# INVESTIGATION OF THE BIOTECHNOLOGICAL ACTIVITY OF DIRECT-SET STARTER CULTURES IN STRUCTURED DAIRY PRODUCTS

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**Abstract:** Galactosidase and proteinase activity of lactic acid bacteria of the DELVO-YOG range has been investigated. Lactic acid bacteria of the DELVO-YOG range have been shown to exhibit maximal galactosidase activity in the presence of the CMC stabilizer Akucell 2785; the activity was minimal when sodium alginate NO4-600 was used as a stabilizer. The proteolytic activity demonstrated by the lactic acid bacteria of the DELVO-YOG range was maximal when sodium pyrophosphate SAPP 28 was used as a stabilizer. Minimal proteolytic activity of lactic acid bacteria of the DELVO-YOG range was registered when CMC 6000-9000 was used as a stabilizer. An increase of galactosidase and proteinase activity concomitant to an increase of the stabilizer content from 0.5 to 1.0 mass. % was demonstrated for all the denominations of lactic acid bacteria of the DELVO-YOG range.

**Key words:** milk, lactic acid bacteria, activity, glycolysis, proteolysis, structure stabilizer

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## INTRODUCTION

Functional food comprises special-purpose products of natural or artificial origin with predefined properties; these products are intended for systematic everyday use and their primary role is to compensate for the deficiency of the regulatory components of food in the organism. Functional foods contribute to health preservation, disease prevention, and enhancement of the body's ability to withstand adverse environmental effects and to endure physical and psycho-emotional stress. The significance of nutrition is emphasized by the Decree № 917 of 10.08.98 "On the Concept of the State Policy Regarding Healthy Nutrition of the Population of the Russian Federation" of the Government of Russian Federation.

Fermented dairy products are manufactured using starter cultures of a defined composition. Milk is an ideal nutrient medium for starter cultures, which are used to direct and control biochemical transformations. Biochemical transformation of milk components includes controlled glycolysis of lactose yielding lactic acid and regulated proteolysis of casein, and therefore both galactosidase activity and proteolytic activity are important characteristics of the starter cultures [1]. The most useful species of starter cultures must display maximal activity during fermentation.

The structure of stabilized dairy products is formed as a result of casein coagulation as the isoelectric point is reached. Structure stabilizers allow for structure formation at pH values deviating from the isoelectric point value. Moreover, the use of stabilizers allows for control over the structural, mechanical, physico-chemical, and organoleptic parameters of the final product, as well as its general quality. Stabilizers are widely used to regulate the structure of fermented milk beverages and achieve the required texture which does not change during storage and remains stable throughout the technological process. Stabilizers should prevent delamination of the product and separation of whey; in case of yoghurts containing fillers and additives stabilizers should provide for uniform distribution of these components in the product during packing and subsequent storage.

The main objectives of the manufacturing of structured fermented dairy products are the optimization of stabilizer content, the selection of starter cultures possessing high acid-forming capacity, and the selection of cultivation conditions providing for rapid propagation of the microorganisms and intensive acid production by them. Cultivation of starter cultures that have significant proteolytic activity accelerates the propagation of bifidobacteria in the structured product and improves the organoleptic properties of the product. A wide range of starter cultures adapted to specific features of the technology used and the biochemical processes of the formation of texture and taste properties of dairy products is available. The qualitative composition of starter cultures is being constantly improved [2].

Food stabilizers are currently considered to be among the most important components of the majority of structured fermented dairy products, since they allow for the manufacturing of a product of required texture [3]. In addition to improving the product quality and increasing its shelf life, the use of stabilizers allows for a decrease of product costs, this improving the economic performance of the enterprise. The experiments performed in the present study revealed the decisive role of the environmental conditions (mass concentration and type of the stabilizer) in regulating the galactosidase and proteinase activity of starter cultures. Investigation of the dependence of galactosidase and proteolytic activity of DELVO-YOG starter cultures on the type and content of the stabilizer can be of practical importance for the production of stabilized dairy products.

