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Influence of mode parameters of heat treatment on moisture binding capacity of metabiotic food systems

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Abstract. Experimental studies of the effect of the method of inactivating active forms of probiotic microorganisms on the intensity of biosynthesis of exopolysaccharides and the moisture-binding ability of metabiotic food systems were carried out. The object of the research was a consortium of probiotic microorganisms based on *Str. thermophiles (B-8328), B. bifidum (AC-1579), B. longum (AC-1257), B. adolescentis (AC-1245), L. acidophilus (B-5097), L. plantarum (B-3962), L. fermentum (B-2875).* The inactivation process was carried out in the temperature range of 75-95 °C and a duration of 30-50 minutes. The water-retaining capacity of inactivated biomasses of probiotic microorganisms was studied using a laboratory centrifuge at a rotor speed of 1000-2500 rpm and a duration of 10-50 minutes. The concentration of exopolysaccharides was determined using the phenol-sulfur method. Thermal exposure in test samples lasting 20-50 minutes caused a change in the amount of separated serum in the range of 8-24 % by weight. In a control sample of biomass not subjected to thermal exposure, the same value was 12-32 % by weight and 10-40 minutes, respectively. The data obtained indicate increased functional and technological properties of inactivated biomasses and are due to the presence of microbial exopolysaccharides synthesized by probiotic microorganisms under conditions unfavorable for life. The concentration and absorbance of exopolysaccharides as a result of inactivation of probiotics varied in the range of 74,0-83,0 mcg/ml and 524-542 A, respectively. In the control sample, these values were 31,5-33,5 mcg/ml and 367-380 A. It was found that the most effective inactivation regimen, providing increased synthesis of microbial metabolites, corresponds to the exposure temperature of (75 ± 2) °C, exposure for 50 minutes and subsequent cooling to (4 ± 2) °C.

1 Introduction

Probiotic food systems, despite their high therapeutic efficacy, are characterized by a number of limitations. The introduction of live microorganisms is associated with certain

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risks, especially for persons with a disturbed composition of microflora, the mucous membrane of the gastrointestinal tract (risk of bacteremia/fungemia). The introduction of live bacteria is also not recommended for undergoing surgical interventions, as well as for children with intestinal dysbiosis and deficiency of beneficial probiotic microorganisms (for example, Lactobacillus and Bifidobacterium). In this regard, an urgent direction in the development of food technologies is the production of metabiotic complexes with a predicted physiological effect on the human body. Metabiotics are a complex of non-viable probiotic microorganisms and products of their lifetime metabolism, biologically active compounds, which include short-chain fatty acids (SCFAs), exopolysaccharides and other metabolites [1]. In addition, metabiotics may include metabolic and secretory products that are formed during culture and immediately prior to the inactivation phase. These biologically active products include various compounds, such as: vitamins necessary for a number of metabolic functions, teichoic acid, which helps maintain the integrity of the cell walls of bacteria, bacteriocins with antimicrobial properties, enzymes and peptides, which are vital for numerous biochemical processes in the body [2].

Biologically active compounds produced by probiotic microorganisms play a crucial role in modulating B-cell immune responses. In particular, according to the results of a number of studies, the immunomodulatory functions of short-chain fatty acids (SCFAs) were confirmed (the hypothesis of the role of SCFAs in enhancing IgA secretion in animal models). In addition to the above, SCFAS are able to stimulate plasma differentiation of B cells by modulating gene expression to produce antibodies such as IgG and IgA. An additional mechanism of immunomodulatory functions is associated with regulation of B-cell activity and correlates with tryptophan metabolites, a group of significant postbiotics [3, 4].

A number of studies confirm the effectiveness of metabiotics in the prevention and treatment of various oral diseases, such as dental caries caused, in particular, by the Streptococcus mutans pathogen [5, 6]. In addition to the above, metabiotics have advantages over probiotics in terms of production and use, since metabiotics practically do not interact with food matrix components during storage, which is guaranteed to prolong the shelf life of the food system. Metabiotic substances maintain stability in a wide range of pH and temperature values, which allows them to be added to food systems before heat treatment, as well as to products with high acidity. The use of metabiotics eliminates the need for storage and transportation under controlled temperature conditions, which reduces the cost of the final product [6].

Metabiotic therapy is based on the introduction into food systems of one or more microbiological metabolites (short-chain fatty acids, metabolites of phenolic origin, enzymes, exopolysaccharides, vitamins, aromatic amino acids, bacterial lysates, as well as cell-free supernatants that contain secreted microbial metabolites). In particular, anti-inflammatory and antioxidant effects on intestinal epithelial cells were exerted by Lactobacillus casei supernatants (decreased secretion of TNF- α and increased secretion of IL-10). The effectiveness of butyrate, a short-chain fatty acid that increases the expression of immunosuppressive cytokines and suppresses some cytokines and pro-inflammatory receptors, has been scientifically confirmed [6, 7].

