Intensification of the production of a plant-based probiotic drink

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Abstract. The quality of the finished probiotic drink is closely related to the completeness and intensity of the fermentation process of lactic acid bacteria in the plant environment of the drink. As part of our research, ways to intensify the fermentation process are proposed due to the additional introduction of vegetable protein and curcumin into the oat drink. The activity of the fermentation process was assessed by the accumulation of biomass, the results of the titrated acidity assessment, the antioxidant properties of probiotic drinks, and the number of probiotic microorganisms. The research results showed that the introduction of additional vegetable protein and curcumin activates the accumulation of microbial biomass by an average of 15-85%, lactic acid – by 40-69% and 5.9 – 12%, respectively, increases antioxidant activity by an average of 2.2 – 6.7%, the increase in protozoan *Paramecium Caudatum* is when introduced into protein samples – 71.8-74.4%, curcumin – 3.1-22.8%. Thus, it was found that an additional increase in the protein component of the nutrient medium of a vegetable drink and the introduction of curcumin have a stimulating effect on biotechnological processes and are promising in the production of probiotic drinks.

1 Introduction

Plant-based drinks are popular alternatives to dairy drinks and are healthy due to the fact that they are an excellent source of plant protein and fiber.

Nevertheless, the plant-based drink has certain disadvantages, in particular, phytic acid binds to essential minerals and trace elements, creating insoluble complexes that prevent their assimilation. Saponins interfere with protein absorption by forming insoluble saponin-protein complexes resistant to digestion. Protease inhibitors in the plant drink also interfere with the digestion of protein and starch by inactivating digestive enzymes. These anti-nutritional substances prevent the absorption of not only minerals, but also vitamins. Fermentation can help eliminate these disadvantages. It improves the solubility of plant protein, amino acid composition and accessibility. In addition, cereals contain microorganisms that promote spontaneous fermentation, and in the production of a lant drink from this raw material, the likelihood of contamination with an obstacle to the development of beneficial microflora increases. In this context, probiotic fermentation, by contributing to an increase in the number of live probiotics, thereby also ensures the microbiological safety of the drink.

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All of the above determines the need and relevance of the development of new probiotic plant-based drinks.

As a basis, we took an oat drink, which is an aqueous extract of oats with a mild, milky taste, with the presence of fatty acids, proteins, minerals, vitamins, dietary fibers and various trace elements in the composition. The ferments of microorganisms, through their complex enzyme systems, generate volatile and non-volatile metabolites that give special taste qualities to probiotic products based on cereals. Lactic acid bacteria are among the most complex organisms in terms of nutrient requirements. For their vital activity, they require the presence of substrates that are a source of energy and substances necessary for them to build a bacterial cell (nucleic acids, polysaccharides, amino sugars, etc.) [1].

The quality of the finished probiotic drink is closely related to the completeness and intensity of the fermentation process of lactic acid bacteria in the plant environment of the drink, which is atypical for them. Organoleptic properties, in particular the consistency and colloidal stability of the finished product, largely depend on it. The problem of many herbal drinks is the instability of their structure, their predisposition to stratification. An analytical review has shown that lactic acid microorganisms are a source of exopolysaccharides. Exopolysaccharides are used as natural and safe thickeners, emulsifiers or stabilizers to improve the texture of food products. The authors have proved the positive effect of fermentation of lactic acid starter culture with the formation of organic acids and exopolysaccharides. Accordingly, the resulting exopolysaccharides in the medium of an oat drink can positively affect its structural characteristics [2, 3].

This fact determines the relevance of research on the study of the conditions for varying the process of probiotic starter culture development in a plant environment, on its intensification, considering the production of fermentation products and the effect on the quality of the finished probiotic drink.

