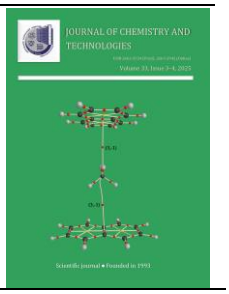
Journal of Chemistry and Technologies, 2025, *33*(3), 831-839

Journal of Chemistry and Technologies

pISSN 2663-2934 (Print), ISSN 2663-2942 (Online).

journal homepage: http://chemistry.dnu.dp.ua
editorial e-mail: chem.dnu@gmail.com



UDC 005.591.6 [579.864+579.873]

INNOVATIVE PROBIOTIC FOOD PRODUCTS USING *LACTOBACILLUS REUTERI*

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Received 25 April 2025; accepted 12 June 2025; available online 20 October 2025

Abstract

The formulation features of popular food products were analyzed and the possibility of improving their quality and ability to positively affect human health by eliminating preservatives from their formulation was investigated. The antimicrobial activity of different types of probiotic microorganisms was determined, and according to the results, the microbial culture of *Lactobacillus reuteri.* was selected as the expedient one for research. When using symbiotic composition of microorganisms with *Lactobacillus reuteri*, higher antimicrobial activity is observed. Comparative evaluation of the sauce samples with preservatives and with microbial culture of *Lactobacillus reuteri* without preservatives by a set of indicators showed an increase in antioxidant activity of samples with probiotic by 10.2 % and 12.0 % for mayonnaise sauce (30 %) and mayonnaise (67 %), respectively; reduction of oxidation level by 25.0–28.6 %; improvement of microbiological safety by a number of indicators, and the absence of food poisoning pathogens; the possibility of prolonging the traditional shelf life of sauce products with probiotic ingredient, as well as creating conditions for the correction of intestinal microbiota while maintaining the main consumer properties of these popular products on the food market. For popular types of ketchup, similar positive results were also observed following the exclusion of preservatives and the incorporation of Lactobacillus reuteri monoculture and Lactobacillus reuteri symbiotic into their formulations. The preservative-free ketchup samples were not inferior to the control ketchup in terms of physicochemical parameters, had a slightly higher concentration of acids, biologically active substances (BAS) and 7.1 % higher antioxidant activity. The given technological scheme of probiotic sauces shows the possibility to produce them on existing technological lines. The results of the studies indicate the effectiveness of probiotic microorganisms *Lactobacillus reuteri* as a preservative for food products, which will allow to expand the work on their industrial application.

Keywords: sauce products; *Lactobacillus reuteri*; probiotics; biosafety; physical and chemical control; microbiological control.

ІННОВАЦІЙНІ ПРОБІОТИЧНІ ХАРЧОВІ ПРОДУКТИ З ВИКОРИСТАННЯМ *LACTOBACILLUS REUTERI*

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Анотація

Проаналізовані рецептурні особливості популярних харчових продуктів і досліджена можливість підвищити їх якість і здатність позитивно впливати на здоров'я людини шляхом виключення консервантів з їх рецептури. Визначена антимікробна активність різних видів пробіотичних мікроорганізмів, за результатами якої обрано доцільну для досліджень мікробну культуру *Lactobacillus reuteri.* Під час використання симбіотичного складу мікроорганізмів з *Lactobacillus reuteri* спостерігається висока антимікробна активність. Порівняльна оцінка контрольних зразків соусної продукції з консервантами і зразків з мікробною культурою *Lactobacillus reuteri* без використання консервантів за комплексом показників показала підвищення антиоксидантної активності зразків з пробіотичним інгредієнтом на 10.2 % та 12.0 % для майонезного соусу (30 %) і майонезу (67 %) відповідно; зниження рівня окиснення на 25.0–28.6 %; покращення мікробіологічної безпеки та відсутність збудників харчових отруєнь; можливість пролонгування традиційних термінів зберігання соусної продукції з пробіотичним інгредієнтом, а також створення умов для корекції кишкової мікробіоти за умови збереження основних споживчих властивостей популярної на продовольчому ринку продукції. Аналогічні позитивні результати виключення консервантів та введення в рецептуру монокультури *Lactobacillus reuteri* і симбіотика з *Lactobacillus reuteri* спостерігали для популярних видів кетчупу. Наведена технологічна схема пробіотичних соусів показує можливість виробляти їх на існуючих технологічних лініях. За результатами досліджень для соусної продукції різних типів вперше визначена доцільність використання пробіотичних мікроорганізмів *Lactobacillus reuteri* як ефективних замінників консервантів у рецептурі.

