

# Substantiated Screening of Functional Ingredients for Extended Shelf Life of Fermented Milk Products

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**Abstract**—The objective is to conduct a substantiated screening of functional ingredients: probiotic cultures, biopolymers and dietary fibers immobilized in gel for fermented specialized milk products. Based on the studies, it was found that the addition of Citri-Fi fiber to a fermented product in a dosage of 1.5% allows you to change the quantitative ratio of water holding forms in the fermented product, while increasing the viability of beneficial microbiota. The use of fermented probiotic cultures immobilized in the mixture consisting of gelatin and pectin, as well as the use of antioxidant Astaxanthin and Citri-Fi fiber as functional ingredients that allows to increase the shelf life of the fermented milk product when stored at a temperature of  $(4 \pm 2) ^\circ\text{C}$  up to 5 days.

**Keywords**—functional ingredients, fermented milk products, probiotic microbiota, prebiotics, dietary fiber.

## I. INTRODUCTION

At the end of 20th and the beginning of 21st centuries, a new promising brand was actively developed – creation, production and use of functional nutrition products; a significant role in this process have the fermented milk products, and first of all – the biological products containing probiotics corresponding to human gastrointestinal microbiocenosis [1].

The dominant factor affecting human health is the diet and the lifestyle. Currently, the preservation and strengthening of human health is the most important task of any country.

In the framework of the foregoing, it is necessary to note the development of regulatory support, both theoretical justification and practical implementation of new fermented milk-based products using functional ingredients that are intended for specialized, therapeutic and sports nutrition, special nutrition for the pregnant and lactating women, etc. [2].

An important link in the formation of functional properties in the fermented milk products is the fermentation starter [3].

At the same time, it is necessary not only to provide the required number of probiotic lactic microorganisms and bifidobacteria in the fermented product, but also to preserve it throughout the shelf life of the product, including in the human gastrointestinal tract [4; 5].

M.F. Fernandez, S. Boris, C. Barbes (Spain) isolated two human strains of *Lactobacillus acidophilus* UO 001 and *Lactobacillus gasseri* UO 002, and conducted a

comprehensive study of the influence of various factors on their viability in the gastrointestinal tract model conditions. Positive results were obtained [6].

I.S. Polyanskaya et al (Vologda State Medical Academy of Agriculture), in the process of studying the acid resistance of probiotics, found that it is important not only to check the acid resistance included in functional food probiotic or in traditional products, but also to check the functional autochthonous properties in the selection of valuable strains for use in the industrial production of fermented milk products [7].

N.A. Glushanova substantiated new approaches to the correction of human intestinal microbiocenosis on the basis of numerous experiments. In the process of analytical and experimental work, she was able to identify new approaches to the substantiated choice of probiotic microorganisms and their use in the therapeutic and post-therapeutic period of dysbiosis treatment in humans of different age groups [8].

On the basis of the study results analysis on the influence of nutrition factors on human health, V.A. Samylina came to the conclusion that certain approaches are needed to educate the population of the concept of a “healthy lifestyle”, including healthy diet using mostly natural products [9].

Based on the foregoing, the objective is formulated – to conduct substantiated screening of functional ingredients: probiotic cultures, biopolymers and dietary fiber immobilized in gel for specialized fermented milk products.

## II. METHODS

The main studies were carried out in the laboratories of the Omsk State Agrarian University; Scientific and Educational Center “Kemerovo Technological Institute of Food Industry” (now – Kemerovo State University), accredited production and testing laboratory “Manros-M” (a branch of “WBD”), Center for New Chemical Technologies, Federal research center “Boreskov Institute of Catalysis” (Russian Academy of Sciences, Omsk branch).

Following objects were studied:

- Biopolymers: gelatin, pectin of SLENDID brand (type 200);
- Association of probiotic cultures of *Lactobacillus acidophilus*, *B. lactis*, *B. longum*, *Str. thermophilus*;
- Fermented milk products;

- Antioxidant “Astaxanthin”;
- Dietary fiber “Citri-Fi 200 FG” of medium grinding, manufactured by the “Georgia” company.

Standard and generally accepted methods for determining the chemical characteristics of products were used.

We used certified measurement procedures for the microbiological studies and a microbiological box with a TENCAN purification system (China).

