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THE EFFECT OF PSYLLIUM FIBER AND CHOKEBERRY FIBRE ADDITION ON THE QUALITY OF PROBIOTIC FERMENTED MILK

Summary

Background. Fiber from fruit, vegetables and other sources of fiber can increase gut microbiota diversity and show anti-inflammatory activity. The use of fiber in the manufacture of probiotic fermented milk has excellent potential, as it can offer several benefits for both consumers and producers. In addition to its well-known physiological benefits, dietary fiber may reduce the risk of diseases of civilization. Consumers expect that current foods are health-promoting. Consumer awareness is increasing through an expanding variety of products and advertising campaigns favoring health-promoting foods, a trend that food manufacturers are also taking advantage of. The aim of the study was to determine the effect of psyllium fiber and chokeberry fiber addition in combination with probiotic cultures *Bifidobacterium animalis* ssp. *lactis* BB-12 and *Lacticaseibacillus casei* 431 on the quality of fermented milk. To investigate the effect of fiber on fermented milk quality, acidity, texture properties, syneresis and colorcolor were measured, moreover, an organoleptic evaluation and microbiological analysis were performed.

Results and conclusion. Natural additives such as chokeberry fiber and psyllium fiber can be used in the production of milk fermented by *Bifidobacterium* BB-12 and *L.casei*. Psyllium fiber can be recommended for its syneresis-reducing properties. Whereas chokeberry fiber, due to its high polyphenol content, can be used as a natural colorcolorant and taste-enhancing additive. Adding fiber as chokeberry or psyllium positively stimulated the growth and survival of *Bifidobacterium* BB-12 and *L. casei* bacteria during storage. The hardness of the milk gel fermented by *Bifidobacterium* BB-12 or *L. casei* was significantly improved by adding psyllium fiber, increasing it by as much as about 2.5 N, and this trend was also maintained after 28 days of storage. Studies on using various fibers in fermented milk production should be continued to determine their health-promoting and technological potential.

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Introduction

Globally, due to COVID-19 and other crises, an increase in all forms of malnutrition could be observed in the future [9]. Even today, an incorrect diet is one of the causes of diseases in the world's population [48]. Therefore, developing new nutrient-enriched products is applicable nowadays and in the future. Dairy products are the most popular category of the food market of the European Union [45]. To provide the population with well-balanced food, traditional ingredients can be used, as well as other plant ingredients with added nutritional value can also be utilized [2,28]. In recent years, many publications have concerned the application of fiber in fermented milk [13,16]. Fiber from fruit, vegetables and other fibers increase gut microbiota diversity and show anti-inflammatory activity [6,34]. The use of fiber in fermented milk has enormous potential, as it can offer a variety of benefits to both consumers and producers.

This study investigated the effects of different fiber types on the physicochemical and organoleptic properties of fermented milk, as well as the health benefits associated with consuming fiber-enriched fermented milk .

The addition of fiber in the form of Psyllium (*Plantago ovata* Forsk) might be considered an alternative method of enriching fermented dairy products [1,7] due to the presence of an active fraction of that it has arabinose 22.6 %, xylose 74.6 % and a branched structure [38].

Psyllium as a soluble fiber has the potential to stimulate bacterial growth in the digestive system [36]. There have been attempts to combine psyllium with various probiotic cultures: *Lactocaseibacillus paracasei*, *Lactobacillus acidophilus* and *Lactobacillus bulgaricus*, *Lactiplantibacillus plantarum*, *S. boulardii* and *Lactocaseibacillus rhamnosus* and others [3, 22, 36, 49].

A potential fruit source of fiber is chokeberry [5]. Chokeberry has a substantial anthocyanin content, indicating its high antioxidant activity [19]. Due to the high content of anthocyanins, chokeberry fruits benefit the cardiovascular system due to their ability to seal blood vessels and provide capillaries with elasticity and proper permeability [27]. Regular consumption of chokeberry improves digestion, stabilizes the cardiovascular and digestive systems and has a disinfectant and antisclerotic effect on the human body [29, 21]. Furthermore, the application of fiber in fermented milk has the potential to reduce production fees, allowing lower-cost ingredients for production, while maintaining the quality of the product. Globally, the potential for applying fiber in fermented milk is substantial, as it can offer a wide range of benefits to both con-

sumers and producers. Therefore, increasing attention and development of fiber-enriched fermented dairy products will probably continue to be observed in the coming years. Thus, the aim of the study was to determine the effect of psyllium fiber and chokeberry fiber addition in combination with probiotic cultures *Bifidobacterium animalis* ssp. *lactis* BB-12 and *Lacticaseibacillus casei* 431 on the quality of fermented milk.

