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Kinetic and microbiological dependencies in the process of fermentation of goat milk, enriched with lactulose

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Abstract

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The obtaining of the fermented dairy product – kefir, based on goat milk and enriched with prebiotic component – lactulose is a quality addition to the existing range of dairy products with functional purpose. It is investigated the influence of lactulose at a concentration of 1%, 3% and 6% on the acidification rate in the fermentation of yogurt and the amount of microorganisms during storage. The results demonstrate that the addition of lactulose, regardless of its concentration, results in the activation process of the propagation of the microorganisms during the fermentation. It has been found that the addition of 3% lactulose provides optimal growth of the main groups of microorganisms during fermentation and more prolonged retention of their number during storage.

Keywords: goat milk; fermentation; kefir; lactulose; acidification rate

Introduction

In recent years, one of the most contemporary trends in the dairy market is the development of the segment so-called enriched foods (Litopoulou-Tzanetaki and Tzanetakis, 2014; Valchkov, 2016). The production of more and more new products enriched with useful elements is encouraged by an increase in the supporters of healthy eating. This stimulates the rapid growth of production and consumption of innovative, functional foods, which are seen as an integral part and a logical continuation of modern lifestyle (Nikolova et al., 2006; Metodieva and Doneva, 2010).

One of the most beneficial products for human health in the group of fermented lactic acid foods is kefir. It has all the beneficial properties of lactic acid beverages providing the needs of the body by calcium and at the same time it is a dietary lactic acid product, with high absorption, rich in many useful bacteria and suitable for all age groups (Roshtunkina, 2010). Enrichment of kefir with prebiotics increases the positive effects on the body. Prebiotics are chemically inert and do not change their properties at wide temperature and pH ranges as well as in contact with other ingredients. They exhibit high stability and remain unchanged in the human gastrointestinal tract, providing conditions and stimulating the development of beneficial microflora. Currently lactulose is considered as one of the most promising substances that stimulates the development of bifidobacteria and lactic acid bacteria (Oliveira et al., 2011).

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 (Olano and Corzo, 2009). Enriching dairy products with lactulose not only does not change their character but also increases their therapeutic and prophylactic properties:

- stimulates the growth of bifido- and lactobacteria in the colon of the human organism;
- inhibits the development and activity of harmful bacteria and ferments;
- normalizes digestion and stimulates the peristalsis of the stomach;

• improves the body's absorption of vitamins and calcium, therefore has a favorable effect on the condition of the circulatory, immune and the locomotor system;

• prevents postoperative complications, protects against intestinal infections, activates the local immunity of the organism, etc.

The purpose of the present study is to investigate the effect of different concentrations (1%, 3% and 6%) of lactulose on the duration of the process of fermentation of goat milk kefir and the amount of microorganisms during its storage.

Materials and Methods

Technological approach

The studies were conducted with goat milk, pasteurized at 85°C for 10 minutes in advance. The heat-treated milk was cooled in an ice bath, transferred under sterile conditions into flasks and stored at 4°C for 24 hours. Natural starter culture of kefir grains is activated in sterile skim milk at 25°C for 24 hours, filtered to remove the coagulated milk and was washed with sterile water. Activated kefir grains were inoculated in pasteurized goat milk and incubated statically at 25°C for 24 hours to obtain a fermented product (kefir). Prior to fermentation, lactulose syrup was added to the milk at concentrations 1%, 3% and 6% to produce experimental variants with different concentrations of prebiotic. The fermentation process is carried out at set temperature range of 22-24°C of up to 24 hours, taking into account the active and titratable acidity of the samples at every 3 hours.

Microbiological approach

Determination of total number of microorganisms from different bacterial groups. A differentiated study was performed for each of the bacteria and yeast groups involved in the mixed starter culture. For the preparation of microbiological examination of samples 1% tryptone water was used according to the method of surface plating of microorganisms.

Cultivation of *Lactobacillus* was performed on MRS medium (pH 6.5 ± 0.2) at incubation temperature of 42-45°C for 48-72 hours, 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 M17 medium (pH 7.2 ± 0.2) at an incubation temperature of 37°C for 48 hours. Yeasts were cultured on Sabouraud medium (pH 7.0 ± 0.2) at 28-30°C for 24-48 hours. The results were statistically processed using MS Office Excel 2007.

Results and Discussion

Table 1 shows data from active and titratable acidity traceability in experimental variants.

The results of the conducted experiments show that the fermentation of all samples was completed by reaching the optimum pH (4.5) and titratable acidity (90÷100) by the 22th hour.