Preliminary studies of the fermentation process of the plant environment by ferments of probiotic cultures have made it possible to establish its dependence on the fermented environment. As part of plant oat-based beverages sold in the Chelyabinsk retail chain: drinking water, oat flour or cereals, salt, some manufacturers introduce rapeseed oil and vitamin and mineral premixes, protein content – 1...1.5%, carbohydrates – 6.5...9%. Nevertheless, studies by Nochebuena-Pelcastre, Alvarez-Contreras have proven the dependence of the development of lactic acid bacteria on the protein and carbohydrate components of the nutrient medium, which suggests the possibility of using additional protein in the production of probiotic drinks as a growth factor [4].

Curcumin is a polyphenol and includes seven carbon linkers and three main functional groups: α, β-unsaturated carbonyl, β-diketone part and aromatic O-methoxyphenol group. Studies by many scientists have established a bidirectional relationship between curcumin and lactobacilli, while curcumin has the ability to increase the number of microorganisms *Bifidobacterium* and lactobacilli and reduce the number of pathogenic species such as *Prevotellaceae, Coriobacteriaceae*, enterococci, enterobacteria [5, 6].

At the same time, lactobacilli play a crucial role in curcumin metabolism, producing various active metabolites such as 1-(4-hydroxy-3-methoxyphenyl)-2-propanol, tetrahydrocurcumin, dihydroferulic acid, bisdemethylcurcumin, demethylcurcumin, hexahydrocurcumin, octahydrocurcumin and other preparations that increase its bioavailability and maximize its potential health benefits [7]. In this regard, it is possible to assume possible prebiotic properties of curcumin during the development of lactic acid bacteria starter culture in the plant environment of an oat drink.

Activation of the development of lactic acid bacteria starter culture, in addition to increasing the probiotic characteristics of a plant-based drink, will presumably affect its organoleptic properties. Among the microorganisms producing exopolysaccharides, lactic acid bacteria are the group that attracts the most attention due to their safe use and potential probiotic properties. Microbiological exopolysaccharides are considered as an alternative to polysaccharides, which are thickening, stabilizing, texture-enhancing and gelling agents of plant origin, which are often used in the food industry. In addition to their technical and functional role, which improves the structural properties of food products, exopolysaccharides also have physiological properties that can positively affect consumer health [8].

In this regard, within the framework of these studies, we have proposed ways to activate the fermentation process by additional introduction of plant protein and the prebiotic curcumin into the plant environment.

2 Materials and Methods

The research was conducted on the basis of the scientific laboratory of the FSAEI HE "South Ural State University (NRU)".

The oatmeal drink "Ovsyasha" (LLC "Southern Juice Company", Belorechensk, Russia) was taken as a vegetable base. The sample contains: drinking water, oat flour, rapeseed oil, vitamin and mineral premix (vitamin D2, vitamin B2 (riboflavin), calcium (tricalcium phosphate), calcium carbonate), iodized salt.

Bacterial starter culture "Bifidum" (TU 10.89.19-026-51070597-2018, LLC "Green Lines", Krasnogorsk MR) was used as a probiotic culture, which includes: *Streptococcus salivarius ssp. thermophilus, Lactobacillus plantarum, Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus fermentum, Bifidobacterium bifidum, Bifidobacterium breve, Bifidobacterium longum ssp. linfantis.*

The combination of *Streptococcus salivarius ssp. thermophilus* strains is responsible for the formation of exopolysaccharides, which play the role of natural thickeners and consistency stabilizers in the production technology of fermented dairy products, as well as perform the functions of self-regulators of the growth and reproduction of microorganisms. *Lactobacillus plantarum* has fermenting properties, as well as the ability to reduce the number of pathogenic microorganisms in the product, in particular, bacteria of the *Escherichia coli* group. *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus fermentum* cause lactic acid fermentation, and are also included in the composition of products to further position them as probiotic. *Bifidobacterium breve* supports the functions of the digestive system, ferments oligosaccharides and synthesizes acetic and lactic acids in the production of a food product, which are able to break down hard-to-digest plant fibers, *Bifidobacterium bifidum* gives the product probiotic properties, *Bifidobacterium longum* strengthens immunity, shows antagonism to pathogenic bacteria. The adaptation and intensive development of these strains of lactic acid bacteria in an oatmeal drink will contribute to the development of a nutritionally complete product, which determines the relevance and significance of the research.