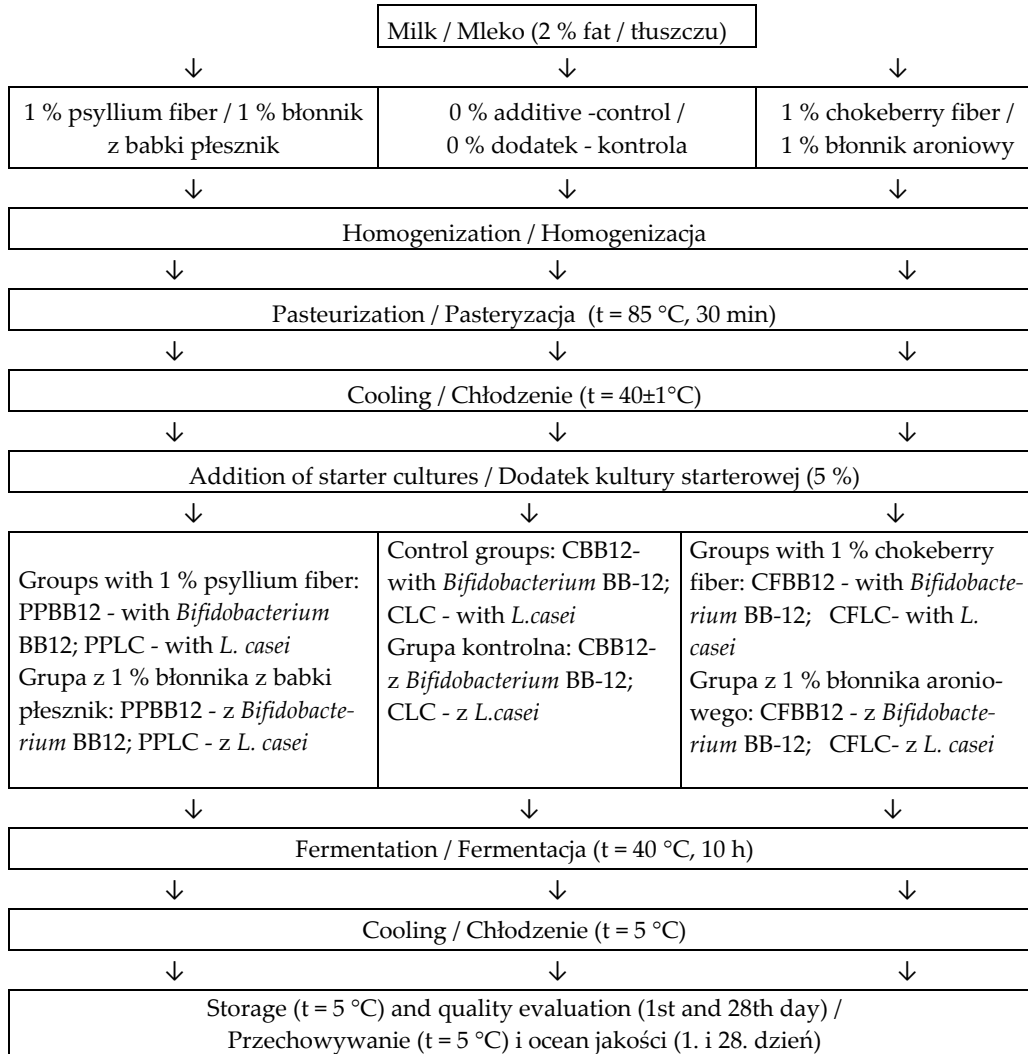
Material and methods

Material

Łaciate milk, 2 % fat (SM Mlekpól, Grajewo, Poland), was used as a raw material for the production of fermented milk. The psyllium fiber (*Plantago psyllium* L.) was purchased from Purasana Ltd. (Komen, Slovenia), and chokeberry fiber was obtained from Bio Planet (Leszno, Poland). Starter cultures of two probiotic strains *Bifidobacterium animalis* ssp. *lactis* BB-12 and *Lacticaseibacillus casei* 431 were purchased from Chr. Hansen (Hoersholm, Denmark). Sodium hydroxide (0.1 M NaOH) and phenolphthalein were purchased from Chempur (Piekary Śląskie, Poland).

Manufacture of fermented milk

Milk with fiber was heated to 60°C and homogenized with a homogenizer Nuoni GJJ-0.06/40 (Zhejiang, China), at the pressure of 20 MPa. A control sample without fiber addition was also prepared. The milk was then repasteurized at 85 °C for 30 min and cooled to 40 °C. The milk samples were divided into batches: control (without the addition of fiber), with a 1 % addition of psyllium fiber and a 1 % addition of chokeberry fiber. At the next stage, a previously activated single starter monoculture of *Bifidobacterium animalis* ssp. *lactis* BB-12 (*Bifidobacterium* BB-12) and monoculture of *Lacticaseibacillus casei* 431 (*L. casei*) were added to each batch of milk (Scheme 1). Starter cultures of *Bifidobacterium* BB-12 and *L. casei* were activated at 40 °C for 5 hours and added to the milk in the amount of 5 %, using the method of Szajnar et al. [41]. After mixing, the milk was poured into 100 ml plastic containers. The fermentation process was carried out at 40 °C for 10 hours. The fermented milk was cooled to 5 °C (Cooled Incubator ILW 115, POL-EKO Aparatura, Wodzisław Śląski, Poland). A total of 60 samples were manufactured and five samples from each group were analyzed on two occasions after 1 and 28 days of storage.



Scheme 1. Production scheme for probiotic fermented milk with psyllium fiber and chokeberry fiber
Schemat 1. Technologia produkcji probiotycznego mleka fermentowanego z dodatkiem błonnika z babki płesznik i błonnika z aronii

Acidity and pH measurement

The pH of the milk with the addition of psyllium raw powder and chokeberry fiber and after the fermentation process was measured with a pH-meter FiveEasy (Mettler Toledo, Greifensee, Switzerland) with InLab®Solids Pro-ISM electrode (Mettler Toledo, Greifensee, Switzerland) [41]. The method described by Jemaa et al. [17] was used to measure lactic acid content: the fermented milk samples were titrated with 0.1

M NaOH solution, using phenolphthalein as indicator, and lactic acid content was expressed as g lactic acid L⁻¹.

Syneresis

The centrifuge method [37], with modifications, was used for measuring syneresis in the fermented milk samples (laboratory refrigerated centrifuge LMC-4200R, Biosan SIA, Riga, Latvia). A 10 g portion of fermented milk was weighed into a 50 mL plastic tube and centrifuged at 4000 rpm for 10 min. Syneresis was determined as the mass percentage of the separated whey in the product.

Evaluation of color

The color was determined with a colorimeter (Precision Colorimeter, Model NR 145, Shenzhen, China) using the CIE L*a*b* system. The following parameters were determined: L*—as lightness (from 0—black to 100—white), a*—as color from red (+) to green (-), b*—as color from yellow (+) to blue (-) [21]. Before measurement, the device was calibrated on a white reference standard [26].

Texture profile

A texture profile analysis, including the measurement of hardness [N], cohesiveness, adhesiveness [mJ] and springiness [mm], was executed using an instrumental method described by Znamirowska et al. [50]. The analysis was performed on a 100 mL fermented milk sample, whose temperature was 8 °C, and the cylindrical dimensions of the sample were 66 mm × 33.86 mm. The test was performed using CT3 Texture Analyzer with Texture Pro CT software (Brookfield AMETEK, Berwyn, PA, USA), acrylic probe TA3/100 (diameter 35 mm), TABTKIT table and settings: measurement speed 1 mm/s, trigger load 0.1 N, test termination distance 15 mm.

