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CHARACTERIZATION OF FERMENTED MILKS AFTER THE PASSAGING PROCESS OF STARTER CULTURES®

Charakterystyka mlek fermentowanych po procesie pasażowania kultur starterowych®

Key words: yoghurt, probiotic, passage, lactose, bacterial viability.

The aim of this study presented in the article was an investigation the influence of the passaging process of starter cultures on selected properties of fermented milks. The study involved fermentation of cow's milk with three starter cultures containing bacteria from the genera Lactobacillus, Streptococcus, and Bifidobacterium. The obtained fermented milk samples were used as starters to perform another round of fermentation and fermentation after 3 days of refrigerated storage of the samples. The pH, number of bacterial cells, and sugar profile of the fermented milk were then determined. The results showed that passage is an important factor determining the dynamics of the lactic acid fermentation process. The passage process significantly influenced the number of bacterial cells in milk. It was also observed that after the first and second passages, the fermented milk samples showed lower lactose content. The present study provides useful references on the metabolism of lactic acid bacteria and bifidobacteria in fermented milks.

Słowa kluczowe: jogurt, probiotyk, pasaż, laktoza, żywotność bakteryjna.

Celem pracy zaprezentowanej w artykule było zbadanie wpływu procesu pasażowania kultur starterowych na wybrane właściwości mlek fermentowanych. Badania obejmowały fermentację mleka krowiego trzema kulturami starterowymi zawierającymi bakterie z rodzajów Lactobacillus, Streptococcus i Bifidobacterium. Otrzymane próbki mleka fermentowanego posłużyły jako startery do przeprowadzenia kolejnej rundy fermentacji i fermentacji po 3 dniach przechowywania próbek w warunkach chłodniczych. Następnie określono pH, liczbę komórek bakteryjnych i profil cukru w próbkach mleka fermentowanego. Wyniki wykazały, że pasaż jest ważnym czynnikiem determinującym dynamikę procesu fermentacji kwasu mlekowego. Proces pasażowania istotnie wpłynął na liczbę komórek bakteryjnych w mleku. Zaobserwowano również, że po pierwszym i drugim pasażu próbki mleka fermentowanego wykazywały niższą zawartość laktozy. Niniejsze badanie dostarcza użytecznych informacji na temat metabolizmu bakterii kwasu mlekowego i bifidobakterii w mleku fermentowanym.

INTRODUCTION

According to the definition of the Food and Agriculture Organization (FAO), the World Health Organization (WHO), and the International Dairy Federation (IDF/FIL), fermented dairy beverages are products made of whole milk, partially skimmed, completely fat-free, concentrated, or regenerated milk powders fermented by specific microorganisms. Microflora ferment lactose present in milk and causes its coagulation, leading to a reduction in pH value. In the final product, these microorganisms must remain viable, active, and abundant until the last day of use [1,3]. Milk beverages are classified into nonfermented and fermented milk products. Nonfermented beverages include pasteurized food milk, sterilized food milk, ultra-high temperature (UHT)-sterilized food milk, milk with added flavors or nutrients. Fermented milk beverages include products such as yogurt, kefir, curdled milk, and new generation dairy products. The criterion to differentiate fermented milk beverages is based on the composition of the beneficial microflora added to them [5,6]. Only products that meet the requirements of the appropriate composition of the microflora and its abundance may be designated as yogurt, acidophilic milk, fermented milk, kefir, and koumiss [1].

According to the guidelines of the FAO/WHO Codex Alimentarius standard, the number of viable lactic acid bacteria (LAB) cells contained in yoghurts should be at

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least 10^7 CFU/g until the last day of the product's shelf-life in terms of technical microflora [1]. The presence of live starter cultures (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*) in yoghurts throughout their declared shelf-life is one of the factors that determine their dietary, preventive, and therapeutic values [8]. Fermented foods have been consumed by humans since several centuries. They are minimally processed and acquire higher health values through the fermentation process. Furthermore, the probiotic bacterial strains have beneficial effects on human health by influencing the regulation of the functions of the intestinal microbiota [2]. They help in faster recovery from previous intestinal infections and enhance immunity. Moreover, their anticancer and antiallergenic activity has been demonstrated. In recent years, because of increased consumer awareness related to healthy eating, there has been a rapid growth in the consumption of this type of food. The fermented food market has been expanding with the introduction of new products. Furthermore, considering all the benefits of fermented foods for the functioning of the digestive system, it has been proposed that they should be included in dietary recommendations [4].

Probiotic is defined as a product or preparation containing live, suitable microorganisms in the required number that alter the microflora (by colonization or implantation) in the host's intestine and consequently have a beneficial effect on the host's health. These microorganisms are mainly LAB that can inhabit various environments, including the human body [7]. The term probiotic is reserved for products or preparations that contain live microbial cells; they improve the health of humans and animals and have a beneficial effect on the digestive tract, the oral cavity, and the genitourinary tract [11]. According to the probiotic criterion of FAO/WHO, the number of viable probiotic microbial cells in fermented milk beverages should be at least 10^6 CFU/g. This condition must be met throughout the product's shelf-life and is referred to as the therapeutic minimum [12].

Therefore, the present study aimed to investigate the role of the passage process of starter cultures on fermented milks and to gain a better understanding of which characteristics of beverages will change and how. To achieve these objectives, we conducted fermentation of milks under different conditions using one type of Zakwaska. During the fermentation process, we monitored the pH value, the number of microbial cells of the culture, and the profile of sugars in the fermented milks. We also assessed these features after the fermentation process when the beverages were refrigerated for 3 days.

MATERIALS AND METHODS

The following three industrial bacterial starters were used in this study: (1) ACIDOLAKT (Vivo, Ontario, Canada) containing lactose-fermenting, sucrose-fermenting, freeze-dried strains of *Bifidobacterium lactis* (2 strains), *Bifidobacterium longum, Bifidobacterium bifidum, Bifidobacterium breve, Bifidobacterium infantis, Lactobacillus bulgaricus, Lactobacillus acidophilus,* and *Streptococcus thermophilus*; (2) BIFIVIT (Vivo, Ontario, Canada) containing lactose-fermenting, sucrose-fermenting, freeze-dried strains of *Bifidobacterium lactis* (2 strains), *Bifidobacterium longum, Bifidobacterium bifidum, Bifidobacterium breve, Bifidobacterium infantis, Lactobacillus bulgaricus, Lactobacillus acidophilus,* and *Streptococcus thermophilus*; and (3) PROBIO JOGURT (Vivo, Ontario, Canada) containing lactose-fermenting, sucrose-fermenting, freeze-dried strains of *Streptococcus thermophilus, Lactobacillus bulgaricus, Lactobacillus acidophilus, Bifidobacterium lactis* (2 strains), *Bifidobacterium longum, Bifidobacterium bifidum, Bifidobacterium breve, Bifidobacterium infantis, Lactobacillus casei, Lactobacillus rhamnosus, Lactobacillus lactis, Lactobacillus gasseri, Lactobacillus brevis, Lactobacillus salivarius, Lactobacillus paracasei, Lactobacillus plantarum,* and *Propionibacterium freudenreichii*.

