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EFFECT OF FUNCTIONAL FIBER ON VIABILITY OF LACTIC ACID BACTERIA AND BIFIDOBACTERIA DURING STORAGE

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Анотація. У представленій роботі показаний вплив гуміарабіка (Nexira, Франція) і олігофруктози (Orafti®P95, Бельгія) на ріст і життєздатність заквасочних культур молочнокислих бактерій і біфідобактерій (Christian Hansen, Данія), що використовуються для виробництва кисломолочних продуктів. Виявлено стимуляцію росту пробіотичних культур в присутності гумміарабіка (в концентрації 1%) і олігофруктози (в концентрації 2-3%). Дані пребіотики покращують синеретичні властивості кисломолочних продуктів, сприяють зниженню їх кислотності і збільшенню влагоутримуючій здатності згустку.

Ключові слова: гуміарабік, олігофруктоза, молочнокислі бактерії, біфідобактерії, життєздатність

Аннотация. В представленной работе показано влияние гуммиарабика (Nexira, Франция) и олигофруктозы (Orafti®P95, Бельгия) на рост и жизнеспособность заквасочных культур молочнокислых бактерий и бифидобактерий (Christian Hansen, Дания), используемых для производства кисломолочных продуктов. Обнаружена стимуляцию роста пробиотических культур в присутствии гуммиарабика (в концентрации 1%) и олигофруктозы (в концентрации 2-3%). Данные пребиотики улучшают синеретические свойства кисломолочных продуктов, способствуют снижению их кислотности и увеличению влагоудерживающей способности сгустка.

Ключевые слова: гуммиарабик, олигофруктоза, молочнокислые бактерии, бифидобактерии, жизнеспособность

Introduction

In the scientific literature concerning the problems of maintenance of a balance of normal microflora and prevention of its violation, much attention is paid to the probiotic microorganisms, which positively affect human health [1]. Such microorganisms are mainly lactic acid bacteria and bifidobacteria. They improve the balance of intestinal microbiota, inhibiting the growth of undesirable microorganisms, reduce the risk of bowel cancer, stimulate the host immune system, help to reduce the level of serum cholesterol etc.

The problem statement

In recent years, a promising direction in medicine and the food industry is development of products and preparations containing prebiotics [2]. These dietary ingredients are not hydrolyzed by the enzymes of the upper gastrointestinal tract, get unchanged into the large intestine and are selectively absorbed by the probiotic microorganisms, stimulating their growth and biological activity, thereby positively affecting the composition of the normal microbiota. The most studied prebiotic is oligofructose (OF) – derivative of inulin. It is a mixture of oligosaccharides consisting of glucose and several fructose residues connected one to the other by ß-(2-1) glycosidic bond. OF is widely used in the treatment of diseases of different etiology [2].

Prebiotics, along with probiotics, are included in the concept of biotherapy. They have been used successfully in the treatment of both acute diseases of the gastrointestinal tract (gastroenteritis caused by various pathogenic and opportunistic microorganisms) and chronic gastrointestinal disorders (gastritis, gastric ulcers, Crohn's disease, colitis, and others) [1].

Scientific researches have shown that the prebiotic effect is a characteristic of many compounds [2]. List of substances having a prebiotic effect is constantly updated. In this regard, gum arabic (GA) – the fiber of acacia gum is of scientific interest. The molecule of GA is a high molecular heteropolysaccharide (about 350 – 850 kDa), containing residues of galactose, rhamnose, glucuronic acid and arabinose, up to 3% protein, and minerals (such as potassium, calcium, magnesium). Prebiotic properties of GA have been shown in [3], but in Ukraine this prebiotic remains insufficiently known.

The problem of today is the creation of so-called symbiotic preparations containing both pro- and prebiotics, as well as the production of functional foods containing probiotics [2]. Therefore, the purpose of this study was to investigate the influence of certain prebiotics – gum arabic and oligofructose – on the growth and viability of the bacteria used for the production of fermented products to determine the optimal combination "probiotic-prebiotic", corresponding to the therapeutic requirements – the presence of 10^7CFU/ml (g) over the period of storage of a symbiotic product.