Microbiological analysis

The pour plate method using MRS agar (Biocorp, Poland) [24, 32] was employed to determine the viable counts of *Bifidobacterium* BB12 and *L. casei*. Incubation was carried out in a vacuum desiccator with GENbox anaer (Biomerieux, Warsaw, Poland). The process was conducted under anaerobic conditions at 37 °C for 72 hours. Following the incubation, colonies were counted with a colony counter (TYP J-3, Chemland, Stagard Szczeciński, Poland). Viable cell count was expressed as log CFU g⁻¹.

Organoleptic evaluation

An organoleptic evaluation of the probiotic fermented milk with the addition of psyllium fiber and chokeberry fiber was carried out by a sensory panel (previously trained ten men and ten women) on the 1st and 28th day of cold storage. Milky-creamy taste, sour taste, a taste of the additives, sweet taste, off-taste, sour odor, an odor of

additives and off-odor [42] were evaluated on a scale with markings at both ends: 1 (undetectable) to 9 (very intense), according to PN-ISO 22935-2:2013-07 [31].

Statistical analysis

The data obtained was processed with Statistica v. 13.1 software (StatSoft, Tulsa, Oklahoma, USA) to calculate the mean, standard deviation, and Pearson's correlation coefficient. A three-way ANOVA was used to investigate the overall effect of additives and storage time (days) and the type of bacteria on the properties of the fermented milk. The significance of differences between the means was estimated with Tukey's test ($p \leq 0.05$).

Results and discussion

Acidity and pH measurement

The milk after homogenization and pasteurization with fibers had significantly different pH values than the control milk (fig.1). Adding psyllium fiber reduced the pH value by 0.1 units and chokeberry fiber by 0.4 units compared to their control counterparts. This was mainly due to the presence of vitamin C and organic acids in chokeberry fruit, which ranges from 1.1 % to 1.4 % [4, 8]. The main acids identified in fresh berries are L-malic (13.1 g/kg), citric (2.1 g/kg) and quinic acid (5.9 g/kg). Shikimic acid, oxalic acid and succinic acid were considered secondary components [8, 21]. The content of free acids in the pomace is low since they transfer into the juice along with other solutes. According to Sójka et al. [39], the predominant organic acid in pomace is galacturonic acid ($5 \div 16$ g/kg).

On the first day of storage, a significantly lower pH value was determined in the milk with the addition of 1 % psyllium fiber (PPBB12, PPLC) compared to the control counterparts. In contrast, adding 1% chokeberry fiber did not significantly change the pH value. As expected, extended storage time reduced the pH value in all the fermented milk samples. Additionally, the addition of psyllium fiber significantly increased the lactic acid content of the fermented milk (PPBB12, PPLC) on the 1st day of storage. However, adding chokeberry fiber did not change the lactic acid concentration of the fermented milk. In the study by Helal et al. [13], yoghurt pH values and titratable acidity were not affected by the addition of different ratios of inulin. On the contrary, the addition of high-density inulin (1 % and 2 %) positively influenced the formation of acetaldehyde, resulting in a higher concentration than low-fat without additives, as well as the apparent viscosity of the yoghurt, which increased with the addition of inulin to 2 % and was comparable to the full-fat yoghurt.

After 28 days of storage, the lactic acid content increased significantly in the control samples CBB12 and CLC (Table 1). However, an insignificant increase in lactic

acid concentration was observed in the samples with fibers (PPBB12, CFBB12, PPLC, CFLC).

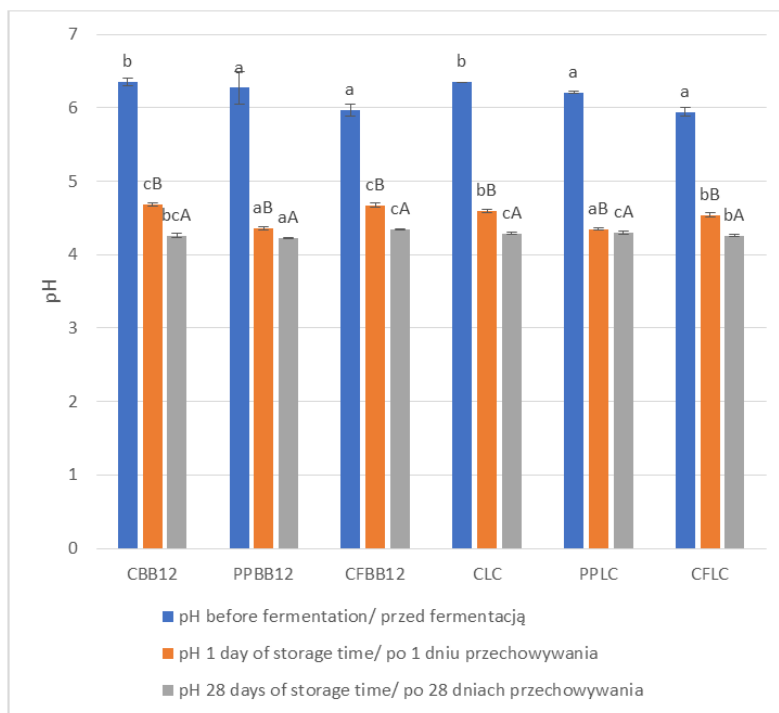


Fig. 1. pH of fermented milk by *Bifidobacterium* BB-12 and *L. casei* before fermentation, after 1 and 28 days of cold storage

Rys. 1. Wartość pH mleka fermentowanego przez *Bifidobacterium* BB-12 i *L. casei* przed fermentacją, po 1 i 28 dniach przechowywania chłodniczego

Explanatory notes / Objasnienia:

CBB12-control milk with *Bifidobacterium* BB-12; PPBB12-milk with 1.0 % psyllium fiber and *Bifidobacterium* BB-12; CFBB1-milk with 1.0 % chokeberry fiber and *Bifidobacterium* BB-12; CLC— control milk with *L. casei*; PPLC-milk with 1.0 % psyllium fiber and *L. casei*; CFLC— milk with 1.0 % chokeberry fiber and *L. casei*. Values are means \pm SD, $n = 5$ for each group. ^{A,B} – means marked with different capital letters indicate statistically significant differences within a given parameter during storage at $p \leq 0.05$; ^{a-c} – mean values with different lowercase letters indicate statistically significant differences at $p \leq 0.05$ depending on the fiber and bacteria type /