## OBJECTS AND METHODS OF THE STUDY

The study was carried out with the following objects:

- Mesophilic aramate-forming DELVO-YOG starter cultures CY-346/347, FVV-21, CY DSL, and FVV-31;
- Structure stabilizers: CMC Akucell 3265, CMC 4500-6000, konjac gum, CMC 6000-9000, pectin ARA 105, carob gum, sodium alginate NO4-600, sodium pyrophosphate SAPP 28, CMC Akucell 2785, sodium pyrophosphate SAPP 40, and xanthan gum;
-Potable water conforming to GOST (State Standard) 2874;
- Structured fermented dairy products with different composition and properties produced either in the laboratory or industrially;
- Raw cow milk (grade II or higher according to GOST (State Standard) 13264);
- Whey conforming to OST (Industrial Standard) 4992;
- Whey powder conforming to TU (Technical Specifications) 49800;
- Granulated sugar conforming to GOST (State Standard) 21;
- Auxiliary materials conforming to the regulatory documents currently in force.

Conventional, standard and original methods were used in the study.

Sampling of milk and milk products and preparation of samples for analysis were performed in accordance with GOST (State Standard) 26809-86.

Sampling for microbiological testing was performed in accordance with GOST (State Standard) 9225-84.

Titration-based acidity assay was performed in accordance with GOST (State Standard) 3524-92.

Active acidity was measured on a potentiometric analyzer according to GOST (State Standard) 26781-85.

Evaluation of taste and smell was performed in accordance with GOST (State Standard) 28283-92.

Total protein content was assayed in accordance with GOST (State Standard) 23327-78.

Activity of the enzyme system referred to the difference between the non-protein nitrogen or lactose concentration measured in non-fermented whey and the respective concentration measured in a system consisting of hydrolyzed whey and native whey pretreated with fermenting microorganisms. The average values of galactosidase activity and proteolytic activity of the lactic acid bacteria of DELVO-YOG series measured in samples with different stabilizer content (mass %) were analyzed in the present work. The stabilizer content was increased from 0.5 to 2.5 mass % in steps of 0.5 mass %.

Galactosidase activity of lactic acid bacteria was determined as follows: a 5% solution of lactose in a buffer solution (pH 4.2 or 7.0) and a 1% solution of the enzyme were prepared, and the enzyme activity was determined using the freezing point depression method. For this, 1 $cm^3$ of the enzyme solution and 4 $cm^3$ of the substrate solution were mixed, and a 1 $cm^3$ aliquot (control) was drawn from the mixture. The remaining solution was incubated at 30° C for 30 minutes, and after this a 1 $cm^3$ test aliquot was drawn and its freezing temperature was measured and used to calculate galactosidase activity.

A method based on the hydrolysis of sodium caseinate by the enzyme preparation under investigation with subsequent quantitation of the peptides formed was used for the determination of proteolytic activity. A unit of proteolytic activity was defined as the ability of the enzyme to convert sodium caseinate into compounds not precipitated by trichloroacetic acid in an amount equivalent to 1 micromole of tyrosine (GOST (State Standard) 20264.2-88) in one minute at 30°C. The activity of proteolytic enzymes in the medium and in the cells was evaluated by measuring the difference in the content of ninhydrin-positive substances in the reaction mixture. The method involves monitoring of the changes in the content of ninhydrin-positive substances in the reaction mixture with subsequent quantitative assessment; the accumulation of the extracellular enzyme of the microorganism under investigation is assayed according to the procedure developed by Chebotarev.

## RESULTS AND DISCUSSION

Stabilizer content of 1.5 mass % was chosen in order to maintain the structure of the fermented dairy product; the choice was based on the analysis of the average values of galactosidase activity of lactic acid bacteria of the DELVO-YOG series.