To intensify the fermentation process, soy protein (Las Flores Group LLC, Moscow) was introduced into a vegetable drink together with a starter culture, consisting of proteins - 90%, fats – 0.62%, carbohydrates – 0% and dietary fiber – 2% – with variations in the amount of application, and in other samples – curcumin (Athlete Arsenal LLC, Samara region), also in different quantities.

Thus, samples were obtained:

1 – control sample of an oat–based plant drink fermented with a bacterial starter culture "Bifidum". The rate of starter applications 0.3 g/100 ml.

2 – sample of an oat-based plant drink fermented with a bacterial starter culture "Bifidum", with the addition of soy protein in the amount of 1% at the fermentation stage.

3 – sample of an oat-based plant drink fermented with a bacterial starter culture "Bifidum", with the addition of soy protein in the amount of 3% at the fermentation stage.

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4 – sample of an oat-based plant drink fermented with a bacterial starter culture "Bifidum", with the addition of 0.05 curcumin at the fermentation stage %

5 – sample of an oat-based plant drink fermented with a bacterial starter culture "Bifidum", with the addition of 0.1% curcumin at the fermentation stage.

The activity of the fermentation process was assessed by the accumulation of biomass, the results of the assessment of titrated acidity, antioxidant properties, and the number of probiotic microorganisms.

The increase in microbial biomass was evaluated using an RTS-1C in vitro bioreactor. The bioreactor software builds graphically the enzymatic kinetics of the process based on determining the intensity of light scattering. At each controlled point, the device records the value of the increase in the biomass of microorganisms.

Titrated acidity was determined by neutralizing acid salts, proteins, free acids and other acidic compounds with an alkali solution in the presence of a phenolphthalein indicator.

The total antioxidant (antiradical) activity was determined by the DPPH method (%). DPPH is a stable free radical compound that is widely used in assays to assess the ability of antioxidants to absorb radicals.

Additionally, the antioxidant and membrane-stabilizing effects of probiotic drinks were evaluated according to the method proposed by E.F. Stepanova [9], on the culture of protozoa – infusoria of the species *Paramecium Caudatum*. The calculation of infusoria was carried out on the BioLaT-3.2 device using the AutoCiliataXP program. Survival rates and relative increase of infusoria were determined according to GOST 31674-2012. *Paramecium Caudatum* has cilia on its entire surface that act as chemoreceptors reacting to dissolved chemicals. By the ability to increase the tolerance of paramecia to cellular poisons under the influence of the studied substances, one can indirectly judge their adaptogenic activity. They are in constant motion, so it is easy to observe the slightest changes in movement under the influence of the substance under study.

The determination and counting of probiotic microorganisms consists in seeding functional foods and ingredients that could contain probiotic microorganisms in certain concentrations into nutrient media, and cultivating them under optimal conditions for growth, as well as subsequently determining their cultural and morphological properties and calculating the quantitative content in the product (according to the GOST R 56139 method). The overall confidence probability of the study results is 0.95.

3 Results and Discussion

Initial studies on the accumulation of microbial biomass during the fermentation of the vegetable medium of an oat drink showed the dependence of the activity of biomass growth on the introduced components (Fig. 1).

