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.
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 immuno-stimulating confectionery products enriched with starters of lactic acid microorganisms seems to be a relevant task for the 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 Leucosanostoc 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. Leucosanostoc 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.
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.

MicroMilk KF 100 starter culture composition:
- Lactococcus lactis;
- Lactococcus cremoris;
- Lactococcus diacetylactis;
- Leuconostoc mesenteroides subsp. cremoris.

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, Envis, 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);
Solids are converted into liquid forms before analysis. by mixing the corresponding substances with the solvent. The correctness and reproducibility of the analysis are ensured (registration and processing of results) takes place in real time. 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.

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. 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].

4. 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 $10^5$ 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

| 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 (acetalddehyde, diacetyl), etc. Cells of lactic acid microorganisms have a spherical or rod-shaped shape.

Fig. 2. Macromorphology of microorganisms 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.

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 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 less lactic acid starters, the opposite effect was obtained, and the absence of lactic acid microorganisms in the finished 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

| Variable factors | Designation | Level     | C1   | C2   | C3  |
|------------------|-------------|-----------|------|------|-----|
| 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    |

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

| Effect                  | One-dimensional significance criterion for C4 |
|-------------------------|-----------------------------------------------|
|                         | SS               | Degree of freedom | MS            | F             | P             |
| Free term               | 4.481481         | 1                 | 4.481481      | 48.400000     | 0.000118      |
| C1                      | 4.962963         | 2                 | 2.481481      | 26.800000     | 0.000284      |
| C2                      | 4.962963         | 2                 | 2.481481      | 26.800000     | 0.000284      |
| C3                      | 1.185185         | 2                 | 0.592593      | 6.400000      | 0.021883      |
| C1*C2                   | 4.932933         | 4                 | 1.148148      | 12.400000     | 0.001654      |
| C1*C3                   | 1.037037         | 4                 | 0.259259      | 2.800000      | 0.100469      |
| C2*C3                   | 1.037037         | 4                 | 0.259259      | 2.800000      | 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. 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:

$$y = b_0 + \sum_{i=1}^{n} b_i \cdot x_i + \sum_{i=1}^{n} \sum_{j=i+1}^{n} b_{ij} \cdot x_i \cdot x_j.$$  \hspace{1cm} (2)

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

$$Y = b_0 + b_1 \cdot x_1 + b_2 \cdot x_2 + \sum_{i=1}^{n} \sum_{j=1}^{n} b_{ij} \cdot x_i \cdot x_j,$$  \hspace{1cm} (3)

where $b_0, b_1, b_{ij}$ 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:

$$X_j = \frac{Z_j - Z_{j,0}}{\Delta Z_j}, \quad j=1, 2, ..., k,$$  \hspace{1cm} (4)

where $X_j$ is the encoded value of the independent variable; $Z_j$ is the eigenvalue of the independent variable; $Z_{j,0}$ is the eigenvalue of the independent variable in the center of the plan; $\Delta Z_j$ 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 | Variance level | ΔZ |
|------------------------------------------------------|----------------|----|
| Whey volume, g (C1) – $x_1$                           | 250            | 375 |
| Starter volume, ml (C2) – $x_2$                        | 0.005          | 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

| 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

| 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.

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

| 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

| Source | Sum of squares | Df | Mean square | F-ratio | P-Value |
|--------|----------------|----|-------------|---------|---------|
| A:C1   | 2.25           | 1  | 2.25        | 63.00   | 0.0042  |
| A:C2   | 2.25           | 1  | 2.25        | 63.00   | 0.0042  |
| A:B    | 2.25           | 1  | 2.25        | 63.00   | 0.0042  |
| Total  | 0.107143       | 3  | 0.035714    | –       | –       |
| Notal  | 6.857144       | 6  | –           | –       | –       |

