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Sinbiotic complexes for production of new fermented functional milk products

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Разработени са синбиотически комплекси за произвеждане на нови ферментирани пробиотически, геродиетически млечени продукти с различни про- и пребиотики.

Sinbiotic complexes for production of new fermented functional milk products

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Sinbiotic complexes for production of new fermented probiotic and gerodietetic milk products with different pro- and prebiotics is developed.

Statement of a problem in a general view. In the modern concept of wholesome nutrition the special role belongs to the products of functional purpose. There are some definitions of functional foodstuffs. These are products which:

- were obtained from natural ingredients and contain significant number of biologically active substances;
- may and shall be a part of everyday food allowance of man;
- at the use shall regulate specific processes in the organism (for example, to stimulate immune reactions, to prevent development of some diseases, etc., in other words, are designed for improvement of consumer's health and decrease of risk of disease rise).

There are some ways of transformation of common product into functional one. The first one is enrichment of products with physiologically functional ingredients (PFI) in the course of production, at that the products can be enriched both with standard set of biologically active substances (BAS), and atypical one (for example, antioxidant, phytocomponents, microelements, etc.). The second one is obtaining of raw materials with prescribed component composition, providing for the regulating of proportion of specific components (for example, fatty acids) in products, obtained from it.

Fermented milk products traditionally are the part of everyday food allowance of each man that is why they have not only to provide its organism with energy and necessary essential components, but also with PFI. That is why the development of scientific bases of biotechnology of new fermented milk products of functional purpose is of current importance on the modern stage.

The work contains the main stages of development of biotechnology of new fermented milk products if functional purpose: probiotic, diabetic, gerodietetic and immuno-modulating ones. Each of represented groups of functional products includes the series of fermented milk drinks, curds and curd products, sour cream.

The author selected the first way of transformation of traditional sour-products into functional ones. Spectrum of workable fermented milk products covers all age-specific categories, excluding children's group. Under the directive effect the gerodietetic and diabetic products belong to the products of special purpose; immuno-modulating ones – to the products of health-improving and preventive purpose; pro- and sinbiotic ones – to the products of treatment-and-preventive effect [6].

One of PFI, used in biotechnology of production of all workable products, is pure and mixed cultures of Bifidobacterium. Introduction of Bifidobacterium just in fermented milk products is the most advisable, as these products already reveal probiotic influence on man organism, and in the case of enrichment of them with Bifidobacterium flora, such effect is increased by some hundreds of times.

At present all products, containing Bifidobacterium, are divided into three groups. The first group includes products, added with viable cells of Bifidobacteria, cultivated on special media. Reproduction of these microorganisms in the products is not provided for. The second group includes the products, ripened with pure or mixed cultures of Bifidobacteria, during the production of which the activation of growth of Bifidobacteria is achieved by enrichment of milk with bifidogene factors of different nature. The third group of bifido-containing products includes the products of mixed fermentation, mostly ripened by joint culture of Bifidobacteria and fermented milk microorganisms.

Wide range of fermented milk products of third group are represented on the world market, and slight assortment of drinks and fermented milk drinks of the first group as well as fermented milk products of the second group are almost not represented.

It is the author's opinion that fermented milk products of the second group have a number of advantages over the products from the first and third groups: products from the first group contain only viable cells of Bifidobacteria and almost doesn't have any products of their activity, which have probiotic effect on man organism; fermented milk products from the third group contain the products of vital activity of Bifidobacteria, but, frequently number of viable cells of Bifidobacteria and their probiotic effect in such products is some times less than in fermented milk products of the second group. That is why it is advisable to conduct development of biotechnology of pro- and sinbiotic fermented milk products on the basis of ripening of milk with pure or mixed cultures of Bifidobacteria, using prebiotics *in vitro*.

Presentation of a base material. The term "prebiotic in vitro" is introduced by the author for indication of bifidogene factors, which stimulate growth and reproduction of Bifidobacteria on nutrient media and foodstuffs, and it is not of necessity to reveal such effect in man organism [5, 15].

With the purpose of compiling of sinbiotic complexes, including Bifidobacteria and prebiotics *in vitro*, the author used the ferments of Bifidobacteria of direct introduction (FD DVS Bb-12, containing oure cultures Bifidobacterium animalis; Liobac BIFI and Liobac BIFIDI, containing mixed cultures Bifidobacterium bifidum, Bifidobacterium breve and Bifidobacterium longum), syrups of lactulose Dufalak and "Lactusan", fructose, glucose, bee honey, and also inulin consisting of puree earth pear [5, 15]. Used ferments have great number of milk-adapted probiotic cultures (not less than $1 \cdot 10^{11}$ cells/g), normalizable organoleptic and microbiological characteristics; they are high-technological and steady.