Ключові слова: соусні продукти; *Lactobacillus reuteri;* пробіотики; біобезпека; фізико-хімічний контроль; мікробіологічний контроль.

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doi: 10.15421/jchemtech.v33i3.327820

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Introduction

Today's global food system is at the crossroads of two key challenges, namely ensuring public health and maintaining environmental sustainability. The intensive urbanization and industrialization that characterize the development of the world economy in the 21st century have significantly transformed the nutritional patterns of the population [1; 2]. The food industry and agriculture must not only produce enough food in terms of calories to feed the growing world population, but also produce a variety of foods that promote human health and can support environmental sustainability [3; 4].

Food and nutrition security is achieved when adequate food (in terms of quantity, quality, safety, socio-cultural acceptability) is available to all people and, accordingly, is used and assimilated by them to lead a healthy and active lifestyle [5; 6]. Food E-additives, including preservatives, are widely used in food production in modern conditions. This leads to prolongation of product shelf life, but has a negative impact on the health of consumers and the microbiota of their gastrointestinal tract [1; 7–9].

A number of analytical sources indicate that in recent years, the number of studies on the human gut metagenome and its impact on the gut microbiome has increased significantly in many countries due to the widespread development of gut microbiome research methods, driven by the active development of high-precision molecular biological methods, in particular next-generation sequencing (NGS) [10–12]. Probiotic microorganisms play a significant role in correcting the intestinal microbiome [13–15]. Scientific studies confirm the positive effects of probiotic cultures not only in modulating the gastrointestinal microbiota, but also within oral medicine and other clinical areas, where they contribute to maintaining microbial balance, strengthening the barrier function of the epithelium and normalizing the body's immune response [16–18]. Since the term “probiotics” refers to a heterogeneous group of live bacteria with species- and strain-specific properties, general claims about their efficacy in affecting the gut microbiota cannot be applied to every probiotic product [19; 20]. Given the number of publications on the effect of Lactobacillus reuteri strains as therapeutic agents capable of being included in the intervention in the mechanisms of attachment of pathogens, as well as interacting with the intestinal microbiota, it is scientifically appropriate and practically important to investigate the possibility of using these probiotic microorganisms in special innovative types of food products.

Assortment analysis of food products systematically produced in significant industrial volumes indicates the presence of a group of goods whose stability during storage depends directly on the use of food additives, in particular preservatives. The use of these compounds is a technological necessity to prevent microbiological spoilage and extend the shelf life of products. This category includes the sauce group of food products, in particular mayonnaises, ketchups and other emulsion and semi-liquid sauces, the formulations of which provide for the inclusion of preservatives in order to ensure the biological stability of the finished product during the declared shelf life [21; 22].

Duration of storage of sauce products in sealed and open containers varies depending on the recipe features and technological process of a particular manufacturer. As an illustrative example, the average values of shelf life for mayonnaise, ketchup and other sauces sold in the Agro-Market™ retail network in Ukraine are given [23].

According to the generalized information specified in the regulatory and technological documentation and on the product labels, the shelf life for different types of sauce products in sealed and open packaging is given in Table 1.

