Prototypes of pastille marmalade products with starters of lactic acid microorganisms have been developed. Pastille marmalade products that contain live cells of probiotic culture (from 1 to 3 CFU/g) help strengthen and maintain immunity. The macro and micromorphology of the probiotic culture were investigated, which confirmed the belonging of microorganisms to lactic acid. The studies reported here were scientifically substantiated by the method of mathematical modeling. Based on the regression equation, it was revealed that the growth of lactic acid microorganisms in the product is affected by the volume of whey (250 ml), the volume (0.02 g) and the time of revival of the starter culture (6 hours). A more significant factor was the volume of application of lactic acid microorganisms, from 0.01 to 0.02 g, which affects the growth of lactic acid microorganisms (increases) in the product. The antimicrobial activity of isolated crops in relation to E. coli was studied. The zones of illumination of the isolated colonies in relation to E. coli range from 0.1 mm to 0.5 mm.

The results show that antagonistic properties affect pathogenic and conditionally pathogenic microorganisms in the gastrointestinal tract.

The antioxidant properties of pastille marmalade products have been established. When applying starters of lactic acid cultures, the volume of antioxidants increased by 1.7 and 2.2 times compared with control.

In this regard, the development of pastille marmalade products with starters of lactic acid microorganisms is a relevant and promising task because they are natural, have an immunostimulating effect, and expand the range of confectionery products

Keywords: starters of lactic acid microorganisms, whey, antimicrobial activity, antioxidant properties, immunity

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IDENTIFICATION OF THE INFLUENCE OF TECHNOLOGICAL FACTORS ON THE GROWTH AND DEVELOPMENT OF LACTIC ACID MICROORGANISMS IN PASTILLE MARMALADE PRODUCTS ENRICHED WITH LACTIC ACID STARTERS

Yuliya Pronina
PhD
Department of Information Support and Patent Research*
Zhanar Nabiyeva
PhD
Science Research Institute of Food Safety*
Olga Belozertseva
Master of Science
Department of Food Safety and Quality*
Saule Shukesheva
Corresponding author
PhD
Accredited Testing Laboratory of Food safety*
E-mail: s.shukesheva@atu.kz
Abdysemat Samodun
Master of Science
Scientific Research Laboratory for Assessing the Quality and Safety of Food Products*
*Almaty Technological University
Tole bi str., 100, Almaty, Republic of Kazakhstan, 050012

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1. Introduction

The need for the development, production and use of pastille marmalade products with probiotic properties is associated with unfavorable environmental conditions of the habitat, with the spread of coronavirus infection. To strengthen and maintain immunity, a healthy sleep, an active lifestyle, a balanced diet are necessary. Proper (healthy) nutrition can activate the reserve forces of the body, contribute to the prevention of diseases, increase efficiency. As additives to strengthen the immune system and to prevent dysbiosis and related diseases, lactic acid microorganisms are important, which help strengthen immunity.

Therefore, the development of pastille marmalade products using lactic acid microorganisms, namely streptococci, is a promising direction in the food industry.

2. Literature review and problem statement

The development of the market is aimed at creating new resource-saving technologies and devising new types of products with high consumer properties. According to statistics from around the world, the popularity of sugar confectionery products is growing. Given this, the specified segment of products can be a carrier of functional ingredients that determine their directed action in the prevention of viral and other mass alimentary diseases [1].

Not less important is the correct selection of ingredients based on plant extracts, vitamins, probiotics, which strengthen the immune system by improving biochemical processes in the body, as well as reducing the sugar intensity of confectionery.

In view of this, work [2] is interesting: the authors analyzed the mass fraction of sugar in the composition of the most common types of marmalade in retail chains. However, they did not

analyze the selected types of fruit and berry puree and other enriching additives of plant origin.

It is scientifically substantiated [3] that excessive consumption of sugar is harmful, but it is impossible to completely deprive the body of glucose since it is necessary to maintain normal carbohydrate metabolism. Therefore, when developing sugar confectionery products, in particular pastille aromatic products and lollipops, it is more expedient to use fruits and berries as a basis because they are natural sweeteners and natural antioxidants [4]. The authors of [3] conducted studies to determine the sugar content in products with the detection of a high fructose content, as well as studies on the preservation of vitamin C in pastille marmalade products. These studies laid the foundation for solving the problem of enriching pastille marmalade products with functional ingredients.

As additives to strengthen the immune system and to prevent dysbiosis and related diseases, lactic acid microorganisms are important. The authors of work [5] conducted a review of studies on the enrichment of pastille marmalade products with probiotic cultures, indicating promising representatives of lactic acid bacteria resistant to high temperatures. The analysis indicates the relevance of the chosen direction of research in view of insufficient data revealing the problems of research in the field of enrichment of pastille marmalade products with probiotics.

Advantages of the use of lactic acid microorganisms in pastille marmalade products: cells of live lactic acid microorganisms contained in the finished product restore and maintain healthy intestinal microflora, stimulate the growth and vital activity of their own microflora (bifido- and lactobacilli). Thus, they contribute to the strengthening of immunity and the development of their own interferon. Lactic acid microorganisms resist intestinal infections and putrefactive bacteria, normalize digestion, improve peristalsis, prevent constipation, are effective when applied locally to combat bacterial and fungal lesions of the skin and mucous membranes.

Adding probiotics to dairy products is a traditional process, so probiotic non-dairy products can contribute to the daily antioxidant diet to improve the quality of life and health of consumers. The study reported in [6] confirmed that *Lactobacillus paraplantarum*, *Lactobacillus plantarum*, *Weissella paramesenteroides* and *Enterococcus faecalis* are ideal bacteria for the probiotic process of grape marmalade. However, it is necessary to expand the choice of probiotic cultures that are resistant to heat treatment and the acidic environment of pastille marmalade products.

Based on the results of the information and patent search, it was proposed to develop sugar confectionery products using lactic acid starters. In this regard, the production of immunostimulating confectionery products enriched with starters of lactic acid microorganisms seems to be a relevant task for the food industry.