Ringer's solution (tablets) was purchased from Merck Millipore (Darmstadt, Germany). BSM supplement, clindamycin hydrochloride, ciprofloxacin hydrochloride, HPLC grade methanol, HPLC purity standard for glucose, HPLC grade galactose standard, HPLC purity standard for sucrose, and HPLC purity standard for lactose were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile for HPLC was purchased from POCH (Gliwice, Poland).

Fermentation process

The contents of the vial with the Zakwaska culture were diluted in 20 mLof UHT milk with 3.2% fat content and left for several minutes at room temperature to hydrate the bacterial cells. Next, 150 g of UHT milk with 3.2% fat content were weighed into the prepared 4 sterile Schott bottles, and the bottles were marked as follows: A, B, C, and D. The milk portion was inoculated with a starter.

- 3 mL starter cultures into samples A and B, and
- 1 mL starter cultures into samples C and D

The prepared milk samples were fermented for 5 h at the following temperatures:

- samples A and C at 37°C
- samples B and D at 45°C.

After the end of fermentation, the fermented milk samples were placed in a refrigerator at 6°C for 3 days. After this time, the trials were used as starters for the fermentation of subsequent portions (at 5% dose) of UHT milk. Schott bottles with milk portions were re-labeled with A, B, C, and D as follows:

- for the new trial A, the starter was trial A of fermented milk,
- for the new trial B, the starter was trial B of fermented milk,
- for the new trial C, the starter was trial C of fermented milk,
- for the new trial D, the starter was trial D of fermented milk.

The parameters of milk fermentation did not change. The fermentation lasted for 5 h. After the completion of fermentation, the Schott bottles with milk portions were placed in the refrigerator at 6°C for 3 days. After this time, the trials were again used as starters for the fermentation of further new portions (at 5% dose) of UHT milk in the same way as that for the first time. The parameters of milk fermentation were kept the same as those for the first time. After the end of this

fermentation step, the passaging and fermentation cycle of the fermented milk samples was completed, and the samples were kept in the refrigerator at 6°C for 3 days.

Determination of the pH value

The pH was measured using a pH meter (CPO-505 model, Elmetron, Zabrze, Poland). The measurement was conducted during the fermentation, passage, and refrigerated storage of the milk samples. Samples for measurement were collected during fermentation (time: 0, 1, 2, 3, 4, and 5 h) and after 3 days of refrigerated storage. Each measurement was performed twice.

Microbiological analysis

The microbiological analysis was performed during fermentation, passaging, and after the fermentation process during refrigerated storage. Five microbiological media were used to conduct the analyses: MRS agar (de Man, Rogosa, and Sharpe agar, a selective medium for the isolation of LAB) (Merck, Darmstadt, Germany) to count lactobacilli cells, BSM agar (Bifidus Selective Medium Agar, Sigma-Aldrich, St. Louis, MO, USA) to count bifidobacteria cells, M17 agar (Merck, Darmstadt, Germany) to count *S. thermophilus* cells, *Propionibacterium* Medium to count *Propionibacterium spp.*, and MRS-CC (de Man, Rogosa, and Sharpe medium with clindamycin and ciprofloxacin addition) to count *Lactobacillus acidophilus*. Microbiological analysis was performed by the traditional plate method. MRS, MRS-CC, BSM, and M17 media were incubated at 37°C for 48 h. *Propionibacterium* medium was incubated at 37°C for 5 days. The Petri dishes with the MRS, MRS-CC, BSM, and *Propionibacterium* medium were placed in anaerobic jars. The results thus obtained are expressed as mean values of colony-forming unit per milliliter of beverage (CFU/mL) in two parallel replicates.

Preparation of Extracts for Carbohydrate Analysis

First, carbohydrate extraction was performed. For this purpose, 8.0 g of the milk sample and 32.0 g of methanol (HPLC grade, >99.9%, Sigma-Aldrich, St. Louis, MO, USA) were measured in a falcon tube, and the contents of the tube were mixed intensively. The tube was then placed in an ultrasonic bath for 30 min (at 30°C). The product thus obtained was then centrifuged in a laboratory centrifuge (MPW-350R, Irmeco, Bielsko-Biała, Poland) at 3000 × *g* for 30 min at 4°C. After evaporating the solvent and concentrating the samples, the contents of the test tubes were mixed intensively and filtered into chromatographic vials through a syringe filter with 0.45 μm pore size.

Chromatographic Analysis of Carbohydrates

The carbohydrates were subjected to chromatographic analysis on an HPLC device consisting of a DeltaChrom Pump Injector (S6020 Needle Injection Valve, Sykam, Fürstenfeldbruck, Germany), a DeltaChrom Temperature Control Unit (Sykam), a refractive index detector (S3580 RI Detector, Sykam, Eresing, Germany), a precolumn Guard Column Sugar-D (10 mm × 4.6 mm, 5 μm; Cosmosil, Nacalai Tesque, Kyoto, Japan), and a column Sugar-D (250 mm × 4.6 mm, 5 μm; Cosmosil, Kyoto, Japan). The mobile phase contained acetonitrile (HPLC grade, >99.9%) and ultrapure distilled water in a weight ratio of 60:40. From the chromatographic vials, 40 μL of the solution was withdrawn using a laboratory microsyringe. The operating parameters of the HPLC device were as follows: flow 1 mL/min, column temperature 30°C; RI detector settings: range 10000 mV, and sample rate 2 Hz. Each sample was analyzed for 20 min. Carbohydrates were identified, and their concentration in the tested samples was calculated based on a comparison of the obtained results with the results obtained for external standards. Relevant external standards of glucose, galactose, sucrose, and lactose (Avantor Performance Materials Poland S.A., Gliwice, Poland) were determined by analyzing the samples. Each measurement was performed twice.

Statistical Analysis

The results were statistically analyzed in Statgraphics XVII software (Statgraphics Technologies, Inc., The Plains, Virginia, USA). A two-way analysis of variance was performed. Tukey's test was used for detailed analysis and evaluation of differences between mean values. Homogeneous groups were designated. Testing was performed at the significance level of $p = 0.05$.

RESULTS AND DISCUSSION

pH values during fermentation

The smallest reduction in pH during the fermentation process was observed for the samples of milk from 1 batch, both for ACIDOLAKT (Figure 1) and PROBIO JOGURT (Figure 3). The pH values for the 2 and 3 settings were remarkably similar for both ACIDOLAKT and PROBIO JOGURT. The most dynamic fermentation process was demonstrated by trials B and D in all types of Zakwaska achieving the lowest pH of approximately 4.5. Trials A and C reached higher pH values, which may be due to lower set point temperature. The changes in the pH value were significantly dependent on the time of the fermentation process, the passage number, and the setting variant. Settings 2 and 3 showed the lowest pH values, which may be due to the presence of active, multiplying microflora that could perform metabolic processes faster than lyophilized cultures. The results obtained in another study showed that the yogurt samples after 5 weeks of refrigerated storage exhibited lower pH values [9]. Similar results were obtained by Nighswonger et al. [9]. The passaging process was not carried out in the abovementioned studies; however, yoghurts exhibited lower pH values after longer storage, which shows analogy to our present study. Settings 2 and 3 were subjected to a longer refrigeration period [15].