Safety of use, high biological (including antimicrobial) activity, unique and complex chemical structure of exopolysaccharides endow them with valuable and significant potential. Exopolysaccharides are currently used as antitumor, immunostimulating, immunomodulating and antioxidant agents in the pharmaceutical industry, as well as functional components in the food industry that contribute to improving the rheological and structural-mechanical properties of food systems [7-8].

Antidiabetic, antiviral, antiulcer and gastro-protective properties of bacterial exopolysaccharides have been established. The gut microbiota is able to use exopolysaccharides to produce short-chain fatty acids (SCFA), which helps to maintain intestinal barrier function, attenuate mucosal inflammation and oxidative damage. Lipid-lowering effects of bacteria producing a significant amount of exopolysaccharides have been proven in animal models [9-11].

The hydrophilic nature of exopolysaccharides makes it possible to reduce oil absorption in the product, which acquires particular relevance for consumers with increased weight, excess cholesterol, suffering from fatty liver, etc. Exopolysaccharides synthesized by lactic acid bacteria have an antimicrobial and antioxidant effect, which increases the potential for their use in the food industry (canning, packaging). In particular, antibacterial activity of exopolysaccharides extracted from probiotic strains of Bifidobacterium bifidum and Lactiplantibacillus plantarum against a wide range of pathogens was noted, including C. sakazakii, E. coli, L. monocytogenes, S. aureus, C. albicans, B. cereus, S. typhimurium, S. sonnei [12].

The potential of industrial use of exoplisaccharides is based on the use of the latter as thickeners, disintegrants, emulsifiers, stabilizers, water-binding, water-retaining, gelling and viscosity-imparting agents. The use of laboratory-produced exopolysaccharides in food can contribute to reducing the use of technological additives with the accompanying optimization of production and satisfying the needs of consumers interested in a healthy diet.

Improvement of textural and sensory characteristics with the help of exopolysaccharides is of particular importance in the production of dairy products with a reduced fat content, as well as products with replacement ingredients (milk of plant origin, etc.) due to the possibility of negative perception of these products by the consumer from a quality standpoint [12].

The synthesis of water-binding metabolites by probiotics has been found to be altered (activated) under adverse environmental conditions, particularly when thermal inactivation is used.

A promising area of research is the use of combined methods of inactivation of probiotic microorganisms, as well as methods of modifying the nutrient medium to synergistically enhance the growth and metabolic activity of probiotics (in particular, bifidobacteria). The development of ways of optimal combination of strains will allow to achieve genomic diversity, and, therefore, diversify metabolic processes in fermentation systems, which will contribute to the expansion of the spectrum and increase functional effects in the food product. Metabolic complementarity contributes to a metabiotic end product with increased biological potential. Due to the fact that the beneficial effects are mediated by biologically active metabolites contained in the metabiotic product, it is necessary to analyze the metabolic profile of this product in order to assess its functional potential and develop directions for further modification.

The aim of the work was to study the effect of inactivation methods of a consortium of probiotic microorganisms on the water-binding ability and intensity of exopolysaccharide synthesis in food systems.

2 Materials and methods

The object of research was a consortium of lacto and bifidobacteria *Str. thermophiles (B-8328), B. bifidum (AC-1579), B. longum (AC-1257), B. adolescentis (AC-1245), L. acidophilus (B-5097), L. plantarum (B-3962), L. fermentum (B-2875),* provided by the bioresource center – « All-Russian collection of industrial microorganisms » (BRC VKPM), SIC «Kurchatov Institute». The concentration of active cells in biomass was not less than 10^9 CFU/ml, titratable acidity was 82-94 ^{o}T, pH was 4,60-4,64. Biomass growth was carried out on skimmed milk in the temperature range 38-40 °C to pH 4,3-4,6, the standard fermentation time was 8-10 hours.

Inactivation of the biomass of the consortium of probiotic microorganisms was carried out using the following regime parameters: experiment 1: temperature (75 ± 2) °C, exposure for 50 minutes, followed by cooling to (4 ± 2) °C; experiment 2: temperature (85 ± 2) ºC, exposure for 40 minutes, cooling down to (4 ± 2) ºC; experiment 3: temperature (95 ± 2) ºC, holding for 30 minutes, cooling to (4 ± 2) ºC. A biomass sample of the consortium of probiotic microorganisms not subjected to inactivation was taken as a control.

The water-retaining capacity of inactivated probiotic food systems was studied using a PE-6900 laboratory centrifuge (Ekros Group) at a rotor speed of 1000-2500 rpm and a duration of 10-50 minutes.

The concentration of exopolysaccharides in inactivated biomasses of the consortium of probiotic microorganisms was determined using the phenol-sulfur method.

