SURVIVAL OF MICROFLORA OF KEFIR FROM GOAT MILK, INCLUDED IN THE BIOPOLYMER MATRICES AFTER LYOPHILIZATION

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Abstract

The effect of three types of biopolymers (pectin, lactulose and carboxymethylcellulose) as cryoprotectors on the survival of microbial groups in goat milk kefir after lyophilization was investigated. The optimal parameters of the freeze drying process of the variant samples were determined. Microbiological analyzes demonstrate increased survival of the microflora of kefir included in biopolymer matrices. Individual groups of lactic acid bacteria and yeast are showing different resistance to freezing and lyophilization, as best results are reported in the use of carboxymethylcellulose and lactulose. The results obtained were statistically processed and prove a high degree of reliability.

Key words: biopolymers, kefir, cryoprotection, lyophilization, microorganisms, survival

Introduction

The impact of low temperatures on various biological objects is subject to long-standing research and increased scientific interest. Revealing the mechanisms of low-temperature adaptation is essential for the vital activity of organisms.

Frequently used technology to preserve the activity and viability of microorganisms is to freeze them at low temperatures and freeze-drying. Resistance of bacterial cultures to cryogenic treatment is different and depends on a complex set of factors of different nature-characteristics of the strain, the culture conditions before freezing, rate and conditions of freezing and composition (type and concentration) of cryoprotectors.

Provoking interest lately are investigations on hydrocolloids of natural and synthetic origin, that influence biochemical processes in the biological system. Some of them can be successfully used as polymer matrices for the immobilization of biologically active compounds, cellular systems, enzymes and others.

Pectin substances belong to the group of high molecular weight heteropolysaccharide, constituting the cell wall and intercellular entities. Their main polymer chain is a D-galacturonate, composed of α- (1,4) -linked units of D- galacturonic acid.

Lactulose is a synthetic disaccharide, produced by the isomerization of lactose, which is not absorbed in the small intestine and has the potential to function as a prebiotic.

The Na- carboxymethylcellulose (Na- CMC) is a cellulose derivative, a cellulose ester with glycolic acid or its sodium salt, respectively.

The aim of the present study is to investigate the possibilities of application of different hydrocolloids as cryoprotectors for freezing and freeze - drying of mixed cultures of microorganisms - lactic acid bacteria and yeast in goat milk kefir.

Materials and methods

1. Technological approach:

- Fermentation - Goat milk is used to produce a fermented milk product (kefir), pasteurized at 85°C for 15 minutes, cooled to 25°C and inoculated with natural starter culture (kefir grains).
- The fermentation process is carried out at 22-24°C of up to 24 hours and is considered to be complete when obtaining a lactic acid product having a pH (4.5) and titratable acidity (90 ÷ 100°T).
- Cryoprotective media - hydrocolloids solutions - pectin, lactulose and sodium carboxymethylcellulose (Na- CMC) were used for the experiment. The method of mechanical incorporation of the cell suspension in a hydrocolloid matrix-gel in a ratio of 1: 1 is applied.
- Four variant samples were prepared: cell suspension without cryoprotector (control), cell suspension, protected with 5% pectin solution, cell suspension, protected with 3% lactulose solution, and cell suspension, protected with 0.5% Na- CMC solution.
- The freezing was performed in refrigerated chambers with forced convection of air, at a temperature of - 35^0 C for 18-20 hours.
- Sublimation drying was carried out in a vacuum sublimation installation of the company "Hochvakuum" - TG 16.50 with contact plate heating and residual pressure in the sublimator, ranging from 10^{-1} - 10^{-2}mmHg. The drying temperature of the tested samples in the technological tests is 31°C and the final products have a minimum residual moisture content (up to 4%).