The object of the study were yogurts made with starter DVS-cultures: *Streptococcus salivarius subsp. thermophilus, Lactobacillus acidophilus and Bifidobacterium animalis subsp. lactis* (starter ABT-1) and *L. delbrueckii subsp. bulgaricus* and *S. salivarius subsp. thermophilus* (starter YC-X11) (Christian Hansen, Denmark). The subject of research was the viability of the starter microorganisms, as well as some of the physico-chemical properties of the test yoghurts.

Materials and methods

*Preparation of dairy products.*0,5 % fat milk was used. Gum arabic (Fibregum, Nexira, France) and oligofructose (Orafti®P95, Belgium) were studied as prebiotics. The prebiotics were added in concentrations of 1 %, 2 % and 3 %. Milk fermented without adding a prebiotic was used as control. Mixtures were placed in a sterile glass vial and heated according to protocol [4] at 85 °C for 30 minutes, then were allowed to cool to a temperature of 40 – 42 °C, followed by inoculating of starter compositions (0,02 g/l). The vials were incubated at 37 ° C for 16 hours. After incubation, the yogurts were stored at 4 °C for 21 days. Studies of the yogurts were performed on the 1st, 7th, 14th and 21th day of storage [4].

Microbiological studies. Samples of yoghurt in an amount of 1 ml were placed in 9 ml sterile 0,85 % solution of NaCl. Serial tenfold dilutions up to 10^{-7}were prepared. Then 1 ml of the dilutions10^{-4} – 10^{-7} was inoculated on the media: M-17 agar for detection of *Streptococcus salivarius subsp. thermophilus*, MRS – for detection of microorganisms of the genus *Lactobacillus*, MRS with 0,05 % cysteine – for detectionof *Bifidobacterium animalis subsp. lactis*. Plates were incubated at 37 °C for 72 hours, lactic acid bacteria – under aerobic conditions and bifidobacteria – under anaerobic conditions using anaerobic system GenBox (BioMerieux, France).

Determination of pH. Active acidity of yogurt was determined using a pH meter "pH-150mA" (Anteh, Belarus).

Titratable acidity was determined titrating 5 ml of the sample solution with 0,1N NaOH, using phenolphthalein as an indicator according to State Standard 4343: 2004.

*Spontaneous syneresis. S*yneresis index was determined as the amount of the separated whey (ml) per 100 ml sample stored at 4 °C.

Induced syneresis (IS). The degree of syneresis was determined by the filtration method [5]. For this purpose, 100 ml of thoroughly mixed clot was placed in a funnel with a paper filter, which was put into a graduated cylinder. After 3 h, the volume ofseparated whey was measured. The degree of syneresis was calculated using the formula:

$$\mathrm{IS}(\%) = \left(\frac{V1}{V2}\right) \times 100\%, \qquad (1)$$

where V1 is volume of whey after filtration,

V2 is an initial volume of yogurt.

Water holding capacity (WHC) was measured by centrifuging a 10 g yoghurt sample at 4500 rpm for 30 min at 4° C. *WHC*was calculated by the formula:

$$\mathrm{BBC}(\%) = \left(1 - \frac{W1}{W2}\right) \times 100\%, \qquad (2)$$

where W1 is the weight of the whey after centrifugation,

W2 is the initial weight of the yogurt.

Statistical analysis. Statistical data processing was performed using the software package "Statistics 6,0" according to conventional techniques, the confidence level was 95 %.

Results and discussion

Gum arabic is a food ingredient, widely used in food and pharmaceutical industries [6]. It is of interest of producers in view of its natural origin, on the one hand, and the low cost, on the other hand. Due to its functional properties, gum arabic is widely used as a stabilizer of structure- and film-former, emulsifier and a natural source of dietary fiber in the confectionery, bakery, meat industry, in the production of flavorings and beverages, as well as dairy products and ice cream. In addition, scientific studies have shown prebiotic properties of gum arabic: the carbohydrate stimulates both indigenous normal human intestinal microbiota and exogenous probiotic microorganisms, promoting the improvement of their survival in functional foods.