Paper [5] reports results that show that lactic acid microorganisms have great biotechnological potential, they ferment carbohydrates with the formation of lactic acid, do not liquefy pectin substances, which is very important in the development of pastille marmalade products. An important property of lactic acid microorganisms is antagonism-suppression of the growth of microorganisms that cause spoilage of the product and unwanted lactic acid microflora, which, along with lactic acid, forms by-products: acetic acid, carbon dioxide, and others [7].

Analysis of patents and research results, for example, reported in [8], show that lactic acid sticks *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus* and others are used in the development of marmalades based on starter cultures. However, there is no information on the use of lactic acid streptococci (*Lactococcus*, *Leuconostoc*, *Streptococcus*).

Lactic acid streptococci, unlike rods, form an aroma, absorb citrates and, in combination, form density. For the growth and reproduction of streptococci, amino acids, vitamins, nitrogenous bases are needed, and for sticks amino acids [9]. Given these characteristics of streptococci, it became necessary to use them in the development of pastille marmalade products. The advantage of developing the intended product is to obtain pastille marmalade products containing living cells of probiotic culture, while it is possible to exclude the use of acidifiers, since lactic acid microorganisms in the process of their vital activity release lactic acid.

Many strains of lactic acid microorganisms are probiotics. Probiotic properties include resistance to human acids and gastric juice, as well as to bile, adhesion to the epithelium and engraftment in the human digestive tract. In addition, probiotic properties include immunostimulation, antagonistic activity against pathogenic microorganisms, antimutagenic properties [9]. The unsolved part of the problem is that, given the beneficial, immunostimulating properties of lactic acid streptococci, their possibility of use is extended to use only in the dairy industry. At the same time, it is necessary to take into consideration the possibility of using them in pastille marmalade products and, on the basis of this, also consider the possibility of their use in a wider range – for the confectionery industry.

Experts distinguish the following main products of the metabolism of lactic acid bacteria, which cause their high antimicrobial activity [10]:

– lactic, acetic, formic, and other organic acids;
– carbon dioxide;
– hydrogen peroxide;
– acetoin, diacetyl;
– nitrogen oxide;
– short-chain fatty acids;
– bacteriocins and bacteriocin-like substances.

It is known that lactic acid microorganisms of even one species can have different antimicrobial activity.

It is shown that *Leuconostoc lactis* are microorganisms that are able to produce bacteriocins (specific proteins that suppress the vital activity of cells of certain types of bacteria). This property endows products that include *Leuconostoc lactis* with a wide spectrum of antibacterial activity, the ability to inhibit the growth and development of certain types of microbes [11]. *Leukonostocs* secrete mucus for homogeneous and viscous products, including for confectionery, emit little lactic acid.

Lactococcus lactis forms bacteriocin, a nisin that has been successfully used to increase the shelf life of food in the food industry in many countries for more than 50 years. This suggests that some cultures may act as antioxidants [12]. The introduction of *Lactococcus lactis* into foods and studies of its antioxidant properties are almost absent.

Lactic acid is the main compound that is formed during the fermentation process but there are other metabolic end products. In particular, the resulting products produced by *Leuconostoc* and other heteroenzymatic lactic acid bacteria are important for obtaining a product with good organoleptic parameters [13]. In addition, small volumes of mannitol, diacetyl, acetaldehyde, and other volatile aromatic compounds can be synthesized during the fermentation process. *Leuconostoc* was not used in the development of sugar confectionery products, its use would improve the taste and aromatic properties of the product due to the synthesis of the above substances.

Properly selected cultures in sourdough contribute not only to the formation of a pleasant taste and aroma of the product, stabilization of color but also to the suppression of the vital activity of putrefactive and sanitary-indicative bacteria. In addition, it has been established that some microorganisms have proteolytic activity, intracellular enzymes of which are able to break down proteins, thereby improving the structural characteristics of the finished product [14].

In addition, the authors of [15] conducted studies to investigate genes associated with the usefulness, virulence, and resistance to antibiotics in 10 well-described bacteriocinogenic strains of lactic acid bacteria. The results of their studies showed that all tested strains showed the ability to auto aggregate at 4 °C and 37 °C, coaggregation with *S. aureus JCM8704*, *S. typhimurium BIOTECH1826*, and *E. coli DH5α*, as well as significant hydrophobicity of the cell surface. *Lactococcus sp. QU12* was characterized by a high frequency of utility and virulence genes: 2 out of 7 genes present encoded a useful factor, and 11 out of 13 genes encoded a virulence factor. The studies conducted by the authors confirm the benefits of some strains of lactic acid bacteria, their resistance in bile juice. In view of this, they can be used in food production.

The improvement of traditional and the development of new technologies that increase the intensity of production of sugar confectionery products, improve their organoleptic properties, increase the guarantee of the production of high-quality products, increase the shelf life, etc. is considered promising.

For example, the authors of [16] developed a recipe for cranberry marmalade with the introduction of a probiotic culture of different mass fractions: sample 1 with probiotics in the free state; sample 2 with immobilized probiotics; sample 3 with pasteurized whey enriched with bacterial concentrate containing 0.001 % acidophilus bacillus. The work is relevant and expands the range of pastille marmalade products of a functional orientation. However, it is necessary to expand this area of research by studying a wider range of lactic acid microorganisms.

The paper reports the results of studies of the influence of technological factors on the growth and development of lactic acid microorganisms in pastille marmalade products enriched with lactic acid starters. Pastille marmalade products enriched with lactic acid starters have antimicrobial and antioxidant properties have been shown. However, the issues related to the partial destruction of living cells of microorganisms during the production of pastille marmalade products remained unresolved. The reason for this may be heat treatment. An option to overcome the relevant difficulties can be different modes of revitalization of starter cultures, the volume of whey, and the volume of culture introduced. It is this approach that was used in the work but in order to identify large quantities of living cells of lactic acid microorganisms, work is underway to improve the technology of pastille marmalade products with the introduction of starter cultures under different heat treatment modes. All this suggests that it is advisable to conduct a study on the development of pastille marmalade products based on fruits and berries using lactic acid streptococci. The created product will be natural, dietary, immunostimulating, and will expand the range of confectionery products.

