Investigation of moisture binding forms of metabiotic food systems

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Abstract. Microbiological production of polysaccharides has undeniable advantages over traditional methods, since it provides an environmentally friendly process for producing functional food. In order to develop methods for modulating and optimizing the synthesis of moisture-binding metabolites of polysaccharide nature, a study of the activity of exopolysaccharide production by a consortium of probiotic microorganisms (Streptococcus thermophiles, Bifidobacterium bifidum, Bifidobacterium breve, Bifidobacterium longum, Lactobacillus acidophilus, Lactobacillus fermentum. Lactobacillus plantarum) under various thermal exposure conditions. An analysis of the effect of temperature inactivation modes (higher and lower values) was carried out, as well as an analysis of the effect of the duration of inactivation of probiotics. The initial concentrations of microorganisms in the probiotic consortium were at least 109 CFU/ml. On the basis of experimental data, a temperature range from 92 to 120 °C and its corresponding endothermic effects were established. The obtained data make it possible to state the presence of additional binding of moisture by microbial polysaccharides. At the end of the thermal inactivation process, the weight of the test samples was 13,65 % and 14,84 %, of the control sample - 12.36 %. The mass fraction of moisture removed in the test samples was 9,48 % and 11,39 %, in the control sample – 6,92 %, which confirms the activation of the production of microbial metabolites. The maximum activity of the synthesis of moisture-binding metabolites was noted with the following parameters of inactivation modes: temperature 75-2 °C; hold for 50 min followed by cooling to 4-2 °C.

1 Introduction

Traditional methods of influencing the gut microbiota are food enrichment with probiotics - live microorganisms that have a positive effect on health, as well as prebiotics - substances that promote the growth and activity of certain types of bacteria. However, advances in high-throughput sequencing and metabolomics have revealed the significance of metabiotics that can be used to directly and specifically control microbiota functions. Metabiotics are the result of the metabolic activity of living bacteria, which can have a positive effect on health through a direct or indirect mechanism [1]. Traditionally,

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metabiotics have been defined as preparations from non-living microorganisms or their components that have a complex of physiological effects on the body [11]. In 2021, the International Scientific Association for Probiotics and Prebiotics (ISAPP) proposed the use of the term "metabiotic" to describe these objects as "soluble factors, including products or by-products of metabolism secreted by live bacteria or released during the lysis of bacterial cells." In addition to this, the statement emphasizes "non-viable metabolites produced by microorganisms that have a biological effect on the host," as well as "compounds that are produced by microorganisms and subsequently released from food ingredients or microbial components, including from non-viable cells."

A metabiotic product consists of two main elements: bacterial cell components and metabolites. Bacterial components mainly include peptidoglycan, phosphomuramic acid, lipomuramic acid, acetalphospholipid, membrane protein, and intracellular polysaccharides. Bacterial metabolites include vitamins, lipids, organic acids, bacteriocins, enzymes, extracellular polysaccharides, and short chain fatty acids. These bacterial components can increase the body's resistance through activation or imitation of the immune system. Intracellular polysaccharides also play a crucial role in maintaining the barrier function of the intestinal mucosa and maintaining a balanced intestinal microbiota [3].

Vitamins, lipids, organic acids and enzymes provide the body with nutrients, facilitate the digestion and absorption of food. Bacteriocins inhibit the growth of pathogenic microorganisms, and as a result, contribute to maintaining the microecological balance of the intestine [6]. Extracellular polysaccharides can promote the growth of probiotics and regulate the immune response [2]. Short-chain fatty acids play an important role in the regulation of intestinal immunity and anti-inflammatory reactions, protecting the health of the intestines and body systems through antioxidant and anti-inflammatory mechanisms [6]. The components of metabiotics contribute to the diversity of their functional component and the completeness of the disclosure of the effectiveness potential.

