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LACTULOSE EFFECT ON VIABILITY OF STARTER CULTURES

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Abstract

Lactulose is non-absorbable sugar, which is used in medicine for the treatment of constipation, hepatic encephalopathy and other liver complications like: salmonellosis, food poisoning, rotavirus infections, for the prevention of tumors, and to enhance immunity. Lactulose as well-known prebiotic is widely used in the fermented milk products production and can give manufactures following benefits: assortment expansion, technology simplification, improvement of products consistency, increase of microflora viability and extension of products shelf life. The aim of this study was to show lactulose effect on viability of different starter cultures.

Materials for research were different starter cultures (for sour cream, cottage cheese, yogurt and probiotic culture *Lactobacterium rhamnosus*). Viability of starter cultures was determined by the standard method for lactic acid bacteria. The quantity of microorganisms after: fermentation, long storage (14 days, t = 4 ± 2 ^{0}C), freezing (t = -18 ± 2 ^{0}C, for 1 day) or freeze drying was compared.

It was established that lactulose increased the viability of sour cream and yogurt microorganisms during storage and freezing. Quantity of bacteria in sour cream samples after 14 days of storage in presence of 1 - 3% lactulose was higher than in control by 15 - 30%. In case of yogurt starter using the viability of microorganisms in samples with 1 - 3% of lactulose after 14 days of storage was higher by 25 - 27%. Positive effects of lactulose on viability of same starter cultures were obtained in case of freezing. Quantity of microorganisms in samples with 1 - 3% of lactulose was higher than in control by 10 - 24%. Viability of sour cream and yogurt starters after freeze-drying and their storage at t = 4 ± 2 ^{0}C, for 2.5 years was higher in samples with 1 - 5% of lactulose, but this effect wasn't established for *Lb. rhamnosus*.

Adding of lactulose in proper concentration increases viability of starter cultures for sour cream and yogurt in storage, freezing and freeze drying conditions. It can be used for functional fermented milk products and ice cream production.

***Key words**: Lactulose, Prebiotic, Microflora, Viability, Starter culture.*

1. Introduction

Disaccharide lactulose is a synthetic and non-digestible sugar, which bifidogenic effect was discovered by Petuely in 1957. Lactulose can be synthesized from lactose by glucose to fructose rearranging methods [1]. In medicine, it can be used as an effective therapeutic compound for treating some disorders like: hepatic encephalopathy and other liver complications, salmonellosis, food poisoning, rotavirus infections, for the prevention of tumors, to enhance immunity and also as a food grade colon-targeted delivery system [2, 3, and 4].

Lactulose induces the growth of health-enhancing probiotics such as *Lactobacillus* and *Bifidobacterium* strains in concomitant with pathogen-growth suppression [5]. Today it is one of the most studied prebiotics [6]. Lactulose as a low-calorie sweetener with functional properties is widely used in the food industry, including the production of confectionery, beverages, dietary and

diabetic foods and dietary supplements [7]. However, the main use of lactulose is the production of functional fermented milk products. Lactulose can be used in the form of syrups, powders or as part of bifidogenic additives from secondary dairy raw materials [8]. Advantages of lactulose using in the production of dairy products are following:

- The opportunity to expand the range of products with healthy properties.

- Technology simplification by eliminating or reducing some operations.

- Increasing of starter microflora viability during long-term storage and freezing.

- Extension of the shelf life of products.

- Improvement of product consistency.

A wide range of starters and food additives are used in the production of fermented milk products [9], but the interaction of microflora and lactulose components is not well understood. Analysis of the literature has shown that lactulose can affect the technological processes and properties of fermented milk products, both directly and by affecting the starter microflora.

If a prebiotic is involved in metabolism of starter microflora, it can affect the biochemical properties of microorganisms, including changes in the morphological and cultural properties of bacteria. Mentioned changes effect on the technological processes of the fermented milk products production, for example, the time of fermentation, as well as the properties of functional products: consistency, flavor and their shelf life.