CBB12-mleko kontrolne z *Bifidobacterium* BB-12; PPBB12-mleko z dodatkiem 1.0 % błonnika z babki płesznik i *Bifidobacterium* BB-12; CFBB1-mleko z dodatkiem 1.0 % błonnika z aronii i *Bifidobacterium* BB 12; CLC— mleko kontrolne z *L. casei*; PPLC-mleko z dodatkiem 1.0 % błonnika z babki płesznik i *L. casei*; CFLC— mleko z dodatkiem 1.0% błonnika z aronii i *L. casei*. Średnia \pm SD, $n = 5$ dla każdej grupy ^{A,B} – średnie oznaczone różnymi dużymi literami wskazują statystycznie istotne różnice time w obrębie danej cechy w czasie przechowywania przy $p \leq 0,05$; ^{a-c} - średnie oznaczone różnymi małymi literami wskazują statystycznie istotne różnice przy $p \leq 0,05$ w zależności od rodzaju błonnika i probiotycznej kultury starterowej

Syneresis

After the first day of storage, milk fermented by *Bifidobacterium* BB-12 showed less susceptibility to syneresis than its counterpart fermented by *L. casei* (Table 1).

Table 1. Properties of fermented milk with psyllium fiber and chokeberry fiber during cold storage
Tabela 1. Właściwości mleka fermentowanego z błonnikiem z babki płesznik i błonnikiem z aronii podczas przechowywania chłodniczego

Properties / Właściwości	Storage time [days] / Czas przechowywania [dni]	<i>Bifidobacterium</i> BB-12			<i>L. casei</i>			
		CBB12	PPBB12	CFBB12	CLC	PPLC	CFLC	
Lactic acid [g·L ⁻¹] / Kwas mlekowy [g·L ⁻¹]	1	0.65 ^{aA} ±0.01	0.82 ^{cA} ±0.01	0.66 ^{aA} ±0.11	0.71 ^{bA} ±0.03	0.84 ^{cA} ±0.03	0.75 ^{bA} ±0.04	
	28	0.75 ^{aB} ±0.02	0.84 ^{bA} ±0.02	0.78 ^{aA} ±0.06	0.80 ^{aB} ±0.05	0.87 ^{bA} ±0.07	0.80 ^{aA} ±0.04	
Syneresis [%] Synereza [%]	1	21.03 ^{bA} ±2.37	14.01 ^{aA} ±0.42	28.52 ^{cA} ±0.79	28.54 ^{cA} ±0.96	21.39 ^{bA} ±1.06	38.09 ^{dA} ±1.55	
	28	42.24 ^{dB} ±0.72	20.85 ^{bb} ±0.83	35.85 ^{cb} ±1.20	46.73 ^{cb} ±1.33	23.75 ^{ab} ±0.50	42.36 ^{dB} ±1.58	
Color / Barwa	L*	1	93.27 ^{cB} ±1.23	81.70 ^{bA} ±0.95	69.98 ^{aA} ±1.28	94.03 ^{cB} ±1.11	79.37 ^{bA} ±1.12	68.12 ^{aA} ±1.01
		28	89.11 ^{dA} ±0.77	80.10 ^{cA} ±0.42	71.01 ^{aA} ±1.77	90.08 ^{dA} ±1.02	77.91 ^{bA} ±1.21	72.09 ^{ba} ±1.52
	a*	1	-2.34 ^{bA} ±0.42	-3.11 ^{aA} ±0.13	16.21 ^{cB} ±0.76	-3.01 ^{aA} ±0.21	-2.39 ^{bb} ±0.16	17.51 ^{cB} ±0.89
		28	-2.07 ^{bA} ±0.31	-3.18 ^{aA} ±0.48	11.92 ^{dA} ±1.08	-2.53 ^{bA} ±0.41	-1.77 ^{cA} ±0.18	13.02 ^{dA} ±1.02
	b*	1	9.48 ^{bA} ±0.82	12.46 ^{cA} ±1.02	0.46 ^{aA} ±0.14	10.02 ^{bA} ±0.82	11.82 ^{cA} ±0.41	0.21 ^{aA} ±0.11
		28	10.68 ^{bA} ±0.12	13.12 ^{dA} ±0.19	1.98 ^{ab} ±0.81	10.81 ^{bA} ±0.83	12.01 ^{cA} ±0.81	0.81 ^{ab} ±0.20

Explanatory notes / objaśnienia:

Values are mean±SD, n = 5 for each group; ^{a-c} – mean values denoted in rows by different lowercase letters differ significantly at $p \leq 0.05$; ^{A,B} – means in columns marked with different capital letters indicate statistically significant differences within a given parameter during storage at $p \leq 0.05$; storage time: 1 – after fermentation, 28 – after 28 days of cold storage; CBB12 – control milk with *Bifidobacterium* BB-12; PPBB12 – milk with 1.0 % psyllium fiber and *Bifidobacterium* BB-12; CFBB12 – milk with 1.0 % chokeberry fiber and *Bifidobacterium* BB-12; CLC – control milk with *L. casei*; PPLC – milk with 1.0 % psyllium fiber and *L. casei*; CFLC – milk with 1.0 % chokeberry fiber and *L. casei*/

Średnia±SD, n = 5 dla każdej grupy; ^{a-c} – średnie oznaczone w wierszach różnymi małymi literami różnią się istotnie przy $p \leq 0,05$; ^{A,B} – średnie w kolumnach oznaczone różnymi dużymi literami wskazują statystycznie istotne różnice w obrębie danej cechy w czasie przechowywania przy $p \leq 0,05$; czas przechowywania: 1 – po fermentacji, 28 – po 28 dniach przechowywania chłodniczego; CBB12-mleko kontrolne z *Bifidobacterium* BB-12; PPBB12-mleko z dodatkiem 1.0 % błonnika z babki płesznik i *Bifidobacterium* BB-12; CFBB1-mleko z dodatkiem 1.0 % błonnika z aronii i *Bifidobacterium* BB-12; CLC – mleko kon-

tolne z *L. casei*; PPLC-mleko z dodatkiem 1.0 % błonnika z babki płesznik i *L. casei*; CFLC – mleko z dodatkiem 1.0 % błonnika z aronii i *L. casei*