2. Microbiological approach:

- Determination of total number of microorganisms by groups in the control and variant samples with added cryoprotectors. Cultivation of *Lactobacillus* was carried out on MRS medium (pH 6.5 ± 0.2) at incubation temperature of 42-45°C for 3 days, the amount of which was read by surface counting of the colonies and recalculation to 1 ml of incubated material. Cultivation of *Lactococcus* was performed on M 17 medium (pH 7.2 ± 0.2) at an incubation temperature of 42°C for 2 days. Yeasts and molds were cultured on Sabouraud medium (pH 7.0 ± 0.2) at 28 -30°C for 7 days.
- The survival rate of the microflora in the kefir in groups after freezing and lyophilization – in % - by the following formula: % Survived cells = (number of surviving cells x / number of cells 0) x100, where: x - survival reporting time one hour after the lyophilization of the samples) 0 - a moment prior to lyophilization (number of viable cells prior to lyophilization).

3. Statistical processing: The results were processed using MS Office Excel 2007.

Results and discussion

The study was conducted by counting the number of viable cells in experimental samples-control (no cryoprotector) and variants with cryoprotectors (pectin, lactulose and Na-CMC) immediately after coagulation, freezing and lyophilization.

The summarized data are presented in Tables 1- 4.

Table 1. Total number of microorganisms by group in control variant after coagulation, freezing and lyophilization (cfu / ml, log)

	Control					
	After coagulation		After freezing		After lyophilization	
	cfu/ml	log	cfu/ml	log	cfu/ml	log
Lactobacilli	$1.7x10^7$	7.2	$0.2x10^4$	3.30	$0.1x10^4$	3.00
Streptococci	$8.9x10^6$	6.94	$0.7x10^4$	3.84	$0.6x10^4$	3.78
Yeasts	$2.9x10^4$	4.47	$0.8x10^3$	2.90	$0.7x10^3$	2.84

Table 2. Total number of microorganisms by group with cryoprotectant pectin after coagulation, freezing and lyophilization (cfu / ml, log)

	Pectin					
	After coagulation		After freezing		After lyophilization	
	cfu/ml	log	cfu/ml	log	cfu/ml	log
Lactobacilli	$3.8x10^7$	7.58	$0.5x10^4$	3.7	$0.4x10^4$	3.60
Streptococci	$1.3x10^7$	7.11	$1.2x10^4$	4.08	$1.0x10^4$	4.00
Yeasts	$6.1x10^4$	4.79	$1.5x10^3$	3.17	$1.4x10^3$	3.15

Table 3. Total number of microorganisms by group with cryoprotectant lactulose after coagulation, freezing and lyophilization (cfu / ml, log)

	Lactulose					
	After coagulation		After freezing		After lyophilization	
	cfu/ml	log	cfu/ml	log	cfu/ml	log
Lactobacilli	$8.8x10^8$	8.94	$1.9x10^5$	5.27	$1.7x10^5$	5.23
Streptococci	$3.4x10^8$	8.53	$4.7x10^5$	5.67	$4.2x10^5$	5.62
Yeasts	$6.3x10^5$	5.79	$1.8x10^4$	4.26	$1.5x10^4$	4.18

Table 4. Total number of microorganisms by group with cryoprotectant Na CMC after coagulation, freezing and lyophilization (cfu / ml, log)

	Na - Carboxymethylcellulose					
	After coagulation		After freezing		After lyophilization	
	cfu/ml	log	cfu/ml	log	cfu/ml	log
Lactobacilli	$1.7x10^8$	8.23	$2.5x10^4$	4.39	$2.5x10^5$	4.39
Streptococci	$3.2x10^7$	7.51	$3.1x10^4$	4.49	$3.0x10^4$	4.47
Yeasts	$2.1x10^5$	5.32	$5.8x10^3$	3.76	$5.4x10^3$	3.73

From the data shown in the tables, it is found that the total number of lactic acid bacteria and yeasts after coagulation in all three variants is high. After freezing, the control sample showed the lowest results, followed by variants with cryoprotectors - pectin and Na-CMC.