Kinetic parameters

In the course of the experiments, the acidity rate (Vmax) in milk in the four variants – control and variants with 1%, 3% and 6% lactulose was calculated. It is a ratio of variations in pH values over a certain time interval (dpH/dt), expressed as 10^{-3} pH units/min. The data on the rate of acidification of goat milk during fermentation in the control and in the presence of 1%, 3% and 6% lactulose are presented in Fig. 1.

For samples with 3% and 6% lactulose, the process is completed at 21st hour. Generally, the addition of lactulose, irrespective of its concentration, does not significantly affect the time of the fermentation process.

Table 1. Change in the values of the active and titratable acidity of kefir fermentation with added 1%, 3% and 6% lactulose

Hour	Active acidity (pH)				Titratable acidity (°T)			
	Control	1% lactulose	3% lactulose	6% lactulose	Control	1% lactulose	3% lactulose	6% lactulose
0	7,01 ± 0,01	7,00 ± 0,02	6,99 ± 0,01	7,00 ± 0,01	16 ± 0,01	17 ± 0,01	18 ± 0,01	18 ± 0,01
3	6,94 ± 0,02	6,90 ± 0,01	6,91 ± 0,00	6,91 ± 0,02	16 ± 0,04	18 ± 0,05	20 ± 0,02	20 ± 0,03
6	6,85 ± 0,01	6,78 ± 0,02	6,73 ± 0,01	6,63 ± 0,04	18 ± 0,01	19 ± 0,01	20 ± 0,03	22 ± 0,02
9	6,64 ± 0,01	6,55 ± 0,01	6,45 ± 0,02	6,29 ± 0,05	22 ± 0,01	23 ± 0,04	25 ± 0,01	28 ± 0,06
12	6,40 ± 0,03	6,28 ± 0,01	6,09 ± 0,01	5,89 ± 0,01	30 ± 0,03	32 ± 0,01	34 ± 0,01	38 ± 0,01
15	6,11 ± 0,02	5,99 ± 0,03	5,65 ± 0,01	5,41 ± 0,01	41 ± 0,01	44 ± 0,01	46 ± 0,05	54 ± 0,01
18	5,78 ± 0,01	5,64 ± 0,01	5,10 ± 0,02	4,89 ± 0,02	68 ± 0,05	68 ± 0,01	70 ± 0,01	78 ± 0,04
21	5,40 ± 0,01	5,24 ± 0,01	4,50 ± 0,01	4,50 ± 0,01	82 ± 0,01	84 ± 0,03	92 ± 0,01	96 ± 0,01
22	4,50 ± 0,02	4,50 ± 0,04			92 ± 0,01	93 ± 0,01	94 ± 0,02	98 ± 0,01
24	4,40 ± 0,01	4,36 ± 0,01			96 ± 0,00	98 ± 0,02	100 ± 0,01	102 ± 0,01

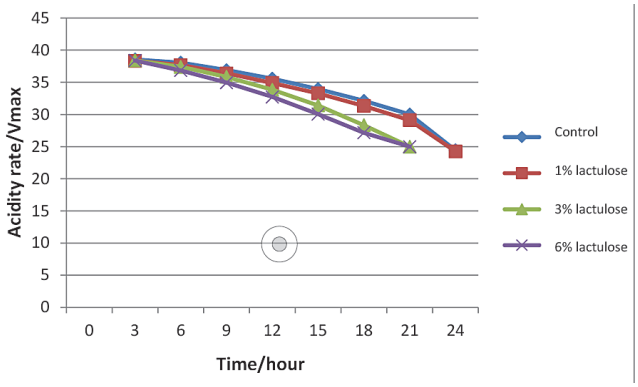

Fig. 1. Acidification rate of goat milk during fermentation – control and variants with lactulose

Microbiological studies

Analyzes are performed immediately after the end of the fermentation process and at every 7 days from the start of storage up to the 21st day. The aim is tracking and determining the survivability of lactic acid bacteria and yeast in kefir in different variations and influence of different concentrations of lactulose on their development during storage.

The results are presented graphically in Fig. № 2, 3, 4 and 5.

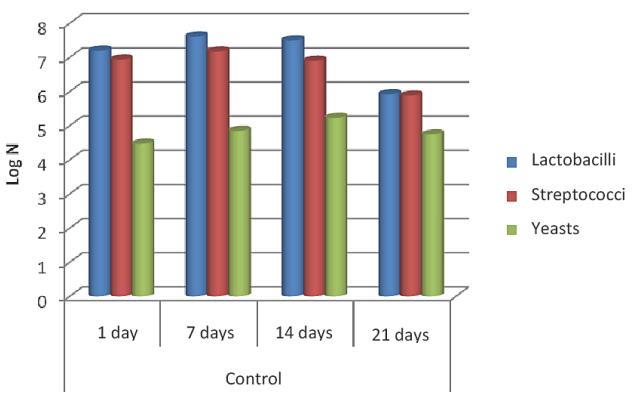

Fig. 2. Survival of microbial groups in kefir of goat milk without lactulose

The addition of 1% lactulose slightly affects the growth of microbial groups (Fig. 1 and 2). A more significant difference is found between the 14th and 21st day of storage, with the control group decreasing the rate of propagation of the microorganisms after 7 days, and in the 1% lactulose variant this effect occurs after the 14th day and continues to the end of the fermentation. In the three microbial groups the survival was higher than the control and ranged from 1 log N in lactobacilli to 0.58 log N and 0.43 log N respectively in lactococci and yeast.