Metabiotics have shown particular promise in the treatment of diseases of the gastrointestinal tract, metabolic disorders, respiratory, oncological, mental and a number of other diseases [14]. Metabiotics have a wide range of beneficial biologically active properties, including anti-inflammatory, antioxidant and anti-tumor effects. The functional potential of metabiotics includes protection of the intestinal epithelial barrier, regulation of immune responses, improvement of metabolism and modulation of neural signaling [5-7]. The therapeutic potential of metabiotics has been proven from the standpoint of influencing the function of B cells and the effectiveness of the use of metabiotics in immune regulation, through interaction with the intestinal microbiota and immune pathways.

Despite the fact that the above properties are inherent in probiotics, the stability and safety of metabiotics allow these objects to be used as a functional alternative to probiotics. As the scientific understanding of processes affecting living organisms by metabiotics continues to grow, it is expected that the application of the latter in clinical practice, in the field of nutrition, as well as in animal husbandry will also expand. In addition to this effect, replacing antibiotics with metabiotics will improve health and productivity with minimal side effects. The above alternative approach has significant potential for improving health conditions, increasing the competitiveness of the food and livestock product industries.

Exopolysaccharides (EPS) synthesized by bacteria are high-molecular carbohydrate polymers endowed with a number of nutrients and mechanisms of protection against bacteriophages [8-10]. Exopolysaccharides have a number of characteristics that can be used in the production of food systems - in particular, high water-retaining capacity, water solubility, potential for emulsification and flocculation [4, 9] A set of health benefits inherent in exopolysaccharides includes antioxidant, antibacterial, hypoglycemic, immunomodulatory and cholesterol-lowering effects. Currently, the problems of synthesis of exopolysaccharides are given increased attention in connection with the possibilities of

their use in various food products. There are many strains producing exopolysaccharides [9].

Lactobacilli and bifidobacteria are the main source of exopolysaccharides of microbial origin, which are traditionally considered safe for human consumption [8, 11]. Due to the fact that most strains of lactic acid bacteria are not productive enough for large-scale production of exopolysaccharides, the relevance of research on the problem of enhancing the synthesis of the latter is increasing. Currently, the demand for functional food products is growing. Developing new products or improving existing food systems based on enrichment with functional post-biotic components is critical in the food industry.

According to the research results of world scientific schools, the influence of external conditions (temperature, pH) on the synthesis of exopolysaccharides with probiotics was confirmed. External influences change physicochemical and functional properties of probiotic food product. In particular, sublethal stresses can increase the viability of probiotics. It is noted that some mesophilic strains produce a greater amount (as well as a wider variety) of exopolysaccharides under suboptimal temperature conditions. The observed increased production of exopolysaccharides was correlated with the mechanism of adaptation of probiotic microorganisms to stressful environmental conditions [8, 9].

In particular, H.T. Nguyen studied stress responses of the strain Bifidobacterium bifidum THT 0101 (exposure to sublethal temperature loads). An increase in the concentration of exopolysaccharides after shock thermal exposure was noted.

The study of the effect of the temperature growth factor on the production of exopolysaccharides by the Lactobacillus paracasei strain, the analysis of changes in the probiotic properties of the product are presented in the work of A.A. Bengoa et al.

According to the results of the study by A.A. Bengoa et al., The effect of partial loss of the exopolysaccharide layer on increasing the susceptibility of bacteria to stress conditions has been proven, which confirms the importance of exopolysaccharides in the mechanism of protection against acid and bile stress. The observed phenomena were identical under different incubation temperature regimes, and the protective effect is not related to the amount and structure of exopolysaccharides. A number of Lactobacillus paracasei strains are capable of producing exopolysaccharides with different levels of polymerization depending on the ambient temperature. Differences in polymerization of exopolysaccharides had no effect on probiotic properties. The preservation of probiotic properties was noted - tolerance to acids and salts of the gastrointestinal tract, adhesion to intestinal epithelial cells and immunomodulatory ability. Thus, the relevance of the use of exopolysaccharide-producing strains in the development of a wide range of functional foods has been confirmed.