In some publications, there is information that lactulose affects the viability of probiotic microflora [10]. The aim of our experiments was to evaluate lactulose influence on the viability of starter microflora in conditions of long-term cold storage, freezing and freeze drying.

2. Materials and Methods

2.1 Sample preparation

Three groups of starter cultures based on mesophilic, thermophilic, and probiotic microflora were chosen for preparation of fermented milk samples. Characteristic of the starters is presented in Table1.

Table 1. Characteristic of the starters

Starter name	Producer	Species composition	Temperature of fermentation, ^{0}C	Time of fermentation, h	Titratable acidity, Turner degrees, no more than
For sour cream KDs	State Scientific Institution «Russian Research Institute of Dairy Industry» (SSI RRIDI), Russian Federation	*Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis* biovar *diacetilactis*	28 ± 2	8 - 12	90
For sour cream LAT CW L	Eco-Com, Bulgaria	*Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris*	28 ± 2	8 - 10	90
For curd MST	IT «Experimental Biofabrika», Russian Federation	*Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris, Lactococcus lactis* subsp. *lactis* biovar *diacetilactis*	28 ± 2	12 - 16	95
For curd LAT CW	Eco-Com, Bulgaria	*Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris, Streptococcus thermophilus*	30 ± 2	10 - 12	95
For yogurt LAT BY	Eco-Com, Bulgaria	*Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus*	40 ± 2	6 - 8	90
For prebiotic products HOWARU Rhamnosus	Danisco, Denmark	*Lactobacillus rhamnosus ATTC SD 5675*	37 ± 2	8 - 12	110

Lactulose syrups contain (in 100 mL) 66.7 g of lactulose, 11.3 g of galactose and 6.7 g of lactose, were used as a source of prebiotic (syrup manufacturers: Solvay pharma, Weesp, Netherlands, and Inalko, Molteni, Italy).

For preparation of test samples, 1, 3 or 5% of lactulose and 5% of activated starter culture were added to sterilized skimmed milk. Control samples were prepared the same, but without the addition of lactulose. Fermentation was carried out under conditions optimal for the development of starter microflora in accordance with the technological instruction. Samples after fermentation were stored at a temperature 4 ± 2 ^{0}C for 14 days. In the process of storage the amount of lactic acid microorganisms, as the most important controlled and regulated indicators determining the quality of fermented milk products was measured.

To study the effect of lactulose on the viability of the starter microflora in freezing conditions samples after fermentation were frozen at temperature -18 ± 2 ^{0}C for 1 day. After that, the quantity of viable cells was determined.

To study the effect of lactulose on the viability of the starter microflora in conditions of freeze drying samples after fermentation were dried in a freeze drier 45.27.U. The samples were packaged in 5 cm^3 in glass vials, which were installed in cassettes and placed in a cooled sublimation chamber with an initial temperature - 47 ^{0}C, final drying temperature was 26 ± 1 ^{0}C, and duration was τ = 3 ± 2 hours. Samples were capped immediately after freeze drying in a dry sterile room (box). Dried samples of fermented milk products were stored for 2.5 years at temperature of 4 ± 2 ^{0}C.The quantity of viable cells in samples was determined at certain intervals of storage.

2.2 Viability of starter microflora

Standard method for determination of the lactic acid bacteria was used for counting the quantity of viable cells [11]. The quantity of starter microflora cells was determined immediately after fermentation and after adverse influences (cold storage, freezing and freeze-drying).

3. Results and Discussion

The results of lactulose effect on the viability of starter microflora for sour cream LAT CW L after 7 and 14 days of storage are presented in Figure 1.

The level of lactic acid microorganisms during the storage period was higher in samples with the addition of lactulose (Figure 1). The presence of lactulose promoted to increase the quantity of cells in the process of starter LAT CW L fermentation. Quantity of lactic acid microorganisms was higher compared to the control by 4 - 7% after fermentation. Cell death in samples with lactulose during storage was less intense. So after 7 days, the quantity of microorganisms in the control decreased by ΔlgN = 1.5 CFU/cm^3, while in samples with 1 and 3% of lactulose by ΔlgN = 0.8 and ΔlgN = 0.6 CFU/cm^3 respectively. In the sample with the addition of 5% prebiotic, the level of viable cells decreased by ΔlgN = 1.7CFU/cm^3, but exceeded the control by 6%. The results suggest that the addition of lactulose has a positive effect on the survival of the microflora for sour cream LAT CW L. Quantity of cells during storage decreased in the control to 10^6 CFU/cm^3, while in samples with lactulose CFU/cm^3 remained at the level of (10^8 - 10^9).