**Table 1.** Galactosidase activity of the DELVO-YOG starter culture at the stabilizer content of 1.5 mass %, Δg lactose/100 mg

| Stabilizer type | Starter culture name | | | |
|---|---|---|---|---|
| | CY-346/347 | FVV-21 | CY DSL | FVV-31 |
| CMC Akucell 3265 | 3.29±0.05 | 3.48±0.05 | 3.02±0.05 | 3.29±0.05 |
| CMC 4500-6000 | 2.81±0.05 | 2.94±0.05 | 2.98±0.05 | 3.14±0.05 |
| Conjac gum | 2.94±0.05 | 2.90±0.05 | 2.97±0.05 | 3.11±0.05 |
| CMC 6000-9000 | 3.06±0.05 | 3.33±0.05 | 3.29±0.05 | 2.96±0.05 |
| Pectin ARA 105 | 3.30±0.05 | 3.30±0.05 | 3.17±0.05 | 3.23±0.05 |
| Locust bean gum | 2.96±0.05 | 3.28±0.05 | 2.74±0.05 | 2.88±0.05 |
| Sodium alginate NO4-600 | 2.77±0.05 | 2.16±0.05 | 2.58±0.05 | 2.79±0.05 |
| Sodium pyrophosphate SAPP 28 | 2.84±0.05 | 2.94±0.05 | 2.81±0.05 | 2.91±0.05 |
| CMC Akucell 2785 | 3.47±0.05 | 3.54±0.05 | 3.32±0.05 | 3.42±0.05 |
| Sodium pyrophosphate SAPP 40 | 3.05±0.05 | 3.11±0.05 | 2.63±0.05 | 2.93±0.05 |
| Xanthan gum | 3.13±0.05 | 2.99±0.05 | 2.83±0.05 | 3.12±0.05 |

The optimal stabilizer type was chosen according to the values of galactosidase activity (Δg lactose/100 mg) of the DELVO-YOG starter culture (subtypes CY-346/347, FVV-21, CY DSL, and FVV-31) measured at a stabilizer content of 1.5 mass %. The results of the experiment are presented in Table 1.

An increase of galactosidase activity by 0.03–0.17 was registered for all types of DELVO-YOG starter culture. The galactosidase activity was maximal when CMC Akucell 2785 was used as a stabilizer; the activity amounted to 3.47 ± 0.05 Δg lactose/100 mg for

CY-346/347, 3.54 ± 0.05—for FVV-21, 3.32 ± 0.05—for CY DSL, and 3.42 ± 0.05—for FVV-31. The activity was minimal when sodium alginate NO4-600 was used as the stabilizer.

The dependence of galactosidase activity of DELVO-YOG lactic acid bacteria on the content of CMC 6000-9000 was analyzed in order to determine the optimal content of the stabilizer. The results of the experiment are presented in Fig. 1.

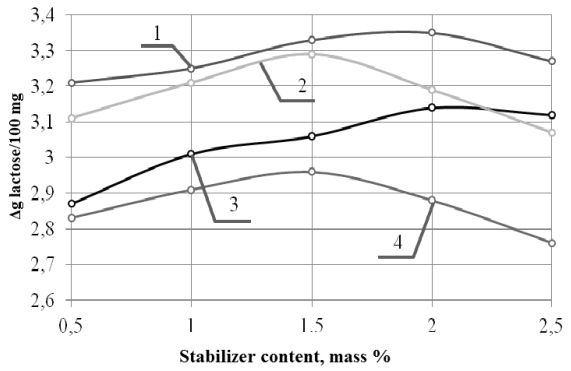


**Fig. 1.** Dependence of galactosidase activity of the DELVO-YOG starter culture on the content of CMC 6000-9000, mass %: 1–FVV-21; 2–CY DSL; 3–CY-346/347; 4–FVV-31.

Maximal increase in galactosidase activity of the DELVO-YOG starter culture was observed as the content of the stabilizer was increased from 1.5 to 2.0%.

The dependence of galactosidase activity of DELVO-YOG CY-346/347 starter culture on the stabilizer type at the stabilizer concentration of 1.5% was analyzed to identify the optimal type of stabilizer.

