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INNOVATIVE TECHNOLOGIES FOR CREATION OF PROBIOTIC FOODS

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Abstract

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Lyophilized probiotic products have been developed by high technology methods for nutrition prophylaxis and healthy nutrition to ensure a maximum working capacity and well- being of each individual. In their composition are included natural sources of essential bioelements and physiologically active substances – a complex of probiotic lactic acid bacteria, oligosaccharides, antioxidants, vitamins and others. Their fine consistency and chemical composition create a possibility for rhythmical introduction in the organism of nutritious mixtures with adequate content of plastic substances and energy. Their proved healthy effect makes them suitable to be included in combined nutrition diets for ensuring of better quality of life in terms of the effective prevention and improvement of the health status of the population

Key words: probiotic foods, artificial neural networks (ANN), lyofilization, nutrition diets

Introduction

The last two decades are characterized by an expressed orientation towards more and more mass application of natural means for protecting of human health under the form of probiotics, functional foods, biological stimulators, regulators and other. This imposes the dynamically growing food industry to develop and implement products meeting the modern requirements for healthy nutrition (Chomakov, 2007).

Every food attributed to the functional foods is assessed by its nutritious value, high-calorie, taste qualities and its effects on human health, expressed in supporting of the organism and healing capacity in case of disease (Georgieva and Tzvetkov, 2001).

Among the multitude of probiotic foods, the interest in the production and consumption of fermented dairy products, containing live microorganisms is particularly great. They have a favorable effect on the intestinal microflora, stimulate selectively its growth and metabolism, strengthening in such a way human health (De Vuyst, 2000; Cash et al., 2005).

The probiotic effect of the lactic acid microorganisms is motivated by a number of physiological characteristics and requirements: the microorganisms of the microflora should be natural inhabitants of the gastrointestinal tract; they should not be pathogenic and toxigenic; they should be capable to survive and grow under the conditions of the digestive system, i.e. to be resistant to low pH and organic acids and to maintain stable population in the gastrointestinal tract (Denkova and Murgov, 2008).

For the development of each new food its function and purpose are determined on the basis of a preliminary elaborated concept for the interaction mechanism between its components and the functions of the organism (Georgieva et al., 2006).

The main requirements to all technologies for food production and storage are to obtain products with unchanged nutritious value, good taste, structure and flavor (Nacheva, 2008).

By the complex combining of the ingredients in the composition of the new functional foods, is a high summary physiological effect is achieved as a result of their application.

The quantitative proportions between the separate ingredients are determined on the basis of an innovative approach in food science, applicable in the development of new foods – the use of artificial neural networks (ANN).

The neural networks also find application in modeling of the growth of various microorganisms, directly related to the food harmlessness, prediction of physical, chemical, functional and sensory characteristics during their production and storage until the purchase by the consumer (Huang et al., 2007).

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The modeling through neural networks can solve relatively complex tasks of the technological production processes management, the simulation of various events and characteristics, which provides for the obtaining of quality and harmless foods.

The main objective of the investigation is the creation of a new class probiotic lyophilized foods with formulated composition by applying of an artificial neural network (ANN) and with multifunctional effect.

Materials and Methods

In the composition of the new foods were included different sources of nutrients and physiologically active substances – probiotic complex of active lactic acid bacteria, polysaccharides, fruits, grain ingredients, honey products, antocyans, and plant oils.

Biotechnological methods

The live microflora in the probiotic products is presented by strains lactic acid bacteria, from the collection of ICFT and NBIMCC: *Streptococcus thermophilus* 1374; *Lactobacillus bulgaricus* 1381; *Lactobacterium acidophilus* 1379; *Lactobacillus casei* 1014, included in a combined lactic acid starter in proportion 1:2 (cocci: rod-shaped lactic acid bacteria). The cultivation of the strains *Lb. bulgaricum* and *Str. thermophilus* was realized at temperature 43^0C for 5-9 h, of *Lactobacterium acidophilus* at temperature 37^0C for 5-8 h and *Lactobacillus casei* subsp. *casei* – 30^0C for 5-8 h.

Technological methods

For the aims of the long term, quality and safe storage of the probiotic foods was used the freeze-drying method (Tzvetkov, 1979). It was realized in a laboratory freeze-drying installation „Gamma 1" – of company „Martin Christ"- Germany with residual pressure in the vacuum chamber in the range of 10^{-1}–10^{-2} mm Hg. The freeze-drying parameters were programmed by applying of differential thermal analysis (Differential thermal analysers STA781 DTA 675 and Differential scanning calorimeter DSC1500 of company "Stanton Redcroft", which guarantees the determining of their optimum values in relation to the end product quality indices).

The data from the experiments were statistically processed with Statistics for Windows.