Initial concentration of probiotic component and composition of prebiotics *in vitro* were varied during compiling of sinbiotic complexes. Determination of number of viable cells of Bifidobacteria in clots, ripened with use of compiled sinbiotic complexes (Table 1) witness that introduction of small number of stimulators of Bifidobacteria growth in milk (0,1%) significantly effects on time of ripening of milk or/and probiotic properties of obtained clots [5-6, 15].

Use of any of the examined sinbiotic complexes is reasonable, but during use of fructose as prebiotic in vitro and increasing of initial concentration of probiotic up to $1 \cdot 10^6$ cells/cm^3 the time if milk ripening significantly reduces, that is caused by fermentative properties of Bifidobacteria [5-6].

Table 1. – Dependence of duration of milk ripening and probiotic characteristics of clots on used complexes of sinbiotics ($P \geq 0,95$, $n = 3...5$)

Probiotic component	Prebiotic in vitro (C=0,1%)	Initial concentration of probiotic, cells/ cm^3	Probiotic concentration in clot, cells/ cm^3	Duration of milk ripening, hour
FD DVS Bb-12	-	$1 \cdot 10^5$	$(0,5\pm0,2) \cdot 10^8$	32 ± 1
		$1 \cdot 10^6$	$(1\pm0,5) \cdot 10^8$	30 ± 1
FD DVS Bb-12	lactulose	$1 \cdot 10^5$	$(2\pm1) \cdot 10^8$	13 ± 1
		$1 \cdot 10^6$	$(3\pm12) \cdot 10^8$	12 ± 1
FD DVS Bb-12	fructose	$1 \cdot 10^5$	$(1,5\pm1) \cdot 10^8$	$12,5 \pm 0,5$
		$1 \cdot 10^6$	$(2,5\pm0,2) \cdot 10^8$	$12,2 \pm 0,3$
FD DVS Bb-12	lactulose + fructose	$1 \cdot 10^5$	$(2\pm1) \cdot 10^8$	$13 \pm 0,5$
		$1 \cdot 10^6$	$(3\pm1) \cdot 10^8$	$12 \pm 0,5$
FD DVS Bb-12	glucose	$1 \cdot 10^5$	$(1\pm0,5) \cdot 10^8$	14 ± 1
		$1 \cdot 10^6$	$(1,5\pm0,5) \cdot 10^8$	13 ± 1
FD DVS Bb-12	glucose + fructose	$1 \cdot 10^5$	$(1,5\pm1) \cdot 10^8$	13 ± 1
		$1 \cdot 10^6$	$(2,5\pm1) \cdot 10^8$	12 ± 1
FD DVS Bb-12	bee honey	$1 \cdot 10^5$	$(0,5\pm0,5) \cdot 10^8$	$13 \pm 0,5$
		$1 \cdot 10^6$	$(1,5\pm0,5) \cdot 10^8$	$12 \pm 0,5$
FD DVS Bb-12	inulin	$1 \cdot 10^5$	$(2\pm1) \cdot 10^8$	13 ± 1
		$1 \cdot 10^6$	$(4\pm1) \cdot 10^8$	12 ± 1
Liobac BIFI (Liobac BIFIDI)	-	$1 \cdot 10^5$	$(0,2\pm0,05) \cdot 10^8$	10 ± 1
		$1 \cdot 10^6$	$(0,4\pm0,03) \cdot 10^8$	$9 \pm 0,5$
Liobac BIFI (Liobac BIFIDI)	lactulose	$1 \cdot 10^5$	$(10\pm2) \cdot 10^8$	$9,5 \pm 0,5$
		$1 \cdot 10^6$	$(15\pm1) \cdot 10^8$	$8,5 \pm 0,5$
Liobac BIFI (Liobac BIFIDI)	fructose	$1 \cdot 10^5$	$(15\pm2) \cdot 10^8$	$9,2 \pm 0,2$
		$1 \cdot 10^6$	$(23\pm3) \cdot 10^8$	$8,2 \pm 0,2$
Liobac BIFI (Liobac BIFIDI)	lactulose + fructose	$1 \cdot 10^5$	$(15\pm2) \cdot 10^8$	$9,5 \pm 0,5$
		$1 \cdot 10^6$	$(19,5\pm1,5) \cdot 10^8$	$8,5 \pm 0,5$
Liobac BIFI (Liobac BIFIDI)	glucose	$1 \cdot 10^5$	$(9\pm1) \cdot 10^8$	$10 \pm 0,5$
		$1 \cdot 10^6$	$(11\pm1) \cdot 10^8$	9 ± 1
Liobac BIFI (Liobac BIFIDI)	glucose + fructose	$1 \cdot 10^5$	$(10\pm2) \cdot 10^8$	$9,5 \pm 0,5$
		$1 \cdot 10^6$	$(14,5\pm1,5) \cdot 10^8$	$9 \pm 0,5$
Liobac BIFI (Liobac BIFIDI)	bee honey	$1 \cdot 10^5$	$(5\pm1) \cdot 10^8$	$9,5 \pm 0,5$
		$1 \cdot 10^6$	$(8\pm1) \cdot 10^8$	$9 \pm 0,5$
Liobac BIFI (Liobac BIFIDI)	inulin	$1 \cdot 10^5$	$(9,5\pm1,5) \cdot 10^8$	$9,5 \pm 0,5$
		$1 \cdot 10^6$	$(14\pm2) \cdot 10^8$	$8,5 \pm 0,5$