Immobilization experiments were carried out in a special box. It was taken into account that

- the optimal temperature of the life of monocultures included in the association is  $(38 \pm 1) ^\circ\text{C}$ ;
- preparation of a mixture of biopolymers was carried out at a temperature of  $20 ^\circ\text{C}$ ;
- in the reactor, the association of probiotic cultures in an activated form at a temperature of  $(33 \pm 1) ^\circ\text{C}$  was combined with a biopolymers gel, mixed for  $(15 \pm 5)$  min;
- then we dosed the resulting mixture into sterile forms;
- the exposure time of the forms in a special box is 15-20 minutes. As a result, thin films (membranes) were formed. Membrane storage temperature  $(4 \pm 2) ^\circ\text{C}$ ;
- the amount of free and bound moisture in fermented milk products was determined using the parameters of transverse nuclear magnetic relaxation (NMR).

To process the obtained results in experimental studies, the program “Math CAD – 14 Professional” was used.

### III. RESULTS AND DISCUSSION

When determining the shelf life of fermented milk products, it is necessary to take into account that this is a multicomponent, active system in which microbiological, biochemical and other reactions can simultaneously occur that affect the complex of organoleptic indicators, including taste and texture.

Fermented milk products, depending on their moisture content, have a different structure: liquid-like, pasty, solid.

The decrease in the quality of fermented milk products can be considered on the basis of factors such as the number of microorganisms (CFU / g), pH, water activity, temperature and moisture content, which includes quantitative indicators of the forms of moisture in the product. In a complex, they are called “barrier” factors [10].

As a functional ingredient, dietary fiber (DF) “Citri-Fi 200 FG” (hereinafter Citri-Fi) was used. To study the fermentation process we used cow milk with mass fraction of fat 2.5%, with added antioxidant “Astaxanthin” in an amount 0.2 g (200 mg) per each 100 g of milk, and Citri-Fi fiber in an amount of 0.5-2.5 g per each 100 g of milk: experiment 1 – 0.5%; experiment 2 – 1.5%; experience 3 – 2.5%. The indicators characterizing the fermentation process of the experimental product in comparison with the control are shown in table 1.

TABLE I. BIOTECHNOLOGICAL INDICATORS OF THE FERMENTATION PROCESS OF THE EXPERIMENTAL PRODUCT

Type of model culture medium	Amount of DF, %	Clot formation time, hours	Acidity		Viable cells concentration, CFU/cm <sup>3</sup>	
			titratable, °T	active, pH units	L. acidophilus	Bifidobacteria
Control	-	4.5±0.5	73.8±5.0	4.49	9.0±0.3	8.2±0.2
Experiment 1	0.5	5.5±0.5	75.3±2.0	4.48	9.2±0.2	8.2±0.2
Experiment 5	1.5	6.0±0.5	77.8±2.0	4.46	9.8±0.3	8.6±0.2
Experiment 3	2.5	6.5±0.5	79.4±2.0	4.45	9.6±0.2	8.4±0.3

The obtained data allowed us to conclude that dietary fiber, due to its moisture-binding properties, somewhat slows down the fermentation of model culture medium, while the volume of probiotic microbiota in experimental samples increases, which can be explained by the prebiotic effect of dietary fiber.

In order to establish the shelf life, the storage capacity of the fermented products was studied in cold storage at a temperature of  $(4 \pm 2) ^\circ\text{C}$ .

A comprehensive analysis of the obtained experimental data, in the process of studying the storage capacity of the experimental products, in comparison with the control made, it possible to note the positive effect of adding Citri-Fi and Astaxanthin to the normalized mixture. So, in experiment 1, 0.5% of DF was added, and as a result, by the 15th day of storage, the mass fraction of moisture in the product decreased by 1.5%; in the control experiment, mass fraction of moisture decreased by 3.0% by the 10th day, an overly sour taste appeared and the product was removed from storage. By the 15th day of storage fractional mass of moisture decreased in the experimental products 2 and 3, compared with the initial (88.0 mass %) by  $(0.5 \pm 0.1)\%$ . All indicators characterizing

the quality of the experimental products (2 and 3) corresponded to the standards.

To determine the degree of influence of the integrated use of antioxidants and dietary fiber in the component composition of fermented products, the state of bound moisture was studied by NMR.

The main goal of this stage of the work was to determine the relative amount of bound and free water in fermented products using the parameters of transverse nuclear magnetic resonance relaxation using the hardware and software of the laboratories of the Federal research center “Boriskov Institute of Catalysis” (Russian Academy of Sciences, Omsk branch). The transverse (spin-spin) nuclear magnetic relaxation time T2 was used as an operating parameter.