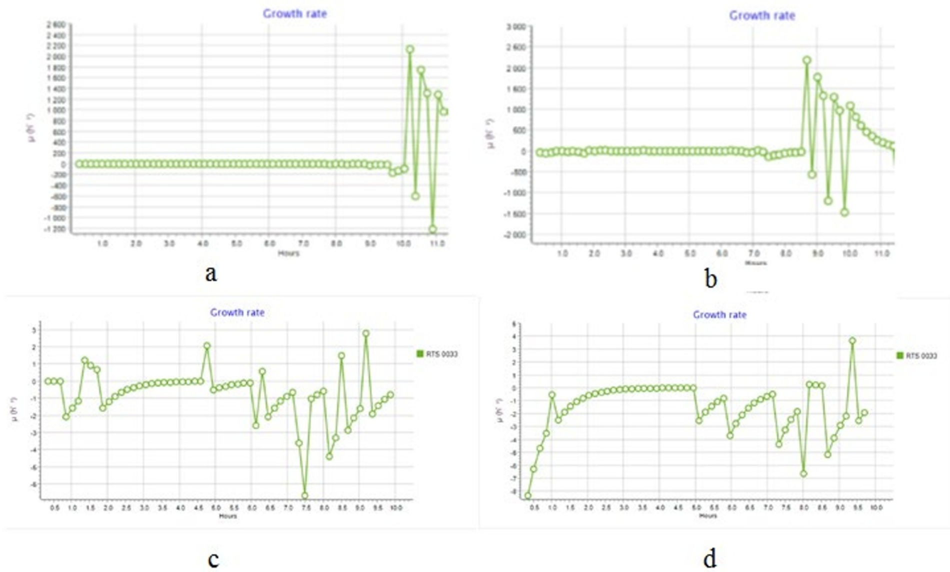

Fig. 1. Dynamics of the biomass growth rate of Bifidum starter culture in samples of a plant-based probiotic drink produced under different conditions: a – control; b – with the addition of soy protein; c – with the addition of curcumin 0.05%; d - with the addition of curcumin 0.1 %.

The results of the assessment of biomass accumulation under different conditions showed that, in general, the introduction of additional plant protein and curcumin activate this process, and this actively affects the growth curves of the bacterial culture.

At the first stage, in the lag phase, the culture adapts to a new environment, its enzyme systems are activated. Cells do not multiply, the concentration of living cells is constant and equal to the number of cells introduced. The duration of the lag phase in the control sample is about 10 hours (Fig. 1, a), then comes the phase of accelerated growth, as evidenced by data on a sharp increase in biomass accumulation.

The introduction of plant protein into the plant medium of oat drink contributes to the intensification of the process, the lag phase is reduced to 8.5 hours, then the growth phase of the starter culture of microorganisms begins. This phase is characterized by the beginning of cell division, an increase in total mass and a constant increase in the growth rate of the culture. The results of biomass accumulation assessment indicate a positive effect of the addition of vegetable protein on the activity of the development of probiotic culture, and exactly the smaller amount of protein is more of a growth factor in the starter culture of lactic acid bacteria.

The ambiguous effect of curcumin on the development of lactic acid bacteria has also been established, its introduction in an amount of 0.05% already during the first 1.5 hours of the fermentation process causes the growth of microbial biomass, but in a smaller volume relative to the control sample and samples with the introduction of plant protein. Curcumin in a volume of 0.1% in the first 5 hours of fermentation slows down the development of the starter slightly, then intensifying it, but also in a smaller volume relative to the control sample and samples with the introduction of plant protein. Moreover, a greater administration of curcumin causes a less active, but constant development of bacteria over time.

There are studies on the interaction between phenolic compounds and lactic acid bacteria in food products, indicating their effect on bacterial growth, which leads to a significant reduction in fermentation time [10]. As a result, the formed gels have a lower viscosity, which could be explained by the increased water content in the gel system due to a decrease

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in syneresis. Nevertheless, on the other hand, polyphenols can covalently or non-covalently interact with proteins. The protein-polyphenolic interaction is maximal at the isoelectric point of the protein and the formation of such bonds can make proteins less accessible to the nutrition of microorganisms [11-14].

The trends obtained in the assessment of biomass accumulation are confirmed by the results of determining the titratable acidity of probiotic drinks (Fig. 2). The results indicate that exactly the introduction of vegetable protein and curcumin intensifies the accumulation of lactic acid relative to the control sample. The introduction of plant protein activated the process by 40-69%, curcumin – by 5.9 – 12%. It is important to note that exactly the smaller amount of additives acts as the preferred growth factors for the starter culture.

