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THE EFFECT OF STARTER CULTURE PRODUCING EXOPOLYSACCHARIDE ON PHYSICOCHEMICAL PROPERTIES OF YOGHURT

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Abstract. The purpose of this research was to investigate the impact of indigenous starter culture capable to synthesize exopolysaccharides (EPSs) on physicochemical properties of yoghurt. Two starter cultures, EPS-producing and non-EPS-producing, were developed from the autochthonous lactic acid bacteria strains by pairwise combining *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* strains. In the present study the ropy strain of *Streptococcus thermophilus* CNMN LB-50 was incorporated in EPS-producing starter culture. The microstructure, viscosity, EPS amount, structural properties and syneresis of yoghurt samples were assessed. It has been established that the EPS-producing starter culture provided a reduction of structural degradation and increased degree of structural recovery after deformation. Besides, it was observed that the use of EPS synthesized starter culture in yoghurt production restrains the syneresis of the gel.

Keywords: yoghurt, starter culture, *Streptococcus thermophilus,* physicochemical properties.

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Introduction

In recent years, the dairy industry of the Republic of Moldova is actively developing, as a result, fermented milk production is growing. Lactic acid bacteria, known as starter cultures, are used in the manufacture of fermented milk products, such as yoghurt, soured cream and others. Therefore, the selection of new strains of lactic acid bacteria suitable for using as a starter culture, especially exopolysaccharide (EPS)-producing strains, becomes a fairly popular research direction in the world.

Different stabilizers are used for improving the rheological properties of dairy products [1]. In connection with this statement, the greatest attention is paid to the EPS-producing strains, as a natural source of food additives that improves the rheological properties of dairy products and as a perspective alternative of stabilizers used in the dairy industry. The physicochemical properties of yoghurt have been vastly studied in the last years [2]. It was shown that EPSs synthesized by lactic acid bacteria play an important role improving the textural characteristics such as thickness in low fat yoghurt [3-5].

The microstructure of the yoghurt is a 3D protein network consisting of casein micelles, water dipoles, fat globules and bacterial cells [6-8]. Due to the relatively weak structure of the casein mesh, the contribution of stabilizer or naturally synthesized EPS has a significant effect on the rheological properties of the product [8,9]. Concerning the water holding capacity, a number of scientists reported that EPS exert high water retention, minimizing syneresis, while other researchers reported on the severe syneresis that occurred when a starter culture was formulated from EPS producing strains [10,11]. Therefore, the aim of the present research was to investigate the influence of the EPS-producing starter culture, consisting of EPS-producing autochthonous *Streptococcus thermophilus* strain, on physicochemical properties of yoghurt.

Experimental

Strains employed

Three single-strain cultures were used for starter culture formation: EPS-producing *Streptococcus thermophilus* LB-50, non-EPS-producing *Streptococcus thermophilus* LB-53 and

Lactobacillus delbrueckii ssp. *bulgaricus* LB-42 (non-EPS-starter culture) belonging to the National Collection of Non-Pathogenic Microorganisms of the Institute of Microbiology and Biotechnology of the Academy of Sciences of Moldova (CNMN IMB ASM), previously selected from raw milk. The selected strains were propagated three times consecutively using 0.1% inoculum volume in 10% reconstituted skim milk (InLac, Republic of Moldova) at 40°C until coagulation. Working strains (pure culture 10 mL) were gradually associated by inserting into 30 mL of sterile skim milk and incubated at 40°C until coagulation (pH 4.6). The two mixed-strain starter cultures were used to make yoghurt samples for studying the influence of the EPS on the quality of the final product as follows: EPS-starter culture consists of *Streptococcus thermophilus* LB-50 and *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-42; non-EPS starter culture – *Streptococcus thermophilus* LB-53 and *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-42. Non-EPS starter culture served as control.

Yoghurt production

Samples of yoghurt were performed under laboratory conditions (Laboratory of Food Biotechnology) using milk produced by JLC Group (Chisinau, Republic of Moldova). The milk was pasteurized at 71°C during 15 s and cooled down to 40°C. Two samples of yoghurt were produced: sample 1 using EPS-producing starter culture and sample 2 with non-EPS starter culture. Samples of yoghurt were incubated at 37°C until pH decreased to a value of 4.5, then kept at 4°C.

Microscopy

Starter culture strains were visualized with a binocular microscope (KRUSS, MBL2000, OPTECH, Germany) using 100x objective lens with immersion oil.

Enumeration of bacteria

1 mL of sample was added to 9 mL of sterile peptone diluents (0.1 g/L) and serial dilutions were made. Enumeration was carried out using pour plate technique. Plates were incubated aerobically at 37°C for 48 h.