The objects of the study were control and experimental fermented products produced using Astaxanthin and Citri-Fi, which were added in amount: experiment 1 – 0.5%, experiment 2 – 1.5%, experiment 3 – 2.5 %, depending on the weight of the product. All products were studied after fermentation (after 24 hours) and at the end of the shelf life, the time of which was determined in previous studies – 15 days, at a temperature of  $(4 \pm 2) ^\circ\text{C}$ .

The obtained relaxation curves were approximated by the sum of one exponential function using the OriginPro software package. The decline curves were simulated using a single-exponential function with varying parameters A and T2. The simulation results are presented in Figures 1-8.

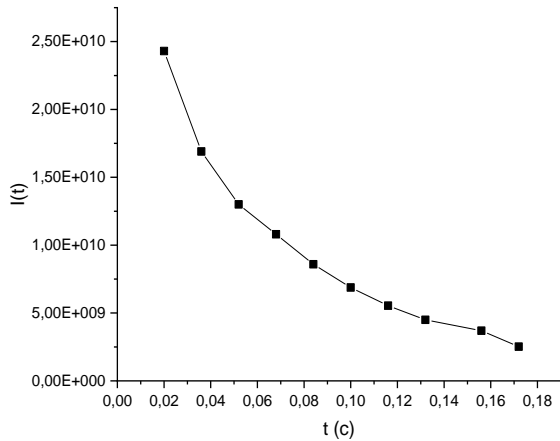


Fig. 1. The decay curve of spin-spin (transverse) magnetization of a fresh fermented product (control).

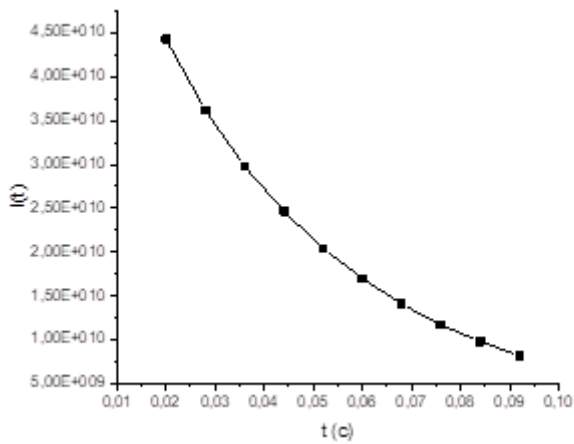


Fig. 2. The decay curve of spin-spin (transverse) magnetization of a fresh fermented product (experiment 1). The decay curve of spin-spin (transverse) magnetization of a fresh fermented product (control).

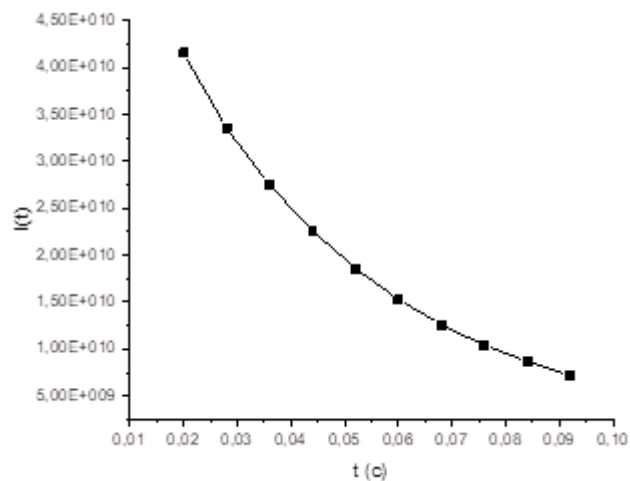


Fig. 3. The decay curve of spin-spin (transverse) magnetization of a fresh fermented product (experiment 2).

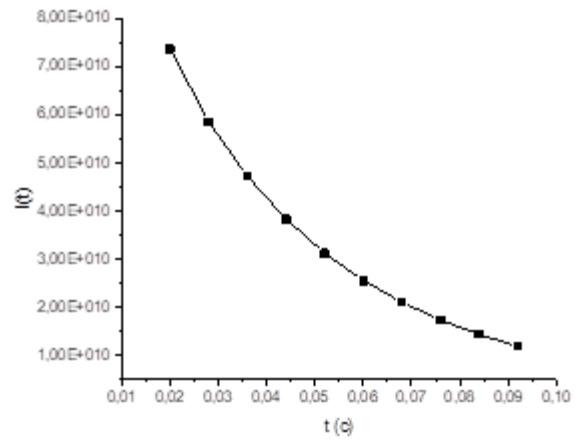


Fig. 4. The decay curve of spin-spin (transverse) magnetization of a fresh fermented product (experiment 3).

