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Technology for Preparing Dried Starters from the Association of Lactic Acid Bacteria to Ferment Beet Juice as a Product of Functional and Clinical Nutrition

I.A. Ratnikova, N.N. Gavrilova, A.K. Sadanov, K. Bayakysheva, Z.Zh. Turlybaeva, V.A. Buzik.,A.S.Kamzaeva

(Institute of Microbiology and Virology of the Ministry of Education and

ABSTRACT: *To produce the fermented beet juice, the association of the cultures Lactobacillus fermentum 27 and Lactobacillus plantarum 22 was recommended. The conditions for preparing a dried starter from the association of lactic acid bacteria L. fermentum 27 and L. plantarum 22 have been selected. It was established that the optimal protective components for freeze drying of the bacteria include 7% glucose together with 5% dried skimmed milk and a combination of 5% sucrose, 5% lactose, and 5% dried skimmed milk. The possibility of using beet residues to enhance the antimicrobial activity of lactic acid bacteria has been demonstrated.*

Keywords: *lactic acid bacteria, antagonistic activity, freeze drying*

I. INTRODUCTION

Major changes in the structure of nutrition, related to the variations in lifestyle, reduction in energy consumption leads to the fact that none of the population groups obtain with the consumed food the adequate amount of vitamins, micro- and macroelements for human health. In this regard, there is an urgent need for developing functional foodstuffs.

D. Potter identified seven major types of functional ingredients: dietary fiber, vitamins, amines, mineral substances, omega-3 fatty acids found in vegetable oils, fish oils, antioxidants (beta-carotene, ascorbic acid, alpha-tocopherol), oligosaccharides (as a substrate for beneficial bacteria), and a group comprising trace elements, amino acids and others [7]. With these food ingredients, some diseases can be prevented, others retarded or their flow facilitated. For example, vitamins-antioxidants C and E, carotenoids and flavonoids, dietary fiber assist in resisting cardiovascular diseases. The use of vitamin C helps to protect the body from the stomach cancer, and beta-carotene – from lung cancer. Osteoporoses may be prevented by introducing foodstuffs that contain calcium and vitamins such as K, C, B_6, D_3 and trace element boron into the diet [4].

Individual ingredients serve as protectors for several diseases. The combined use of vitamins as part of functional foodstuffs at doses comparable to their daily requirement enhances their effect. Unsaturated fatty acids are involved in the degradation of low density lipoproteins, cholesterol, take part in hydrogenation processes, prevent aggregation of haematocytes and blood clot organization, relieve inflammatory processes, etc. To prepare a variety of functional foodstuffs, vegetables can be used since they are one of the main sources of vitamins, mineral salts, organic acids, aromatic compounds and, to a considerable degree, easily available carbohydrates. At the same time, table beet juice deserves special consideration, since it has a positive effect on the body as a whole and activates functioning of a number of organs, exerts a positive impact on the blood, normalizes metabolism of fats, clears the body of chemical waste, has an antibacterial activity. The presence of polyphenols in the juice, being free radical acceptors and inhibitors of chain reactions, determines its use in radiation damages, cancer and cardiovascular diseases, atherosclerosis. It was proved that beet is a rich source of physiologically essential substances for the human body.

Despite the useful properties of beet juice, its production is limited. This is due to the fact that it has inharmonious taste and a low acidity. Furthermore, existing production technologies do not always provide the preservation of biologically active substances in the juice, including betanidin.

One way to enrich juices with biologically active substances and stabilize them is lactic-acid fermentation. Beet juice prepared by lactic-acid fermentation, contains natural lactic acid, high content of essential amino acids, has an intense dark red color, good dietary properties [1, 2, 3]. At that, the quality of the juice depends on the bacteria used in its fermentation.

Therefore, the purpose of this research is the technology for preparing dried starters from the association of lactic acid bacteria to ferment beet juice as a product of functional and clinical nutrition.

II. MATERIAL AND METHODS

The association of lactic acid bacteria (Lactobacillus fermentum 27 and Lactobacillus plantarum 22) was the object of the research aimed to prepare beet juice with high organoleptic properties and enriched with vitamin C and the B group vitamins, essential amino acids.

