

Relevance of application of irradiated starter cultures to production of fermented milk products

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Abstract. Nowadays yogurt, fermented milk products, enriched with biologically active substances, acquire increasingly important significance in people's diets. The traditional method for producing fermented milk products and yogurt is to ferment the milk using starter cultures. The purpose of this research was to figure out if it is possible to use suspensions of probiotic microbial cultures irradiated with ionizing radiation to produce yoghurt products. Liquid live suspensions of *bifidobacteria* and *lactobacilli*, as well as a mixture of *bifidobacteria*, *lactobacilli*, *propionibacteria* and *lactic acid streptococci* were taken as a research model. The goal was achieved by receiving the yoghurt products enriched with active secondary metabolites due to using the suspensions of lysed cells of different microorganisms. We studied physico-chemical and organoleptic properties of the received products after 1, 7 and 14 days of storage.

Keywords: fermented milk products; probiotic microorganisms; secondary metabolites; ionizing radiation

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Introduction

Currently, it is generally accepted to use probiotics to produce a variety of dietary supplements, fermented food products and drinks, and medicaments. It is enough to mention that almost all fermented milk products are obtained using lactic acid and probiotic microorganisms. All over the world, for decades this approach has been defined as fully justified in terms of preserving and improving the health of people and animals. At the same time, most researchers, as well as wide public trained by them, undoubtedly identify probiotic microorganisms

with exceptionally useful ones, which do not cause any side effects and have no contraindications to their unlimited use [1, 2]. Few people realize that production strains of *bifidobacteria*, *lactobacilli*, *propionibacteria* and other probiotic microorganisms are selected from specific donors, such as astronauts, or from some environmental objects. It means that biomaterial, which represents just a part and is endowed with specific individual properties, applies to the entire community of animal and human populations without any restrictions or control. But, after all, it is known

that there is a huge variety of races and strains of probiotic microorganisms, and every human or animal has got its unique microbial “landscape” and it is not really a correct measure to artificially plant someone else’s heterogeneous microflora [3].

On the other hand, it is true that the benefits of liquid probiotics have been proven by numerous studies and experi-

Experimental

Liquid live suspensions of *bifidobacteria* and *lactobacilli*, as well as a mixture of *bifidobacteria*, *lactobacilli*, *propionibacteria* and *lactic acid streptococci* were taken as a research model. A positive impact of liquid probiotics on the organism is diverse. Improvement of all parts of the gastrointestinal and urogenital tracts, skin surface, and mucous membranes of the upper respiratory tract; normalization of digestive functions; immunostimulating and anti-allergic effects — it is not a complete list of health-improving properties of the abovementioned probiotics in various age groups of the population, and also of domestic and farm animals, confirmed by us with a dozen patents for inventions [4].

We have attempted to convert liquid probiotics into more physiological forms with the elimination of any possible alien influence on the organisms of humans and animals, while increasing the nutritional value, as we have confirmed in our earlier works, and maintaining antimicrobial activity against opportunistic and pathogenic microflora [5]. This was achieved by lysis live cellular probiotic cultures of microorganisms through the irradiation and with subsequent application of the obtained cell-free suspensions as starter cultures to produce sour-milk yogurt-like products. The processing of the probiotic cultures

with ionizing radiation was carried out on the linear electron accelerator model UELR-10-10C2 in the Innovative-introduction Center of Radiation Sterilization of the Physical-Technological Institute (Ural Federal University, Yekaterinburg).

There were used liquid probiotics, received at the production equipment, since in almost all cases there is a statistically significant difference in the quality and safety criteria of any products, received using laboratory and industrial methods.

The BR-30 bioreactor (Fig. 1) was used to produce liquid live probiotics. This reactor allows carrying out sterilization of initial nutrient hydrolysate-milk medium in automatic mode (temperatures 106–108 °C and excess pressure 0.3–0.4 atm for 60 min); cooling till 38–40 °C and holding at this temperature of mixture of nutrient medium with preliminarily prepared liquid starter material for 24 hours, based on the following proportions: 3–5% of starter culture to the volume of seeded sterile environment [6].