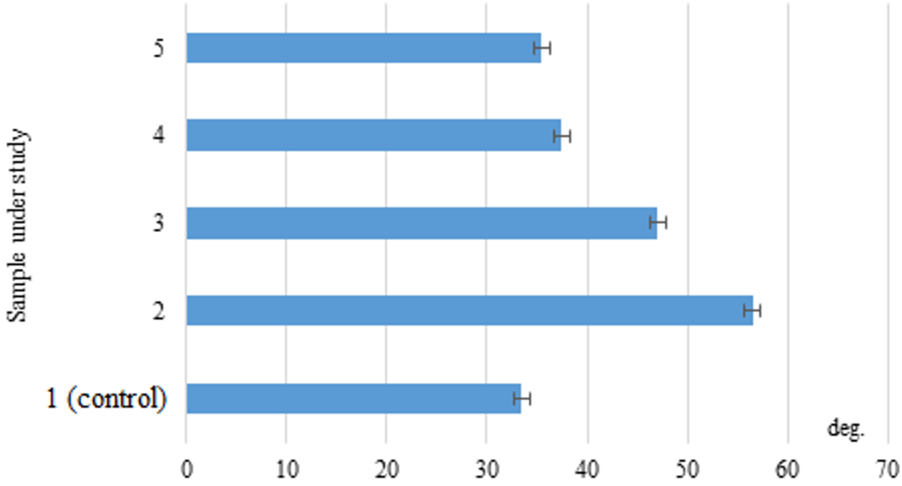

Fig. 2. The results of the assessment of the titrated acidity of the studied samples of probiotic plant-based drinks, deg.

The introduction of protein and curcumin also has a positive effect on the antioxidant properties of probiotic drinks (Fig. 3), the increase in antioxidant activity averaged 2.2 – 6.7%.

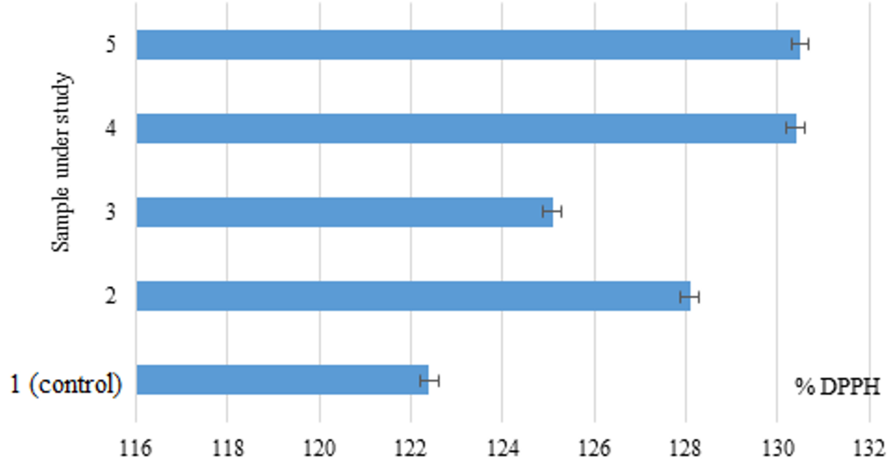

Fig. 3. The results of the evaluation of the antioxidant activity of the studied samples of probiotic plant-based drinks, % (DPPH).

These results can be explained both by fermentation activation and by the additional antioxidant properties of the administered curcumin.

The next stage of biological research on *Paramecium Caudatum* consisted in the fact that their reproduction and growth were analyzed during the experiment. Among the variety of microorganisms, paramecia are one of the most convenient test objects for medical, environmental, pharmacokinetic and toxicological studies, as they are sufficiently studied and have large sizes that allow working with individual organisms.

Cultures of protozoan *Paramecium Caudatum* have selective digestibility of food substances, are very sensitive to the choice of nutrient medium, combine the biological complexity of eukaryotes and the availability of unicellular organisms.

The assessment of the increase in protozoan *Paramecium Caudatum* also allows to note the actively developing antioxidant properties of probiotic drinks produced using modified technologies (Fig. 4).