Adding 1 % psyllium fiber to the milk fermented by *Bifidobacterium* BB-12 reduced syneresis by 7 % on the 1st day of storage, while adding 1 % chokeberry fiber increased syneresis by 7 %. Regarding the milk fermented by *L. casei*, the addition of psyllium fiber also reduced syneresis by about 7 %, but the addition of chokeberry fiber increased syneresis by as much as 9.5 %. The phenomenon of decreased syneresis is due to the presence of mucilage in psyllium. The mucilage of psyllium seeds is often referred to as husk or psyllium husk. Ground mucilage obtained from seeds is a hydrophilic white fibrous substance, indicating that its molecular shape causes it to absorb and bind water. Once water is absorbed, it forms a transparent, colorless, mucilaginous gel that increases in size by ten times or more [10]. Psyllium fiber containing a water-soluble hydrophilic mucilage fiber are abundant in various primary and secondary metabolites and, in addition, many bioactive compounds [43]. Extending storage time to 28 days increased syneresis in the control milk by 21.21 % in CBB12 and 18.19 % in CLC. Milk with fibers also showed higher syneresis compared to the first day of cold storage. Significantly lower syneresis was shown in the samples with psyllium fiber (PPBB12 and PPLC) by 21.39 % and 22.98 % compared to the controls. After 28 days of storage, milk with 1 % added chokeberry fiber CFBB12 had lower syneresis by about 6.4 %, and the milk with *L. casei* by approximately 4.4 % compared to their controls. However, it should be noted that milk fermented by *Bifidobacterium* BB-12 has a higher water retention capacity than milk with *L. casei*. These differences, due to the use of different strains, may be related to the production of exopolysaccharides under certain environmental conditions. Bacterial exopolysaccharides are characterized by high molecular weight polysaccharides, which are secreted by some bacteria into the culture medium and consist of monosaccharides [20, 25, 33, 48]. Like water-binding agents, exopolysaccharides exhibit textural properties such as viscosity, stabilization, emulsification, sweetening and gelation. Jovanovi et al. [18] studied a new probiotic yoghurt fortified with apple pomace flour, where syneresis was reduced up to 1.8 times compared to the control.

Evaluation of color

Adding fiber in the form of psyllium and chokeberry significantly influenced the $L^*a^*b^*$ color components compared to their control counterparts, both in the milk fermented by *Bifidobacterium* BB-12 and *L. casei* (Table 1). The addition of chokeberry fiber (CFBB12, CFLC) caused the strongest color darkening on the 1st and 28th day of storage. The milk fermented by *Bifidobacterium* BB-12 with the addition of psyllium fiber (PPBB12) was characterized by a higher proportion of green and yellow color than the control (CBB12). In contrast, the milk fermented by *L. casei* with psyllium

fiber (PPLC) was characterized by a lower proportion of green color and a higher proportion of yellow color compared to the control (CLC). Extending the storage period to 28 days increased the proportion of yellow in all milk groups, which was influenced by syneresis ($r = 0.989$, $p \leq 0.05$) and the lactic acid content ($r = 0.996$, $p \leq 0.05$).

The color of the milk with chokeberry fiber results from the presence of polyphenolic compounds in chokeberry fruit. Anthocyanins present in chokeberry are the second largest group of phenolic compounds. Anthocyanins give the fruit a color that varies from orange to red and purple to blue. The concentration of hydrogen ions influences the type of the color of anthocyanins and their stability. They show greater stability in acidic environments. However, in technological processes, anthocyanins are degraded by light, oxygen and heating [31]. Several studies [4, 9, 10] demonstrated that the stability of anthocyanins, apart from food processing, depends on numerous factors, including storage temperature, pH, light, the presence of metal ions, oxygen, enzymes, ascorbic acid, sugars, sulfur dioxide or sulfites and copigments belonging to different classes of compounds. Nevertheless, the color of milk enriched with fiber from psyllium was probably influenced, among other things, by the presence of flavonoids which provide a yellow color [16].

Microbiological analysis of fermented milk

Figure 2 shows the number of viable bacterial cells of *Bifidobacterium* BB-12 and *L. casei* after 1 and 28 days of storage of the control milk and with the addition of chokeberry fiber or psyllium fiber. The control milk fermented by *Bifidobacterium* BB-12 (CBB12) had comparable bacterial cell count to the control milk fermented by *L. casei* (CLC), and the observed differences were not significant. On the first day of storage, the milk fermented by *Bifidobacterium* BB-12 with psyllium fiber (PPBB12) showed the highest number of viable bacterial cells. On the contrary, the other fiber-fermented milk samples (CFBB12, PPLC, CFLC) had a significantly higher number of viable bacterial cells by about 1 log cfu g⁻¹ than their control counterparts. After 28 days of storage, the number of bacterial cells decreased by approximately 2 log cfu g⁻¹ compared to the first day. In the PPLC and CFLC samples, the number of viable *L. casei* bacterial cells was about 3 log cfu g⁻¹ lower compared to the first day of storage. The milk with fibers fermented by *Bifidobacterium* BB-12 (PPBB12 and CFBB12) after 28 days of storage had significantly higher bacterial cell count than the other groups. However, it should be noted that even on the 28th day of storage, all the fermented milk samples met the requirements of the International Dairy Federation's Recommendation that probiotic products should contain at least 7 log CFU g⁻¹ of lactic acid bacteria [9].

Studies by some authors report that phenolic compounds can also stimulate the growth of beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* and can there-

fore modulate the intestinal microflora [23, 44]. In the study by Szajnar et al. [40], the addition of chokeberry fiber affected the stimulation of the growth of bacteria. In the milk sample without fiber addition, more viable cells were counted for *L. acidophilus* by $0.5 \log \text{CFU g}^{-1}$ more than the milk fermented with *L. rhamnosus*. Furthermore, in milk fermented by *L. acidophilus* with 1.5 % chokeberry fiber, the number of viable bacterial cells was higher than that in milk fermented by *L. rhamnosus* with the same addition of fiber.