The following raw materials were used to prepare unfermented products: cow milk “Irbitsky” 2.5% fat, pasteurized, homogenized, standardized in the package volume of 1 l was manufactured by JSC “Irbitsky milk factory” (Yekaterinburg, Russia) according to the Russian State Standard 31450–2013 [7]. It was bought

from supermarkets in Yekaterinburg, Russia. The milk ingredients were marked on the package as the following: fat — 2.5%, protein — 3.0%, and carbohydrate — 4.7%. Biologically active additives “Euflo-rine-L” (liquid Lactobacillus), (liquid Bifidumbacterin) (Fig. 2a,b); non-alcoholic drink “Euflo-rine-plus” (protein hydrolysate — metabolic) (Fig. 2c) in 100 ml dark glass bottles were selected as probiotic starter culture for yoghurt products preparation. They were produced by LLC NPC “PRIORITY” (Yekaterinburg, Russia).

Weighing was performed on the laboratory analytic balance AND HR-60 of the I accuracy class. The pH was measured according to the requirements of State Standard 33776–2016 [8] using electronic pH-meter Kelilong PH-911. The inoculation process was carried out under a sterile condition of microbiological laminar flow (II class, B2 type BMB-II — “Laminar-S-1.2”, Lamsystem Company, Russia) (a thermostable incubator). The milk was fermented in Yogurt maker Marta MT-1854 MARTA TRADE INC., United Kingdom, equipped with the timer and thermometer. All experiments and sample analysis were carried out in two parallels.

Results and discussion

Comparative chemical analysis to determine the qualitative and quantitative spectrum of amino acids, containing in whole cell and lysed by irradiation of the respective microbial cultures, was carried out in the scientific-research laboratory of united laboratory complex at Ural State University of Economics using highly efficient liquid chromatograph Agilent 1260 Infinity II (Germany), equipped with multi-wave detector and analytic tube with



Fig. 1. The BR-30 bioreactor to produce liquid live probiotics



Fig. 2. Models of starter cultures for fermented milk products:
a — Euflo-rine-L, b — Euflo-rine-B,
c — Euflo-rine-plus

reversed phase Agilent ZORBAX Eclipse AAA 4.6 * 150 mm 3.5-Micron. Gradient elution with two eluents was used during the research process. We used the phosphate buffer based on Na_2HPO_4 with 0.5 M concentration as the first eluent, and a mixture of acetonitrile:methanol:water with 45:45:10 ratio was used as the second eluent. Elution speed was 2 ml/min, and temperature of the tube was maintained at the level of 40 °C. Before start-

ing the measurements, the chromatograph was calibrated using the standard of amino acids produced by Agilent Technologies company. In the calibration the following non-essential amino acids were used: Alanine, Aspartic and Glutamic acids, Serine; conditionally essential — Arginine, Cysteine, Glycine, Proline, Tyrosine, and essential amino acids, such as Histidine, Threonine, Valine, Methionine, Phenylalanine, Isoleucine, Leucine, Lysine.

The performed research showed that in the irradiated liquid probiotic microbial cultures, the total content of the above-mentioned amino acids, expressed in mg per 100 g of sample, is 1.6–2.2 times higher than in live whole-cell monocultures of *bifidobacteria*, *lactobacilli* and their mixtures in the 1:1 ratio. In the average values, cellular microbial cultures, destroyed by radiation, contained 1.8 times more amino acids than the corresponding live whole cell cultures.

Besides, studies were carried out to determine the number of live colony-forming microorganisms in liquid probiotic cultures after various modes of their irradiation. During this, the NMAFAM parameter was determined (number of mesophilic aerobic and facultative anaerobic microorganisms) [9]. When determining this value, we used the scales VMK-622, automatic single-channel dispenser BIOHIT, pH-meter “Anion 7000”.