The results of scientific researches indicate the beneficial effects of oligofructose on the composition of the intestinal microflora and microorganism [7-9].

On the other hand, according to available data, in Ukraine gum arabic and oligofructose are still not widely used as prebiotic additives for dairy products. Therefore,it was interesting to investigate the influence of the presence of these prebiotics in the milk on the physiological properties of probiotic cultures of microorganisms, in particular their number and viability, adding these substances to the milk for the production of dairy products.

The result of the experiment have shown that the addition of gum arabic and oligofructose into the milk in whole positively affects the number of starter organisms, although statistically significant stimulation of growth of starter cultures was not observedcomparing to control (in the absence of prebiotics) (Fig. 1,2) .

In the presence of gum arabic at concentrations of 1 – 3 % after 1 day of storage the amount of *L. acidophilus* averaged 5,0x10^{8}CFU/ml, *S. salivarius subsp. Thermophilus* – 4,7x10^8 CFU/ml (for both starters), *L. delbrueckii subsp. Bulgaricus* – 4,0x10^8 CFU/ml, *Bifidobacterium animalis subsp. Lactis* – 2,5x10^8 CFU/ml.

After addition of oligofructose in amount of 2 %,a slight growth stimulation of *L. acidophilus* (4,6x10^{8}CFU/ml), *S. salivarius subsp. thermophilus* (4,6x10^{8}CFU/ml), *L. delbrueckii subsp. bulgaricus* (4,0x10^{8}CFU/ml) was observed, at 3 % of the prebiotic growth stimulation of *Bifidobacterium animalis subsp. lactis* (4,4h108 CFU / ml) was detected, which satisfies the requirements for the dairy products.

At the same time, at the end of shelf-life (21 days) of both types of yogurts with prebiotics studied increased content of the probiotic cultures have been observed(as compared with control), but these results were different depending on the concentration of prebiotics in the medium (Fig. 1,2). In the presence of gum arabic amount *L. acidophilus* averaged 5,5x10^{7}CFU/ml, *S. salivarius subsp. Thermophilus* – 2,9x10^8 CFU/ml (for both starters), *L. delbrueckii subsp. Bulgaricus* – 2,6x10^{7}CFU/ml, *Bifidobacterium animalis subsp. Lactis* – 2,2x10^{7}CFU/ml (in control: 1,1x10^{7}CFU/ml, 1,2x10^{8}CFU/ml, 1,0x10^{7}CFU/ml, 1,6x10^7 CFU/ml, respectively). Furthermore, the results show an increase of viability of starter cultures after addition of gum arabic to yoghurt in a concentration of 1 %, there was an increase in the content of lactobacilli 5,7 – 8,7 times, other cultures – 1,5 – 2 times. With increasing of concentration of gum arabic to 2 and 3 % statistically significant increase in viability was not observed. Moreover, at the end of the shelf life of gum arabic at a concentration of 3 % reduction of *Streptococcus thermophilus, Lactobacillus bulgaricus* and *Bifidobacteriumanimalis subsp. lactis* quantity was observed, as compared with the control.