Isolation of EPS

100 mL of yoghurt were centrifuged at 837 rad/s for 10 minutes and 17 mL of 85% trichloracetic acid (Chimprom, Ukraine) were added to each sample. Samples were cooled up to 4°C and again centrifuged at 837 rad/s for 10 minutes. Precipitation of EPS from samples was provided using cold ethanol (1:3). The samples were kept in the fridge for 24 h and then centrifuged (40°C, 837 rad/s, for 10 min). EPSs were dried at room temperature and weighed.

Determination of viscosity

The viscosity of yoghurt samples was studied with a digital rheometer DV-III (BROOKFIELD, Canada) using the Rheocalc 32 software. Measurements were performed at different rotation speeds up to 21 rad/s. The viscosity measurement was carried out at a temperature of 25°C.

Determination of damage and recovery degree of yoghurt gels

Degree of damage α (%) was calculated according to the Eq. (1):

$$\alpha = \frac{100 - \eta_d}{\eta_i} \quad (1)$$

where, η_d – the viscosity of deformed gel, Pa·s;
η_i – the viscosity of undeformed gel, Pa·s.

Degree of recovery β (%) was calculated according to the Eq. (2):

$$\beta = \frac{100 - \eta_d}{\eta_i} \quad (2)$$

where, η_d – the viscosity of deformed gel, Pa·s;
η_i – the viscosity of undeformed gel, Pa·s.

Determination of spontaneous syneresis

The method was adapted from Lucey *et al.* [12]. In our study, cooled yogurt samples (4±1°C) were weighed and kept at an angle of approximately 45° to allow whey collection at the side of the cup. The whey was collected from the surface of the sample using a syringe, and the cup of yogurt was weighed again. Syneresis was expressed as the percent weight of the whey over the initial weight of the yoghurt sample.

Determination of microstructure of the samples

Small drops of yogurt samples were smeared on the microscope slide and stored at minus 18°C for 5 min, then dried at room temperature and stained with Ehrlich's hematoxylin for 10 minutes and with an aqueous Eosin solution. Microscopic study was performed using the binocular microscope (KRUSS, MBL2000, OPTECH, Germany) at 40x objective lens.

Determination of acidity in yoghurt

The titratable acidity measurements were performed as follows: 10 g of yoghurt was weighed into a titration beaker, then diluted with 20 mL of distilled water, 3-5 drops of phenolphthalein indicator were added and the

solution was titrated using NaOH 0.1 mol/L. pH of yoghurt was measured using pH meter HI 110 (HANNA Instruments, USA).

Statistical data analysis

The determination of physicochemical properties of yoghurts was conducted in 3 replications. For each sample the confidence interval was calculated [13].

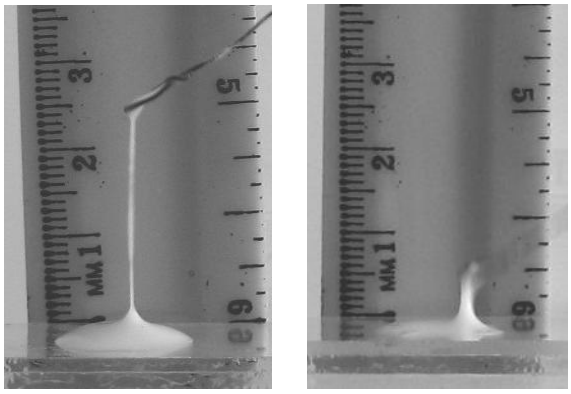
Results and discussion

Properties of the starter culture

For yogurt production, the starter culture must contain at least one *Streptococcus thermophilus* strain and one *Lactobacillus delbrueckii* ssp. *bulgaricus* strain.

The ability of the single strain *Streptococcus thermophilus* LB-50 to produce EPS was preliminarily confirmed in skim milk incubated at 40°C being 52.1±1 mg/100 mL (Figure 1). The strain LB-50 produced high amount of ropy EPSs, whereas at the strain LB-53 and LB-42 this functional capacity was absent.

Also, two starter cultures were tested on EPS production. The results showed that EPS-starter culture produced 58.43±1.9 mg/100 mL of EPS that was higher with 12% in comparison to the result obtained at single strain cultivation in skim milk. It could be explained by symbiotic proto-cooperation factors between *Streptococcus thermophilus* and *Lactobacillus bulgaricus* due to the fact that each one of them produces substances favourable for the other [14] (Figure 2).



(a) ***(b)***

Figure 1. Appearance of the ropy strand formed by Streptococcus thermophilus LB-50 *(a)* and non-ropy – LB-53 *(b)*.