Furthermore, in the study by Helal et al. [13], the viability of *L. delbrueckii* ssp. *bulgaricus* was enhanced by adding 1 % and 2 % of inulin, while adding 3 % had a negative effect. However, no effect was reported on *Streptococcus thermophilus* viability.

An ANOVA analysis of variance indicated that the number of bacterial cells in the fermented milk was affected by factors such as storage time ($p = 0.000$), type of bacteria ($p = 0.001$), fiber addition ($p = 0.000$), and interactions between these factors ($p = 0.001$).

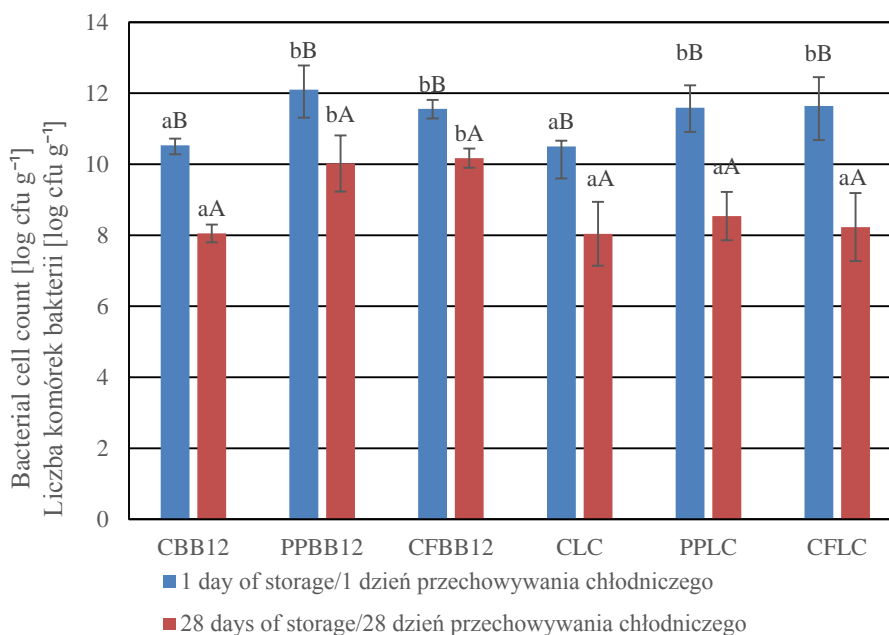


Fig. 2. Viable count in milk fermented by *Bifidobacterium* BB-12 and *L. casei* after 1 and 28 days of cold storage

Rys. 2. Liczba żywych komórek bakterii w mleku fermentowanym przez *Bifidobacterium* BB-12 i *L. casei* po 1 i 28 dniach przechowywania chłodniczego

Explanatory notes / objaśnienia:

CBB12 – control milk with *Bifidobacterium* BB-12; PPBB12-milk with 1.0 % psyllium seed husks and *Bifidobacterium* BB-12; CFBB1 – milk with 1.0 % chokeberry fiber and *Bifidobacterium* BB-12; CLC –

control milk with *L. casei*; PPLC – milk with 1.0 % psyllium seed husks and *L. casei*; CFLC – milk with 1.0 % chokeberry fiber and *L. casei*. Values are means \pm SD, $n = 5$ for each group. ^{A,B} – means marked with different capital letters indicate statistically significant differences within a given parameter during storage at $p \leq 0.05$; ^{a-c} – mean values with different lowercase letters indicate statistically significant differences at $p \leq 0.05$ depending on the fiber and bacteria type/

CBB12 – mleko kontrolne z *Bifidobacterium* BB-12; PPBB12-mleko z dodatkiem 1.0 % błonnika z babki płesznik i *Bifidobacterium* BB-12; CFBB1 – mleko z dodatkiem 1.0 % błonnika z aronii i *Bifidobacterium* BB-12; CLC – mleko kontrolne z *L. casei*; PPLC – mleko z dodatkiem 1,0% błonnika z babki płesznik i *L. casei*; CFLC – mleko z dodatkiem 1.0 % błonnika z aronii i *L. casei*. Średnia \pm SD, $n = 5$ dla każdej grupy. ^{A,B} – średnie oznaczone różnymi dużymi literami wskazują statystycznie istotne różnice w obrębie danej cechy w czasie przechowywania przy $p \leq 0,05$; ^{a-c} – średnie oznaczone różnymi małymi literami wskazują statystycznie istotne różnice przy $p \leq 0,05$ w zależności od źródła błonnika i probiotycznej kultury starterowej

Texture analysis

Figure 3 shows a texture profile of the milk fermented by *Bifidobacterium* BB-12 and *L. casei* after 1 and 28 days of cold storage. The hardness of the milk gel fermented by *Bifidobacterium* BB-12 or *L. casei* was significantly improved by adding psyllium fiber, increasing it by as much as about 2.5 N, and this trend was also maintained after 28 days of storage. The increase in hardness in the milk with the addition of psyllium fiber may be due to the fact that psyllium husk (polysaccharides) acts as a stabilizer in the fermented milk because it can absorb moisture, thus improving the texture of the finished fermented milk [12, 14]. A significant correlation was found between milk hardness and lactic acid content ($r = 0.969$).

The addition of chokeberry fiber to the milk, regardless of the type of probiotic strain used, resulted in a decrease in hardness compared to the control on the first day of storage. The study indicates that the addition of psyllium fiber or chokeberry fiber decreases the adhesiveness or results in similar adhesiveness to the control milk throughout the storage period. Furthermore, the determined cohesiveness in the milk with fiber addition was similar to their control counterparts and ranged from 0.41 (for CFLC) to 0.53 (for PPB12), and the differences shown were significant only in the particular cases.

In contrast, the addition of fiber and the type of probiotic strain used did not significantly affect the springiness of the fermented milk. In the study by Szajnar et al. [40], the milk with chokeberry fiber fermented with *L. acidophilus* was characterized by harder gel than its analogues fermented with *L. rhamnosus*.