Positive results were obtained also for sour cream starter KDs. The amount of cells in the samples with lactulose after 7 and 14 days of storage was higher than in control by 11 - 20%.

Next group of experiments was carried out using starter for curd. The results of 1% lactulose effect on the viability of curd starter MST after 7 and 14 days of storage are presented in Figure 2.

Addition of lactulose does not have a significant effect on the viability of MST curd starter. The quantity of cells in the control and experimental samples was almost at the same level at all stages of the study. Similar conclusion was made in case of adding 3 and 5% of lactulose and using the starter for curd LAT CW.

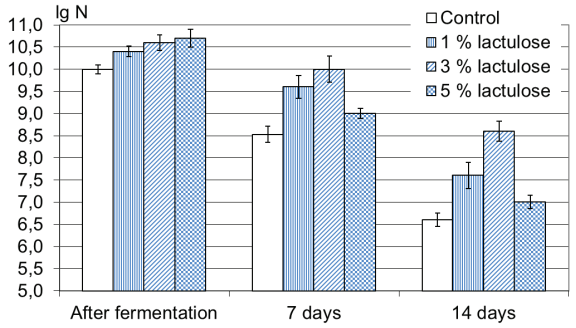

Figure 1.The effect of lactulose concentration on the viability of starter microflora for sour cream LAT CW L

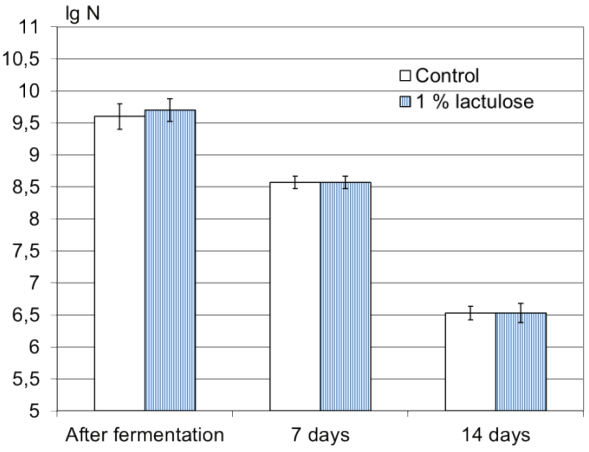

Figure 2. The effect of lactulose on the viability of the starter microflora for curd MST

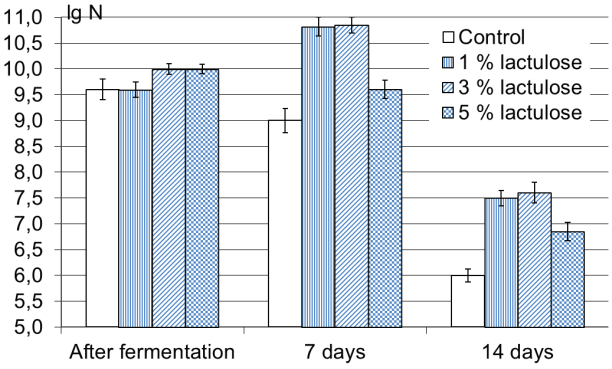

Figure 3. The effect of lactulose on the viability of the starter microflora for yogurt LAT BY