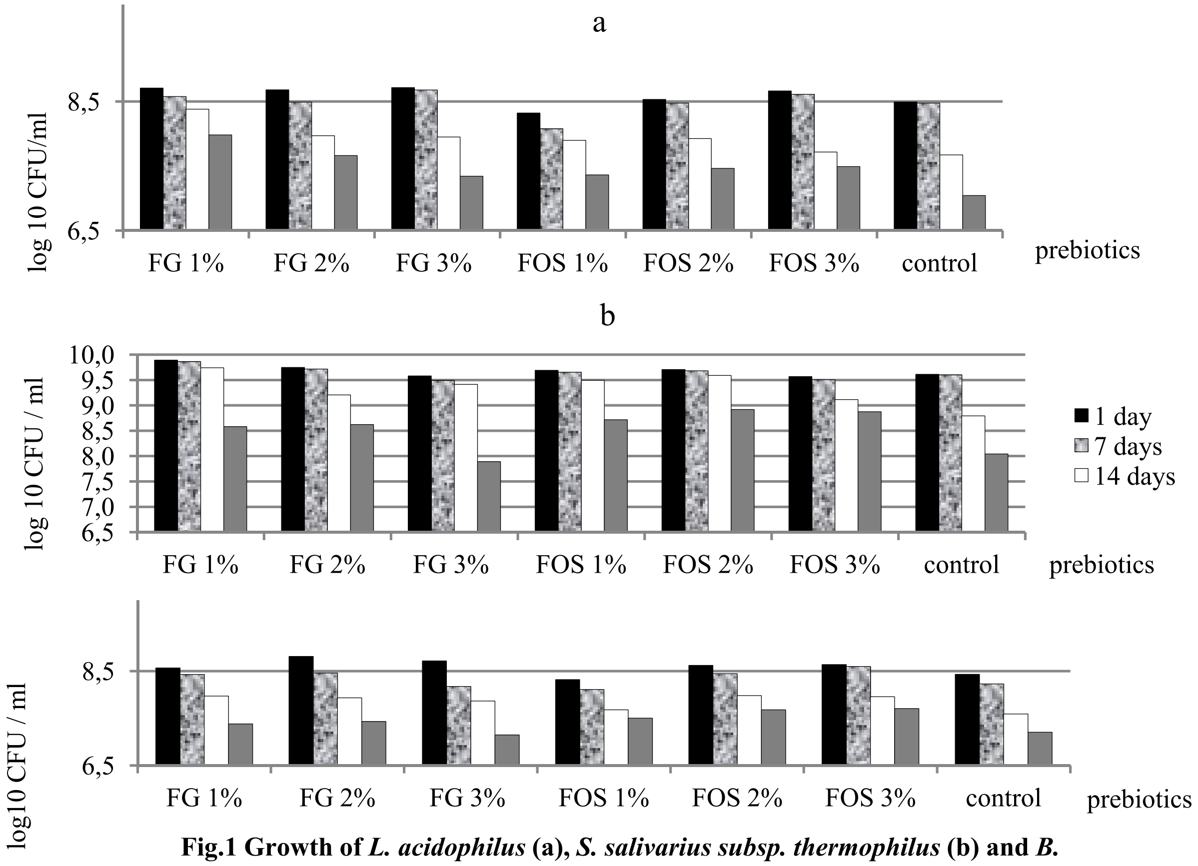

Fig.1 Growth of *L. acidophilus* (a), *S. salivarius subsp. thermophilus* (b) and *B. animalis subsp. lactis* (c) at different concentrations of prebiotics in yoghurt ABT-1 (FG - gum arabic, FOS - oligofructose) . p≤0,05