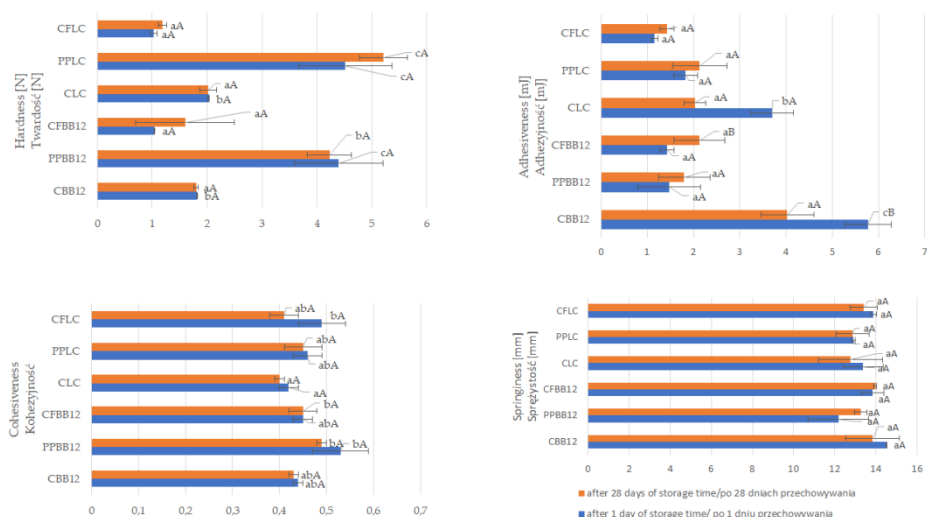


Fig. 3. Effect of fiber addition on texture profile of milk fermented by *Bifidobacterium* BB-12 and *L. casei* after 1 and 28 days of cold storage

Rys. 3. Wpływ dodatku błonnika na profil teksturometryczny mleka fermentowanego przez *Bifidobacterium* BB-12 i *L. casei* po 1 i 28 dniach przechowywania chłodniczego

Explanatory notes / objaśnienia:

CBB12 – control milk with *Bifidobacterium* BB-12; PPBB12 – milk with 1.0 % psyllium fiber and *Bifidobacterium* BB-12; CFBB1 – milk with 1.0 % chokeberry fiber and *Bifidobacterium* BB-12; CLC – control milk with *L. casei*; PPLC – milk with 1.0 % psyllium fiber and *L. casei*; CFLC – milk with 1.0 % chokeberry fiber and *L. casei*. Values are means \pm SD, $n = 5$ for each group. ^{A,B} – means marked with different capital letters indicate statistically significant differences within a given parameter during storage at $p \leq 0.05$; ^{a-c} – mean values with different lowercase letters indicate statistically significant differences at $p \leq 0.05$ depending on the fiber and bacteria type /

CBB12 – mleko kontrolne z *Bifidobacterium* BB-12; PPBB12 – mleko z dodatkiem 1.0 % błonnika z babki płesznik i *Bifidobacterium* BB-12; CFBB1 – mleko z dodatkiem 1.0 % błonnika z aronii i *Bifidobacterium* BB-12; CLC – mleko kontrolne z *L. casei*; PPLC – mleko z dodatkiem 1.0% błonnika z babki płesznik i *L. casei*; CFLC – mleko z dodatkiem 1.0 % błonnika z aronii i *L. casei*. Średnia \pm SD, $n = 5$ for each group. ^{A,B} – średnie oznaczone różnymi dużymi literami wskazują statystycznie istotne różnice w obrębie danej cechy w czasie przechowywania przy $p \leq 0,05$; ^{a-c} – średnie oznaczone różnymi małymi literami wskazują statystycznie istotne różnice przy $p \leq 0,05$ w zależności od źródła błonnika i probiotycznej kultury starterowej

Organoleptic analysis

The results of the organoleptic analysis are presented in Figure 4a and Figure 4b. The control milk, without fiber addition, fermented by *Bifidobacterium* BB-12 or *L. casei*, was characterized by a more intense milky-creamy taste than the milk with psyl-

lium fiber or chokeberry fiber. The addition of psyllium fiber or chokeberry fiber neither enhanced the sour taste nor affected the differences in the intensity of the sweet taste. Furthermore, off-taste and off-odor in the control milk and the milk with fiber additives were not detectable, regardless of the type of probiotic strain used. As expected, the additive taste and odor were perceptible in the milk with psyllium fiber and in the milk with chokeberry fiber. A consumer panel evaluating the fermented milk samples on days 1 and 28 of the organoleptic evaluation indicated that the storage time did not significantly affect the quality of the product, as the marks given were similar to those on the 1st day. This was also confirmed by an ANOVA analysis of variance, which indicated that the factor that significantly affected the taste of the product was the addition of fiber ($p < 0.007$), while the storage time was found to be insignificant.

Furthermore, in a study by Sadowska et al. [35] on the functional properties of fruit fiber preparations and their application in wheat bakery products (Kaiser Rolls), it was found that chokeberry fiber product influenced the color and was the darkest with a purplish-brown hue. The fibers used showed a fruity taste and odor characteristic of the fruit species used, with no off-taste or aftertaste. In a study by Bhat et al. [3], yoghurt with 0.5 % psyllium fiber was found to be the most acceptable during a sensory evaluation.