An analysis of the decay curves of the spin-spin (transverse) magnetization of the studied samples of fermented products that were not subjected to storage indicates that the mobility of hydrogen protons is limited in the experimental samples 1, 2, 3, since part of the free moisture is bound by dietary fibers, as evidenced by the decay time of the magnetization of the experimental products as compared with the control.

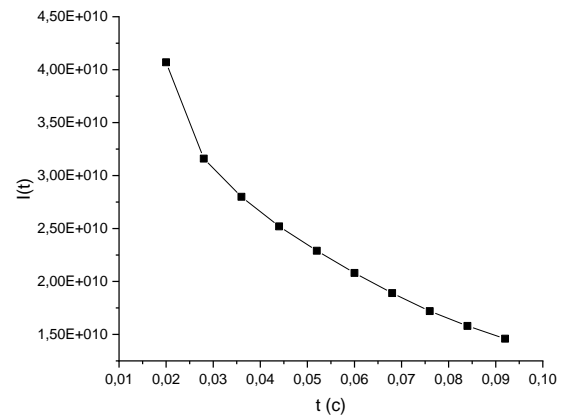


Fig. 5. The decay curve of the spin-spin (transverse) magnetization of the fermented product subjected to storage (control).

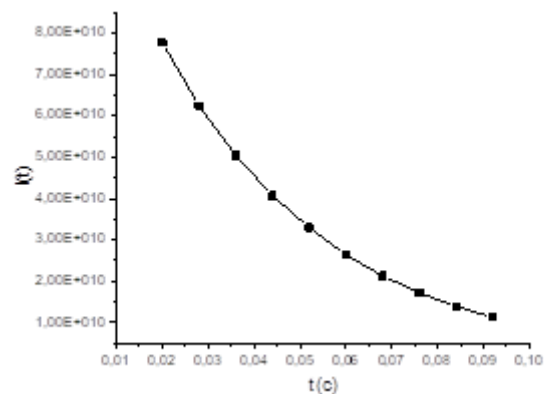


Fig. 6. The decay curve of the spin-spin (transverse) magnetization of the fermented product subjected to storage (experiment 1).

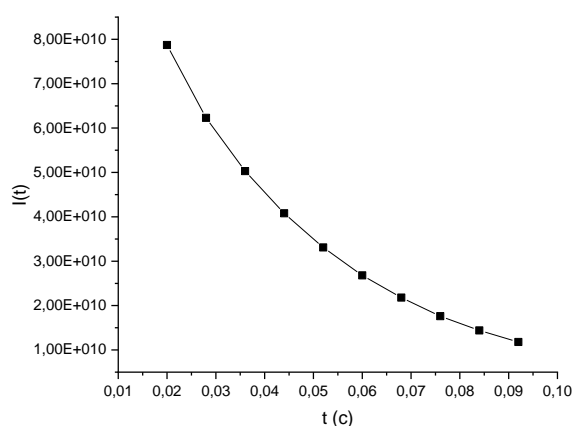


Fig. 7. The decay curve of the spin-spin (transverse) magnetization of the fermented product subjected to storage (experiment 2).

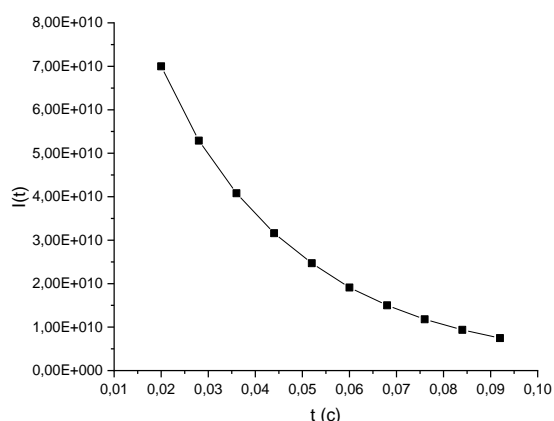


Fig. 8. The decay curve of the spin-spin (transverse) magnetization of the fermented product subjected to storage (experiment 3).

According to the analysis of the decay curves of the spin-spin (transverse) magnetization of the studied samples of products subjected to storage, the magnetization signal decreases faster in the experimental samples 1, 2, 3 (compared to the control), due to the binding of free water during the swelling of dietary fiber.

A comparative analysis of the relaxation decay of the experimental fermented products subjected to and not subjected to storage indicates that the decay of the magnetization signal in the samples subjected to storage proceeds an order of magnitude faster than in the samples that are not subjected to storage.