It was found that before irradiation the content of probiotic microorganisms in liquid live whole-cell microbial cultures was from $1 \cdot 10^8$ to $1 \cdot 10^{10}$ CFU/cm³. After irradiation with the dose 10 kGr the NMAFAM parameter was at the level of values from $1.6 \cdot 10^4$ to $2.0 \cdot 10^2$ CFU/cm³. At the same time, colony-forming microbial units in the culture of lactobacilli were not determined at all (the parameter

matched the value less than $1.0 \cdot 10^1$ CFU/cm³). At a higher radiation dose of 15 kGr the NMAFAM parameter in all studied cultures of microorganisms was less than $1.0 \cdot 10^1$ CFU/cm³; it means that the microbial cells have been completely lysed in this case.

The next stage of our work was to study the possibility of using biologically active additives of brands “Euflo-rine-B”, “Euflo-rine-L” and “Euflo-rine-plus”, produced by LLC NPC “PRIORITY”, as a starter in the production of yoghurt products using probiotic cultures lysed by ionizing radiation and their secondary metabolites, as well as living microorganisms.

At first it was identified that the pH value of the original milk was $6.65 \pm 0.75\%$. The milk was pasteurized at 85 °C for 15 min and subsequently cooled to 38–40 °C (fermentation temperature), then 125 ml of it was poured into clean, numbered 150 ml containers. After that the milk was divided in models inoculated by the dose 15 kGr and non-irradiated models of starter cultures, with a dose of 100 g/l of milk. Containers with fermented milk and with reference samples were closed with lids, put into a yogurt maker at 40 °C for 8 hours. The control sample was prepared using the previous method without the addition as a starter culture to the pasteurized milk neither the living culture, nor the suspension of lysed cells of microorganisms, their secondary metabolites.

Analysis of the appearance, organoleptic and physical parameters of quality of yoghurt products, made by adding irradiated, as well as live cultures of microorganisms, was performed after 1, 7 and 14 days of storage.

While determining the pH of the samples after 8 hours of fermentation, it was established that only in case of milk fer-

mentation by not irradiated Eufloirine-L the value lowered to pH = 4.4, the texture of this product was dense, homogeneous (Fig. 3), while the other samples were less dense, and the pH value varied from 4.9 to 5.8.

Consequently, the samples were again placed into the yogurt maker for another 3 h and suddenly cooled to 4 °C after the fermentation time was determined. After 6-hour cooling, gelation was observed in all samples (Figs. 4a, 4b, 5a, 5b), although the pH values remained at the same level.

Therefore, milk fermentation in the samples with not irradiated starters is carried out due to the activity of microorganisms, as well as the activity of their metabolites; when using sterile starters, fermentation process is conditioned by solely the interaction of secondary metabolites and milk components.

The control sample during fermentation and the consequent cooling has not changed even for 24 hours, so it can be argued the fermentation due to microorganisms contained in milk, without adding the model starters, does not occur.

During the research there were determined physical and organoleptic indicators of yoghurt product samples quality, received using irradiated starters and mixtures with living microorganisms and during 1, 7 and 14 days of storage (see Table 1). Determination of sensory indicators — appearance and consistency, taste and smell, color of received products was performed according to the requirements of State Standard 31981–2013 [10].

The sensory evaluation of yogurt product samples was provided by 5 volunteers expert panel members from professors and students in the department of technologies of organic synthesis according

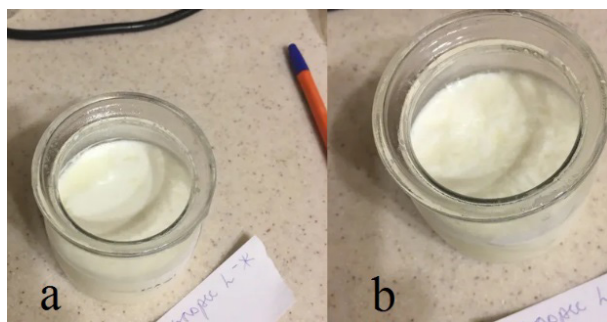


Fig. 3. Yoghurt products with the addition of not irradiated (a) and irradiated (b) Eufloirine-L