Bacterial population

The milk samples after fermentation with ACIDOLAKT ZAKWASKA (Figure 4) showed a greater number of cells of *Lactobacillus*. For 1 setpoint in each variant of the trial, the initial number of cells was lower than that in sets II and III and was 6.5 log (CFU/mL) for trials A, B, and C and 6.0 log (CFU/mL) for trial D. The final cell count after the fermentation process was similar in all variants of the trials and was 8.5-9 log (CFU/mL). This indicates that the type of starter does not significantly affect the number of bacterial cells from the *Lactobacillus* genus, which was confirmed by statistical analysis. The number of *S. thermophilus* cells (Figure 4) was

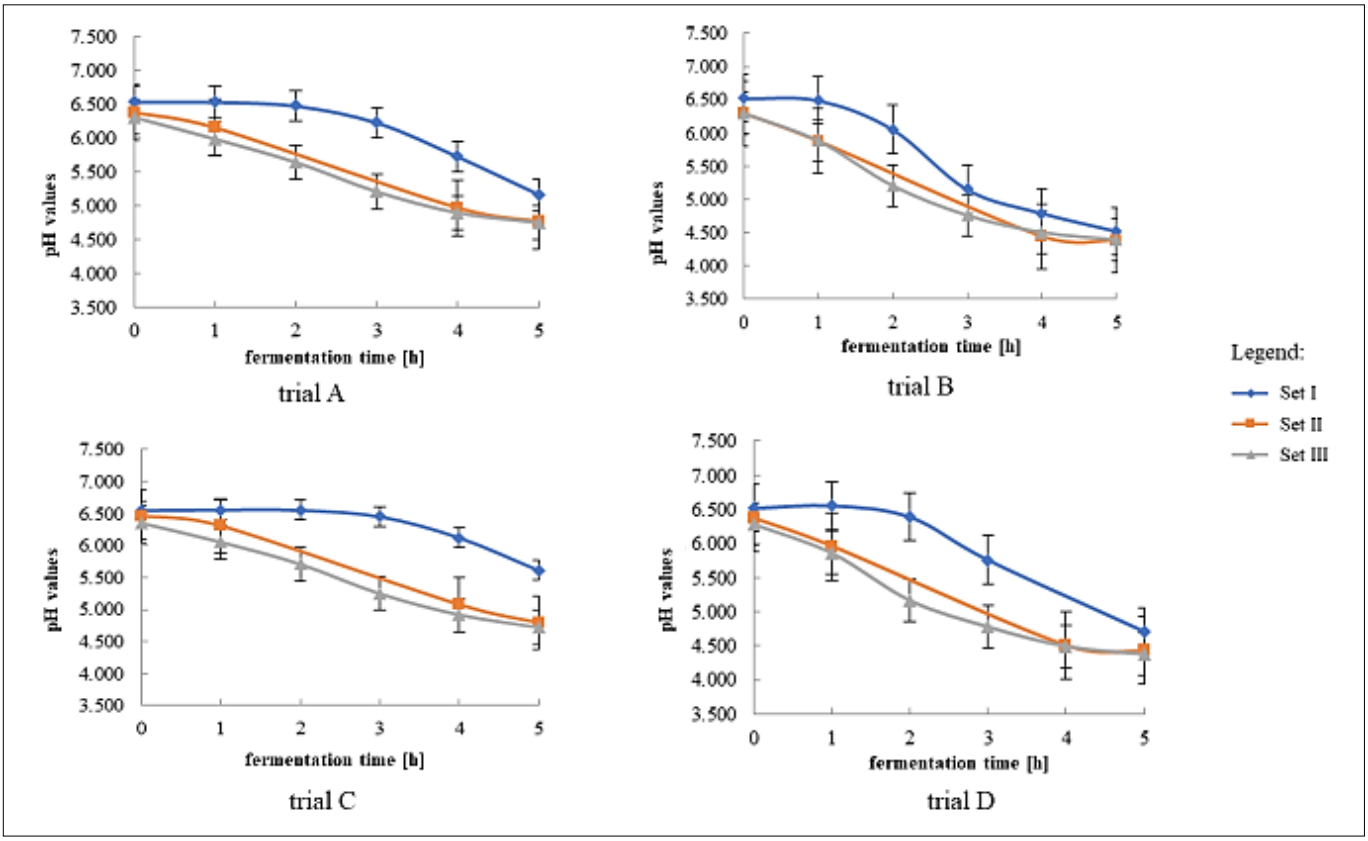

Fig. 1. Changes in the pH value (mean values and standard deviations)- ACIDOLAKT.
Rys. 1. Zmiany wartości pH (wartości średnie i odchylenia standardowe) – ACIDOLAKT.
Source: The own study
Źródło: Badania własne

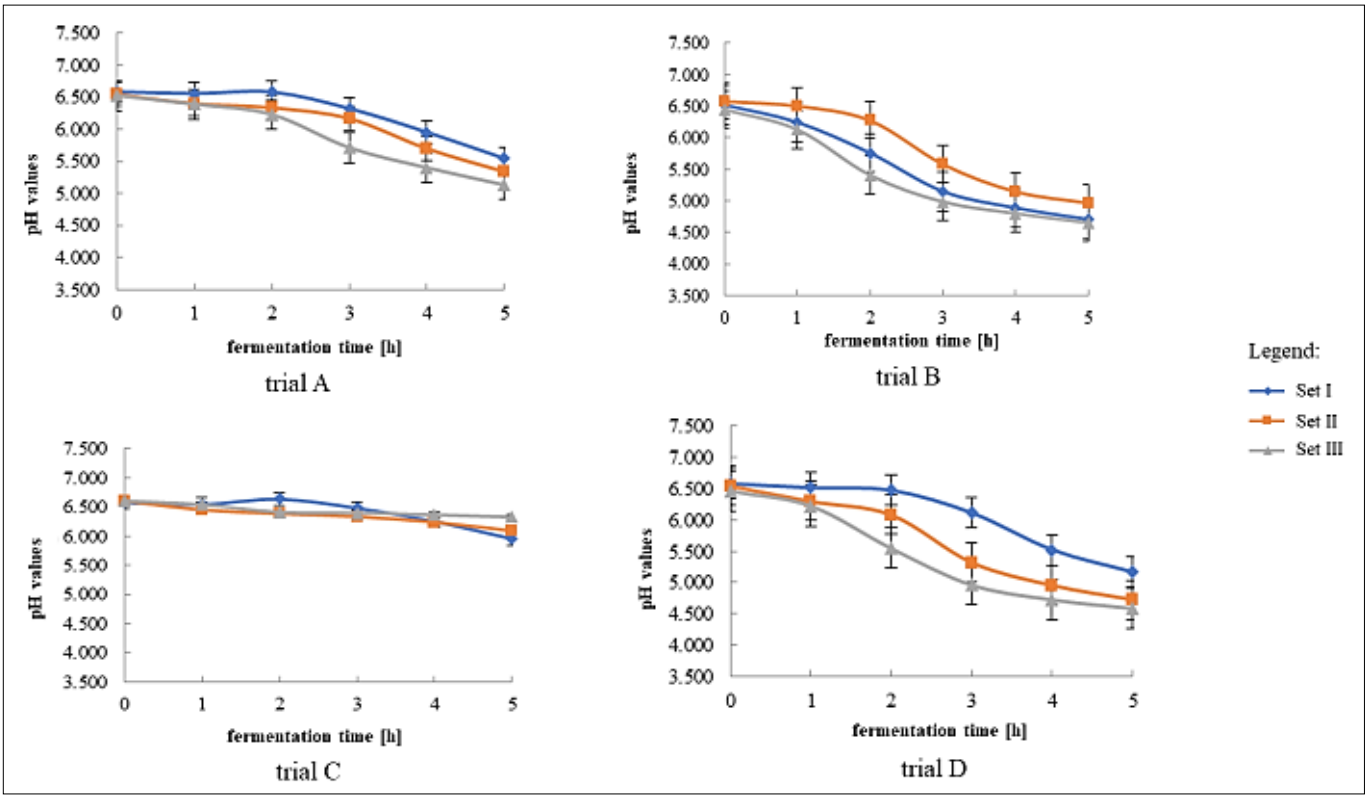

Fig. 2. Changes in the pH value (mean values and standard deviations)- BIFIVIT.
Rys. 2. Zmiany wartości pH (wartości średnie i odchylenia standardowe)- BIFIVIT.
Source: The own study
Źródło: Badania własne