Table 1

Assortment and shelf life of sauce products

Name of sauce	Packaging condition	Shelf life	Notes
Ketchup	Sealed up	to 2	
Ketchup	Opened	not more than 6 months	
Sweet-and-sour sauce	Sealed	6 months	
Sweet-and-sour sauce	Opened	not more than 30 days	
Mustard	Sealed	1.5–2 years	
Mustard	Open	not more than 30 days	
Barbecue sauce	Sealed	up to 1 year	With seasoning
Barbecue sauce	Open	not more than 4 months	
Tartar sauce	Sealed	up to 18 months	
Tartar sauce	Open	not more than 6 months	
Pesto sauce	Sealed	14–16 months	

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Continuation of the table 1

Pesto sauce	Open	not more than 1 week	
Mayonnaise	Sealed	4–6 months	
Mayonnaise	Open	1–2 weeks	Prone to rapid spoilage

Most of the listed products have various E-additives in their particular, preservatives. For example, tomato sauce "Ketchup Soft" (mass fraction of solids 13.4%) contains preservatives sodium benzoate and potassium sorbate [24]; mayonnaise "Provençal Traditional" besides acidity regulator (lactic acid), contains a preservative (potassium sorbate) and other E-additives (TM "Torchin", TM 'Nestle' [25]; in the recipe of mayonnaise Jubilee TM "Norma" besides acetic (E260) and lactic acids (E270) also includes potassium sorbate (E202) [26]. The potassium sorbate preservative (E202) is also included in the high-calorie table mayonnaise "Provençal 67 % fat" "McMay" [27], low-calorie salad mayonnaise [28] and other types of sauce products.

Taking into account the above-mentioned and modern trends of healthy nutrition, the aim of the work was: to develop an assortment of sauce products without the use of preservatives, with long shelf life and probiotic properties.

The main objectives were:

- to select an appropriate probiotic culture of microorganisms according to analytical and experimental research results;
- to determine the range of popular food products for enrichment and characterize their residual microbiota;
- to establish the influence of the selected probiotic microbial culture on the microbiological landscape (contamination) of sauce products;
- to characterize the shelf life of sauces with probiotic ingredient.

Materials and Methods

For the study of antimicrobial activity, the following probiotic microorganisms were selected from the collection of microbial cultures of the Department of FCEandB ONTU: *Lactobacillus reuteri* DSM 17938, *Lactobacillus reuteri* ATCC PTA 6475, *Bifidobacterium bifidum, Lactobacillus plantarum, Lactococcus lactis ssp. Lactis.* Microorganisms were cultured on standard and modified MRS medium [29; 30].

As a test culture to establish antagonistic activity, *E. coli*, *Enterococcus faecalis*, *Bacillus cereus, Bacillus subtilis* were used also from the collection of microbial cultures of the Department of FCEandB. Test cultures were grown on agar [30].

Sauce products were manufactured in accordance with the technological instructions for the corresponding types of products with the introduction of an activated probiotic culture, in particular DSTU 4487:2015 [31]. Ketchups were tested as canned goods of group B [32]. Microbiological control of new types of products was carried out based on the following indicators: the presence of yeast, mold fungi, saccharolytic clostridia, gas-forming mesophilic bacilli according to [29; 30; 32]. Microbiological indicators were determined using classical and accelerated PCR methods and using Compact Dry microbiological media (manufacturer Nissui Pharmaceutical CO. LTD, Japan) [5; 33].

The main physicochemical and biochemical indicators of the samples were determined using classical methods [34]. Antioxidant activity was determined using a volumetric method, and the total concentration of antioxidants was estimated by the induction period of isopropylbenzene oxidation [35].

The introduction of a monoculture and symbiotic with *Lactobacillus reuteri* was carried out in pasteurized cheese whey during the production of mayonnaise products and in the plasma from tomato fruits for ketchups according to the modes given in [36, P. 109–110]. When inoculating the developed types of products, the initial concentration of viable *Lactobacillus reuteri* cells was 8.5×10^7 CFU/cm^3.

The research results were processed using mathematical statistics methods.

