperature 38 °C			
K1	85 ±1	5.5 ±0.3	8.7 ±0.2
D1	90 ±3	5.0 ±0.1	9.4 ±0.1
K2	84 ±2	5.6 ±0.2	8.8 ±0.1
D2	89 ±3	5.4 ±0.2	9.3 ±0.2
K3	88 ±1	5.3 ±0.5	8.5 ±0.2
D3	92 ±1	4.8 ±0.3	9.3 ±0.1
K4	87 ±2	5.4 ±0.3	8.6 ±0.1
D4	91 ±3	4.9 ±0.1	9.3 ±0.1
Cultivation temperature 40 °C			
K1	86 ±1	5.4 ±0.1	8.8 ±0.1
D1	91 ±1	4.7 ±0.1	9.5 ±0.2
K2	85 ±1	5.5 ±0.2	8.9 ±0.3
D2	89 ±2	4.9 ±0.1	9.3 ±0.3
K3	89 ±2	5.2 ±0.1	8.7 ±0.2
D3	94 ±2	4.5 ±0.2	9.3 ±0.2
K4	88 ±3	5.3 ±0.3	8.5 ±0.3
D4	92 ±1	4.6 ±0.2	9.3 ±0.1
Cultivation temperature 42 °C			
K1	88 ±1	4.8 ±0.1	8.5 ±0.1
D1	93 ±2	4.0 ±0.1	9.3 ±0.3
K2	86 ±2	4.9 ±0.2	8.5 ±0.2
D2	90 ±1	4.2 ±0.2	9.2 ±0.1
K3	90 ±2	4.6 ±0.2	8.4 ±0.1

Table 7 Characteristics of products obtained under different fermentation regimes.

Sample	Fermentation temperature								
	38 °C			40 °C			42 °C		
	score	viscosity, sec.	pH	score	viscosity, sec.	pH	score	viscosity, sec.	pH
K1	5	83 ±1	4.7 ±0.03	4.9	83 ±2	4.7 ±0.02	4.9	84 ±1	4.6 ±0.09
D1	5	84 ±1	4.6 ±0.07	5.0	84 ±2	4.6 ±0.05	4.9	84 ±3	4.6 ±0.03
K2	4.9	70 ±2	4.7 ±0.05	4.9	71 ±3	4.7 ±0.03	4.8	73 ±2	4.7 ±0.02
D2	4.9	79 ±1	4.6 ±0.08	4.9	80 ±3	4.6 ±0.07	4.8	80 ±2	4.6 ±0.07
K3	4.4	80 ±2	4.6 ±0.09	4.4	81 ±1	4.6 ±0.08	4.3	80 ±3	4.6 ±0.07
D3	4.7	83 ±1	4.6 ±0.04	4.7	83 ±3	4.6 ±0.03	4.6	83 ±1	4.6 ±0.01
K4	4.5	80 ±1	4.7 ±0.01	4.5	80 ±2	4.6 ±0.09	4.4	81 ±1	4.6 ±0.08
D4	4.8	82 ±2	4.6 ±0.05	4.7	82 ±2	4.6 ±0.04	4.6	82 ±1	4.6 ±0.01

accumulate biomass decreases, and the number of viable cells decreases, both in control and in the experiment.

For example, in the control of acidity 85 °T Log CFU ml⁻¹ is 8.7, and at an acidity of 88 °T – 8.5, and the time of clot formation at the specified acidity is reduced by 13 %. Similar dynamics are observed in the experimental samples, but the loss of cell concentration for every 1 °T is twice lower. It should be noted that coagulation should not be accelerated while neglecting the probiotic properties of the sour-milk drink. Therefore, the recommended fermentation regimes are 39 ±1 °C for 5.0 ±0.3 hours of fermentation. Analyzing the results of the model associations of microorganisms, we can conclude that the best results were achieved by those who had a congruent ratio of cultures (K1, D1), both in environments with apiprodukt and without them.

Studies of organoleptic, physicochemical, and rheological properties of cultures of lactic acid organisms under different cultivation conditions are presented in **Table 7**.

The data obtained indicate that with increasing fermentation temperature, there is a tendency to accelerate the decrease in the concentration of hydrogen ions and a slight increase in viscosity. A more pronounced difference was observed in the number of points scored between control and experimental samples, fermented under different conditions (**Woo et al., 2019**). An increase in the cultivation temperature by 2 °C causes such negative phenomena as excessively sour taste, intense serum secretion, minor defects in the consistency of the sour-milk clot (flakes, etc.) and it leads to a decrease in the overall score by an average of 0.4 points for all samples.

Thus, the results of the research of organoleptic, physicochemical, and rheological properties of LAB cultures under different cultivation conditions indicate that fermentation should be carried out at a temperature of 38 – 40 °C.

CONCLUSION

During the study of the fermentation process of three species associations of lactic acid bacteria in technological systems with a complex of apiprodukt and without them, it was noted that the sour-milk process is more active in the experimental environment because the hydrogen index was lower than in control ones.

Sensory evaluation of sour-milk drinks showed that the best taste properties had experimental samples. This is because the sweet taste of honey was harmoniously combined with sour-milk, and the floral aroma of the apiprodukt successfully emphasized the characteristic smell of fermented dairy products. The highest score among the experimental samples scored D1 and D2, but the relative viscosity of the sample D1 was higher by 6 % and was 83 – 85 seconds.

All experimental samples (D1 – D4) in the set of indicators prevailed control ones (K1 – K4), but the leader was the sample D2, fermented with an equal ratio of cultures at a temperature of 38 – 40 °C.

Therefore, according to the results of the study of the set of indicators of finished products for the production of sour-milk beverages with a complex of apiprodukt, a three-strain poly fermentation product with a congruent ratio of cultures was selected and optimal fermentation regimes were established: 39 ±1 °C for 5.0 ±0.3 hours.

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