2 Materials and methods

A consortium of probiotics was adopted as the subject of the study (*Bifidobacterium bifidum, Streptococcus thermophiles, Bifidobacterium longum, Lactobacillus acidophilus, Bifidobacterium breve, Lactobacillus plantarum, Lactobacillu sfermentum*).

The activated starter was added to the culture medium - normalized cow's milk. Culture mode: temperature t=40±2ºC, duration 8-10 hours, followed by cooling to t=4±2ºC. During cultivation, control measurements of the active and titratable acidity of the medium were carried out until the growth of the values of the indicators in the final product slowed down, which amounted to 6.3 pH units and 90 ^{0}T, respectively.

The optimal initial number of starter cultures varied in the range of 0,029-0,031 billion cells/kg (0,6-0,65 ml/kg bacterial suspension). During the experiment, there was no excess of the specified number of bacterial units. The concentration of probiotic microorganisms in

the test samples was not less than 10^9 CFU/ml (inoculation on Petri dishes with dense culture medium).

Inactivation of the biomass of the consortium of probiotic microorganisms was carried out using the following regime parameters: experiment 1: temperature (95±2) °C, exposure for 30 minutes, cooling to (4±2) °C; Experiment 2: temperature (75±2) ºC, followed by holding for 50 minutes and cooling to (4±2) ºC. The control sample of lacto - and bifidobacteria biomass was not inactivated.

The study of the forms of bound moisture was carried out using a synchronous thermal analysis device (STA 449 F3 Jupiter) in the temperature range of 25-300 °C, recording of structural and physicochemical transformations, as well as changes in the mass of the test samples.

3 Results and discussion

Numerical values of temperature intervals related to phase transformations, mass change parameters of samples (in %) and enthalpy are given in Table 1.

Table 1 - Thermal effects of biomass samples of probiotic microorganisms during thermolysis

Sample name	Temperature band, DT, ^{0}C	Enthalpy, J/kg	Change in sample weight, %
1	30-131	1,821	86,35
2	30-150	1,724	85,16
3 (control)	30-122	1,886	87,64

In conditions of increasing the temperature range, the test samples are distributed in the following order: No. 3, control (92 °C) - No. 1 (101 °C) - No. 2 (120 °C). The result is explained by the involvement of the mechanism of moisture binding by exopolysaccharides produced by additionally synthesized probiotic microorganisms under sublethal stress exposure. The use of thermal exposure contributed to a change in the weight of samples. The residual weight was: No. 1 – 13,65 %, No. 2 – 14,84 %, No. 3, control – 12,36 %, which indicates the greatest degree of water retention by sample No. 2, due to additional binding of moisture by exopolysaccharides of probiotic microorganisms.

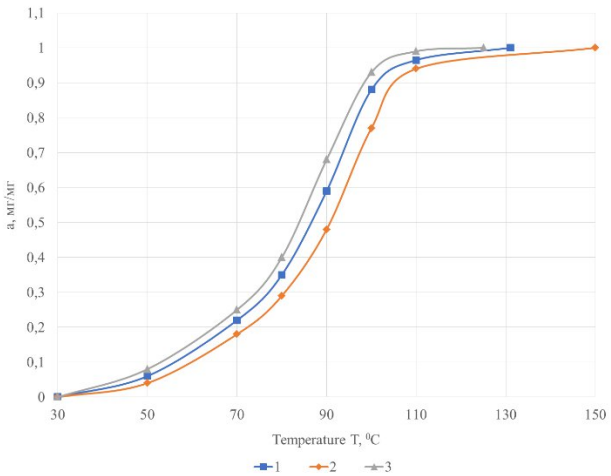

Fig.1 - Graphical dependence of the a (mg/mg) on the temperature T (0C) of inactivated biomass samples (1, 2 - experiment, 3 - control)

On the basis of experimental data, graphs of the dependence of the values of the *a* index (degree of conversion of the substance, mg/mg) on factor T (sample temperature, °C) were plotted (Fig. 1).

The obtained *a=f(T)* relationships are S-shaped, correspond to a complex process of dehydration of samples with sequential release of a fraction of free, physically, physicochemically and chemically bound moisture (Table 2).