Results and discussion

Modern technologies diversify consumer properties and increase the nutritional value of sauce products by adjusting the composition of products. This allows to significantly expand the range of its positive effects on the human body, but additives that prolong the shelf life of these products are undesirable ingredients. To eliminate undesirable E-additives of preservative action, an appropriate probiotic culture of microorganisms was selected based on both analytical and experimental research results. Screening of strains producing antagonistically active substances to microbial cultures of various morphological and tinctorial properties was carried out (Table 2).

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The obtained results show that *Lactobacillus reuteri* strains have significantly higher antimicrobial activity for both spore-forming cultures of microorganisms and for their non-spore-forming species, as well as for both gram-positive and gram-negative microorganisms. It should be noted that when using a symbiotic composition of microorganisms, this indicator increases from the state of "sensitive", when the diameter of the growth inhibition zone is 15–24 mm, to "highly sensitive" – with a diameter of 25 mm or more. Therefore, these microorganisms were used in further studies.

Table 2

Comparative antimicrobial activity of probiotic cultures of microorganisms

Type of probiotic	Diameter of the growth inhibition zone of the microbial test culture, mm			
	E.coli	***Enterococcus faecalis***	***Bacillus cereus***	***Bacillus subtilis***
Lactobacillus reuteri DSM 17938	23.0	21.5	19.0	21.5
Bifidobacterium bifidum	13.0	14.5	13.5	12.0
Lactobacillus plantarum	14.5	16.0	12.5	13.5
Lactococcus lactis ssp. lactis	9.0	10.5	7.5	8.0
Lactobacillus reuteri ATCC PTA 6475	21.5	22.0	19.5	21.0
Lactobacillus reuteri + Lactobacillus plantarum + Lactococcus lactis	26.5	25.5	22.5	24.0

Among the popular types of food products that use preservatives and that can be dangerous due to significant contamination by microorganisms are sauce products – mayonnaise with different fat content, fruit and vegetable sauces, etc. Taking into account the relatively mild pasteurization regimes of mayonnaise and the content of components with significant contamination in them, samples of mayonnaise with low (30 %) and high fat content (67 %) were studied.

To develop a technological scheme for the production of innovative probiotic products using *Lactobacillus reuteri*, preliminary preparation of the microbial culture was carried out by inoculation into one of the ingredients of mayonnaise sauce – cheese whey, and for probiotic ketchups – into tomato plasma. This made it possible to adapt microorganisms to the peculiarities of the polycomponent composition of the sauce assortment. The technological scheme for the production of an innovative probiotic products using *Lactobacillus reuteri* is presented in Fig. 1.