Lactic acid bacteria were cultivated in MRS medium with the following composition (g/l): yeast extract – 5.0; meat extract - 10.0; peptone - 10.0; glucose - 20.0; ammonium citrate - 2.0; sodium acetate - 5.0; Tween 80 - 1.0; KH_2PO_4 – 2.0; $MgSO_4 \cdot 7H_2O$ - 0.2; $MnSO_4 \cdot 4H_2O$ – 0.05; $CoCl_2$ – 0.01; $(NH4)_2SO_4$ – 1.0

In the preparations, the number of viable cells and antagonistic activity against pathogenic and opportunistic microorganisms, promoting fermentation of beet juice, were determined [4].

In vegetable juices, the following parameters were evaluated: solids content using refractometer RPL-3 according to GOST 8756.14-70; 6etanidin by photometric method [5]; vitamin C using iodometric method, based on the ability of ascorbic acid to reduce the potassium iodate to free iodine, the amount of which was measured in the reaction with starch [6]; mass fraction of titratable acids in terms of malic acid (GOST 25555.0-82); ash content.

In the process of drying, the following variants of protective media were used:

1) 10% sucrose + 5% sodium citrate.
2) 10% sucrose + 1% gelatin.
3) 7% glucose + 5% dried skimmed milk.
4) 5% sucrose + 5% lactose + 5% skimmed dried milk.
5) 10% sucrose + gelatin 1.0-1.5% + 0.05-0.2% agar.
6) 10% sucrose + 5% sodium citrate + 0.5% ammonium chloride + 0.05% magnesium sulfate.

The pH of the dried mixture was adjusted in the range of 6.0-6.5. Drying was carried out using LyoBeta-35-35 freeze dryer. The preparation in the vials was frozen at -50 ^{0}C for 5-6 hours. Drying of the frozen product was started at -30^{0}C, the temperature of the final drying was of +25 - +27^{0}C. Duration of the whole process was 28-36 hours.

As the objects of the research aimed to study dietary fiber (table beet residues fermented by lactic acid bacteria and cellulose) that selectively stimulates the growth and antimicrobial activity of lactic acid bacteria, L. fermentum 27 and L. plantarum 22, as well as pathogenic and opportunistic microorganisms including Escherichia coli, Salmonella gallinarum, Stahyelococcus aureus, Pasteurella multocida, Klebsiella pneumoniae, Candida albicans, Mycobacterium B-5, Fusarium vasinfectum, Aspergillus niger, Bacillus subtilis, were chosen.

III. RESULTS AND DISCUSSION

In the present study we have selected strains of lactic acid bacteria promoting the preparation of beet juice with a pleasant taste and aroma, the producers of vitamins C, B_5, B_{12}, B_1, B_2, PP, essential amino acids, hydrolytic enzymes, antagonists against Bacillus subtilis, Bacillus mesentericus, Escherichia coli, which can be specifically used to develop products of functional and clinical nutrition.

In the production of fermented beet juice, it is necessary to have an association of lactic acid bacteria with stable properties, since it affects the quality of the finished product. Therefore, in the juice production, it is desirable to use freeze-dried starter with predetermined properties.

To prepare the fermented beet juice, the association of the cultures L. fermentum 27 and L. plantarum 22 has been recommended, which we developed in the process of purposeful selection by the necessary criteria.

Dried starters to ferment juices were prepared from the cultures of L. plantarum 22 and L. fermentum 27, cultivated together. To culture lactic acid bacteria, the MRS nutrient medium was used, the cultivation continued for 24 hours at a temperature of 37 ^{0}C.

Dried preparations of lactic acid bacteria depending on the composition of protective media were cream to dark brown in color.

It was established (□**able I.**), that the protective media No. 3 (7% glucose + 5% dried skimmed milk), and No. 4 (5% sucrose + 5% lactose + 5% dried skimmed milk) have proved to be the best for the selected association; when using these, the initial dry preparations contain 18-68 billion cells/g, and after reactivation in sterile tap water for 4 hours - 730-1150 billion cells/g.

Variants of protective media	Number of viable cells, billion CFU/g			
	in the initial preparation	after reactivation, h		
		2	4	6
1	5.7	32.0	104.0	104.0
2	11.0	101.0	157.0	161.0
3	68.0	530.0	1150.0	1210.0
4	18.0	270.0	730.0	800.0
5	12.0	102.0	144.0	148.0
6	4.4	35.0	75.0	80.0

□able I. Number of viable cells of lactic acid bacteria in dried preparations

Variants of dried starters were tested when preparing beet juice compared with the initial cultures. To this effect, the dried starters were diluted with tap water up to the initial titer of the liquid culture. After two-hour reactivation, a bacterial suspension was introduced into the beet pulp in an amount of 10%. The test results are given in **□able II.**