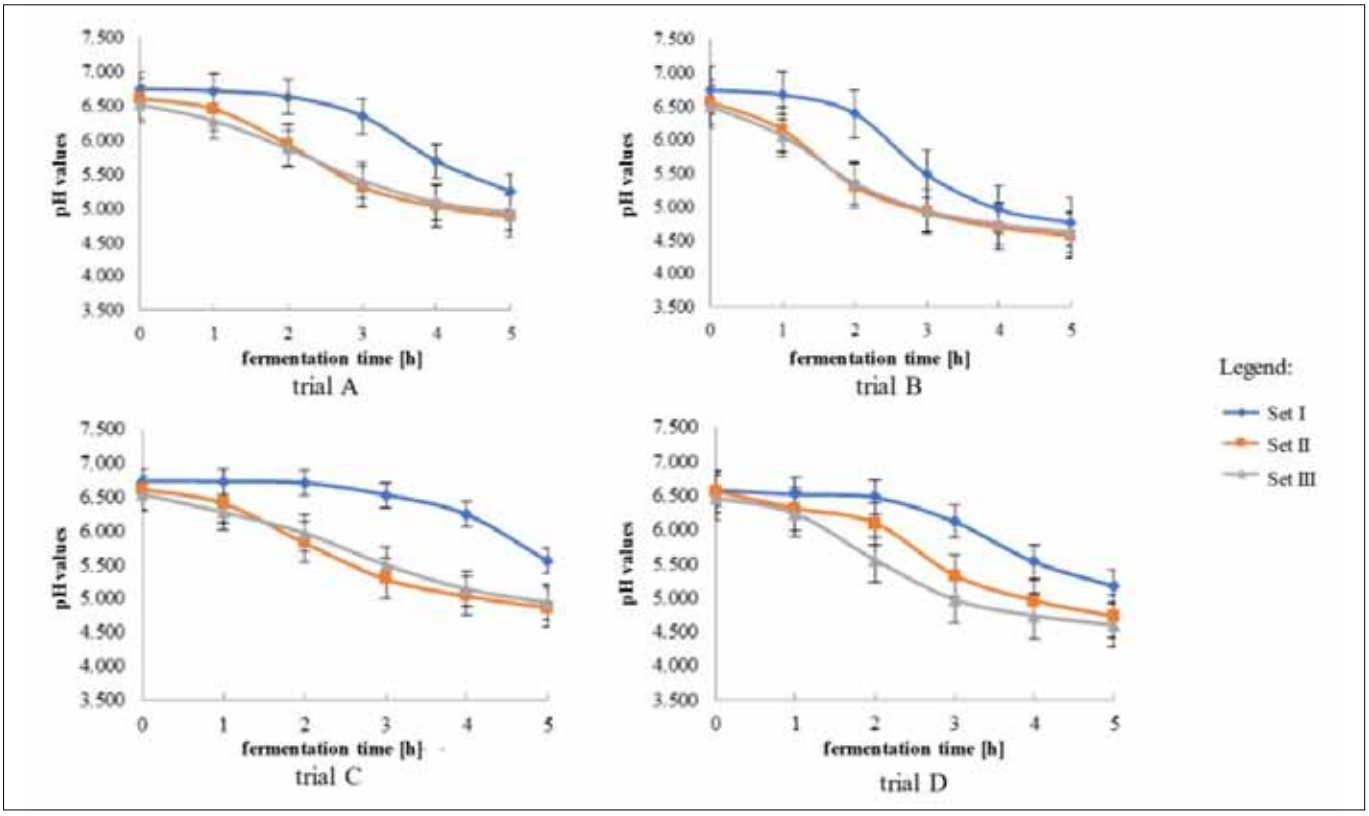

Fig. 3. Changes in the pH value (mean values and standard deviations) – PROBIO JOGURT.
Rys. 3. Zmiany wartości pH (wartości średnie i odchylenia standardowe) – PROBIO JOGURT.