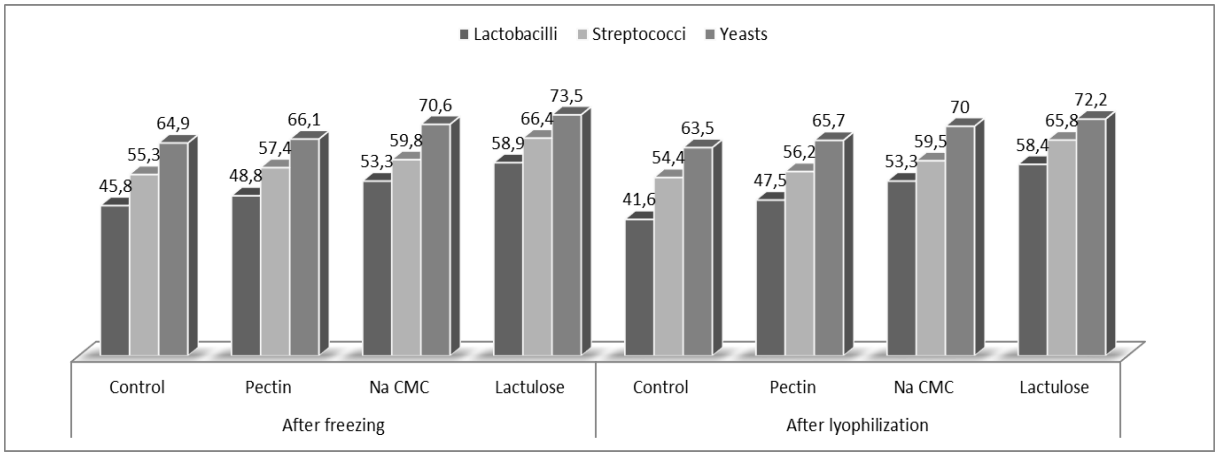


Figure 1. Survival of the different groups microorganisms in kefir, %

The survival of individual groups of microorganisms in кefir is calculated based on 100% amount of living cells at the first day after coagulation. The graph shows that the lowest survival was observed in the control and using cryoprotectant pectin. *Lactobacillus* are characterized by a higher sensitivity, than streptococci and yeast.

The lactobacilli group showed low survival without cryoprotectant and using pectin - 45.8% and 48.8%, respectively. Upon addition of the CMC, after freezing, survival increased by 7.5%, compared to the control and when adding lactulose, survival was 13.1% higher. This trend is preserved in the survival of lactobacilli after lyophilization.

Streptococcus are more stable and their survival is higher in both- control and variant with cryoprotectors. Upon addition of pectin, their survival after freezing increased by 2.1%, in the presence of CMC by 4.5% and by lactulose by 11.1%. After lyophilization, almost the same levels remained.

In yeast, we observed relatively stable survival after freezing - 64.9% in control and 73.5% in lactulose. They show the highest survival rate, but at the same time are affected the least by the inclusion of cryoprotectant in the growth medium. The best result was achieved with the use of lactulose as a cryoprotector - an increase in survival by 8.6%, compared to control. But it is lower than the result for lactobacilli - 13.1% and streptococci - 11.1%. From the results

obtained, it is evident that the group of lactobacilli is the most sensitive and the use of cryoprotectors significantly increases its survival.

The three groups of microorganisms a more substantial loss of viability is observed after freezing, while lyophilization process slightly decreased or even no influence on their survival.

Conclusions

Biopolymers pectin, lactulose and carboxymethylcellulose applied as protective medium have a stabilizing effect and increase the numbers of viable cells in the various microbial groups of kefir.

Highest values were obtained using 3% lactulose, whereby the survival of the lactobacilli, lactococci and yeast group increased by 16.8%, 11.4% and 8.7%, respectively, compared to the control.

Good cryoprotective effect was also achieved with the use of a 0.5% solution of Na-CMC, but the results were lower than those for lactulose.

The use of 5% pectin has the least effect on cells and their survival ranging, from 5.9% in the lactobacil group and about 2% in the *lactococcus* and yeast.

The convection freeze process reduces significantly cell survival in individual microbial kefir groups in the control variant, followed by a variant including pectin, Na-CMC and lactulose.

The lyophilisation process slightly affects the number of viable cells, keeping their ratio and results achieved after freezing.

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