3. The aim and objectives of the study

The purpose of this study is to identify the patterns of influence of technological factors on the growth and development of lactic acid microorganisms in pastille marmalade products enriched with lactic acid starters. This will make it possible to develop pastille marmalade products with an immunostimulating effect.

To accomplish the aim, the following tasks have been set:

– to develop prototypes of pastille marmalade products with the introduction of different concentrations of lactic acid starters;

– to investigate the affiliation of the grown colonies isolated from the prototypes of pastille marmalade products to lactic acid microorganisms;

– to substantiate the influence of technological factors on the growth and development of lactic acid microorganisms by the method of mathematical modeling;

– to investigate the antimicrobial and antioxidant activity of prototypes of pastille marmalade products enriched with lactic acid starters.

4. The study materials and methods

4. 1. Object and hypothesis of the study

The objects of lactic acid microorganisms were a complex of lactic acid streptococci – Camembert starter culture and microMilk KF 100 starter culture.

Camembert starter culture composition:

– *Lactococcus lactis*;
– *Lactococcus cremoris*;
– *Lactococcus diacetylactis*;
– *Leuconostoc mesenteroides* subspecies cremoris.

MicroMilk KF 100 starter culture composition:

– *Streptococcus thermophiles;*
– *Lac.lactis;*
– *Lac.cremoris;*
– *Leuc. mesent. subsp.cremoris;*
– *Lac.biovardiacetylactis.*

The working hypothesis of the study assumed that the influence of technological factors on the growth and development of lactic acid microorganisms in pastille marmalade products can be assessed on the basis of the development of prototypes of pastille marmalade products using lactic acid starters.

In the study of the microflora of products, classical methods of microbiology were used: methods of sampling and sample preparation according to GOST 32751–2014 Confectionery products. Sampling methods for microbiological analyses and methods of culturing microorganisms according to GOST 26670–91. Determining the content of lactic acid microorganisms in the finished product was carried out by the method of maximum dilutions on nutrient media according to GOST 10444.11-2013 (ISO 15214:1998) Microbiology of food and animal feed. Methods for identifying and counting the number of mesophilic lactic acid microorganisms.

4. 2. Methods of mathematical treatment of experimental research results

We processed experimental data (calculation of numerical sample characteristics, construction of tables and frequency graphs) and performed calculations by using application software packages (PPP) STATISTICA, Envies, and Microsoft Excel [17].

When using mathematical methods of planning and analyzing the experiment, research was conducted according to a certain plan, consisting of several successive stages [18]:

– collection and preliminary analysis of the initial data;
– construction of a system of indicators (factors);

– processing of experimental data: calculation of numerical characteristics of the sample;

– construction of tables and graphs of frequencies;

– correlation analysis (construction of a matrix of pair correlation coefficients);

– selection of the type of model and numerical assessment of its parameters;

– checking the quality of the model;

– assessment of the influence of individual factors on the basis of the model;

– construction of an optimization problem based on the regression model.

4. 3. Methods for determining antagonistic activity

Antagonistic activity was determined by diffusion into agar from wells. Cultivation regime: 30–37 °C, 10 days. The assessment of the antagonistic activity of cultures was determined on the 2nd, 5th, 10th day of incubation by the diameter of the sterile zones formed around the wells. Strains of lactic acid microorganisms were grown on the nutrient medium MRS-agar for the isolation of lactobacilli. Statistical processing of the results of the studies was carried out according to the standard methodology using the Student's criterion for the significance level of $p<0.05$.

4. 4. Determining antioxidant activity by amperometry

We determined AOA in the raw materials and products on the device "TsvetYauza-01-AA", based on the amperometry measurement method according to GOST R 54037-2010.

The amperometry method for determining antioxidants is based on the measurement of the electric current in the cell that occurs when the analyte is oxidized on the surface of the working electrode when a certain potential is applied to it. The signal is recorded as differential output curves. With the help of special software, the areas or heights of peaks (differential curves) of the analyzed and standard substance are calculated. For the analysis, the average value of a series of three to five consecutive measurements is used. As standard substances, one can use well-known antioxidants: quercetin, dihydroquercetin, mexidol, trolox, gallic acid, etc. The amperometry method has a number of advantages in determining antioxidant activity: without taking into consideration sample preparation, the time of separate determination takes several minutes; analysis (registration and processing of results) takes place in real time. The correctness and reproducibility of the analysis are ensured by precise dosing with a six-way crane. The root mean square deviation (RMS) of tap dosing is less than 0.5 %; COEX of sequential measurements of analyzed samples is less than 5 %; the limit of detection of polyphenols and flavonoids is at the level of nano-, picograms (10^{-9}–10^{-12} g). At such low concentrations, there is less likelihood of the mutual influence of different antioxidants in their joint presence, in particular the manifestation of synergy [19].

The preparation of the analyzed samples is carried out by mixing the corresponding substances with the solvent. Solids are converted into liquid forms before analysis.

Before starting work on the day of measurements, the calibration of the device is carried out. To exclude random results and average the data, five consecutive measurements are performed for each of the five calibration solutions of quercetin (a natural antioxidant from the group of flavonols). The result is taken as an arithmetic mean of five measurements (relative root mean square deviation is not more than 5 %). According to the data obtained, a calibration characteristic is constructed (Fig. 1) according to GOST R 54037-2010 [20].