Produced fermented milk products retains high probiotic properties within the whole shelf life (14 days), that stipulates their positive influence on organism if experimental animals in medical-biological investigations.

Necessity of development of fermented milk products of gerodietetic purpose is dictated by global senescence of population in the world. Author proposes to introduce two groups of fermented milk products: the first group – on milk basis, the second group – on combined milk-plant basis. It is advisable to use mixture of buttermilk and non-fat milk or buttermilk, *non-fat milk and cheese whey as milk basis in the ratio 85:15 and 50:45:5 accordingly [3].*

As the combined basis one can use mixture of non-fat milk with cheese whey and rice, or buckwheat, or oatmeal for children's and dietary nutrition in the ratio 80,00:14,26:5,74 or 80,00:16,14:3,86 or 80,00:15,74:4,26 accordingly [14]. Composition of milk and combined bases was optimized taking into account requirements of gerodietetics: mass part of protein in them is 3,0-3,1%; protein is of full value, doesn't contain limited amino acids, its amino acid composition is to the maximum brought to the composition of ideal protein [3, 14].

As fat component in the production of fermented milk products of gerodietetic purpose it is proposed to use milk-plant cream, 50% of fat of which is consisted of milk fat, 45% - olive oil, and 5% - soybean oil. Such cream has ratio of saturated fatty acid (SFA), mono-nonsaturated fatty acids (MNFA) and poly-nonsaturated fatty acids (PNFA): 0,39:0,47:0,14, close to recommended by the norms of gerodietetics. Besides, such cream has by 50% less of cholesterol, that is also very important in the nutrition of greybeards. It is proposed to establish mass parts of fat in fermented milk drinks on the level 1,0 and 2,5%; in curds – 5,0 and 11,0%; in sour cream – 10,0 and 15,0%. The modes of thermo-mechanical treatment of milk-fat mixtures are perfected that allows obtaining of kinetically stable mixtures with high organoleptic and normalized physical-chemical and microbiological indices.

Carbohydrate composition of fermented milk geroproducts is proposed to be corrected by means of introduction of lactulose and ferment preparations of β-galactosidase [10]. Use of lactulose allows increase of probiotic properties of worked out products, and β-galactosidase provides decrease of lactose contents by the way of its fermentative hydrolysis up to mono-saccharides (glucose and galactose). The mono-saccharides, obtained during hydrolysis, are the prebiotics in vitro for microorganisms used in sinbiotic complexes, in particular, for Bifidobacteria.

Enrichment of sour-milk geroproducts with antioxidants is necessary for intensification of activity of antioxidant system of greybeards. In the technologies of geroproducts on milk basis it is provided for to use milk extract of roots of licorice bare (way of obtaining of extract is proposed and worked out by the author [16], vitamins E and C, β-carotene and selenium, in the technologies of products on combined basis – of vitamin E, C and selenium [11, 16].

Enriched milk and combined mixtures contain complex of BAS, promoting growth and reproduction of microorganisms. Experimentally determined optimal proportions of yeast, bifido- and lacto-cultures in sinbiotic complexes, designed for biotechnological treatment of enriched mixtures (they are marked out in the table 2), allow obtaining of geroproducts with normable physical-chemical and microbiological indices, high organoleptic, antioxidant and probiotic properties [6].

Conclusions. The influence of biotechnological treatment of milk and cream by means of sinbiotic complexes on physical-chemical, organoleptic, microbiological and synergetic indices of ready clots is examined [5, 15, 7, 13]; the modes of production of fermented milk drinks with pro- and sinbiotic properties without excipients, with excipients and fruit-berry juices are developed [4]; modes of production of sour cream with pro- and sinbiotic properties, curds and curd products with pro- and sinbiotic properties [8]; modes of production of whey and milk-whey drinks with probiotic properties [12]; industrial approbation of proposed technologies is carried out.

The modes of production of fermented milk gerodrinks on milk and combined bases are perfected; the modes of production of gero-sour cream and gerocurds on milk basis; the industrial approbation of proposed technologies is carried out [1, 9]. Worked out fermented milk geroproducts preserves high antioxidant and probiotic properties during the whole shelf time (not less than 14 days), that stipulates their positive effect on organism of experimental animals in medical-and-biological investigations [2].