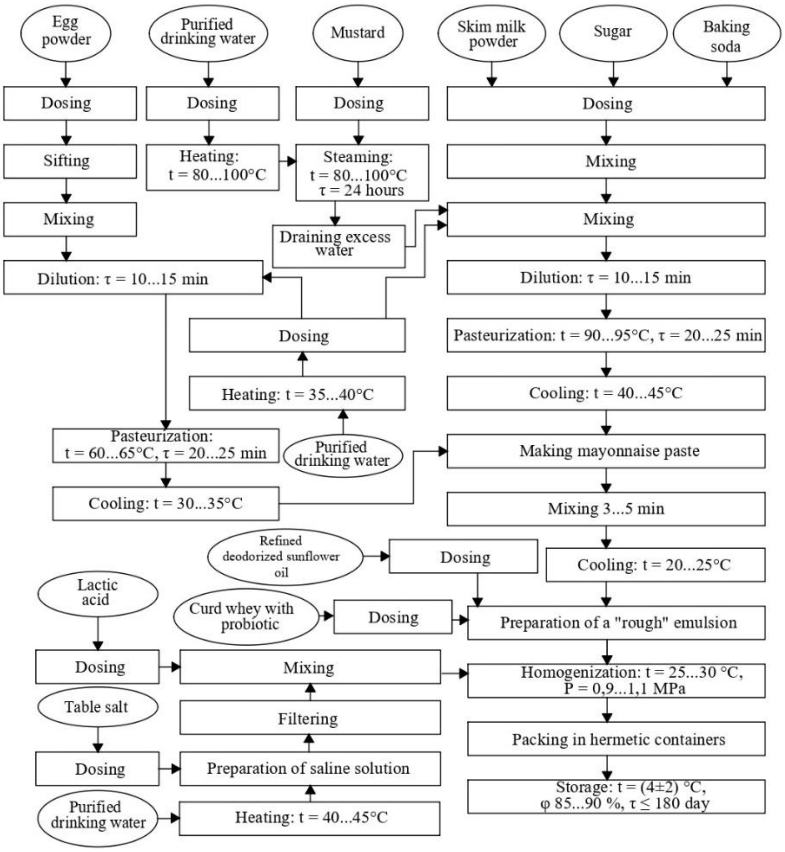

Fig. 1. Technological scheme for the production of an innovative probiotic mayonnaise products

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A characteristic feature of the given technological scheme is the preparation and addition of the microbial component to the mayonnaise sauce recipe and the exclusion of preservatives. All other technological operations are classical, but lactic acid was partially replaced by cheese whey with the addition of an activated culture of *Lactobacillus reuteri*, which allows the production of new products on existing technological equipment and does not require additional economic costs.

The results of comparative studies of control and developed samples of mayonnaise products in terms of safety, quality, stability and sensory analysis are presented in Table 3.

Table 3

Characteristics of mayonnaise products according to a complex of physicochemical, biochemical, microbiological and organoleptic indicators (n=3; p≤0.05)

Indicators	Mayonnaise sauce with preservative (control)	Mayonnaise with preservative (control)	Mayonnaise products from *Lactobacillus reuteri*	
			Mayonnaise sauce	Mayonnaise
Physico-chemical and biochemical indicators				
Fat mass fraction,%	30.0	67.0	30.0	67.0
Sedimentation stability of the emulsion,%	98.0 ± 0.7	99.0 ± 0.5	98.1 ± 0.5	98.9 ± 0.6
Viscosity, kPa·s	3105.0 ± 30.1	2860.0 ± 15.8	3197.0 ± 27.1	2900.3 ± 10.5
Acidity,%	0.85 ± 0.02	0.88 ± 0.02	0.93 ± 0.01	0.98 ± 0.03
Active acidity, pH	4.11 ± 0.02	3.91 ± 0.02	3.92 ± 0.01	3.85 ± 0.02
Vitamin E, 10^{-3}%	10.1 ± 0.2	25.7 ± 0.1	10.1 ± 0.2	25.7 ± 0.1
Stability and safety indicators				
Peroxide value, mgO_2/g	0.07 ± 0.01	0.12 ± 0.01	0.05 ± 0.01	0.09 ± 0.01
Oxidation induction period, s	89.5 ± 2.5	117.9 ± 5.7	98.6 ± 3.5	132.0 ± 5.0
Concentration of antioxidants [In H], mol/kg	(0.608 ± 0.02) · 10^{-5}	(0.802 ± 0.07) · 10^{-5}	(0.670 ± 0.04) · 10^{-5}	(0.898 ± 0.09) · 10^{-5}
Lactobacillus reuteri, CFU/g	absent	absent	(4.8 ± 0.3) ·10^7	(2.9 ± 0.4) ·10^7
Mold fungi, CFU/g	6.0 ± 1.0	4.0 ± 1.0	1.0 ± 1.0	відсутні
Yeast, CFU/g	(4.3 ± 0.1) ·10^2	(3.8 ± 0.1) ·10^2	(3.5 ± 0.1) ·10^2	(3.2 ± 0.2) ·10^2
BGCP, including Shiga toxin-producing *Escherichia coli* (STEC), CFU/g	None	None	None	None
Pathogenic microorganisms, including *Salmonella* bacteria in 25 g	None	None	None	None
Bacillus cereus, CFU/g	None	1.0 ± 1.0	None	None
Clostridium perfringens, CFU/g	None	None	None	None
Staphylococcus aureus, CFU/g	None	None	None	None
Listeria monocytogenes in 25 g	None	None	None	None
Organoleptic indicators				
Consistency and appearance	Sour cream-like, homogeneous.	Thick, homogeneous, emulsified	Sour cream-like, homogeneous.	Thick, homogeneous, emulsified
Taste and smell	Sour taste with a slight mustard flavor. The smell is characteristic of mayonnaise sauce.	Oily taste with a sour taste. The smell is characteristic of mayonnaise.	Sour taste with a slight mustard flavor. The smell is characteristic with a pronounced aroma of lactic acid.	Oily taste with a sour taste. The smell is characteristic of mayonnaise with a slight lactic-sour tinge.
Color	White, homogeneous throughout	Yellowish-cream, homogeneous throughout	White, homogeneous throughout	Yellowish-cream, homogeneous throughout