Source: The own study

Źródło: Badania własne

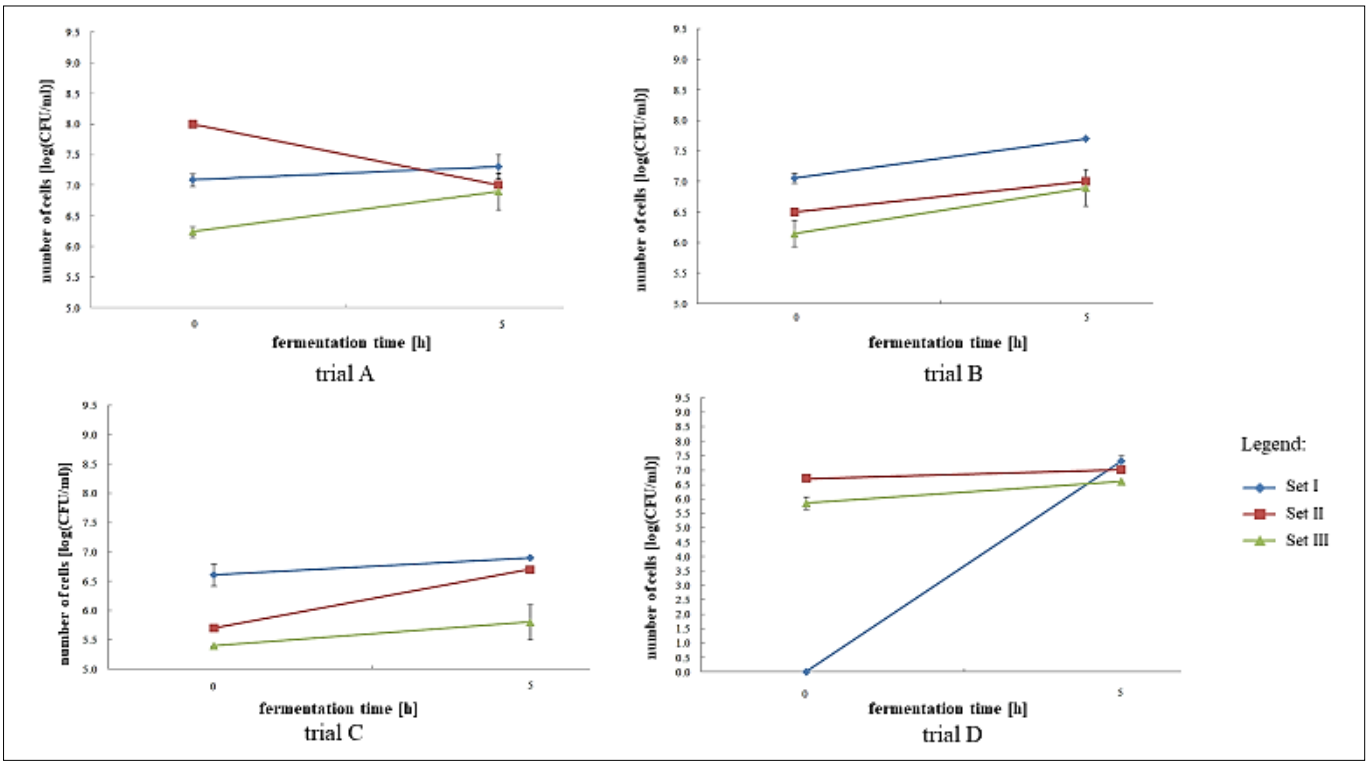

Fig. 4. Changes in the number of *Streptococcus thermophilus* cells in milk fermented with ACIDOLAKT ZAKWASKA (mean values and standard deviations).
Rys. 4. Zmiany liczby komórek *Streptococcus thermophilus* w mleku fermentowanym kulturą starterową ACIDOLAKT ZAKWASKA (wartości średnie i odchylenia standardowe).

Source: The own study

Źródło: Badania własne

remarkably similar, except for trials A and C of set II; this finding suggests that bacterial cells die due to competition for nutrients and the production of metabolites. The final bacterial cell count of the remaining trial variants ranged from 8.0 to 9.0 log (CFU/mL). Thus, the passage number has a statistically significant effect on the number of cells.

For *Lactobacillus acidophilus*, the highest number of cells after fermentation was found in set I and amounted to 7.7 log (CFU/mL) for trial A, 7.5 log (CFU/mL) for trial B, and 7.0 log (CFU/mL) for trial C. The lowest number of cells was found in trials after 2 passages. There was little or no increase in the number of cells of the genus *Bifidobacterium*. Changes in the number of bacterial cells depended significantly on the setting variant, but not on the passage number.

The increase in the number of bacterial cells of the *Bifidobacterium* genus after fermentation in all samples within each setting was exceedingly small or was not observed.

It was noted that for each sample of milk fermented with BIFIVIT ZAKWASKA, the milk after fermentation from set III showed the highest number of *Lactobacillus* spp. cells, and it was approximately 8.0 log (CFU/mL) in samples A, B, and D and approximately 7.5 log (CFU/mL) in sample C. This was probably because after the second passage, bacterial cells showed high metabolic activity, and therefore, it was easier for them to multiply quickly. The fermented milk from sets I and II of all samples showed a similar content of *Lactobacillus* spp. cells, and this value was from approximately 6.0 to 6.5 log (CFU/mL).

In all milk samples in almost all settings, immediately after the addition of the starter, the number of *L. acidophilus* cells was lower than that after the fermentation process. The observed phenomenon occurred because the bacteria grew and multiplied during fermentation, and therefore, their increased population was observed after the end of the process. For trial C, after the second passage, a reduction in the population of *L. acidophilus* cells was observed after the fermentation. In samples B and D, the lowest number of cells after fermentation was found in samples after the second passage, and this value was approximately 5.7 log (CFU/mL). There was no trend indicating an association of cell population and passage across all samples, which was confirmed by statistical analysis.

The samples from the first set had the lowest number of bacterial cells of the genus *Bifidobacterium* (Figure 5) in fermented milk in all variants. The lowest number of cells was recorded in sample D, and it was approximately 5.6 log (CFU/mL). The fermented milk samples B and D after the second passage and trial B after the first passage showed the highest number of cells of the genus *Bifidobacterium* in all variants, and the value was approximately 8.5 log (CFU/mL). After fermentation in trial A, the highest value was found in milk after the first passage, i.e., approximately 8.3 log (CFU/mL), and in trial C, the highest value was found in milk after the first and second passages, i.e., approximately 7.2 log (CFU/mL). Therefore, it can be concluded that the incubation temperature and the dose of the set primer affected the number of *Bifidobacterium* cells in the final product, which was confirmed by statistical analysis.

The A sample of fermented milk after the second passage showed the highest number of *S. thermophilus* cells among all variants, and the value was approximately 9.0 log (CFU/mL). The lowest number of bacterial cells in fermented milk was recorded in the third setting of test C, with a value of approximately 7.7 log (CFU/mL). No relationship was observed between the cell population and the passage number in all samples, which was confirmed by statistical analysis.

For PROBIO JOGURT, the highest number of *L. acidophilus* cells after fermentation was found in sample B after the second passage, and the value was approximately 8.0 log (CFU/mL); the lowest number of cells was observed in sample A after the first passage and amounted to approximately 6 log (CFU/mL). It was also noted that in variants C and D, all samples after the fermentation process achieved a similar population of *L. acidophilus* cells, and the values ranged from approximately 6.0 to 7.0 log (CFU/mL).

Thus, it can be concluded that the largest number of *Lactobacillus* spp. cells after fermentation was contained in sample B after the second passage, and it was approximately 8.3 log (CFU/mL); the smallest number of cells was noted in sample A after the first passage and was approximately 5.7 log (CFU/mL). For each sample, the milk after fermentation from set III had the largest population of *Lactobacillus* spp. cells, which amounted to approximately 8.3 log (CFU/mL) in sample A, approximately 8.3 log (CFU/mL) in sample B, and approximately 8.0 log (CFU/mL) in samples C and D. This is most likely because after the second passage, bacterial cells exhibited high metabolic activity; therefore, it was easier for them to multiply quickly. The fermented milk from sets I and II of all samples showed a similar cell population of *Lactobacillus* spp. ranging from approx. 7.0 to 7.8 log (CFU/mL).

The highest number of *Bifidobacterium* cells after fermentation was found in sample B after the second passage, which amounted to approximately 8.6 log (CFU/mL), while a similar population of cells was found in sample D after the first passage. The smallest number of cells was found in trial C from the 1st set point and amounted to approximately 5.7 log (CFU/mL).

In all variants and in all settings, after fermentation, the number of *S. thermophilus* cells (Figure 6) reached a similar value in the range of approximately 8.5 to 9.0 log (CFU/mL). A slightly larger population of these bacterial cells, at the level of approximately 9.2 log (CFU/mL), was observed in trial A after the first passage.

The highest number of *Propionibacterium* spp. cells after fermentation was present in samples A and C after the second passage, and this value was approximately 8.5 log (CFU/mL); the smallest number of cells was noted in sample D after the first passage, i.e., approximately 5.7 log (CFU/mL). No correlation was observed between the cell population of the tested bacteria and the passage number in all samples, which was confirmed by statistical analysis.