Variants of protective media	Titratable acidity	Juice yield, %	Solids, %	Vitamin C content, %	Betanidin content, %	Organoleptic properties	
						pH	Organoleptic parameters
1	0.5	109	8.2	4.6	84	4.3	Grey-brown color
2	0.5	109	8.2	4.7	78	4.3	Grey-brown color
3	0.5	109	8.3	4.8	221	4.2	Satisfactorily
4	0.5	109	8.3	4.8	215	4.2	Satisfactorily
5	0.5	109	8.2	4.7	148	4.2	Satisfactorily
6	0.5	109	8.2	4.8	76	4.2	Grey-brown color
Control – the initial liquid culture	0.5	109	8.3	4.8	215	4.2	Satisfactorily

□able II. Comparative characteristics of juices fermented by the dried lactic acid starters

As seen from Table II, when using dried starters prepared from the association of lactic acid bacteria and dried with the protective media Nos.1, 2, 6, the juice had a brown tint. This demonstrates the unacceptability of these protectors in the production of dried starters to ferment beet juice.

When beet juice is fermented by dried starters, its acidification to pH 4.3-4.1 occurs with formation of organic acids (0.4-0.5%). Juice yield in all variants was the same, but in variants with starter cultures, dried with protective media Nos.1, 2, 5 and 6, solids content was, as a rule, lower than in variants Nos. 3 and 4. By the content of vitamin C and betanidin, the juices, prepared with the starters and dried with protective media Nos. 3 and 4, have proved to be the best.

When comparing data, it can be seen that the juices prepared with freeze-dried lactic acid bacteria with protective media Nos. 3 and 4, by the content of solids, betanidin, and vitamin C are superior to those made with the initial cultures. Therefore, the best protective media for drying the selected association of lactic acid bacteria are Nos. 3 and 4, in which the carbohydrates in combination with dried skimmed milk are used as protective components. To enhance the starter activity, it should be reactivated in the drinking water for 2-4 hours. Reactivation over 4 hours had no advantages. The possibility of using beet residues obtained during the juice extraction to enhance the antimicrobial activity of lactic acid bacteria was studied as well.

As is well known, dietary fiber has numerous physiological effects, which determines its importance for the normal functioning of the body. It retains water, thereby affecting the osmotic pressure in the lumen of the gastrointestinal tract, electrolyte composition of intestinal contents. Dietary fiber has a high adsorption capacity, which accounts for its detoxifying effect. It is an important regulator of the intestinal microflora composition. Digestion of fermentable and partially fermentable dietary fiber entering the intestine is realized by the colon microflora that receives in this way the energy and plastic material.

The aim of further research was to study dietary fiber (table beet residues fermented by lactic acid bacteria and cellulose) that selectively stimulate the growth and antimicrobial activity of lactic acid bacteria.

Lactic acid bacteria were cultivated in the MRS medium, pathogenic and opportunistic microorganisms - Gauze-2, in which dietary fiber served as a source of carbon: table beet residues fermented by lactic acid bacteria - production waste of the therapeutic dietary beet beverage prepared by the technology developed at the Institute of Microbiology and Virology, and cellulose as well. The medium with glucose served as the control.

In nutrient medium containing fermented beet residues, lactic acid bacteria grew well, reaching a titer predominantly of n x 10^{10} CF/mL, and in some cases - n x 10^{11}. Antagonistic activity was established virtually against all examined test cultures. No activity was detected against C. albicans. Antimicrobial activity of the two strains of lactic acid bacteria taken in the experiment and cultivated in a medium with residues of the fermented table beet, exceeded the activity of those grown in medium in which glucose served as a carbon source (Table 3).

Media	Inhibition zones for test cultures, mm								
	S. gallinarum	E. coli	P. multocida	B. subtilis	A. niger	F. vasinfectum	C. albicans	Mycobacterium B_5	S. aureus
	L. plantarum 22								
1	14	15	15	19	19	13	0	25	14
2	12	14	14	12	15	0	0	17	13
	L. fermentum 27								
1	19	17	14	24	18	20	0	23	28
2	12	14	14	12	15	0	0	17	13
Note: 1 – medium with the residues of fermented table beet 2 – medium with glucose									

able III. Antagonistic activity of lactic acid bacteria on the table beet residues

IV. CONCLUSION AND FUTURE WORK

It can be concluded that the introduction of the fermented table beet residues in the nutrient medium as a source of carbon appreciably enhances the antimicrobial activity of lactic acid bacteria.

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