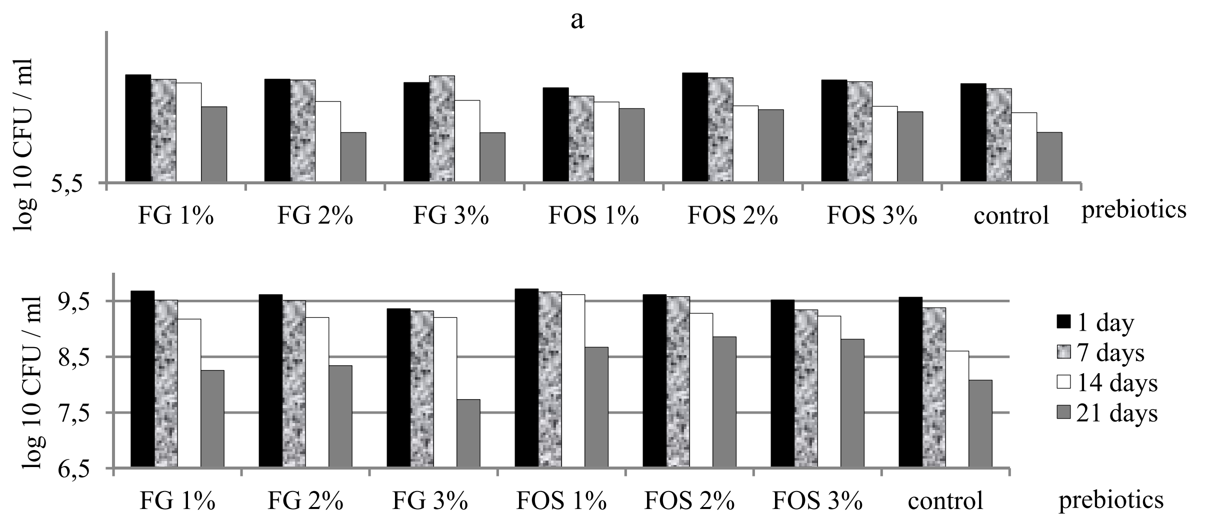

Fig. 2 Growth *of L. delbruekii subsp. bulgaricus* (a) and *S. salivarius subsp. thermophilus* (b) at different concentrations of prebiotics in yoghurt YC-X11 FG - gum arabic, FOS - oligofructose) p≤0,05

According to the results, the addition of 2 % oligofructose to the milk contributed to a small increase of the viability of *Lactobacillus bulgaricus,Streptococcus thermophilus*and bifidobacteria(4,7x10^7 CFU/ml and 7,7x10^8 CFU/ml, respectively), and 3 % oligofructose promoted the number of *Lactobacillus acidophilus* and bifidobacteria (3,1x10^7 CFU/ml 5,1x10^7 CFU/ml, respectively).

It should be noted that in the presence of 1 % gum arabic the reduction of the starter cultures titres occurred much more slowly as compared with the control and on the 14th day of the product storage the number of viable cells of starter microorganisms was practically unchanged.

Since the use of any additives in the manufacture of the dairy product can change its physical and chemical properties [10], it was interesting to study the influence of gum arabic and oligofructose on such characteristics as spontaneous and induced syneresis (IS), an active and titratable acidity, water-holding capacity of the clot (WHC).

In all of the investigated yoghurts,spontaneous syneresis was absent or less than 0,1 % throughout the storage period. Therefore, this characteristic was not considered in further work.

It has been found that the degree of the IS depends on both the concentration of the prebiotic and the starter. Thus, reduction in the degree of IS 1,5 times at the end of storage in the case of yoghurt ABT-1 was observed by adding 1 % gum arabic, and 2 % of oligofructose, and in the case of yoghurt YC-X11 – adding a 1 % gum arabic and 1 % oligofructose (Table 1). In other cases, a statistically significant difference in the degree of IS was not observed as compared with the control.

Table 1 – Inducible syneresis of studied yoghurts during storage

starter	prebiotics	Degree of inducible syneresis, %			
		1 day	7 day	14 day	21day
ABT-1	FG 1%	15,33±0,52	17,67±0,52	18,00±1,00	20,67±0,52
	FG 2%	18,01±0,28	20,67±0,52	23,33±0,52	27,00±1,00
	FG 3%	27,67±0,52	28,00±1,00	28,67±1,37	30,67±0,52
	FOS 1%	18,00±1,00	20,00±0,00	24,00±2,00	31,00±0,00
	FOS 2%	15,33±0,52	15,33±0,52	16,67±1,03	20,00±1,00
	FOS 3%	16,00±0,00	17,33±1,03	19,00±0,00	25,67±0,52
	control	22,33±0,52	24,67±0,52	27,00±2,00	32,00±1,00
YC-X11	FG 1%	19,67±1,03	20,00±1,00	19,33±0,52	24,00±0,00
	FG 2%	23,00±0,00	25,00±2,00	29,33±0,52	35,67±0,52
	FG 3%	19,00±1,00	20,00±1,00	28,67±0,52	32,00±1,00
	FOS 1%	17,67±1,03	17,67±0,52	19,67±1,03	22,00±0,00
	FOS 2%	21,67±0,52	21,00±0,00	24,00±1,00	26,67±0,52
	FOS 3%	26,00±2,00	27,33±0,52	30,67±0,52	35,33±0,52
	control	15,00±0,00	17,33±0,52	27,67±1,03	36,33±0,52