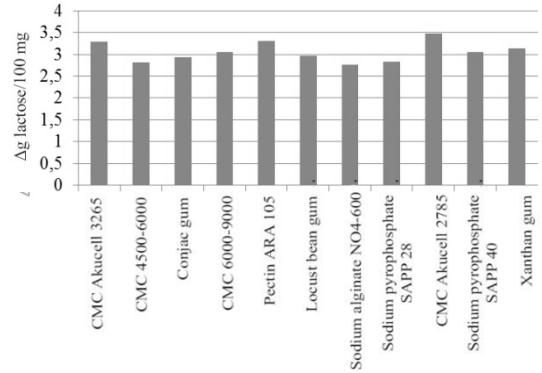


**Fig. 2.** Galactosidase activity of DELVO-YOG CY-346/347 lactic acid bacteria at a stabilizer content of 1.5 mass %.

DELVO-YOG CY-346/347 starter culture exhibited maximal galactosidase activity when CMC Akucell 2785 was used as a stabilizer.

Stabilizer content of 1.5 mass % was chosen according to the results of the analysis of the average proteolytic activity values of lactic acid bacteria. The proteolytic activity of DELVO-YOG starter culture at a stabilizer content of 1.5 mass % was analyzed in order to choose the optimal stabilizer. The results of the experiments are presented in Table 2.

**Table 2.** Proteolytic activity of lactic acid bacteria of the DELVO-YOG series at a stabilizer content of 1.5 mass %, $10^{-2}$ Δmg non-protein nitrogen/100 mg

| Stabilizer type | Name | | | |
|---|---|---|---|---|
| | CY-346/347 | FVV-21 | CY DSL | FVV-31 |
| CMC Akucell 3265 | 2.57±0.05 | 2.52±0.05 | 2.70±0.05 | 2.54±0.05 |
| CMC 4500-6000 | 2.93±0.05 | 2.88±0.05 | 2.98±0.05 | 3.06±0.05 |
| Konjac gum | 2.70±0.05 | 2.63±0.05 | 2.76±0.05 | 2.71±0.05 |
| CMC 6000-9000 | 2.51±0.05 | 2.43±0.05 | 2.31±0.05 | 2.52±0.05 |
| Pectin ARA 105 | 3.16±0.05 | 3.02±0.05 | 3.03±0.05 | 3.24±0.05 |
| Locust bean gum | 2.78±0.05 | 2.63±0.05 | 2.82±0.05 | 3.00±0.05 |
| Sodium alginate NO4-600 | 2.93±0.05 | 2.73±0.05 | 2.70±0.05 | 2.86±0.05 |
| Sodium pyrophosphate SAPP 28 | 3.29±0.05 | 3.06±0.05 | 3.11±0.05 | 3.27±0.05 |
| CMC Akucell 2785 | 3.00±0.05 | 2.89±0.05 | 2.94±0.05 | 2.98±0.05 |
| Sodium pyrophosphate SAPP 40 | 2.59±0.05 | 2.42±0.05 | 2.65±0.05 | 2.79±0.05 |
| Xanthan gum | 2.79±0.05 | 2.80±0.05 | 2.76±0.05 | 2.70±0.05 |

The values of the proteolytic activity of lactic acid bacteria increased by 0.01–0.05; maximum proteolytic activity of starter cultures, amounting to 3.29 ± 0.05 for CY-346/347, 3.06 ± 0.05 for FVV-21, 3.11 ± 0.05 for CY DSL, and 3.27 ± 0.05 for FVV-31, was registered when sodium pyrophosphate SAPP 28 was used as a stabilizer. Proteolytic activity was the lowest when CMC 6000-9000 was used as a stabilizer: in this case it amounted to 2.51 ± 0.05 for CY-346/347, 2.43 ± 0.05 for FVV-21, 2.31 ± 0.05 for CY DSL, and 2.52 ± 0.05 for FVV-31.

The dependence of the proteolytic activity of the DELVO-YOG starter culture on the content of CMC Akucell 3265 was analyzed in order to determine the optimal content of the stabilizer. Results of the experiment are presented in Fig. 3: 1–CY-346/347; 2–FVV-31, 3–FVV-21, 4–CY DSL.