3 Results and discussion

In the course of experimental studies, the use of various operating parameters of the centrifugation process made it possible to establish the water-retaining capacity of prototypes of inactivated biomasses under dynamic loads (Table 1).

Table 1 - Volume of separated serum in inactivated biomasses of the probiotic microorganism consortium

Centrifugation mode, rpm	Duration of centrifugation, min	Inactivation mode / Mass fraction of separated whey, % by weight			
		1	2	3	Control
1000	5	2,8	3,5	5,1	6,2
	10	3,9	4,6	5,8	6,7
	15	4,5	5,4	6,6	7,1
	20	5,2	6,1	7,2	8,2
	25	6,3	6,8	7,7	9,4
	30	6,8	7,0	8,1	11,8
	35	7,1	7,5	8,8	12,9
	40	7,3	8,0	9,3	13,4
	45	7,6	8,5	10,0	13,5
	50	8,0	8,6	10,0	13,5
1500	5	4,7	6,2	7,8	12,4
	10	5,3	7,8	9,5	13,8
	15	7,7	10,4	11,3	15,5
	20	9,6	11,5	12,8	16,2
	25	10,7	13,3	14,7	18,7
	30	12,4	14,4	15,6	18,7
	35	13,2	14,5	15,7	18,8
2000	5	8,6	9,3	10,5	17,2
	10	9,8	10,5	12,2	18,5
	15	10,8	12,8	14,8	20,6
	20	12,6	15,3	17,6	23,8
	25	16,3	19,2	20,3	24,6
	30	17,7	19,3	20,5	24,7
2500	5	10,5	12,4	15,3	24,3

	10	13,4	15,5	17,8	30,8
	15	16,3	19,6	22,4	32,0
	20	20,4	22,6	24,0	32,0
	25	21,0	22,8	24,1	32,1

Experimental studies have shown that prototypes of inactivated biomass of the consortium of probiotic microorganisms show resistance to external centrifugal loads. It was found that the amount of separated serum in the test samples at a centrifugation speed of 1000 rpm is 8-10% by weight with a duration of exposure of up to 45-50 minutes. In a control sample with a similar centrifugal load, the amount of separated serum corresponds to 12-14 % by weight the duration of centrifugation was 35-40 minutes. For test sample No. 1, inactivated at a lower temperature (75 ^{0}C) and a longer duration of the process (50 minutes), the test parameters were 8 % by weight and 50 minutes, respectively. The experimental data obtained are due to the presence of microbial exopolysaccharides in the composition of inactivated biomasses synthesized by probiotic microorganisms under conditions unfavorable for life.

Increasing the centrifugation speed of the test samples to 2500 rpm allowed to increase the volume of isolated serum to 21-24 % by weight with a decrease in duration to 20-25 minutes. At the same time, for prototype No. 1, the studied indicators amounted to 21 % by weight and 25 minutes, respectively, indicating a higher content of water-binding metabolites in the inactivated biomass. In the control, similar values were 30-32 % by weight and 10-15 minutes, respectively. Thus, it was found that carrying out the process of inactivation of biomasses of probiotic microorganisms at a lower temperature and longer duration of thermal exposure is guaranteed to provide higher functional and technological properties of the final food system, which is resistant to physical stress of various intensity.

In the course of further experimental studies, the content of moisture-binding agents - microbial exopolysaccharides, in the composition of the obtained metabiotic food systems was established. Absorbance values of exopolysaccharide solutions for test and control samples were revealed, which is 524-542 A and 367-380 A, respectively.

The concentration of exopolysaccharides in inactivated biomass solutions was determined by means of a calibration curve obtained on the basis of a set of optical density data and the concentration of the D-glucose solution (Table 2).

Table 2 - Concentration of exopolysaccharides in solutions of inactivated biomasses of the probiotic microorganism consortium

Inactivation mode	Exopolysaccharide concentration, mcg/mL		
	Replicate 1	Replicate 2	Replicate 3
1	81,5±2,0	83,0±2,0	80,0±2,0
2	77,0±2,0	77,0±2,0	77,5±2,0
3	74,5±2,0	75,0±2,0	74,0±2,0
Control	31,5±2,0	32,5±2,0	33,5±2,0

Based on the analysis of the results of experimental studies, the values of exopolysaccharide concentrations for test samples of inactivated biomasses were established, the numerical values of which, depending on the applied regimen, vary in the range of 74,0-83,0 mcg/ml. For the control sample, similar values were 31,5-33,5 mcg/ml. The use of lower temperature modes of inactivation of active forms of probiotic microorganisms in the range of 75-80 ^{0}C and the corresponding duration of the process in the range of 45-50 minutes provides an increased content of microbial exopolysaccharides in the test samples compared to the control by 1,4-1,6 times.