Table 2 - Dehydration characteristics of test samples

Sample name	Dehydration stage number	*DT, °C*	*Da*	Mass fraction of moisture removed,%
1	1	30-65	0-0,18	12,97
	2	65-108	0,18-0,90	63,85
	3	108-131	0,90-1,0	9,53
2	1	30-72	0-0,14	12,14
	2	72-116	0,14-0,86	62,71
	3	116-150	0,86-1,0	10,31
3 (control)	1	30-62	0-0,20	14,83
	2	62-104	0,20-0,94	65,90
	3	104-122	0,94-1,0	6,91

The curve fitting method produces three-line splines. The identified areas indicate a stepwise (step-by-step) removal of moisture, in accordance with the forms of its connection with the biopolymers of the experimental samples (Fig. 2).

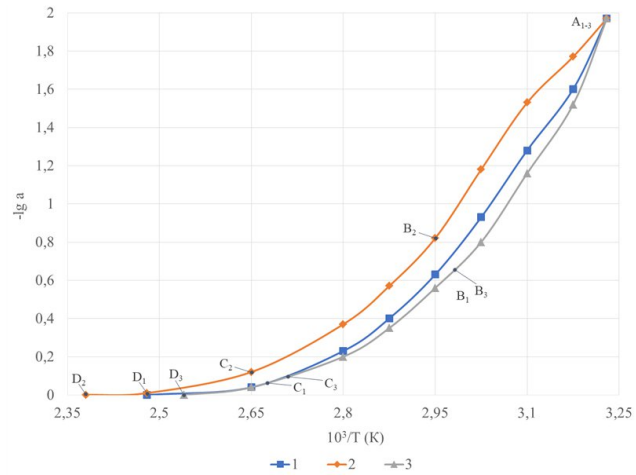

Fig. 2 - Graph of -lg*a*=f(103/T(K)) inactivated biomass samples (1, 2 - experiment, 3 - control)

It was found that at the initial stage of heating (25-30 °C, A_iB_i sites), free moisture contained in the cells of casein-polysaccharide gels is removed. With a subsequent increase in temperature (31-70 °C), the destruction of water-water bonds is observed. The amount of moisture removed at this stage was 12,92 %, 12,25 %, 14,69 % in samples No. 1-3, respectively. At a temperature of 71-105 °C (B_iC_i sections,), mechanically bound moisture, including capillary and adsorption moisture, is removed. For test samples No. 1-3, the mass fraction of moisture removed was 63,91 %, 63,75 %, 64,82 %, respectively. Removal of

physico-chemically bound moisture was initiated in the temperature range of 106-150 °C (C_iD_i sites,). The mass fraction of moisture removed was 9,48 % (sample No. 1), 11,39 % (sample No. 2) and 6,92 % (sample No. 3). It was found that the test samples contained 1,36-1,64 times more bound moisture compared to the control, due to the synthesis of polysaccharides. It should be noted that the average values of water retention indicators for each strain varied depending on the incubation time and the characteristics of the strain. As the incubation period progresses, the bacterial cells establish stronger cell-cell interactions. The results of the observation highlight the role of specific surface molecules in modulating the hydrophobicity and hydrophilicity of probiotics.

4 Conclusion

In the course of experimental studies, the residual weight of the test and control samples was 13,65 %, 14,84 % and 12,36%, respectively. The mass fraction of moisture removed in the test samples was 9,48 % and 11,39 %, in the control sample – 6,92 %, respectively, which indicates the activation of the production of microbial metabolites by a consortium of lacto and bifidobacteria. The use of a low-temperature inactivation regime of a consortium of probiotic units (temperature (75±2) °C, exposure for 50 minutes, followed by cooling to (4±2) °C) ensures maximum synthesis of metabolites of bacterial origin. Taking into account the therapeutic efficacy of foods containing metabiotics, we note the relevance of conducting research in areas related to the engineering of biosynthesis in probiotic systems.

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