An increase of the proteolytic activity of DELVO-YOG starter cultures (with the exception of FVV-21) was observed as the stabilizer content was increased from 1.5 to 2.0 mass %.

The dependence of the proteolytic activity of DELVO-YOG CY-346/347 lactic acid bacteria on stabilizer type at the stabilizer concentration of 1.5 mass % was analyzed in order to identify the optimal type of stabilizer.

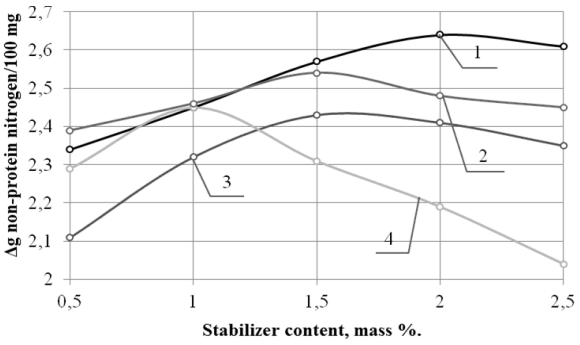


**Fig. 3.** The dependence of the proteolytic activity of DELVO-YOG lactic acid bacteria on the content of CMC Akucell 3265, mass %: 1–CY-346/347; 2–FVV-31, 3–FVV-21, 4–CY DSL.

Notably, both galactosidase activity and proteolytic activity of all types of DELVO-YOG starter culture increased as the stabilizer content was increased from 0.5% to 1.0 mass %.

As a rule, use of the stabilizer at a content of 1.5 mass % provided for the highest values of galactosidase activity and proteolytic activity of DELVO-YOG starter cultures

Maximal values of proteolytic activity of DELVO-YOG starter cultures were registered when sodium pyrophosphate SAPP 28 was used as stabilizer; these values amounted to 3.29 ± 0.05 for CY-346/347, 3.06 ± 0.05 for FVV-21, 3.11 ± 0.05 for CY DSL, and 3.27 ± 0.05 for FVV-31. The minimal values of the proteolytic activity of DELVO-YOG starter cultures were registered when CMC 6000-9000 was used as a stabilizer and amounted to 2.51 ± 0.05 for CY-346/347, 2.43 ± 0.05 for FVV-21, 2.31 ± 0.05 for CY DSL, and 2.52 ± 0.05 for FVV-31, as shown in Fig. 4.

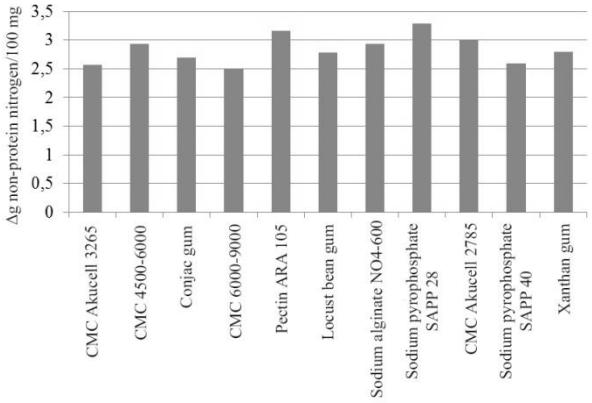


**Fig. 4.** Proteolytic activity of the DELVO-YOG CY-346/347 starter culture at a stabilizer content of 1.5 mass %.

The increase of galactosidase activity and proteolytic activity of lactic acid bacteria upon the increase of the stabilizer content above 1.5 mass % was insignificant (not more than 0.09 units), and in some cases a slight decrease of galactosidase activity and proteolytic activity of lactic acid bacteria was observed.

Maximal galactosidase activity of DELVO-YOG starter cultures was detected when CMC Akucell 2785 was used as a stabilizer; the activity values amounted to 3.47 ± 0.05 for CY-346/347, 3.54 ± 0.05 for FVV-21, 3.32 ± 0.05 for CY DSL, and 3.42 ± 0.05 for FVV-31, and minimal activity was registered when sodium alginate NO4-600 was used as the stabilizer.

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