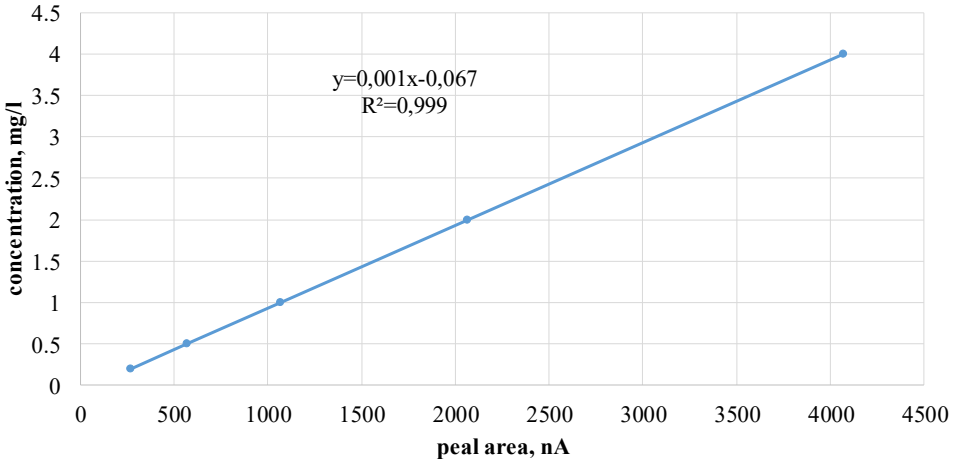


Fig. 1. Quercetin calibration chart

According to the calibration chart, the content of AO was determined, the calculation is carried out according to (1).

$$X = \frac{X_r \cdot V_n}{m_n \cdot 1{,}000}, \quad (1)$$

X_r is the SSA value found from the calibration curve, equivalent to quercetin, mg/dm^3;

V_n is the volume of the analyzed sample solution, cm^3;

M_n is the batch of the analyzed sample, g;

N is the dilution factor of the analyzed sample.

5. Results of the study of prototypes of pastille marmalade products

5. 1. Development of prototypes of pastille marmalade products with the introduction of different concentrations of lactic acid starters

Based on the literature data [5], prototypes of pastille marmalade products using lactic acid microorganisms were developed.

According to the technological regimes of starters, the duration of milk fermentation is 9–11 hours. To produce prototypes of pastille marmalade products, starter cultures were revived in a milk whey from the company "Amiran". Next, milk whey with cultures of live lactic acid microorganisms were transferred to the production workshop for the development of pastille marmalade products based on IP "VM". To determine the immunostimulating properties of pastille marmalade products, the effect of starter cultures on the growth of lactic acid microorganisms in the finished product was investigated.

The objects of our study were the following prototypes of marmalade:

1. Sample No. 1 with the Camembert starter (0.005 g of culture per 500 ml of whey, duration of revival 12 hours).

2. Sample No. 2 with the starter microMilk KF 100 (0.01 g of culture per 500 ml of whey, duration of revival 12 hours).

3. Sample No. 3 with the Camembert starter (0.01 g of culture per 500 ml of serum, duration of revival 6 hours).

4. Sample No. 4 with the starter microMilk KF 100 (0.02 g of culture per 500 ml of whey, duration of revival 6 hours).

5. Sample No. 5 with the Camembert starter (0.01 g of culture per 250 ml of whey, duration of revival 6 hours).

6. Sample No. 6 with the starter microMilk KF 100 (0.02 g of culture per 250 ml of whey, duration of revival 6 hours).

5. 2. Investigation of the belonging of grown colonies from prototypes of pastille marmalade products to lactic acid microorganisms

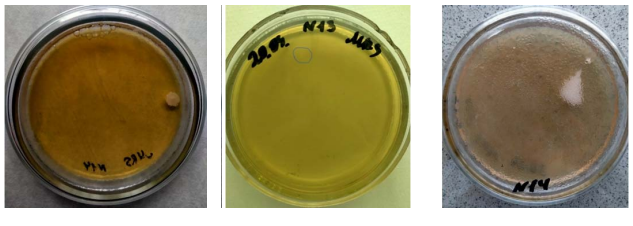
Prototypes of marmalade from 10^1 degrees were sown on the nutrient media MRS. The cultures were incubated at 37 °C, 48–72 h. The growth of lactic acid microorganisms was determined by the presence of colony-forming units. The results are given in Table 1, and macromorphology is shown in Fig. 2.

Table 1

Growth of lactic acid microorganisms on MRS medium

No.	Sample designation	CFU/g
1	Sample 1	Not detected
2	Sample 2	Not detected
3	Sample 3	Not detected
4	Sample 4	1
5	Sample 5	1
6	Sample 6	3

Lactic acid microorganisms are mostly immobile, according to Gram they are painted positively, they do not form spores. Along with the main metabolite, these bacteria accumulate other products: acetic acid, ethanol, carbon dioxide, aromatic substances (acetaldehyde, diacetyl), etc. Cells of lactic acid microorganisms have a spherical or rod-shaped shape.

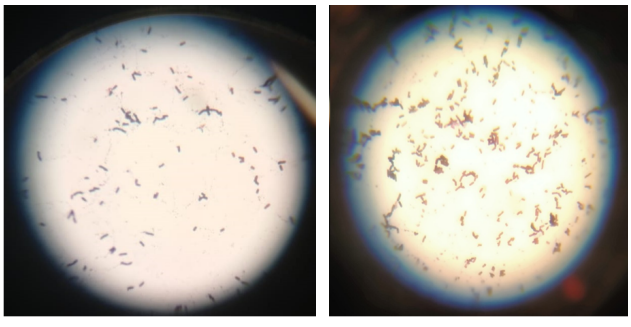


a *b* *c*

Fig. 2. Macromorphology of microorganisms on the nutrient medium MRS, isolated from the prototypes of marmalade: *a* — growth of 1 CFU; *b* — growth of 1 CFU; *c* — growth of 3 CFU

To confirm the grown colonies belonging to lactic acid microorganisms, their microscopy was carried out. The results are shown in Fig. 3.

The micromorphology of microorganisms isolated from the prototypes of pastille marmalade products shows their belonging to lactic acid. The isolated microorganisms are gram-positive, mainly oval in size (0.5–1.2) (0.5–1.5) μm, connected in pairs (diplococci) or in the form of short chains. These characteristics indicate the membership of lactic acid microorganisms to the *Streptococcaceae* family, which unites *genera Lactococcus, Streptococcus, Pediococcus, and Leuconostoc.*



a *b*

Fig. 3. Micromorphology of microorganisms isolated from the prototypes of marmalade at 100 times magnification using the trinocular microscope MS 300 Vision (Austria): *a* — micromorfology Fig. 2, *a*; *b* — micromorfology Fig. 2, *c*

5. 3. Substantiation of the influence of technological factors on the growth and development of lactic acid microorganisms by the method of mathematical modeling

The technological process of production of pastille marmalade products is the boiling of the recipe mass to a certain volume of dry matter. The developed method for the production of pastille marmalade products is based on the use of low esterification pectins and a final dry matter of not more than 64 %. According to the above-described features of the technology, the product retains its freshness for nine months. To enrich the product with lactic acid organisms, it is necessary to introduce a suspension with a refractive index of 12 %, which is a feature in which there is a need for additional heat treatment of the product and bringing the total mass to 62–64 % of dry substances.