As shown in the results in Table 3, the exclusion of preservatives from the formulation and the introduction of a microbial probiotic ingredient led to an improvement in almost all the studied indicators. In samples with a probiotic culture, a high number of microorganisms is recorded (from $2.9 \cdot 10^7$ to $4.8 \cdot 10^7$ CFU/g), which is not present in the control samples, and this confirms the viability of the added probiotics and the probiotic properties of this popular food product.

Considering the possibility of long-term storage of sauce products, it is especially

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important to increase the antioxidant activity and microbiological stability of the product. According to the results obtained, the antioxidant activity of the samples increases when a probiotic ingredient is introduced into the product by 10.2 and 12.0 % for mayonnaise sauce (30%) and mayonnaise (67 %), respectively. Comparing the obtained results with the known literature data [37–41], *Lactobacillus reuteri* has a medium-high antioxidant property according to in vitro radical neutralization tests (DPPH, ABTS, FRAP) and protects intestinal cells from oxidative stress. Along with antioxidant activity, significant antimicrobial activity is observed as shown in Table 3.

According to the results of microbiological studies: the number of mold fungi in probiotic sauces is low (up to 1.0 CFU/g), and there are no fungi in mayonnaises; yeast is stored in quantities significantly lower than the permissible level ($1 \cdot 10^3$ CFU/g), which indicates a stable quality of sanitary control; pathogenic microorganisms were not found; and *Bacillus cereus* is only in the control sample of mayonnaise.

As can be seen from the results presented in Table 3, the use of probiotic cultures of microorganisms *Lactobacillus reuteri* can be considered as an effective substitutes for preservatives in the recipe of sauce products. It should also be noted that in terms of safety, quality, stability and sensory analysis of mayonnaise products, the obtained samples even exceed the control samples, namely – the addition of *Lactobacillus reuteri* to mayonnaise products contributes to an increase in antioxidant activity, a decrease in the level of oxidation, an improvement in microbiological safety, and the creation of conditions for the correction of intestinal microbiota while maintaining the main consumer properties.

The studied samples were stored and analyzed after 3 and 6 months for a set of quality indicators. Based on the results of the analysis of the peroxide value after product preparation, in samples with *Lactobacillus reuteri,* this indicator is lower than in the control ones by 25.0 - 28.6%, and this allows predicting better antioxidant stability of probiotic products during storage, which is also confirmed by a number of studies [37; 42–44].

The results of the analysis of quality, safety and stability of the developed samples after storage confirmed this theoretical prediction. Sensory analysis also showed better organoleptic indicators for probiotic products compared to the control ones.

Ketchups are quite popular both in terms of assortment and in terms of quantitative consumption. Preservatives (most often potassium sorbate and sodium benzoate) are added to most of their types according to the recipe. Therefore, a preliminary series of studies was conducted with the exclusion of preservatives and the introduction of a probiotic monoculture of microorganisms and a symbiotic with *Lactobacillus reuteri* into their recipe (Table 4).