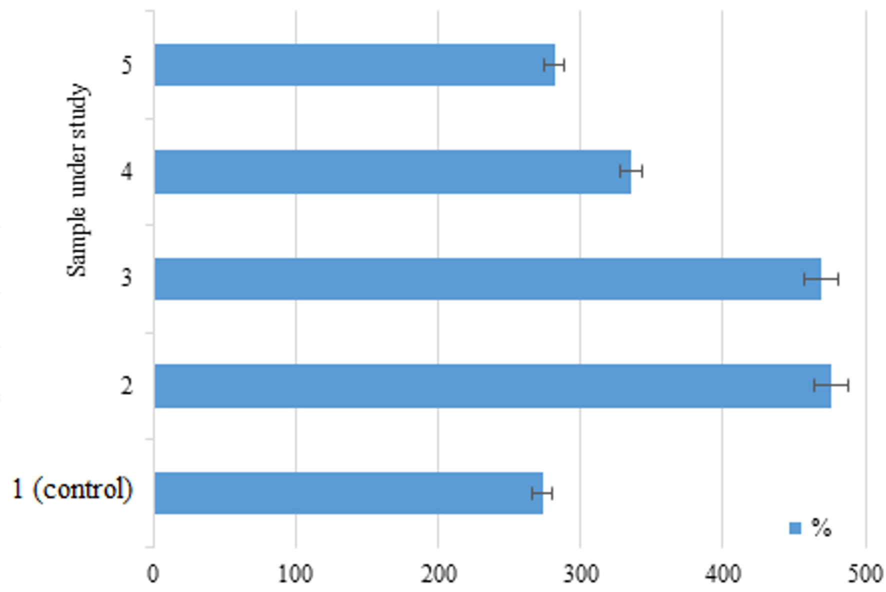

Fig. 4. The results of the assessment of the increase in the culture of protozoa *Paramecium Caudatum* in the studied samples of probiotic plant-based drinks, %.

The results obtained suggest an increase in the growth of protozoan *Paramecium Caudatum* when using experimental samples of probiotic drinks. The increase is clearly reflected in the figure and amounts to 71.8 – 74.4% when introduced into plant protein samples, and 3.1 – 22.8% curcumin. There is also a more pronounced positive effect of protein supplementation on the development of the starter culture of microorganisms, which indicates the preference for using an increased amount of protein as a nutrient medium for the development of lactic acid bacteria starter culture.

The activation of the growth of protozoan *Paramecium Caudatum* culture also indicates the safety of plant-based probiotic drinks produced.

At the final stage, the number of probiotic cultures of microorganisms was estimated according to the GOST R 561396 method by determining the MPN index.

The results obtained indicate that the number of probiotic cultures of microorganisms in all produced samples of a plant-based beverage is 3.8...5.2 $\times 10^7$, which makes it possible to classify the resulting drinks as functional products with probiotics and additionally indicates the ability of microorganisms of the starter culture used to develop in the plant environment of oat drink [15, 16].

4 Conclusion

Thus, the research results confirm the possibility of using oat-based plant drink to produce probiotic products. The stimulating effect on biotechnological processes is provided by an additional increase in the protein component of the nutrient medium of the plant drink and the introduction of curcumin.

An assessment of the accumulation of microbial biomass under different conditions showed that the introduction of additional vegetable protein and curcumin activates biomass growth by an average of 15-85%, intensifies the accumulation of lactic acid by 40-69% and 5.9-12%, respectively, increases the antioxidant activity of prepared drinks by an average of 2.2-6.7%.

The increase in the growth of protozoan *Paramecium Caudatum* is 71.8 – 74.4% relative to the control sample when protein is introduced into the samples, and curcumin is 3.1 – 22.8%. The increase in the culture of protozoan *Paramecium Caudatum* in all samples also indicates the safety of the food products produced. The number of probiotic cultures of microorganisms in all plant-based beverage samples produced is 3.8...5.2 $\times 10^7$, which makes it possible to classify the resulting drinks as functional products with probiotics.

Thus, it was found that an additional increase in the protein component of the nutrient medium of a vegetable drink and the introduction of curcumin have a stimulating effect on biotechnological processes and are promising in the production of probiotic drinks.

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