A study was conducted on the effect of the volume of starter culture applied on the consistency and texture of the finished product. Based on the experiment, the optimal dosage of lactic acid starters was chosen, which made it possible to obtain a product with probiotic properties with pleasant flavoring properties and a soft consistency characteristic of fruit-eating jelly marmalade. When applying more lactic acid starters, the opposite effect was obtained and the absence of lactic acid microorganisms in the final product, since additional long-term heat treatment, which is necessary to obtain the structure, adversely affected the growth of microorganisms. Accordingly, based on the results of the study, an average indicator was derived, which made it possible to obtain a homogeneous, highly elastic structure with a significant viscosity component and the presence of lactic acid microorganisms in the finished product. Boiling of the mass occurs at a temperature of 105 °C for 20–25 minutes but when the starter is applied, the boiling time increases to 40 minutes. In order to reduce the duration of boiling, it was decided to increase the proportion of starter microorganisms with a decrease in the proportion of whey and the period of revival of the starter. At the same time, the introduction of this animated starter culture was carried out at the end of the heat treatment of the pastille marmalade mass. As a result of this operation, positive results of the study on the survival of lactic acid microorganisms were obtained.

In order to design the optimal recipe and technology of pastille marmalade products, the degree of influence of technological factors on the growth of lactic acid microorganisms was studied. The effect of the volume of the starter

culture microMilk KF 100 (C1), the volume of milk whey (C2), the revival time of the starter microMilk KF 100 (C3) on the growth of lactic acid microorganisms in the finished product (C4) was studied (Table 2).

Table 2

Levels of variable factors

Variable factors	Designation	Level		
Number of lactic acid microorganisms (starter microMilk), g	C1	0.005	0.01	0.02
The amount of whey, ml	C2	250	500	1000
MicroMilk KF 100 starter revival time, h	C3	6	12	24
Growth of lactic acid microorganisms, CFU/g	C4	0	1	3

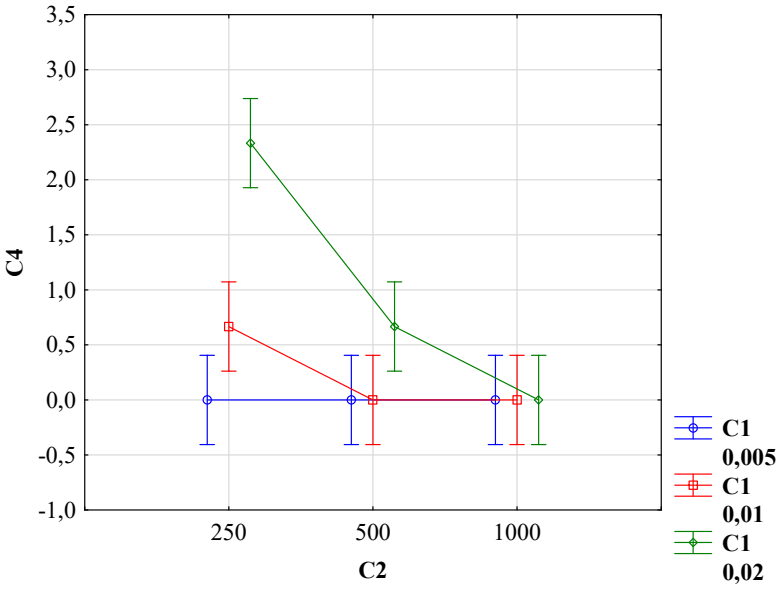


Fig. 4. Graph of "Averages" for levels of factors C1 and C2

As a result of processing the data obtained, a mathematical model was built that characterizes the effect of the volume of the microMilk KF 100 starter culture and the volume of whey on the number of lactic acid microorganisms in pastille marmalade products. Table 3 gives the results of factor analysis of variance with a one-dimensional significance criterion for C4.

Table 3

Results of factor analysis of variance with a one-dimensional significance criterion for C4

Effect	One-dimensional significance criterion for C4				
	SS	Degree of freedom	MS	F	P
Free term	4.481481	1	4.481481	48.40000	0.000118
C1	4.962963	2	2.481481	26.80000	0.000284
C2	4.962963	2	2.481481	26.80000	0.000284
C3	1.185185	2	0.592593	6.40000	0.021883
C1*C2	4.592593	4	1.148148	12.40000	0.001654
C1*C3	1.037037	4	0.259259	2.80000	0.100469
C2*C3	1.037037	4	0.259259	2.80000	0.100469
Error	0.740741	8	0.092593	–	–

Table 3 shows that the relationship of factors C1 and C2 significantly affects the target variable C4, which can be clearly seen in Fig. 4, 5.

It is also established that the parameters of the model are statistically significant since the actual value for the relationship of factors C1 and C2 is p=0.001654.

To determine the influence of independent variables and optimize the process, the reflective surface (RSM) method was applied. This method gives a change in the dependent variable y with a change in the independent variables (x_1, x_2, ..., x_n), so the reflection surface equation can be written as follows:

$$y=f(x_1, x_2, ..., x_n). \quad (2)$$

To describe the processes in the food industry, a polynomial of the second power is most often used, which takes the following form:

$$Y = b_0 + \sum_{i=1}^{n} b_i \cdot x_i + \sum_{i=1}^{n} \sum_{j=1}^{n} b_{ij} \cdot x_i \cdot x_j, \quad (3)$$

where b_0, b_i, b_{ii} are the coefficients of the regression equation.

For each of the dependent variables, an equation in polynomial form is obtained.

To achieve the set goal, a full factor experiment (FFE) of the FFE 2^m type with three replications at the center of the experiment was used in this work.