Modelling

The neural model was realized as a software instrument with flexible structure which can be easily adapted to the objectives of the investigation. The obtained model was used to formulate the composition of the experimental samples.

Analytical methods

Physical-chemical and biological investigations – residual moisture content after lyophilization (BSS 1109-89); pH; titrated acidity, total protein – (BSS 9374-82); content of reducing sugars (incl. lactose – method of Alin), vitamin C (by the method of Muri).

Microbiological investigation –determining of the number of viable lactic acid bacteria – by the method of the limit dilutions with consequent calculations by the table of McCrady; *recording of the lactic acid microflora survival* - in % - by the following formula: % survived cells = (number of survived cells x /number of cells 0) x100, where: x – moment of the survival recording (at the 48^{th} hour after the lyophilization of the samples) 0 – moment before the lyophilization (number of viable cells before lyophilization).

Granulating, packing and storage conditions: After the lyophilization, the freeze-dried samples were granulated with a granulator "ERWEKA", packed in a three-layer /aluminum - polyamide - polyethylene/ folio, hermetically vacuum sealed and stored in premises with relative air humidity not above 35 % at temperature - 20-22^0C.

Results and Discussion

The bioformulas of the new foods are in accordance with the requirements for physiological activity, harmlessness, safety and microbial stability valid in principle for the probiotic foods. Of great importance for their good assimilation is also their taste acceptability, resp. their organoleptic properties /taste, aroma, stability of the consistency, good solubility/ which is taken in consideration in the process of formulation. An organoleptic assessment was made, after rehydratation, by the 9-grade Hedonic scale.

Despite the not typical taste-aroma properties due to the specificity of their recipe compositions, the new foods are with an excellent organolepsy and with a pleasant sour taste. The physical-chemical indices of the investigated samples showed the following trends (Table 1).

As it is seen from the results, the new probiotic foods are food concentrates with low residual moisture content – 2.46 - 3.00%, which is a proof for an optimally realized technological process of freeze-drying. The titrated acidity of the control, before and after lyophilization is in the standard norms (up to 120^oT). The colloid character of the remaining variant samples, causing a slight slow up of the lactic acid bacteria life intensity, determines the decreased titrated acidity values (72 - 90^0T). In respect to the active acidity (pH), sudden changes in the values of the investigated samples are not observed – they are in the limits– 4.02 – 5.12, i.e. in the standard norms for fermented foods. It is pH of the medium that also affects to a great extent

the enzyme and the biochemical activity of the microorganisms, which is of importance for their viability and survival and for their probiotic effect, respectively.

The increased content of total protein, reducing sugars and vitamin C is a result of the introduced additional nutrients in the new foods formulas.

The quantitative content and the viability of the lactic acid bacteria are the main criteria determining the biological activity and probiotic qualities of the experimental samples.

As it is seen from Figure 1 the survival is lowest after lyophilization of the control without included cryoprotector 50.2% for the lactic bacteria and 68.5% for Str.thermophilus. For the lactic acid microorganisms in the variant samples the survival is higher – 85.4% for Str.thermophilus и 78.6% for the lactic bacteria because of the presence in their composition of oligo- and polysaccharides, which have a cryoprotective effect as well. They increase the colloid linked water for the sake of the osmotic pressure as a result of the filling of the micro- and macrocapillaries (Figure 1).

For the control and the variant samples, after lyophilization was observed was observed high resistance of Str.thermophilus to freeze-drying – from 68.5 up to 85.4%, compared to *Lb. bulgaricus, L. acidophilus* and *L. casei*, which are more thermolabile and with a lower level of survival from 50.2 up to 78.6%.

A possible reason for the different resistance of the microorganisms to drying is the different sensibility and the cell wall to the strains arising during freezing and drying or the different capacity f the cell enzymes for denaturation.

The survival of the pathogenic microflora during lyophilization is affected by the moisture content of the product, the temperature parameters and the vacuum values during storage.

The carried out by the standard methods investigations of the microbiological characteristics of the lyophilized foods proved an absence of microorgnism seeding and of pathogenic microflora (Table 2).

Therefore, the applied storage conditions of the studied lyophilized probiotics - 20-22^{0}C and relative humidity up to 35% are favorable for their long storage.