Table 2. – Dependence of number of cells of fermented milk and probiotic cultures in clots on ratio of initial concentrations of the cultures ($P \geq 0{,}95$, $n = 3...5$)

Name of product	Type of enriched base	Lacto-cultures (LK)	Bifido-cultures (PBK)	Yeast (DR)	*Initial concentration of cultures in enriched base/concentration of cultures in clot, cells/cm³, for* LK	BK	DR
Gero-kefir	Milk bases	S.lactis+ S.diaceti-lactis+ L.mesen-teroides	B.bifidum+ B.breve + B.longum	Saharomices	**$1 \cdot 10^6 / (70 \pm 5) \cdot 10^8$**	**$1 \cdot 10^5 / (2 \pm 0{,}5) \cdot 10^8$**	**$1 \cdot 10^2 / (1{,}1 \pm 0{,}1) \cdot 10^4$**
					$1 \cdot 10^6 / (25 \pm 5) \cdot 10^8$	$1 \cdot 10^6 / (4 \pm 0{,}5) \cdot 10^8$	$1 \cdot 10^2 / (0{,}6 \pm 0{,}1) \cdot 10^4$
	Combined milk plant bases	S.lactis+ S.diaceti-lactis+ L.mesen-teroides	B.bifidum+ B.breve + B.longum	Saharomices	**$1 \cdot 10^6 / (110 \pm 5) \cdot 10^8$**	**$1 \cdot 10^5 / (3 \pm 0{,}5) \cdot 10^8$**	**$1 \cdot 10^2 / (1 \pm 0{,}1) \cdot 10^5$**
					$1 \cdot 10^6 / (70 \pm 5) \cdot 10^8$	$1 \cdot 10^6 / (4{,}5 \pm 0{,}5) \cdot 10^8$	$1 \cdot 10^2 / (0{,}7 \pm 0{,}1) \cdot 10^5$
Gero-curdled milk (gero-curds, gero-sour cream)	Milk bases	S.lactis+ S.diaceti-lactis+ L.mesen-teroides	B.bifidum+ B.breve + B.longum	-	**$1 \cdot 10^6 / (70 \pm 4) \cdot 10^8$**	**$1 \cdot 10^5 / (2 \pm 1) \cdot 10^8$**	-
					$1 \cdot 10^6 / (25 \pm 3) \cdot 10^8$	$1 \cdot 10^6 / (3{,}5 \pm 1) \cdot 10^8$	-
	Combined milk plant bases	S.lactis+ S.diaceti-lactis+ L.mesen-teroides	B.bifidum+ B.breve + B.longum	-	**$1 \cdot 10^6 / (110 \pm 5) \cdot 10^8$**	**$1 \cdot 10^5 / (3 \pm 1) \cdot 10^8$**	-
					$1 \cdot 10^6 / (70 \pm 5) \cdot 10^8$	$1 \cdot 10^6 / (4{,}5 \pm 0{,}5) \cdot 10^8$	-
Gero-acido-philin	Milk bases	L.acido-philus (probiotic cultures)	B.bifidum+ B.breve + B.longum	-	$1 \cdot 10^5 / (13 \pm 3) \cdot 10^8$	$1 \cdot 10^5 / (1{,}6 \pm 0{,}4) \cdot 10^8$	-
					$1 \cdot 10^6 / (60 \pm 5) \cdot 10^8$	$1 \cdot 10^6 / (3{,}5 \pm 0{,}5) \cdot 10^8$	-
					$1 \cdot 10^5 / (25 \pm 5) \cdot 10^8$	**$1 \cdot 10^6 / (60 \pm 3) \cdot 10^8$**	-
					$1 \cdot 10^6 / (25 \pm 5) \cdot 10^8$	$1 \cdot 10^5 / (6 \pm 1) \cdot 10^8$	-
	Combined milk-plant bases	L.acido-philus (probiotic cultures)	B.bifidum+ B.breve + B.longum	-	$1 \cdot 10^5 / (25 \pm 5) \cdot 10^8$	$1 \cdot 10^5 / (2 \pm 0{,}5) \cdot 10^8$	-
					$1 \cdot 10^6 / (60 \pm 5) \cdot 10^8$	$1 \cdot 10^6 / (4{,}5 \pm 1{,}5) \cdot 10^8$	-
					$1 \cdot 10^5 / (25 \pm 5) \cdot 10^8$	**$1 \cdot 10^6 / (30 \pm 2) \cdot 10^8$**	-
					$1 \cdot 10^6 / (25 \pm 5) \cdot 10^8$	$1 \cdot 10^5 / (7 \pm 1) \cdot 10^8$	-

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