The encoded values of the input factors are related to their natural values by the following ratio:

$$X_j = \frac{Z_j - Z_j^o}{\Delta Z_j}, \; j=1, 2,...,k, \quad (4)$$

where X_j is the encoded value of the independent variable;

Z_j is the eigenvalue of the independent variable;

Z_j^o – the eigenvalue of the independent variable in the center of the plan;

ΔZ_j is the interval of change of the coefficient Z_j.

The levels of variance of the corresponding input factors (independent variables) are given in Table 4.

Table 4

Levels of variance and names of explanatory variables

Independent variable	Variance level			ΔZ_j
	–1	0	+1	
Whey volume, g (C1) – x_1	250	625	1,000	375
Starter volume, ml, (C2) – x_2	0.005	0.0125	0.02	0.0075

As a dependent variable, the rate of lactic acid microorganisms, CFU/g (C4) is selected.

The matrix in natural and coded form, on which the experiments were conducted, is given in Tables 5, 6.

Table 5

Experiment design in its natural form PFE 2^2 +3

No.	x_1, whey volume, g, (C1)	x_2, starter volume, ml, (C2)
1	250	0.005
2	1,000	0.005
3	250	0.02
4	1,000	0.02
5	625	0.0125
6	625	0.0125
7	625	0.0125

Table 6

Experiment design in coded form PFE 2^3 +3

No.	x_1, whey volume, g, (C1)	x_2, starter volume, ml, (C2)
1	–1	–1
2	1	–1
3	–1	1
4	1	1
5	0	0
6	0	0
7	0	0

Table 7 gives the results of experiments to determine the effect of the volume of the microMilk KF 100 starter culture and the volume of whey on the growth of lactic acid microorganisms in pastille marmalade products.

Table 7

Results of experiments to determine the effect of the volume of the microMilk KF 100 starter culture and the volume of whey on the growth of lactic acid microorganisms in pastille marmalade products

No.	The number of lactic acid microorganisms (the micromilk starter culture), g (C1)	The amount of whey, ml (C2)	Survival time of lactic acid microorganisms, h (C3)	Growth of lactic acid microorganisms, CFU/g (C4)
1	250	0,005	6	0
2	1000	0,005	6	0
3	250	0,02	6	3
4	1000	0,02	6	0
5	625	0,0125	6	1
6	625	0,0125	6	1
7	625	0,0125	6	0

Table 8

Analysis of variance for C4

Source	Sum of squares	*Df*	Mean square	F-ratio	P-Value
A:C1	2.25	1	2.25	63.00	0.0042
B:C2	2.25	1	2.25	63.00	0.0042
AB	2.25	1	2.25	63.00	0.0042
Total error	0.107143	3	0.0357143	–	–
Notal (corr.)	6.85714	6	–	–	–

R^2=98.4375 percent.

R^2 (corrected for d.f.) =96.875 percent.

Standard estimation error=0.188982.

Average absolute error=0.122449.

The calculation of the coefficients was carried out in the STATISTICA software and is given in Table 9. Parallel experiments were conducted, and 3 experiments were performed for each set of parameters.

The following normalized regression equation is obtained:

$$y=0.58633-0.5833x_1+0.5833x_2-0.5833x_1 \cdot x_2, \quad (5)$$

where y is the output variable corresponding to the growth of lactic acid microorganisms, CFU/g (C4).

The coefficient of determination R^2=0.82 indicates a good statistical significance and reliability of the regression equation.

On the basis of the above equation, the response surface is constructed (Fig. 5).

Fig. 5 shows that an increase in C2 and a decrease in C1 increase the target variable. The highest value – the number of lactic acid microorganisms from 1.0 to 3.2 CFU/g – was detected when applying the starter culture in a volume from 0.01 to 0.02 g. The optimal concentration for the revival of lactic acid streptococci is the volume of whey from 200 to 300 ml. Such values indicate a maximum increase in the number of colony-forming units in pastille marmalade products. A study was also conducted to determine the time of the revival of lactic acid microorganisms in the period from 6 to 24 hours. This study did not show a significant dependence on the growth of lactic acid microorganisms, and therefore this factor was not considered in the analysis since the use of 6 hours is sufficient.

Table 9

Calculation of coefficients in the STATISTICA software, to check their significance

Factor	effects estimator R^2=0,82123; Speed 0,75419 Two-level plan 2k=2; Residual (SS – sum of deviation squares) *SS*=0,3333333									
	Effect	Standard error	*T* (8)	*P*	–95 % Confidence limit	+95 % Confidence limit	Coefficient	Standard error coefficient	–95 % Confidence limit	+95 % Confidence limit
Mean/ Free term	0.58333	0.166667	3.5	0.008079	0.19900	0.967667	0.583333	0.166667	0.198999	0.967667
C1	–1.16667	0.333333	–3.5	0.008079	–1.93533	–0.397999	–0.583333	0.166667	–0.967667	–0.198999
C2	1.16667	0.333333	3.5	0.008079	0.39800	1.935335	0.583333	0.166667	0.198999	0.967667
C1 per C2	–1.16667	0.333333	–3.5	0.008079	–1.93533	–0.397999	–0.583333	0.166667	–0.967667	–0.198999