Table 1
Physical-chemical indices of the experimental samples – in native status and after lyophilization

Indices	Control		Variant 1		Variant 2		Variant 3	
	before lyophilization	after lyophilization	before lyophilization	after lyophilization	before lyophilization	after lyophilization	before lyophilization	after lyophilization
Total moisture %	87,90±0.01	2,98±0.00	83,1±0.00	2,46±0.01	84,88±0.07	3,00±0.05	82,8±0.01	2,95±0.06
Titrated acidity °T	110 ±0.00	95 ±0.01	82±0.01	78±0.2	79±0.012	72±0.11	90±0.011	83±0.014
pH	4,20±0.03	4,16±0.04	5,12±0.03	4,32±0.03	4,43±0.11	4,02±0.00	4,82±0.09	4,11±0.01
Total protein %	4.12±0.01	16.21±0.01	14,05±0.23	29,05±0.01	16,78±0.01	35,01±0.01	18,96±0.00	38,11±0.02
Reducing sugars % (incl. lactose)	5,16±0.02	8,97±0.02	7,08±0.01	8,58±0.06	6,12±0.00	7,00±0.02	8,09±0.01	9,39±0.03
Vit.C mg %	1,206±0.01	2,542±0.02	3,123±0.00	3,670±0.01	2,185±0.00	3,225±0.01	2,312±0.15	3,412±0.01

Table 2
Values of the microbiological seeding of the experimental samples in CFU/g and log - in native status and after lyophilization

Sample type	Total number mezophylic microorganisms		Moulds		Yeasts		Coliforms	
I . Native	CFU/g	log	CFU/g	log	CFU/g	log	CFU/g	log
Control	$2,8.10^1$	1.45	2.8	0.45	$0,9.10^1$	0.95	ND	-
Variant 1	$2,3.10^3$	3.36	$1,8.10^1$	1.25	$1,3.10^1$	1.11	ND	-
Variant 2	$1,45.10^3$	3.16	$1,6.10^1$	1.2	$1,8.10^1$	1.26	ND	-
Variant 3	$2,7.10^3$	3.43	$1,4.10^1$	1.15	$1,7.10^1$	1.23	ND	-
II. Lyophilized								
Control	$1,2.10^1$	1.08	1.5	1.18	4.3	0.63	ND	-
Variant 1	$1,9.10^2$	2.28	$1,2.10^1$	1.08	$0,7.10^1$	0.85	ND	-
Variant 2	$1,38.10^2$	2.14	$1,1.10^1$	1.04	$1,1.10^1$	1.04	ND	-
Variant 3	$2,6.10^2$	2.41	0.8	0.9	$1,2.10^1$	1.08	ND	-

ND – Not determined

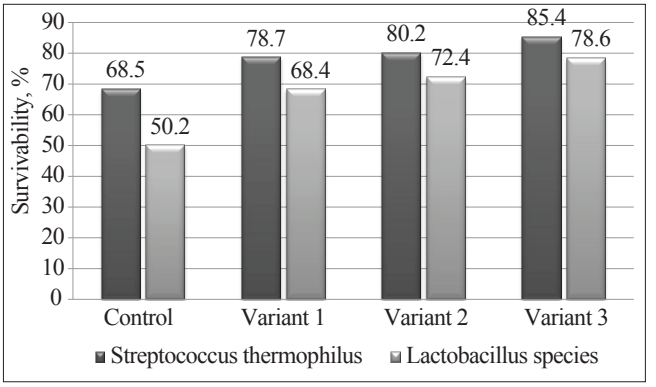

Fig. 1.

EFFECT OF THE WATER ACTIVITY (a_w) – The water activity was introduced as an important controlling and variable quantity in the technology of foods with long shelf life. For the microorganisms it was established that their growth is in correlation with a_w under certain conditions.

The results obtained from the investigation of the lactic acid bacteria survival in lyophilized probiotic foods prove that the water activity in them s optimum, i.e. relatively low - 0.28 -0.47, which can be explained both with the appropriate physical-chemical and biochemical characteristics of the products and by the contained therein cryoprotective water soluble biopolymer matrices, and also as a result of the carried out cryogenic cryogenic treatment of the source material – fast freezing and freeze-drying. Probably the lactic acid bacteria adapt themselves to low a_w easily under the effect of the mentioned factors. This can be attributed to the possible direct effect of the added substances on the microorganisms.

A proof for the low water activity values in our probiotic foods is the resistance to the applied heat treatment – freezing and freeze-drying, determined by the achieved high survival of the lactic acid bacterial microflora during freezing and lyophilization.

Conclusions

A complete technology for production of a new range probiotic products based on freeze-drying, by applying of a modern, innovative method of formulation of their composition – neural networks (ANN) has been developed.

The applied technology ensures the obtaining of multicomponent food concentrates with maximum preservation of their nutritious value and bioactive complex, in combination with a viable and active useful lactic acid microflora.

The new lyophilized probiotic foods, technologically processed without introducing of chemical reagents in the biosystem are 100% organic foods with a wide application spectrum in the bioprophylaxis of a number of modern diseases – gastro-intestinal, metabolic, professional and other as well as for increasing of the adaptability of the organism to unfavorable environment conditions.

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