Note: (FG — gum arabic, FOS - oligofructose).*p*≤0,05

According to official documents, titratable acidity of yogurt should be 7 –140 °T. It has been shown that after addingof gum arabic titratable acidity of the yogurt was normal but significantly lower than that in the control (Table 2). Moreover, unlike the control, it slightly decreased during storage. It should be noted that this property depends on the starter. Thus, the titratable acidity of the yogurt ABT-1 was significantly lower than that of yogurt YC-X11.

Table 2 – Titratable acidity of studied yoghurts during storage

starter	prebiotics	Titratable acidity, °T			
		1 day	7 day	14 day	21day
ABT-1	FG 1%	84,67±0,52	84,33±0,52	83,00±0,00	81,00±1,00
	FG 2%	80,33±0,52	79,00±1,00	76,33±1,03	75,33±1,03
	FG 3%	84,00±0,00	82,33±0,52	77,33±0,52	73,67±0,52
	FOS 1%	100,67±0,52	92,00±1,00	85,67±0,52	72,00±2,00
	FOS 2%	80,00±0,00	79,67±0,52	75,00±0,00	71,00±0,00
	FOS 3%	100,00±0,00	97,33±1,03	86,33±1,03	70,33±1,03
	control	126,00±1,00	125,00±2,00	97,33±0,52	86,33±0,52
YC-X11	FG 1%	108,67±0,52	105,00±0,00	96,33±0,52	76,67±0,52
	FG 2%	98,00±2,00	98,00±2,00	93,00±0,00	92,33±0,52
	FG 3%	96,33±1,03	92,33±1,03	82,67±0,52	73,00±1,00
	FOS 1%	114,33±0,52	110,67±0,52	105,33±1,03	80,33±0,52
	FOS 2%	102,33±0,52	101,67±0,52	90,00±1,00	75,33±1,03
	FOS 3%	96,67±0,52	98,00±0,00	88,33±0,52	73,67±0,52
	control	120,00±1,00	116,67±0,52	106,33±1,03	90,00±2,00

Note: (FG — gum arabic, FOS - oligofructose).$p \leq 0,05$

The pH is important for dairy products, since the growth of starter microorganisms and their influence on the clot formation depend on it. pH influence the speed of formation of components that affect the taste and smell of dairy products, their enzymes' activity. By pH the quality of dairy products is estimated. After adding of prebiotics active acidity of yogurts ABT-1 and YC-X11 differed significantly (Table 3): from 4,03 ± 0,02 to 4,24 ± 0,00 in the first day of yogurt storage, and from 3,90 ± 0,00 to 4,07 ± 0,02 – on the 21st day of storage.

Table 3 – Active acidity of studied yoghurts during storage

starter	prebiotics	pH			
		1 day	7 day	14 day	21day
ABT-1	FG 1%	4,11±0,01	4,08±0,00	4,02±0,01	3,99±0,01
	FG 2%	4,23±0,02	4,20±0,02	4,12±0,00	4,10±0,02
	FG 3%	4,24±0,00	4,20±0,00	4,19±0,01	4,14±0,00
	FOS 1%	4,11±0,01	4,05±0,01	3,90±0,00	3,90±0,01
	FOS 2%	4,13±0,02	4,12±0,01	4,09±0,02	4,06±0,01
	FOS 3%	4,21±0,01	4,20±0,00	4,15±0,01	4,07±0,02
	control	4,08±0,01	4,07±0,02	4,05±0,01	4,01±0,01
YC-X11	FG 1%	4,03±0,02	4,02±0,02	4,00±0,01	3,97±0,02
	FG 2%	4,06±0,00	4,01±0,02	4,00±0,02	3,99±0,00
	FG 3%	4,07±0,01	4,05±0,01	4,04±0,02	4,04±0,01
	FOS 1%	4,03±0,01	4,02±0,02	4,02±0,01	3,99±0,01
	FOS 2%	4,08±0,01	4,07±0,02	4,03±0,01	4,01±0,02
	FOS 3%	4,10±0,00	4,09±0,01	4,07±0,00	4,06±0,01
	control	4,09±0,01	4,07±0,02	4,04±0,01	3,99±0,00