Identifying parameters of the studied fermented products are presented in table 2.

TABLE II. IDENTIFICATION PARAMETERS OF THE STUDIED FERMENTED PRODUCTS

Product	Time of spin-spin relaxation $T_2$ , ms	Chemical shift of the bound water signal, ppm
Fresh fermented products		
Control	66.412	5.157
Experiment 1	42.264	5.140
Experiment 2	40.732	5.137
Experiment 3	38.738	5.182
Fermented products subjected to storage		
Control	70.930	5.090
Experiment 1	37.273	5.131
Experiment 2	37.611	5.142
Experiment 3	31.376	5.111

Based on the analytical evaluation of the data, it can be concluded that the  $T_2$  spin-spin relaxation time in fresh fermented products and in products that are subjected to storage decreases, which indicates a decrease in the number of free hydrogen protons and moisture binding in experimental products 1, 2, 3 with dietary fibers, which goes for the samples not subjected to storage, as well as the stored samples.

According to the methods, we can conclude as longer the spin-spin relaxation time  $T_2$  is – the greater the amount of free moisture will be in the product.

#### IV. CONCLUSION

1. Adding Citri-Fi dietary fiber to the fermented product helps to reduce the amount of free moisture, which allows increasing the shelf life of the product.

2. The substantiated screening of experimental data allows us to conclude that the most rational dosage of introducing dietary fiber into the product is the 1.5%. Specifically this dosage helps the product to retain the largest amount of free water (as evidenced by the longer spin-spin relaxation time  $T_2$  in comparison with the other samples).

3. Co-use of probiotic cultures associations of the *Lactobacillus acidophilus*, *B. lactis*, *B. longum*, *Str. thermophilus* in an immobilized form in a biopolymer gel of gelatin and pectin, taken in the ratio 1: 2, the antioxidant Astaxanthin and Citri-Fi dietary fiber as functional ingredients, allowed us to increase the shelf life of fermented milk products by 5 days when stored at a temperature  $(4 \pm 2) ^\circ\text{C}$ , that is, the shelf life of fermented products in total became 15 days.

#### REFERENCES

- [1] Functional products, Global technology trends, Trendler, 2015, Vol. 15. <https://clck.ru/Jj6JH>
- [2] N.B. Gavrilova, M.P. Schetinina and N.L. Chernopolskaya, "Experimental and scientific formulation development of a specialized (sport) product, enriched with probiotic microorganisms," *Voprosy Pitaniia (Problems of Nutrition)*, Vol. 86, No. 5, 2017, pp. 22-28.
- [3] T.N. Orlova, I.A. Funk, R.V. Dorofeev, E.F. Ott and K.E. Shevchenko, "Isolation of the lactic acid bacteria for milk products," *Dairy Industry*, Vol. 7, 2019, pp. 20-21.
- [4] E. Dudriková, M. Nagyová and Z. Dičáková, "Survival of *Lactobacillus bulgaricus* and *Bifidobacterium animalis* in yoghurts made from commercial starter cultures during refrigerated storage," *Potravinárstvo Slovak Journal of Food Sciences*, Vol. 11, 2017, pp. 597-601. doi: <https://dx.doi.org/10.5219/758>
- [5] N.V. Shakovets and T.N. Terekhova, "Significance of probiotics for health and microbiota of oral cavity (literature review)," *Voenmaya medicina*, Vol. 2, 2011, pp. 134-139.
- [6] M.F. Fernandez, S. Boris and C. Barbes, "Probiotic properties of human lactobacilli strains to be used in the gastrointestinal tract," *Journal of applied Microbiology*, Vol. 94, pp. 449-455, 2003.
- [7] T.A. Berseneva, A.S. Berskaya and I.S. Polyanskaya, "Acid Resistance of Probiotics," Chisinau: New Science: Problems and Prospects, 2016, pp. 60-64. [https://elibrary.ru/download/elibrary\\_28009243\\_94774300.pdf](https://elibrary.ru/download/elibrary_28009243_94774300.pdf)
- [8] N. A. Glushanova, "Experimental substantiation of new approaches to the correction of intestinal microbiocenosis," Moscow: Authors Abstract of D.Sci.(Med.) Thesis, 2005. (in russ.)
- [9] V.A. Samylina, "Influence food products enrichment pro- and prebiotics on microecology status of man," *Voprosy Pitaniia (Problems of Nutrition)*, Vol. 80, No. 2, 2011, pp. 31-36.
- [10] Understanding and measuring the shelf-life of food, R. Steele, Eds. Cambridge : Woodhead publ., 2004.