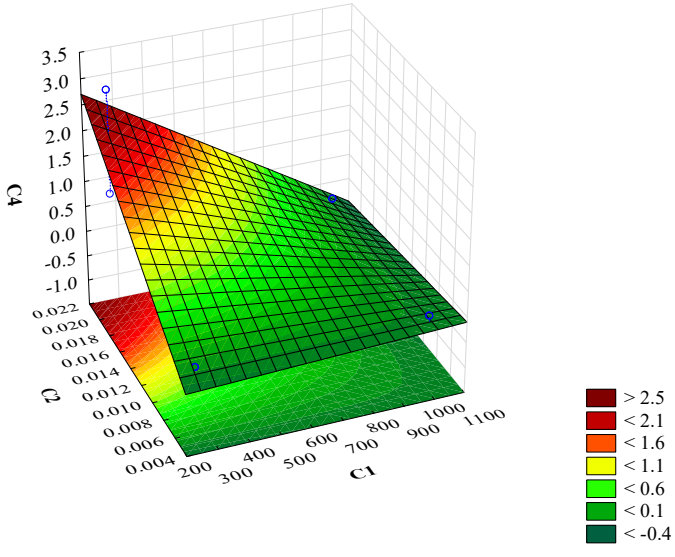
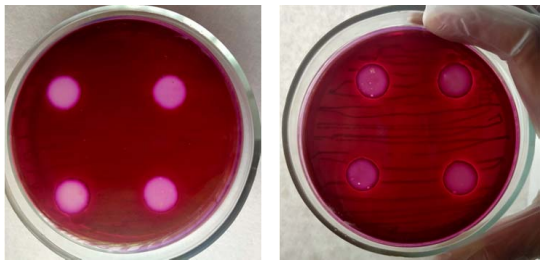
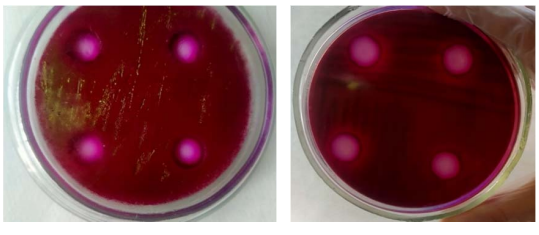


Fig. 5. Natural response surface demonstrating the effect of the microMilk KF 100 starter culture quantity and whey volume on the growth of lactic acid microorganisms in pastille marmalade products



a *b*

Fig. 6. Antimicrobial activity of lactic acid microorganisms isolated from the prototypes of marmalade in relation to *E. coli* (stroke culture). Zone of clarification — 0.1 0.3 mm: *a* — sample No. 4 in relation to *E. coli*; *b* — sample No. 5 in relation to *E.coli*



a *b*

Fig. 7. Antimicrobial activity of lactic acid microorganisms isolated from the prototypes of pastille marmalade products in relation to *E.coli* (Method of seeding with a depleting stroke). Zone of clarification — 0.3—0.5 mm: *a, b* — sample No. 6 in relation to *E. coli*

5. 4. Investigation of immunostimulating properties of the prototypes of pastille marmalade products enriched with lactic acid starters

Many strains of lactic acid microorganisms are probiotics. It has been established that probiotic strains of microorganisms give a multifaceted effect. For example, probiotics have a beneficial effect on diarrhea caused by clostridia or rotaviruses, as well as associated with antibiotics or chemotherapy. Probiotics can affect certain immunological parameters, for example, enhance the activity of phagocytes (macrophages) and lymphocytes.

It is scientifically substantiated that lactic acid microorganisms contribute to the restoration of intestinal microflora: stimulate intestinal motility, reduce gas formation, and improve the digestibility of calcium, phosphorus and iron [21].

The main technological properties of starter cultures include the fermentation of carbohydrates with the formation of lactic acid, antagonistic activity to the sanitary-indicative microflora, the synthesis of bacteriocins and antibiotic-like compounds. Antioxidant activity (due to the release by cells of enzymes such as catalase, peroxidase, and superoxide dismutase, necessary to eliminate the toxic effect of oxygen) can also be attributed [19].

Taking into consideration that in the prototype of pastille marmalade product No. 6, the growth of 3 colonies of lactic acid microorganisms was detected, it can be judged that this product has useful properties. To substantiate the immunostimulating properties of the prototype of pastille marmalade product No. 6, the antimicrobial properties of the isolated colonies in relation to *E.coli* were determined. The results are shown in Fig. 6.

These figures indicate the antimicrobial activity of lactic acid microorganisms isolated from the prototypes of marmalade in relation to *E.coli*. The sowing was carried out by the stroke method, the clarification zones are from 0.1 mm to 0.3 mm. To obtain a more reliable result, the antimicrobial activity of lactic acid microorganisms was determined by sowing a depleting stroke. The results are shown in Fig. 7.

The method of sowing with a depleting stroke made it possible to identify the zones of suppression of E. coli more clearly. On the surface of the nutrient medium Endo there is a slight increase in *E. coli*, and the zones of clarification are clearly visible and range from 0.3 mm to 0.5 mm. This result indicates the probiotic properties of marmalade with the starter microMilk KF 100, which helps increase immunity.

The main cause of many diseases and premature aging of people is the formation of an excessive volume of free radicals – particles of molecules of some substances containing oxygen of high reactivity. It was found that the human body can resist their destructive effect only with the help of antioxidants (antioxidants). Antioxidants block free radicals, prevent destructive oxidative processes in the body, stimulate the human immune system and prevent the risk of occurrence and risk of reducing diseases, including cancer [19].

Further studies were aimed at studying the effect of lactic acid starters on antioxidant activity, the results of which are demonstrated in Fig. 8.

As can be seen from Fig. 8, the introduction of lactic acid cultures into the formulation of pastille marmalade products has a positive effect on increasing the antioxidant properties of the product. When using the Camembert starter culture, the volume of antioxidants increased by 2.2 times compared to the control, and when using the microMilk KF 100 starter culture – by 1.7 times. This indicates that the developed pastille marmalade products, in addition to having probiotic properties, additionally have antioxidant properties. This result is further evidence that the developed pastille marmalade products have a probiotic and probiotic and immunostimulating effect on the human body.