All sourdoughs showed good survival of both technical and probiotic yoghurt microflora. During the study, the vast majority of the obtained products met the therapeutic minimum criterion, i.e., the population of probiotic microflora ranged between 6-8 log (CFU/mL). Similar results for the probiotics *Bifidobacterium animalis* subsp. *lactis*, *L. acidophilus*, and *L. casei* were obtained by Ziarno et al. [15]. In this study, the

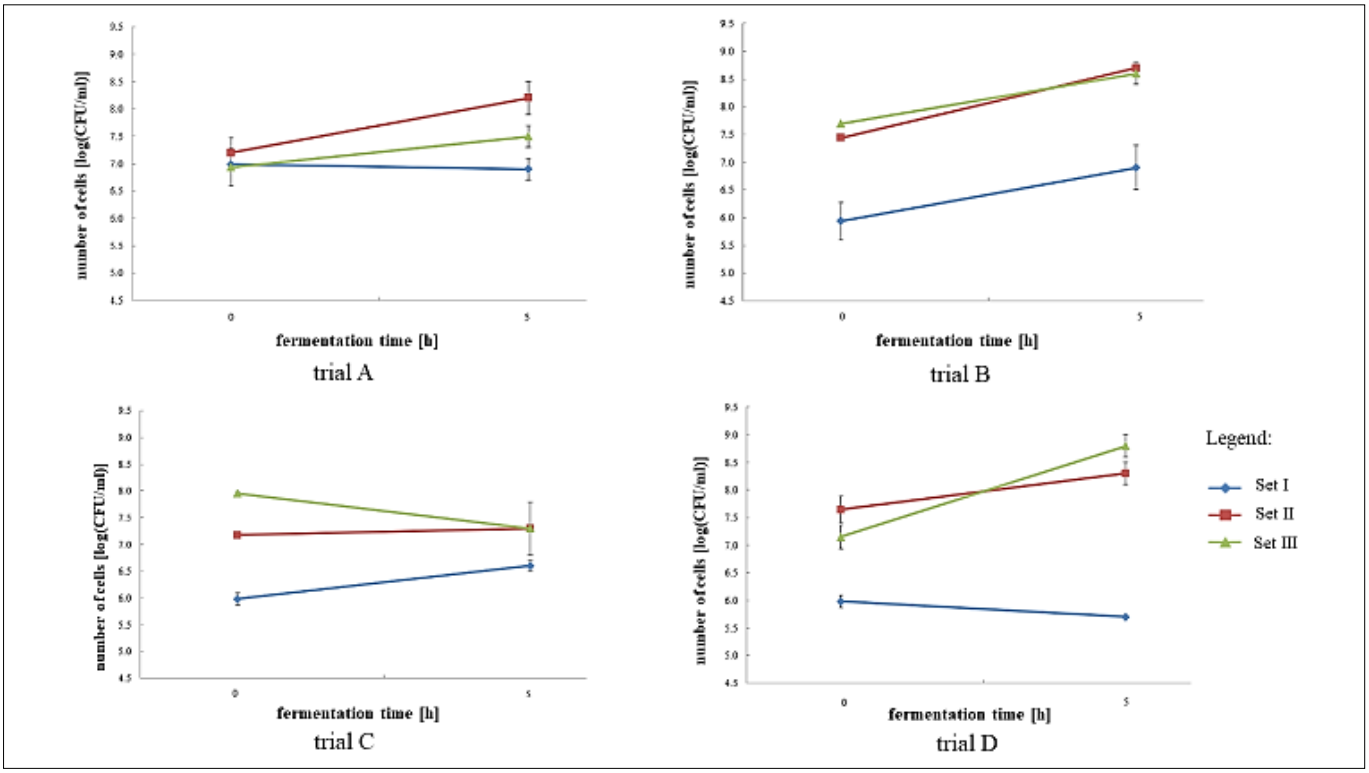

Fig. 5. Changes in the number of *Bifidobacterium* cells in milk fermented with BIFIVIT ZAKWASKA (mean values and standard deviations).

Rys. 5. Zmiany liczby komórek *Bifidobacterium* w mleku fermentowanym kulturą starterową BIFIVIT ZAKWASKA (wartości średnie i odchylenia standardowe).

Source: The own study

Źródło: Badania własne

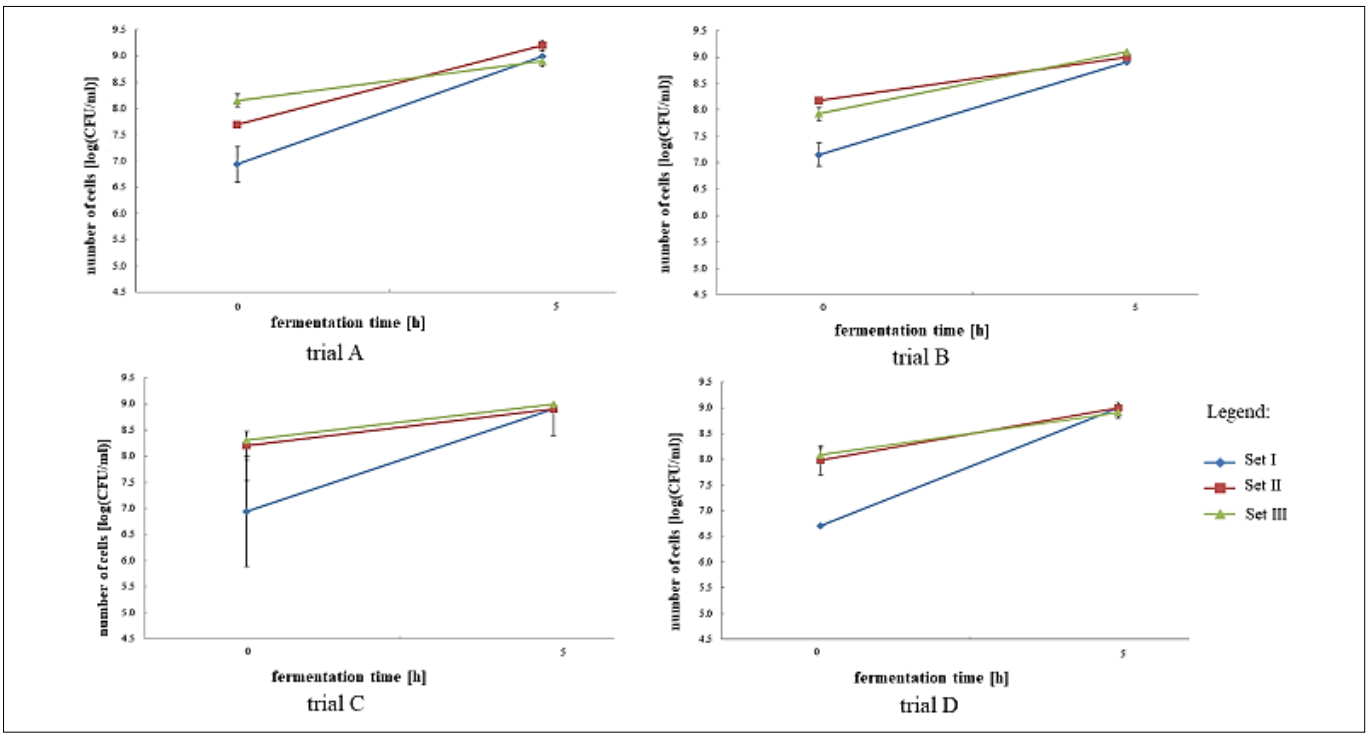

Fig. 6. Changes in the number of *Streptococcus thermophilus* cells in milk fermented with PROBIO JOGURT ZAKWASKA (mean values and standard deviations).