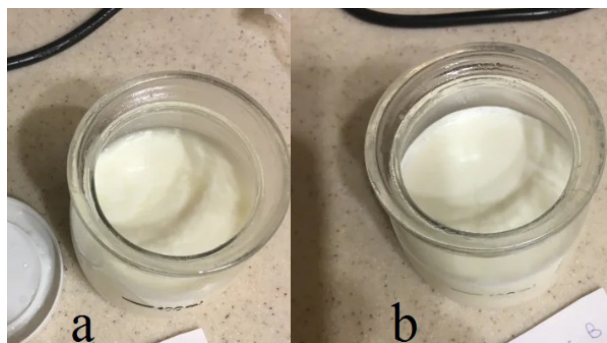


Fig. 4. Fermented products with the addition of not irradiated (a) and irradiated (b) Eufloirine-B

to scoring scale (0–5) for its appearance (color and syneresis), flavor (aroma and taste), consistency (firmness and texture) and overall acceptability. The samples were served to panelist cold at 4 °C by random order. Samples No. 2 and No. 6 have shown the best organoleptic parameters during 7 days of storage; after 14 days of storage the taste characteristics of all the samples have been decreased.

It was determined that with the increase in the storage period of all the samples, pH value decreased, titratable acidity (from 55 °T to 145 °T [11]) and whey separation (syneresis) increased. This is well correlated with the fact that during storage, the protein-lipid complex is destroyed due to acidification of the medium by the forming lactic acid, which results in compaction of the yoghurt product structure and separation of the whey [12].

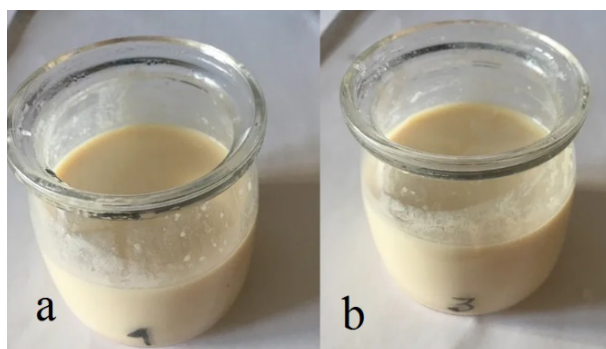


Fig. 5. Yoghurt products, made of not irradiated (a) and irradiated (b) Eufloirine — plus

Table 1
Results of determination of pH, density and sensory indicators of fermented products

Samples number	Eufloirine type	pH			Density, g/ml	Evaluation of sensory indicators (1, 7 days)		
		1 day	7 days	14 days		taste	smell	appearance
1	Eufloirine-B	5.8	5.6	5.2	0.9780	3.0	3.4	3.6
2	Eufloirine-B irradi.	4.9	4.7	4.3	0.9820	4.0	4.0	4.4
3	Eufloirine-L	4.4	4.0	3.8	0.9860	3.6	3.8	3.8
4	Eufloirine-L irradi.	5.3	5.0	4.8	0.9750	3.4	3.4	3.6
5	Eufloirine-plus	5.1	4.7	4.6	0.9630	4.0	4.4	3.8
6	Eufloirine-plus irradi.	5.4	4.9	4.5	0.9450	3.8	4.0	4.6

Conclusions

In conclusion, it should be noted that during the performed research live cell probiotic cultures of the microorganisms were irradiated by different doses at the linear electron accelerator. Microbiological

analysis showed that microbe cells were completely lysed after radiation by the dose 15 kGr. Fermented products were received using irradiated mixtures instead of traditional starters. It should be also noted

that irradiated Euflorin-B and Euflorin-plus are preferable for this purpose. They can improve the texture, viscosity, and the rheological characteristics of yogurt effectively, ensure the formation of a denser and more uniform structure and consistency of yogurt products. This fact can be explained, apparently, the formation by *bifidobacteria* of exopolysaccharide (EPS), which can act as thickeners and stabilizers

[13]. We produced fermented milk products soured by unirradiated live probiotic mixtures as control samples. Thus, we have shown the basic possibility to transform liquid probiotics into more physiological forms and eliminate any possible unwanted influence on the organisms of people and animals along with the increase in their nutritional value.

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