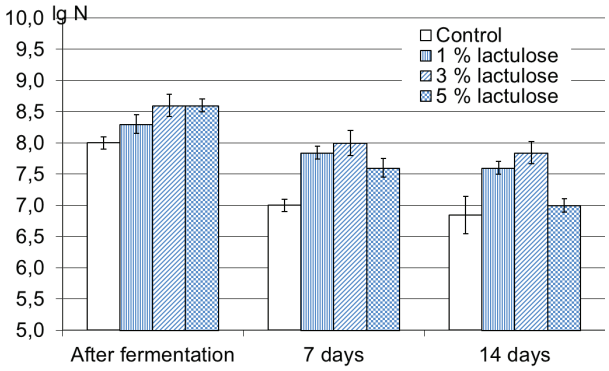

Figure 4. The effect of lactulose on the viability of *Lb. rhamnosus*

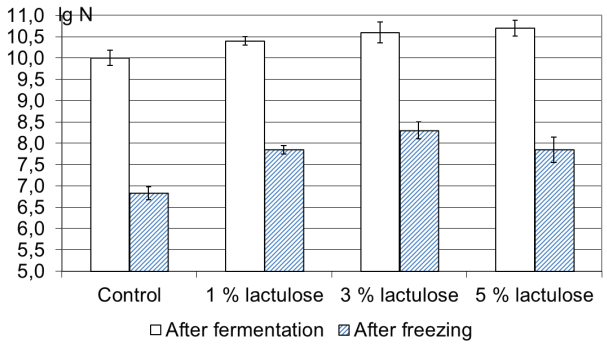

Figure 5. Lactulose effect on the viability of sour cream starter LAT CW L

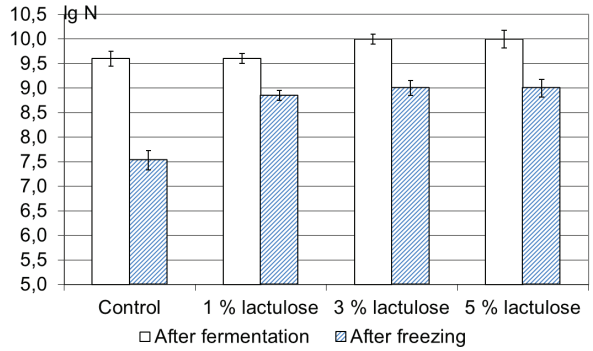

Figure 6. Lactulose effect on the viability of yogurt starter LAT BY

The same experiments were carried out using the starter for the yogurt LAT BY, which results are shown on Figure 3.

The quantity of cells in samples with 3 and 5% of lactulose after the fermentation of yogurt was insignificantly higher than in the control, but this difference was not exceeded 5%. During storage, the level of lactic acid microorganisms decreased in the all samples.

After 7 days, the quantity of microorganisms in samples with 1, 3 and 5% lactulose was higher than in the control by 20, 21 and 7%, respectively. After 14 day of storage, the amount of cells in the control significantly decreased up to 10^6 CFU/cm^3, but in samples with 1, 3 and 5% of lactulose, their quantity was higher by 25, 27 and 14% and remained at the level of (10^7 - 10^8) CFU/cm^3.

The results of lactulose influence on the viability of *Lb. rhamnosus* after 7 and 14 days of storage are presented on Figure 4.

It was established that the addition of lactulose had a positive effect on the development of *Lb. rhamnosus*, since the quantity of cells in the samples containing the prebiotic was higher after fermentation than in the control by 4 - 7.5%. After 7 days of storage, the highest level of *Lb. rhamnosus* was observed in samples with 1 and 3% lactulose and exceeded control by 12 and 14%, respectively.

After 14 days, the quantity of cells in the control and in the sample with the addition of 5% prebiotic was almost at the same level - 10^7 CFU/cm^3. The amount of microorganisms in samples with 1 and 3% of lactulose was at level 10^7 - 10^8 CFU/cm^3 and exceeded the same value of control by 11 and 15%. The most significant results were obtained in case of addition of 1 and 3% of prebiotic.

The results of lactulose effect on the viability of sour cream starter microflora (LAT CW L) under the conditions of freezing are shown on the Figure 5.

Addition of lactulose had a positive effect on maintaining the viability of the sour cream LAT CW L microorganisms under freezing conditions.