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

Effects estimator \( R^2 = 0.82123 \); Speed 0.75419

Two-level plan 2k=2; Residual (SS – sum of deviation squares) \( SS = 0.3333333 \)

| Factor | Effect | Standard error | \( T (8) \) | \( P \) | -95 % Confidence limit | +95 % Confidence limit | Coefficient | Standard error | -95 % Confidence limit | +95 % Confidence limit |
|--------|--------|----------------|-------------|-------|------------------------|------------------------|-------------|----------------|------------------------|------------------------|
| Mean/ Free term | 0.583333 | 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 | 1.93533 | 0.397999 | 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 |

\[ R^2 = 98.4375 \text{ percent.} \]

\[ R^2 (corrected for d.f.) = 96.875 \text{ percent.} \]

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:

\[ y = 0.58633 - 0.5833x_1 + 0.5833x_2 - 0.5833x_1x_2, \] (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.
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.
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 Streptococcus thermophilus, 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 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-
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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References

1. Nigam, D. (2018). Probiotics as Functional Foods in Enhancing Gut Immunity. Functional Food and Human Health, 59–82. doi: https://doi.org/10.1007/978-981-13-1123-9_4
2. Alkhadith, A. (2020). Antiviral Functional Foods and Exercise Lifestyle Prevention of Coronavirus. Nutrients, 12 (9), 2633. doi: https://doi.org/10.3390/nu12092633
3. Bozhkova, S. E., Pugorelets, T. P., Gaivoronskaya, N. S., Plipenko, D. N., Surkova, S. A. (2019). Technology of production of granulated cottage cheese with usage of dietary fiber. Agrarian-And-Food Innovations, 5 (1), 77–83. doi: https://doi.org/10.31208/2018-7353-2019-5-77-83
4. Stambeckova, A. K., Elemesova, A. A., Bayakhan, A. A., Alimardanova, M. K., Petchenko, V. I., Levochkina, N. A. (2022). Development of the recipe, technology of a functional fermented milk product with dill greens and “Dzhusay.” BIO Web of Conferences, 43, 03043. doi: https://doi.org/10.1051/bioconf/20224303043
5. Cronin, P., Joyce, S. A., O’Toole, P. W., O’Connor, E. M. (2021). Dietary Fibre Modulates the Gut Microbiota. Nutrients, 13 (5), 1655. doi: https://doi.org/10.3390/nu13051655
6. Oripova, M. Z., Kuzieva, Z. N., Oshechekov, Y. I., Salikhov, S. I. (2022). Obtaining Chitosan from Artemia Cysts and Studying its Sorption Properties. Pharmaceutical Chemistry Journal, 55 (11), 1234–1239. doi: https://doi.org/10.1007/s11094-022-02563-9
7. Fatullayeva, S., Tagiyev, D., Zeynalov, N. (2021). A review on enterosorbents and their application in clinical practice: Removal of toxic metals. Colloid and Interface Science Communications, 45, 100545. doi: https://doi.org/10.1016/j.jcolcom.2021.100545
8. Sarnatskaya, V., Mikhailenko, V., Prokopenko, I., Gerashchenko, B. I., Shevchuk, O., Yushko, L. et. al. (2020). The effect of two formulations of carbon enterosorbents on oxidative stress indexes and molecular conformation of serum albumin in experimental animals exposed to CCl4. Heliyon, 6 (1), e03126. doi: https://doi.org/10.1016/j.heliyon.2020.e03126
9. Kucher, S. V., Lototska, O. V. (2021). Inclusion of enterosorbents in anti-inflammatory therapy improve treatment effectiveness in copd patients during exacerbations. The Ukrainian Biochemical Journal, 93 (2), 107–114. doi: https://doi.org/10.15407/ubj93.02.107