Production of the yoghurt samples

The milk (2.5% fat content) was inoculated by 5% (wt/wt) starter cultures. The fermentation process was stopped after 4 h, when gel formation occurred. Properties of yoghurt samples produced in this study using the EPS and non-EPS starter cultures are presented in Table 1.

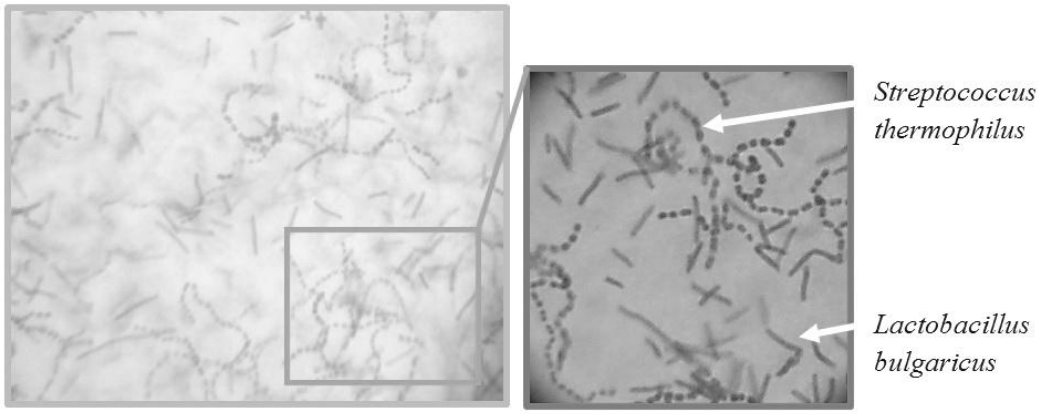


Figure 2. Observation of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* strains in a starter culture.

Table 1

Characteristics of yoghurt samples during fermentation at 40°C.

Parameters	*0 h*		*2 h*		*4 h*	
	EPS	*non-EPS*	*EPS*	*non-EPS*	*EPS*	*non-EPS*
pH	6.5±0.1	6.5±0.0	5.7±0.1	5.5±0.1	4.6±0.1	4.5±0.1
Titratable acidity, °T	16 ±0.0	16±1	29±3	34±2	71±3	75±1
EPS, mg/100 mL	0	0	28.6±0.5	0	66.6±1.2	0
Cell count, log CFU/mL	6.39±0.3	6.07±0.1	7.57±0.4	6.61±0.3	9.2±0.1	8.34±0.3

The pH value and titratable acidity were similar for two samples of yoghurt. The EPS amount produced in yoghurt by EPS-producing starter culture after 4 h of fermentation was 66.6 mg/100 mL that is with 14% greater than that found in skimmed milk and with 27% in fermented milk with single *Streptococcus thermophilus* LB-50 strain. The increase in synthesized EPSs could be caused by milk (2.5% fat content) that contains more nutrient substances in comparison with skimmed milk. Viable cell concentration of lactic acid bacteria was different. The lactic acid bacteria concentration in yoghurt produced by the EPS-starter culture was higher (9.1 log CFU/mL) than in yoghurt produced by non-EPS-producing starter culture (8.1 log CFU/mL). The result of this study suggests the protective properties of EPS on the bacterial cells under acid medium (pH 4.5-4.6) [15].

Physicochemical properties of the gel

Both long fermentation process and high concentration of lactic acid had a negative impact on the quality indicators of fermented dairy products. Lactic acid accumulated due to the activity of the microorganisms reduces the electrical charge of the proteins, thus reducing their hydrophilic properties the proteins easily dehydrogenate and the gel eliminates the whey. This spontaneous separation of moisture is explained by free water that fills only the inner volume of the gel structure without the formation of close physical-chemical bonds. The consistency evaluation was performed after measuring the apparent (dynamic) viscosity of yoghurts, the deformation and recovery degree of the gel structure (Table 2 and Figure 3). The destruction of the yoghurt gel structure was performed during 3 minutes.

Table 2

Structural properties of yoghurt.

Starter culture	*Apparent viscosity of the gel at shear rate 48.6 s^{-1} Pa·s*			*Damage degree of the gel, %*	*Recovery degree of the gel, %*	*Syneresis, %*
	Deformed	*Undeformed*	*Recovered*			
EPS	1.58±0.02	1.08±0.02	1.30±0.01	31.1	83.1	0.0
non-EPS	1.43+0.03	0.90±0.02	1.07±0.03	36.5	75.2	1.4

Our study showed that relaxation time of 30 minutes is sufficient for recovery of the gel structure, increasing the relaxation time did not lead to an increase in the degree of recovery of the initial structure of the gels. As a result it was determined that the use of EPS-producing starter culture blocks the process of syneresis and increases the water retention capacity of the gel, due to the fact that EPS strengthens the water connections with the milk components.