The lowest cell death was observed in the sample with 3% of lactulose. The quantity of microorganisms in this sample was higher than in the control by 22%. In samples containing 1 and 5% of prebiotic, the content of starter microflora cells was almost the same, but exceeded control by 15%.

In case of using starter for sour cream KDs the amount of cells in the samples with lactulose was higher

compared to the control by 10 - 24%. The best results were obtained when 1 and 3% of prebiotic was added.

These results confirmed the data obtained on viability of same starters in conditions of cold storage.

Positive effect of lactulose on viability of starters for curd (MST and LAT CW) in freezing conditions wasn't found, therefore, these starter cultures were not used in further studies.

Influence of lactulose concentrations on viability of yogurt starter LAT BY is shown on Figure 6. After freezing, the quantity of microorganisms in all samples with lactulose was almost at the same level. For example, in samples with 1 and 3% of prebiotic, the content of viable cells was higher than in the control by $\Delta lg = 1.32$ and $\Delta lg = 1.5$ CFU/cm^3 or 17.5 and 19.5%, respectively. The amount of microorganisms in samples with 3 and 5% of the prebiotic was the same.

It was established that addition of lactulose didn't effect on the viability of *Lb. rhamnosus* under freezing conditions. The quantity of cells in samples with prebiotic was higher than in the control, however, this result was a consequence of a higher level of microorganisms immediately after fermentation.

The results of experimental studies of lactulose effect on the starter's microflora viability in the conditions of freeze-drying were analyzed by the graph-analytical method. The surface and the isolines of its sections, which characterized the quantity of viable cells depending on the concentration of lactulose and the duration of storage, were designed.

The results of the lactulose effect on dried starter viability for sour cream LAT CW L during storage are presented on the Figure 7.

The presence of lactulose promoted to the increasing of the microorganisms viability. The amount of lactic acid microorganisms in samples with 1 - 5% of lactulose on the first stages of storage was higher compared to the control by $\Delta lgN = 1 - 3$ CFU/cm^3.

During storage time the quantity of starter microflora cells decreased in all samples. After 180 days of storage: in control - from 6 to 5 units, in the samples with 1% lactulose - from 7 to 6 units, with 3% - from 9 to 8 units. The same process occurred in a sample with 5% of lactulose less intensively, where the quantity of microorganisms decreased from 9 to 8.5 units. The quantity of microorganisms after 360 days of storage was: control - 10^5 CFU/cm^3, 1% lactulose - 10^6 CFU/cm^3, 3 and 5% of the prebiotic - 10^8 CFU/cm^3. At the final stage of storage (980 days), the amount of cells in the control was at the level of 10^3 CFU/cm^3, and in samples with 1, 3 and 5% lactulose - 10^4, 10^6, 10^6 CFU/cm^3, respectively.

Addition of lactulose had a positive effect on the viability of microorganisms of starter LAT CW L under freeze-drying conditions. This fact confirmed the data obtained during refrigerated storage and freezing. However, the addition of 1% prebiotic was insufficient to ensure a high level of viable cells during the storage of dried samples.

Positive effect of lactulose presence was also found for yogurt starter LAT BY. The quantity of viable cells in presence of lactulose decreased slowly, than in control samples. At the initial stage of storage (after 180 days) the value of microorganisms higher than 10^7 CFU/cm^3 was provided in samples with concentration of prebiotic from 1.45%.The addition of lactulose in the amount of 4 - 5% contributed to increase the quantity of starter cells to 8 - 8.2 units.

After 360 days of storage, the amount of microorganisms in samples with 1, 3 and 5% of prebiotic was higher than in the control (10^6 CFU/cm^3), and amounted 10^7 CFU/cm^3 and 10^8 CFU/cm^3. At the end of storage period the quantity of yogurt microorganisms was as follows: control - 10^4CFU/cm^3, 1, 3 and 5% of lactulose - 10^5 CFU/cm^3.