Rys. 6. Zmiany liczby komórek *Streptococcus thermophilus* w mleku fermentowanym kulturą starterową PROBIO JOGURT ZAKWASKA (wartości średnie i odchylenia standardowe).

Source: The own study

Źródło: Badania własne

level of yoghurt microflora was similar; however, comparing their population in all milk samples, it was concluded that the milk samples had slightly more number of *S. thermophilus* cells, and our research team also noted a similar phenomenon. The cited authors did not perform the passaging process but stored the produced yoghurts for 12 weeks at 6°C [14]. In another study, the authors determined the number of viable LAB cells in samples of fermented and nonfermented milk with the addition of various starter cultures [13]. In that study, the authors did not perform the passaging process; however, they determined the number of cells, including yoghurt bacteria, after 1 day of storage of the samples. The number of yoghurt bacteria obtained by those authors (9.1 log (CFU/mL)) was similar to the results obtained in the present study for milk fermented with the ACIDOLAKT culture, where the number of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* fluctuated in the range of 8.0-9.0 log (CFU/mL) after the first setting in all trials [13]. A similar high value of the number of cells was recorded after the first passage in milk fermented with the culture of PROBIO JOGURT in trial A.

Carbohydrate content

In all variants of the trials of milk fermented with ACIDOLAKT ZAKWASKA (Table 2) before fermentation, a similar lactose content at the level of approximately 4.50 g/100 g of the milk sample was observed with the starter dose. This is because the bacteria had not yet started with their metabolic activity, and therefore, they had not yet used lactose as a nutrient. The lowest lactose content was found in the trials after the first passage after the fermentation process in all trial variants. This proves that the bacteria most intensively used lactose contained in milk as a food substrate. A similar situation was observed for BIFIVIT (Table 3) and PROBIO JOGURT (Table 1). In BIFIVIT, the lowest content of lactose after the fermentation process was found in trial B after the first passage, and the sample contained 3.44 g of lactose in 100 g of milk. The lowest lactose decomposition was observed in the first setting of trial D, and the concentration of lactose in the milk after fermentation was 3.98 g/100 g of milk. No correlation was observed between the setting variant or the

Table 1. Sugar content of the analyzed samples of milk fermented with the PROBIO JOGURT culture (mean values and standard deviations, *n*=2)

Tabela 1. Zawartość cukru w analizowanych próbkach mleka fermentowanego kulturą starterową PROBIO JOGURT (wartości średnie i odchylenia standardowe, n=2)

carbohydrates (g/100 g)	PROBIO I 0h A	PROBIO I 0h B	PROBIO I 0h C	PROBIO I 0h D
glucose	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
galactose	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
lactose	4.58 ± 0.12	4.53 ± 0.18	4.51 ± 0,15	4.5 ± 0,08
carbohydrates (g/100 g)	PROBIO I 5h A	PROBIO I 5h B	PROBIO I 5h C	PROBIO I 5h D
glucose	0.12 ± 0.01	0.16 ± 0.03	0.17 ± 0.00	0.25 ± 0.00
galactose	0.40 ± 0.08	0.50 ± 0.10	0.31 ± 0.03	0.38 ± 0.07
lactose	3.74 ± 0.05	3.45 ± 0,04	3.95 ± 0.03	3.75 ± 0.05
carbohydrates (g/100 g)	PROBIO II N 0h A	PROBIO II N 0h B	PROBIO II N 0h C	PROBIO II N 0h D
glucose	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
galactose	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
lactose	4.53 ± 0.18	4.48 ± 0.01	4.58 ± 0.12	4.52 ± 0.18
carbohydrates (g/100 g)	PROBIO II 5h A	PROBIO II 5h B	PROBIO II 5h C	PROBIO II 5h D
glucose	0.30 ± 0.03	0.34 ±0.04	0.37 ± 0.07	0.32 ± 0.03
galactose	0.34 ± 0.04	0.34 ± 0.04	0.19 ± 0.01	0.18 ± 0.00
lactose	3.69 ± 0.12	3.40 ± 0.09	3.49 ± 0.10	2.43 ± 0.04
carbohydrates (g/100 g)	PROBIO III 0h A	PROBIO III 0h B	PROBIO III 0h C	PROBIO III 0h D
glucose	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
galactose	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
lactose	4.59 ± 0.12	4.44 ± 0.07	4.53 ± 0.11	4.45 ± 0.07
carbohydrates (g/100 g)	PROBIO III 5h A	PROBIO III 5h B	PROBIO III 5h C	PROBIO III 5h D
glucose	0.27 ± 0.02	0.26 ± 0.02	0.13 ± 0.00	0.17 ± 0.00
galactose	0.23 ± 0.01	0.18 ± 0.00	0.37 ± 0.03	0.15 ± 0.00
lactose	3.20 ± 0.08	2.64 ± 0.03	3.30 ± 0.06	1.88 ± 0.02

Source: The author's own study

Źródło: Opracowanie własne

passage number and the tendency for the highest or lowest lactose content, which was confirmed by statistical analysis. In PROBIO JOGURT, the lowest lactose content was found in the trials after the second passage after the fermentation process in all trial variants. This proves that the bacterial cells most intensively used lactose contained in milk as a food substrate, and therefore, their metabolic changes exhibited the greatest dynamics after the second passage. The lowest concentration of lactose after the completed fermentation process was recorded in trial D after the second passage, and this value was 1.88 g/100 g of milk; the highest concentration of lactose at the level of 3.951 g/100 g of milk was observed in trial C.

The available literature data indicate the ability of bacteria contained in the used yoghurt starters to degrade lactose into glucose and galactose [10]. This is in line with the obtained results: after the fermentation process, a decrease in the lactose content in the milk sample was observed, together with the appearance of appropriate amounts of glucose and galactose. Sarkar [10] showed that the lactose content was much lower in yoghurts obtained from concentrated milk by ultrafiltration (approximately 3.82% lactose) than in those obtained from milk to which skimmed milk powder was added (approximately 4.66% lactose). The results obtained for condensed milk were identical to those obtained in the present study, where the level of lactose after fermentation depended on the level of yoghurts obtained from condensed milk by the researchers and was even lower after the first and second passages.