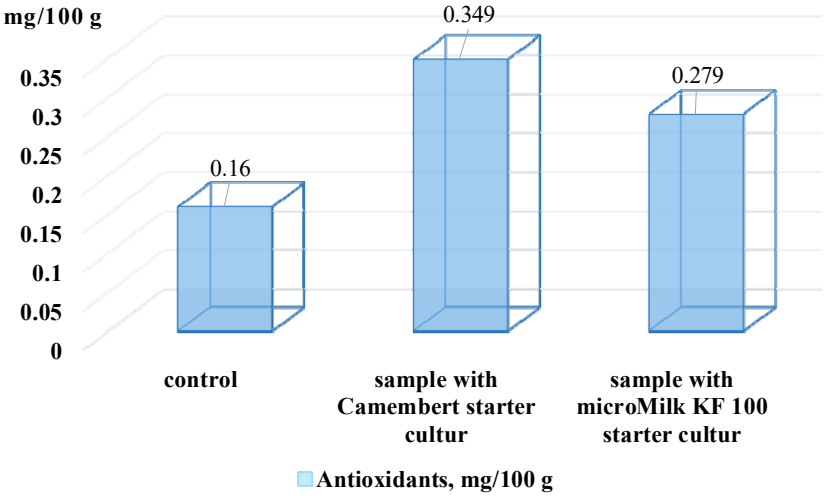


Fig. 8. Antioxidant content in pastille marmalade products in the control sample and in samples enriched with the lactic acid starters microMilk KF 100 and Camembert

In terms of quality and safety, the developed product had high consumer properties and met the requirements of TR CU 021/2011 "On food safety".

6. Discussion of the results of the study of pastille marmalade products enriched with lactic acid starters

The obtained results confirm the immunostimulating effect of pastille marmalade products enriched with starters of lactic acid microorganisms. According to the results given in Table 1, it can be seen that the more active starter is the microMilk KF 100. For this starter culture, the rational conditions were the introduction of 0.02 g of culture per 250 ml of whey, the duration of the revival of 6 hours at a temperature of 37 °C.

It is known that active strains of lactic acid streptococci coagulate milk in 4–6 hours, forming an even dense clot. The effect of the volume of starter culture on the growth of living cells of lactic acid streptococci in pastille marmalade products is due to a large concentration of culture (0.02 g per 250 ml of whey), respectively, active revival in milk whey. In addition, the fruit and berry base of marmalade serves as a substrate for lactic acid microorganisms. The affiliation of the grown colonies isolated from the prototypes of pastille marmalade products to lactic acid microorganisms was investigated. This is evidenced by the macro- and micromorphology of the grown colonies (Fig. 2, 3).

The activity of the microMilk KF 100 starter culture compared to the Camembert starter culture is characterized by the fact that the microMilk KF 100 starter culture contains *Streptococcusthermophiles,* which have high thermal stability. It withstands a temperature of 75 °C for 15 minutes, as a result of which it makes up a significant part of the residual microflora in pastille marmalade products after heat treatment.

The multifactorial experiment made it possible to substantiate and identify the main patterns affecting the growth of lactic acid microorganisms in the finished product of such factors as the number of lactic acid microorganisms (microMilk starter), the volume of whey, and the time of the revival of lactic acid microorganisms.

The immunostimulating properties of the prototype of pastille marmalade product No. 6 were determined. The zones of clarification of the isolated colonies in relation to *E.coli* are from 0.1 mm to 0.5 mm (Fig. 6, 7).

It has been experimentally proven and shown that the developed pastille marmalade products, in addition to probiotic properties, also have antioxidant properties (Fig. 8).

Analysis of patents and research results show that lactic acid bacilli, *Lactobacillus plantarum, Lactobacillus plantarum, Lactobacillus acidophilus, Lactobacillus acidophilus, Lactobacillus bulgaricus,* and others are used in the development of pastille marmalade products. However, information on the use of lactic acid streptococci (*Lactococcus, Leuconostoc, Streptococcus*) is missing. Lactic acid streptococci, unlike rods, form a fragrance, absorb citrates, and in combination form density. For the growth and reproduction of streptococci, amino acids, vitamins, and nitrogenous bases are necessary, and for rods mainly amino acids. The peculiarity of the results obtained in comparison with those reported in [9] is that lactic acid streptococci were used in our work.

This study has limitations on the reproducibility of the results because microbiological studies are conducted manually, it is important to observe the conditions of asepsis, to control the sterility of the nutrient medium, the conditions of cultivation of microorganisms, and conduct a biochemical analysis of the grown colonies. Moreover, in order to identify lactic acid microorganisms, it is important to carry out microbiological seeding of pastille marmalade products immediately after readiness for use of the product. It is necessary to plan the time of preparation of the starter, the production of pastille marmalade products, and microbiological seeding. To obtain reliable results, it is important to conduct at least a three-time study, and if necessary, more, which takes time.

The following shortcomings of the study were noted: this is the heat treatment of pastille marmalade products with the starters of lactic acid microorganisms, which partially destroys the living cells of microorganisms. In order to obtain more living cells of lactic acid microorganisms, the technology of pastille marmalade products for introducing starter cultures into different modes is being worked out. It is important to observe the vital activity of lactic acid microorganisms in milk whey. The interval between the revival of lactic acid microorganisms and its further introduction into pastille marmalade products should not exceed three days.

Research into the shelf life of a product with lactic acid microorganisms continues. The analyzed samples are in the aging cabinet and undergo periodic testing.

The development of this study is to create dietary immunostimulating pastilles for children.

7. Conclusions

1. Prototypes of pastille marmalade products with the starters of lactic acid microorganisms have been devel-

oped. The active starter chosen was the microMilk KF 100, the rational conditions for the revival of which were the introduction of 0.02 g of culture per 250 ml of whey, the duration of the revival of 6 hours at a temperature of 37 °C.

2. Prototypes of pastille marmalade products contained live cells of lactic acid microorganisms (from 1 to 3 CFU/g). Biochemical studies (micromorphology) have confirmed the belonging of microorganisms to lactic acid.

3. Based on the regression equation, it was revealed that the growth of lactic acid microorganisms in the product is affected by the volume of whey (250 ml), the volume (0.02 g), and the fermentation of the starter (6 hours). Significant factors are the volume of application of the starter culture of lactic acid microorganisms from 0.01 to 0.02 g and the volume of whey 250 ml, which affect the growth of lactic acid microorganisms (increases) in the product.

4. The prototypes of pastille marmalade products contained an increased volume of antioxidants compared to the control (an increase of 1.7 and 2.2 times). In addition, they contained live cells of lactic acid microorganisms (from 1 to 3 CFU/g), which showed antimicrobial activity against *E. coli* (the zones of clarification are from 0.1 mm to 0.5 mm), which characterize their immunostimulating properties.

Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this paper.

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