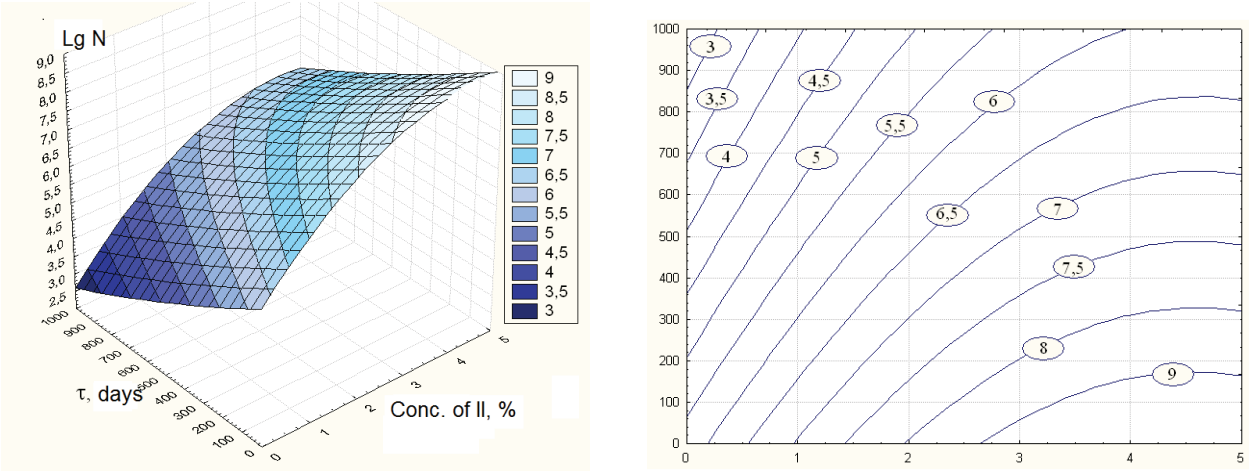

Figure 7. Dependence of the cells quantity (N, CFU/cm^3) in dried sour cream starter LAT CW L on storage time (τ, days) and concentration of lactulose (Conc. II, %): a) surface, b) isolines of its sections

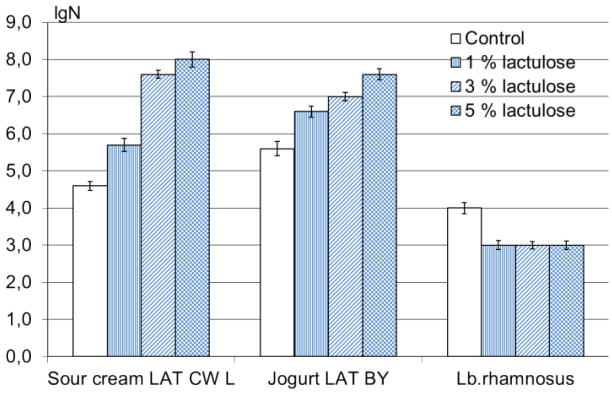

Figure 8. Effect of lactulose concentration on viability of different starters

Summarized results of lactulose effect on the viability of various starters microflora after 360 days of dried samples storage are presented in Figure 8.

After 360 days of storage, the quantity of cells in the samples with 3 and 5% of lactulose, fermented by sour cream starter was higher than in the samples, prepared using yogurt starter and probiotic culture. The lowest quantity of viable cells was observed in *Lb. rhamnosus* samples.

4. Conclusions

- The addition of lactulose contributed to the microflora viability enhancement of the starters for sour cream LAT CW L, KDs, yogurt LAT BY and probiotic culture *Lb.rhamnosus* during storage. The highest level of viable cells was obtained by adding 1% and 3% lactulose and using the LAT CW L starter. This positive effect of lactulose presence can be used in the technology of products with long shelf life.

- The positive effect of lactulose on the survival of the starter microflora for sour cream and yogurt in the conditions of freezing and freeze-drying had been established. The most effective results were obtained with the addition of 1 and 3% lactulose. The obtained results can be used in the development of technology of ice cream with functional properties.

- It was established that presence of lactulose didn't effect on the viability of *Lb. rhamnosus*, either during the freeze-drying process or during the storage of dry samples. It should be noted that the process of cell death in samples with prebiotic, proceeded faster than in the control.

5. References

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