SUMMARY AND CONCLUSIONS

Presently, food products with beneficial effects on human health are becoming increasingly popular. Fermented milk beverages are an example of a food with a high content of minerals, and it is also a source of probiotic strains with a positive effect on the intestinal microbiota. Moreover, the occurrence of intolerance to lactose is currently more frequent among people. The final product obtained in the present study showed a lower lactose content because of the passaging

Table 2. Sugar content of the analyzed samples of milk fermented with the ACIDOLAKT culture (mean values and standard deviations, n=2)

Tabela 2. Zawartość cukru w analizowanych próbkach mleka fermentowanego kulturą starterową ACIDOLAKT (wartości średnie i odchylenia standardowe, n=2)

carbohydrates (g/100 g)	ACIDO I 0h A	ACIDO I 0h B	ACIDO I 0h C	ACIDO I 0h D
glucose	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
galaktoza	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
lactose	4.52 ± 0.10	4.51 ± 0.10	4.51 ± 0.10	4.54 ± 0.11
carbohydrates (g/100 g)	ACIDO I 5h A	ACIDO I 5h B	ACIDO I 5h C	ACIDO I 5h D
glucose	0.18 ± 0.00	0.24 ± 0.01	0.17 ± 0.00	0.19 ± 0.00
galactose	0.33 ± 0.04	0.38 ± 0.02	0.17 ± 0.00	0.27 ± 0.02
lactose	3.69 ± 0.12	3.91 ± 0.15	4.19 ± 0.05	4.03 ± 0.04
carbohydrates (g/100 g)	ACIDO II 0h A	ACIDO II 0h B	ACIDO II 0h C	ACIDO II 0h D
glucose	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
galactose	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
lactose	4.51 ± 0.10	4.49 ± 0.08	4.52 ± 0.10	4.55 ± 0.11
carbohydrates (g/100 g)	ACIDO II 5h A	ACIDO II 5h B	ACIDO II 5h C	ACIDO II 5h D
glucose	0.18 ± 0.00	0.26 ± 0.02	0.26 ± 0.02	0.27 ± 0.02
galactose	0.24 ± 0.01	0.34 ± 0.04	0.31 ± 0.03	0.34 ± 0.04
lactose	2.57 ± 0.05	3.48 ± 0.07	3.53 ± 0.06	3.29 ± 0.06
carbohydrates (g/100 g)	ACIDO III 0h A	ACIDO III 0h B	ACIDO III 0h C	ACIDO III 0h D
glucose	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
galactose	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
lactose	4.58 ± 012	4.60 ± 0.13	4.51 ± 0.10	4.52 ± 0.10
carbohydrates (g/100 g)	ACIDO III 5h A	ACIDO III 5h B	ACIDO III 5h C	ACIDO III 5h D
glucose	0.26 ± 0.02	0.26 ± 0.02	0.25 ± 0.01	0.28 ± 0.02
galactose	0.34 ± 0.04	0.36 ± 0.04	0.35 ± 0.04	0.38 ± 0.05
lactose	3.85 ± 0.05	3.61 ± 0.08	3.69 ± 0.12	3.69 ± 0.12

Source: The author's own study

Źródło: Opracowanie własne

Table 3. Sugar content of the analyzed samples of milk fermented with the BIFIVIT culture (mean values and standard deviations, n=2)

Tabela 3. Zawartość cukru w analizowanych próbkach mleka fermentowanego kulturą starterową BIFIVIT (wartości średnie i odchylenia standardowe, n=2)

carbohydrates (g/100 g)	BIF I 0h A	BIF I 0h B	BIF I 0h C	BIF I 0h D
glucose	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
galactose	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
lactose	4.59 ± 0.12	4.52 ± 0.11	4.49 ± 0.10	4.50 ± 0.10
carbohydrates (g/100 g)	BIF I 5h A	BIF I 5h B	BIF I 5h C	BIF I 5h D
glucose	0.20 ± 0.00	0.26 ± 0.02	0.13 ± 0.00	0.23 ± 0.01
galactose	0.20 ± 0.00	0.25 ± 0.02	0.12 ± 0.00	0.25 ± 0.02
lactose	3.88 ± 0.11	3.63 ± 0.12	3.74 ± 0.12	3.98 ± 0.10
carbohydrates (g/100 g)	BIF II 0h A	BIF II 0h B	BIF II 0h C	BIF II 0h D
glucose	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
galactose	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
lactose	4.56 ± 0.11	4.46 ± 0.1	4.57 ± 0.11	4.50 ± 0.10
carbohydrates (g/100 g)	BIF II 5h A	BIF II 5h B	BIF II 5h C	BIF II 5h D
glucose	0.24 ± 0.01	0.26 ± 0.02	0.15 ± 0.00	0.24 ± 0.01
galactose	0.25 ± 0.02	0.30 ± 0.03	0.12 ± 0.00	0.32 ± 0.03
lactose	3.77 ± 0.04	3.44 ± 0.05	3.82 ± 0.06	3.54 ± 0.05
carbohydrates (g/100 g)	BIF III 0h A	BIF III 0h B	BIF III 0h C	BIF III 0h D
glucose	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
galactose	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
lactose	4.52 ± 0.10	4.57 ± 0.11	4.52 ± 0.10	4.51 ± 0.10
carbohydrates (g/100 g)	BIF III 5h A	BIF III 5h B	BIF III 5h C	BIF III 5h D
glucose	0.26 ± 0.02	0.30 ± 0.03	0.12 ± 0.00	0.29 ± 0.03
galactose	0.26 ± 0.02	0.31 ± 0.02	0.10 ± 0.00	0.34 ± 0.03
lactose	3.54 ± 0.05	3.74 ± 0.06	4.20 ± 0.07	3.57 ± 0.05

Source: The author's own study

Źródło: Opracowanie własne

process. This feature can provide a great advantage of milk products for people with diagnosed lactose intolerance. Moreover, the passaging process influences the number of bacterial cells. In most of the products obtained, the number of bacterial cells was higher after passaging and met the therapeutic minimum criterion of 6 log (CFU/mL). In the present study, the passaging process was used to understand the influence of bacterial count on the characteristics of fermented milk.

Our study shows how to obtain a high-quality product with most economical approach possible. The important factors that influence the dynamics of the lactic acid fermentation process are incubation temperature, the initial dose of the primer, and passage. The passaging provides the possibility of obtaining a full-fledged product in a cheaper way than that using a commercial starter.

In the future, research studies should be undertaken to confirm the validity of this hypothesis and to conduct similar experiments on other dairy products and fermented plant products.

PODSUMOWANIE I WNIOSKI

Obecnie coraz większą popularność zyskują produkty spożywcze o korzystnym wpływie na zdrowie człowieka. Jogurt jest przykładem pokarmu o wysokiej zawartości składników mineralnych, a także jest źródłem szczepów probiotycznych pozytywnie wpływających na mikrobiotę jelitową. Co więcej, obecnie wśród ludzi częściej występuje nietolerancja laktozy. Otrzymany w niniejszych badaniach produkt końcowy wykazywał niższą zawartość laktozy ze względu na proces pasażowania. Ta cecha może stanowić ogromną zaletę produktów

mlecznych dla osób ze zdiagnozowaną nietolerancją laktozy. Ponadto proces pasażowania wpływa na liczbę komórek bakteryjnych. W większości otrzymanych produktów liczba komórek bakteryjnych była wyższa po pasażowaniu i spełniała kryterium minimum terapeutycznego 6 log (CFU/mL). W pracy wykorzystano proces pasażowania do poznania wpływu zawartości bakterii na cechy mleka fermentowanego.

Ważnymi czynnikami wpływającymi na dynamikę procesu fermentacji mlekowej są temperatura inkubacji, początkowa dawka startera i pasaż. Nasze badanie pokazuje, jak uzyskać produkt wysokiej jakości przy możliwie najbardziej ekonomicznym podejściu. Pokazaliśmy również, że pasażowanie pozwala uzyskać pełnowartościowy produkt w tańszy sposób niż przy użyciu komercyjnego startera.

W przyszłości należy podjąć badania naukowe w celu potwierdzenia słuszności tej hipotezy i przeprowadzić podobne eksperymenty na innych produktach mlecznych i fermentowanych produktach roślinnych.

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