4 Conclusion

The positive effects of varying the temperature regimes of the process of inactivation of biomass of consortia of probiotic microorganisms in the range of (75-95) °C and the corresponding time intervals (30-50 minutes) of the process were revealed.

Resistance of inactivated probiotic food systems to action of external centrifugal loads in the range of 1000-2500 rpm and duration of action of 10-50 minutes is shown. When carrying out the centrifugation process in the specified speed and time range, it was found that the amount of separated serum in test samples of inactivated biomasses varies in the range of 8-24 % by weight with a duration of exposure of 20-50 minutes, in the control sample the same value was 12-32 % by weight and 10-40 minutes, respectively.

The values of optical density and concentration of exopolysaccharides of inactivated biomass of probiotic microorganisms, which vary in the range of 524-542 A and 74,0-83,0 mcg/ml, respectively, were revealed. Similar values for the control sample were 367-380 A and 31,5-33,5 mcg/ml. It was found that the most effective inactivation regimen, providing increased synthesis of microbial metabolites, corresponds to the exposure temperature of (75 ± 2) °C, exposure for 50 minutes and subsequent cooling to (4 ± 2) °C.

It has been established that the use of a low-temperature inactivation mode with a simultaneous increase in the duration of thermal exposure allows providing increased functional and technological properties of inactivated biomasses of probiotic microorganisms resistant to external physical factors.

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References

1. N.M. Maftei, C.R. Raileanu, A.A. Balta, L. Ambrose, M. Boev, D.B. Marin, et al., The potential impact of probiotics on human health: an update on their health-promoting properties, Microorganisms **12,** 234 (2024), https://doi.org/10.3390/microorganisms12020234
2. C.M. Carmen, S. Seppo, G. Vinderola, Postbiotics: defining the impact of inactivated microbes and their metabolites on promotion of health, in: The Human Microbiome in Early Life, Academic Press, Cambridge, MA, pp. 257–268 (2021). https://doi.org/10.1016/B978-0-12-818097-6.00011-0
3. Y.F. Yu, C.X. Lu, W.R. Yu, Y. Lei, S. Sun, P. Liu, et al., B cells dynamic in aging and the implications of nutritional regulation, Nutrients **16**, 487 (2024), https://doi.org/10.3390/nu16040487
4. R.A. Homayouni, H. Pourjafar, E.A. Mirzakhani, A comprehensive review of the application of probiotics and postbiotics in oral health. Frontiers in Cellular and Infection Microbiology **8 (13).** 1120995 (2023). https://doi.org/10.3389/fcimb.2023.1120995
5. Q. Liu, T. Ma, et. al., Adjuvant postbiotic administration improves dental caries prognosis by restoring the oral microbiota/ Food Science and Human Wellness **13**(5), 2690-2702 (2023). https://doi.org/10.26599/FSHW.2022.9250217

6. A.H. Rad, A. Abbasi, et al., Potential pharmaceutical and food applications of postbiotics: A review. Current Pharmaceutical Biotechnology **21** (15), 1576–1587 (2020). https://doi.org/10.2174/1389201021666200516154833
7. R. Pujari, G. Banerjee. Impact of prebiotics on immune response: from the bench to the clinic. Immunology and Cell Biology **99**(3). 255–273 (2021), https://doi.org/10.1111/imcb.12409
8. S.Y. Sun, Y.X. Han, et. al., Hematopoietic stem cell: regulation and nutritional intervention. Nutrients **15**. 2605 (2023), https://doi.org/10.3390/nu15112605
9. E. Vassilopoulou, A. Comotti, N. Douladiris, G.N. Konstantinou, T. Zuberbier, I. Alberti, et al., A systematic review and meta-analysis of nutritional and dietary interventions in randomized controlled trials for skin symptoms in children with atopic dermatitis and without food allergy: an EAACI task force report, Allergy **79**, 1708–1724 (2024), https://doi.org/10.1111/all.16160
10. Anjana, S. Tiwari, Bacteriocin-producing probiotic lactic acid bacteria in controlling dysbiosis of the gut microbiota. Frontiers in Cellular and Infection Microbiology **12**. 851140 (2022). https://doi.org/10.3389/fcimb.2022.851140
11. M. Lin, Y.J. Cong, Research progress on the mechanism of probiotics regulating cow milk allergy in early childhood and its application in hypoallergenic infant formula, Front. Nutr. **11**, 1254979 (2024), https://doi.org/10.3389/fnut.2024.1254979
12. M.K. Liu, J.J. Chen, I.P.W. Dharmasiddhi, S. Chen, Y. Liu, H. Liu, Review of the potential of probiotics in disease treatment: mechanisms, engineering and applications, Processes **12**, 316 (2024), https://doi.org/10.3390/pr12020316