Table 4

Complex of composition and safety indicators for ketchups (n=3; p≤0.05)

Indicators	Ketchup with preservative (control)	Ketchup with *Lactobacillus reuteri*
Physico-chemical and microbiological parameters		
Mass fraction of dry matter, %	27.0 ± 0.37	27.6 ± 0.20
Acidity, %	0.97 ± 0.05	1.1 ± 0.03
Active acidity, pH	4.08 ± 0.03	3,95 ± 0.04
Chlorides, %	2.1 ± 0.15	2.0 ± 0.1
Vitamin C, 10^{-3}%	10.0 ± 0.2	10.6 ± 0.1
Carotenoids, 10^{-3}%	0.55 ± 0.01	0.57 ± 0.02
Oxidation induction period, s	84.0 ± 2.0	90.0 ± 1.0
Concentration of antioxidants [In H], mol/kg	$0.57 \cdot 10^{-5}$	$0.61 \cdot 10^{-5}$
Lactobacillus reuteri, CFU/g	None	$(2.8 \pm 0.3) \cdot 10^7$
Mold fungi, CFU/g	None	None
Yeast, CFU/g	None	None
BGCP, including Shiga toxin-producing Escherichia coli (STEC), CFU/g	None	None
Pathogenic microorganisms, including bacteria of the genus *Salmonella*, in 25g	None	None
Bacillus cereus, CFU/g	1.0 ± 1.0	None
Clostridium perfringens, CFU/g	None	None
Staphylococcus aureus, CFU/g	None	None

Continuation of the table 4

Listeria monocytogenes in 25 g	None	None
Organoleptic indicators		
Consistency and appearance	Thick homogeneous consistency, without impurities	Thick homogeneous consistency, without impurities
Taste and smell	Taste and smell typical of ketchup	Taste and smell typical of ketchup with a pleasant sour aftertaste
Color	Red homogeneous with a brownish tint	Red homogeneous with a barely noticeable brownish tint

As can be seen from Table 4, the ketchup samples with *Lactobacillus reuteri* are not inferior to the control sample in terms of physico-chemical parameters and do not contain prohibited microorganisms. Like the control sample, they are characterized by appropriate organoleptic parameters, have a homogeneous consistency, pleasant taste and aroma properties, better color and 7.1 % higher antioxidant activity compared to the control. A significant difference of ketchup with *Lactobacillus reuteri* is the absence of preservative in the presence of probiotic properties in the number of microbial cells. The microbiological indicators of ketchup samples obtained without preservatives met sanitary requirements. This made it possible to store the obtained samples for further research in order to determine the optimal storage periods.

Thus, the analysis of control and test samples of ketchup in terms of a set of safety, quality and sensory properties, as well as for mayonnaise products, supported the possibility of refusing preservatives.

Conclusions

According to the results of the antimicrobial activity of various types of probiotic microorganisms, the microbial culture *Lactobacillus reuteri* was selected as appropriate for research.

The recipe features of popular food products were analyzed and the need to exclude preservatives from a certain range of multi-component sauce products was substantiated.

A comparative assessment of control samples of sauce products with preservatives and samples with the *Lactobacillus reuteri* microbial culture without preservatives showed an increase in the antioxidant activity of samples with a probiotic by 10.2 % and 12.0 % for mayonnaise sauce (30 %) and mayonnaise (67 %), respectively; a decrease in the level of oxidation by 25.0–28.6 %; improvement of microbiological safety by a number of indicators; absence of food poisoning pathogens; the possibility of prolonging the traditional shelf life of sauce products with a probiotic ingredient, as well as creating conditions for correcting the intestinal microbiota while maintaining the basic consumer properties of these popular products with consumers. Similar positive results regarding the exclusion of preservatives and the introduction of a probiotic monoculture of microorganisms and a symbiotic with *Lactobacillus reuteri* into the recipe were observed for popular types of ketchup. Samples of ketchup without preservatives were not inferior to the control ketchup in terms of physico-chemical parameters, had a slightly higher concentration of acids, bacterial barriers, and 7.1 % higher antioxidant activity.

The results of the studies indicate the effectiveness of probiotic microorganisms *Lactobacillus reuteri* as a preservative for food products, which will allow to expand the work on their industrial application.

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