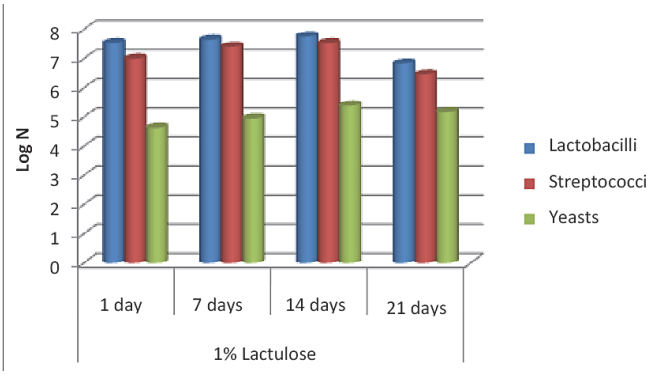

Fig. 3. Survival of microbial groups in kefir of goat milk with 1% lactulose

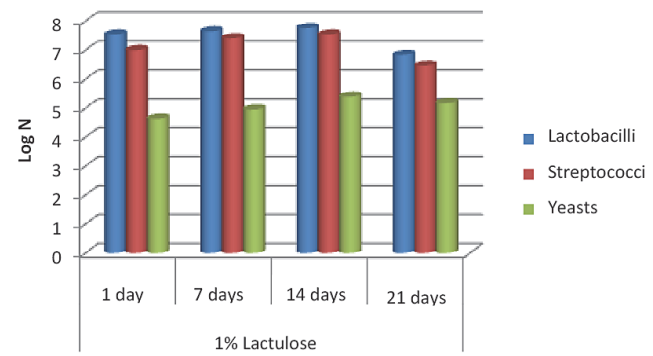

Fig. 4. Survivability of microbial groups in kefir from goat milk with 3% lactulose

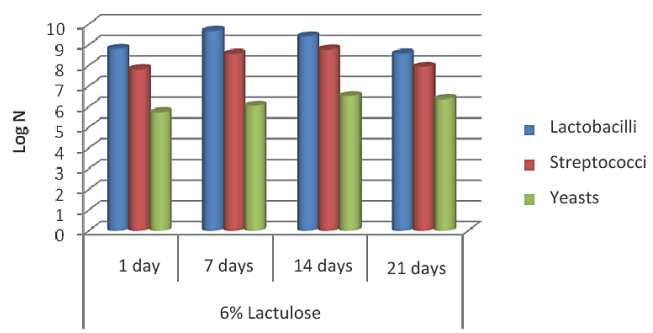

Fig. 5. Survivability of microbial groups in kefir from goat milk with 6% lactulose

In the variants with added 3% and 6% lactulose the difference in the number of microorganisms compared to the control was more pronounced (Fig. 4 and 5). The tendency to decrease the activity of microorganisms after 14 days of storage is maintained. In the *Lactobacillus* group at the end of the storage period (21 days), the difference between the

control and the variant with 3% lactulose was 2 log, and in the variant with 6% lactulose – 2.64 log. Similar results are obtained for *Streptococcus* – the difference with the control group is 1.8 log (3% lactulose) and 2 log (6% lactulose). In yeast, at day 21 of storage, we recorded an increase of 1.4 and 1.6 log, respectively, in both concentrations compared to the control.

Conclusions

The addition of the prebiotic component – lactulose at the beginning of the fermentation of kefir, irrespective of its concentration, leads to activation of the process of propagation of microorganisms during fermentation and longer and qualitative preservation of their number during storage.

In all three variant samples of lactulose there is an increase in survival of microorganisms up to the 14th day and a slight decrease to the 21st day of storage whereas in the control sample the number of microorganisms after the 7th day of storage begins to decrease. Therefore, prebiotic kefir has a longer shelf life (about 7 days) and a higher quality than Kefir without lactulose.

The difference in microflora survivability between samples with 3% and 6% lactulose is not significant and it is inappropriate and economically unprofitable to use higher prebiotic concentrations. The addition of 3% lactulose syrup can be considered as an optimal dose that best affects the major groups of microorganisms both during fermentation and during storage.

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