10. Tsukanov, V. V., Vasyutin, A. V., Tonkikh, J. L., Gorchilova, E. G., Rzhavicheva, O. S., Borisov, A. G. (2020). Possibilities of application of enterosorbent in combined therapy of opisthorchiosis patients with skin syndrome. Meditsinskii Sovet = Medical Council, 5, 70–76. doi: https://doi.org/10.21518/2079-701x-2020-5-70-76
11. Budnyak, T. M., Vlasova, N. N., Golovkova, L. P., Slabon, A., Tertykh, V. A. (2019). Bile acids adsorption by chitoan-fumed silica enterosorbent. Colloid and Interface Science Communications, 32, 100194. doi: https://doi.org/10.1016/j.jcolcom.2019.100194
12. He, Y., Wang, B., Wen, L., Wang, F., Yu, H., Chen, D. et. al. (2022). Effects of dietary fiber on human health. Food Science and Human Wellness, 11 (1), 1–10. doi: https://doi.org/10.1016/j.fshw.2021.07.001
13. Alimardanova, M. K., Baybolova, L. K., Zh. al., Mutushev, A. Zh. et. al. (2019). Pat. No. 4406 RK. Sposob proizvodstva yogurta. No. 2019/0316.2
14. Shunekeyeva, A. A., Alimardanova, M., Albertovich, M. A. (2021). Chemical Composition, Texture and Sensory Evaluation of Yogurts Supplemented with Amaranth Flour. American Journal of Animal and Veterinary Sciences, 16 (2), 136–143. doi: https://doi.org/10.3844/ajavsp.2021.136.143
15. Tavakoli, M., Habibi Najafi, M. B., Mohebbi, M. (2019). Effect of the milk fat content and starter culture selection on proteolysis and antioxidant activity of probiotic yogurt. Heliyon, 5 (2), e01204. doi: https://doi.org/10.1016/j.heliyon.2019.e01204
16. Irvine, S. L., Hummelen, R., Hekmat, S. (2011). Probiotic yogurt consumption may improve gastrointestinal symptoms, productivity, and nutritional intake of people living with human immunodeficiency virus in Mwanza, Tanzania. Nutrition Research, 31 (12), 875–881. doi: https://doi.org/10.1016/j.nutres.2011.10.005
17. Sarangi, M., Bhattacharyya, S., Behera, R. C. (2009). Effect of temperature on morphology and phase transformations of nano-crystalline silica obtained from rice husk. Phase Transitions, 82 (5), 377–386. doi: https://doi.org/10.1080/01411590902978502
18. Munotihar, A. S. (2002). Utilization of uncontrolled burnt rice husk ash in soil improvement. Dimensi Teknik Sipil, 4 (2), 100–105. Available at: https://tekniskipil.umy.ac.id/wp-content/uploads/2011/06/CIV02040207.pdf
19. Sapei, L., Suseno, N., Riadi, L., Padmawijaya, K. S., Wurarah, T. S., Dewi, V. (2018). Biosilica recovery from pulped rice husk by acid precipitation. In: Chemeca 2018: Chemical Engineering in Australasia. Available at: http://repository.ubaya.ac.id/34069/
20. Todkar, B. S., Deorukhkar, O. A., Deshmukh, S. M. (2016). Extraction of Silica from Rice Husk. International Journal of Engineering Research and Development, 12 (3), 69–74. Available at: http://www.ijerd.com/paper/vol12-issue3/Version-2/H12326974.pdf
21. Karavay, L. V., Levochkina, L. V. (2008). Gidrolizovannaya risovaya shelukha dlya proizvodstva muchnykh izdeliy. Pschevaya promysshlennost’, 11, 53. Available at: https://cyberleninka.ru/article/n/gidrolizovannaya-risovaya-sheluha-dlya-proizvodstva-muchnyh-izdeliy
22. Kruglova, A. S., Selina, A. A., Minakova, P. S. (2020). Issledovanie aktivnogo kremniya v otkhodakh plodovykh obolochok risa i solomy. Eurasian Scientific Association, 9-3 (67), 168–170. Available at: https://www.eilib.ru/item.asp?id=44122569
23. Galchenko, A. V., Sherstneva, A. A., Levina, M. M. (2021). Conditionally essential trace elements in nutrition of vegetarians and vegans: fluorine, silicon, bromine, boron. Trace elements in medicine, 22 (1), 32–43. doi: https://doi.org/10.19112/2413-6174-2021-22-1-32-43
24. Ailmardanova, M., Tlevlessova, D., Bakiyeva, V., Akpanov, Z. (2021). Revealing the features of the formation of the properties of processed cheese with wild onions. Eastern-European Journal of Enterprise Technologies, 4 (11 (112)), 73–81. doi: https://doi.org/10.15587/1729-4061.2021.239120