Note: (FG — gum arabic, FOS - oligofructose).$p \leq 0,05$

WHC ofdairy product is an indicator of its ability to retain the gel structure of whey. The ability of the product to ensure a minimum separation of whey is an important factor for retail success, because the separationof the product negatively affects the perception of the consumer. The results indicate that the gum arabic and oligofructose help to increase WHCof prepared dairy product throughout the whole period of storage (Table 4). On the first day of storage it was 72,33 ± 0,52 to 73,33 ± 1,03 % with the addition of

gum arabic and from 72,67 ± 0,52 to 77,33 ± 0,52 % with the addition of oligofructose, whereas in the control WHC was 64,00 ± 0,00 to 65,67 ± 0,52 %. At the end of the storage WHC of yogurt with gum arabic was 69,00 ± 0,00 to 73,67 ± 0,52 %, and that with oligofructose – up 66,67 ± 0,52 to 75,33 ± 0,52 %, in the control – up 56,67 ± 0,52 to 59,33 ± 0,52 %.

Table 4 – Water-holding capacity ofclot ofstudied yoghurts during storage

starter	prebiotics	WHC,%			
		1 day	7 day	14 day	21day
ABT-1	FG 1%	72,33±0,52	72,33±1,03	71,00±0,00	69,00±0,00
	FG 2%	73,33±1,03	73,33±0,52	72,67±0,52	69,33±1,03
	FG 3%	76,33±0,52	75,00±1,00	74,33±0,52	70,33±0,52
	FOS 1%	75,00±0,00	73,33±1,03	72,33±0,52	67,00±0,00
	FOS 2%	73,33±1,03	71,00±0,00	78,00±1,00	67,33±0,52
	FOS 3%	73,33±0,52	72,67±0,52	71,33±0,52	66,67±0,52
	control	65,67±0,52	62,00±1,00	61,33±1,03	59,33±0,52
YC-X11	FG 1%	75,67±0,52	75,33±0,52	74,67±0,52	71,67±0,52
	FG 2%	76,00±0,00	75,33±1,03	74,33±0,52	71,00±0,00
	FG 3%	77,33±1,03	76,33±0,52	75,67±0,52	73,67±0,52
	FOS 1%	72,67±0,52	72,33±0,52	70,00±0,00	70,00±1,00
	FOS 2%	77,33±0,52	76,33±1,03	74,33±0,52	71,33±1,03
	FOS 3%	76,33±1,03	76,33±0,52	76,67±0,52	75,33±0,52
	control	64,00±0,00	63,5,67±0,52	58,33±0,52	56,67±0,52

Note: (FG — gum arabic, FOS - oligofructose).$p \leq 0,05$

Conclusions

Thus, the studies have revealed the stimulation of growth of probiotic cultures in the presence of gum arabic (at 1% concentration) and oligofructose (at a concentration of 2-3%). These prebiotics can improve syneresis properties of fermented milk products, reduce their acidity and increase WHC. It should be noted that the expression of these properties depends onthe prebiotic and the starter used. Therefore, the composition of the prebiotic and its concentration should be selected experimentally. Taking into account the fact that gum arabic and oligofructose have official status GRAS («generally regarded as safe») [2], these prebioticscan be recommended as growth promoters for studied probiotic cultures.

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