The consistency evaluation of the yoghurt produced with non-EPS starter culture demonstrated that the destroyed gel exhibits thixotropy to a lower degree because of lactic acid present in the yoghurt that contributes to the protein hydrolysis process, accompanied by the destruction of the product structure [16].

Investigations concerning the damage and recovery degree of yoghurt gels demonstrated that the least degree of destruction was 31.1% and the highest rate of recovery was 83.1% at the yoghurt produced using EPS-starter culture. Yoghurt produced with non-EPS starter has been characterized by the lowest indicators: the degree of destruction was 34.3% and degree of the structure recovery was 80.4%. Thus, the use of EPS-producing starter cultures for yoghurt can increase their viscosity (1.57 Pa·s) and thixotropic characteristics.

Thus, the use of EPS-producing starter cultures contributes to the regulation of the structuring process and improves the mechanical properties of yoghurt.

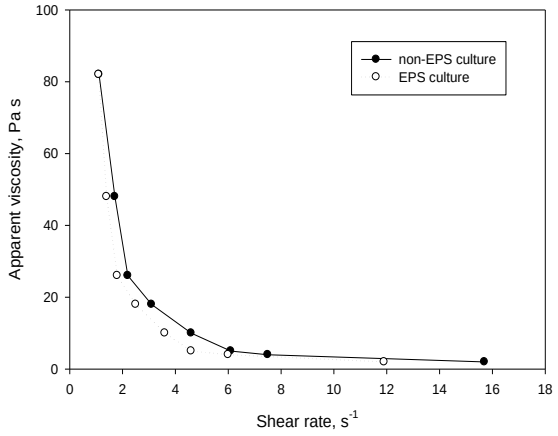
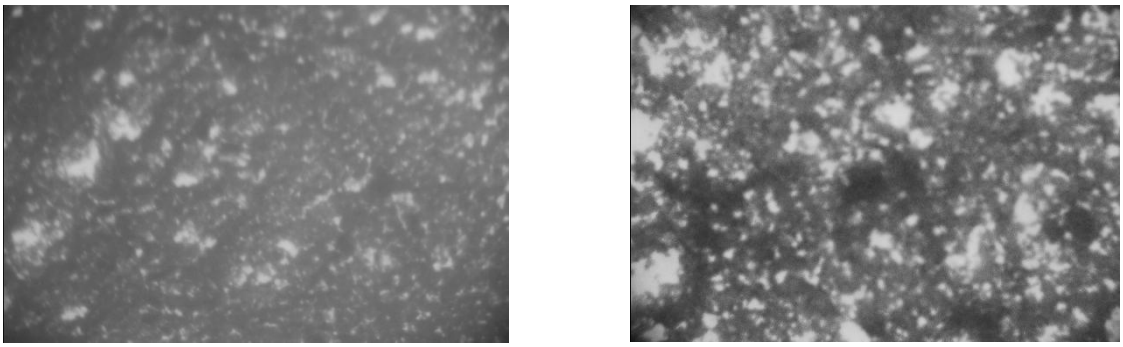


Figure 3. Apparent viscosity (dynamic) variation according to the shear rate for yoghurt samples.

Microstructure of yoghurt gel

The microscopic images (Figure 4) have shown that the yoghurt produced with the EPS starter culture had a homogeneous compact structure without the elimination of whey (Figure 4*(a)*). Milk proteins were grouped in a

sufficiently large complex and the structure bounded free water, meanwhile preventing the separation of the whey. It is obvious that EPS contributes to the water binding in the product. Yoghurt produced with non-EPS-producing starter culture had weak structure, protein aggregation in small complexes, which were separated and distributed unevenly (Figure 4*(b)*) and caused a small capacity of water retention and whey separation.



(a) ***(b)***

Figure 4. Microstructure of yoghurt samples producing with: *(a)* EPS-starter culture, *(b)* non-EPS-starter culture.

Conclusions

The developed EPS-producing starter culture consisting of autochthonous strains of *Streptococcus thermophilus* CNMN LB-50 and *Lactobacillus delbrueckii* ssp. *bulgaricus* CNMN LB-42 have demonstrated positive effect on physicochemical properties of yoghurt in comparison with yoghurt made with non-EPS-starter under laboratory conditions. The EPS starter prevents syneresis of yoghurt forming viscous compact structure milk gel with the lowest degree of destruction (31.1%) and the highest rate of recovery (83.1%). The yoghurt samples microstructure analysis confirmed the beneficial offset of EPS on rheological properties of the final product.

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