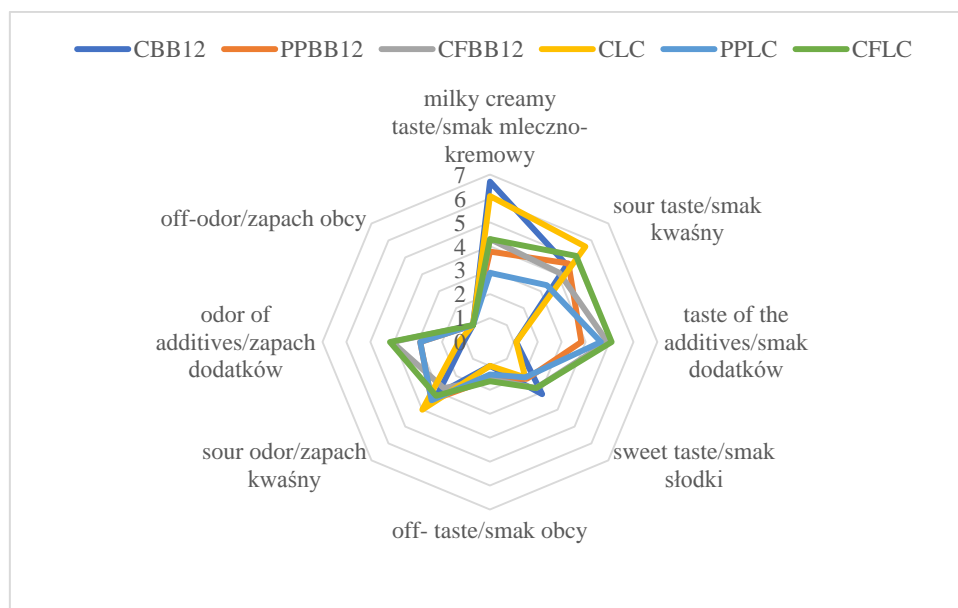


Fig. 4a. Effect of fiber addition on organoleptic parameters of fermented milk after 1 day of cold storage
Rys. 4a. Wpływ dodatku błonnika na właściwości organoleptyczne mleka fermentowanego po 1 dniu przechowywania chłodniczego

Explanatory notes / objaśnienia:

CBB12 – control milk with *Bifidobacterium* BB-12; PPBB12 – milk with 1.0 % psyllium fiber and *Bifidobacterium* BB-12; CFBB1-milk with 1.0 % chokeberry fiber and *Bifidobacterium* BB-12; CLC – control milk with *L. casei*; PPLC – milk with 1.0 % psyllium fiber and *L. casei*; CFLC – milk with 1.0 % chokeberry fiber and *L. casei*; n = 20 for each group

CBB12 – mleko kontrolne z *Bifidobacterium* BB-12; PPBB12 – mleko z dodatkiem 1.0 % błonnika z babki płesznik i *Bifidobacterium* BB-12; CFBB1 – mleko z dodatkiem 1.0 % błonnika z aronii i *Bifidobacterium* BB-12; CLC – mleko kontrolne z *L. casei*; PPLC – mleko z dodatkiem 1.0 % błonnika z babki płesznik i *L. casei*; CFLC – mleko z dodatkiem 1.0% błonnika z aronii i *L. casei*; n = 20 dla każdej grupy

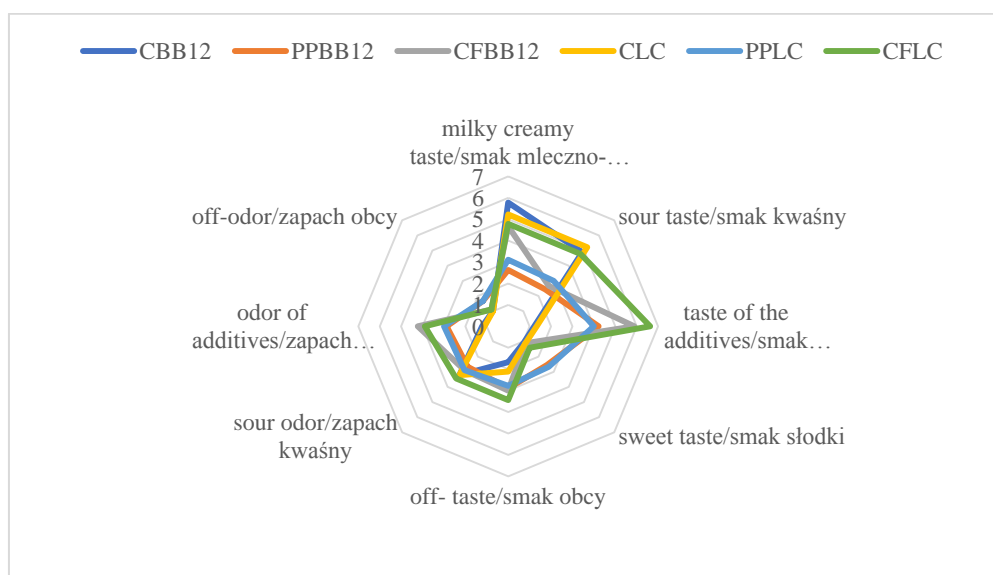


Fig. 4b. Effect of fiber addition on organoleptic parameters of fermented milk after 28 days of cold storage

Rys. 4b. Wpływ dodatku błonnika na właściwości organoleptyczne mleka fermentowanego po 28 dniach przechowywania chłodniczego

Explanatory notes / Objasnienia:

CBB12 – control milk with *Bifidobacterium* BB-12; PPBB12 – milk with 1.0 % psyllium fiber and *Bifidobacterium* BB-12; CFBB1-milk with 1.0 % chokeberry fiber and *Bifidobacterium* BB-12; CLC – control milk with *L. casei*; PPLC – milk with 1.0 % psyllium fiber and *L. casei*; CFLC – milk with 1.0 % chokeberry fiber and *L. casei*; n = 20 for each group

CBB12 – mleko kontrolne z *Bifidobacterium* BB-12; PPBB12 – mleko z dodatkiem 1.0 % błonnika z babki płesznik i *Bifidobacterium* BB-12; CFBB1 – mleko z dodatkiem 1.0 % błonnika z aronii i *Bifidobacterium* BB-12; CLC – mleko kontrolne z *L. casei*; PPLC – mleko z dodatkiem 1.0 % błonnika z babki płesznik i *L. casei*; CFLC – mleko z dodatkiem 1.0 % błonnika z aronii i *L. casei*; n = 20 dla każdej grupy

Conclusions

1. Psyllium fiber reduced syneresis, increased gel hardness, induced color darkening, improved the proportion of yellow and had a favorable effect on the growth and

survival of the *Bifidobacterium* BB-12 and *L. casei* strains, however, did not affect the organoleptic characteristics of the fermented milk.

2. Chokeberry fiber increased syneresis only on the first day of storage, reducing syneresis by 4.0 ÷ 6.0 % on the 28th day. The addition enhanced the proportion of red color, reduced the hardness of the gel and contributed to the desired flavor. The chokeberry fiber provided favorable conditions for the growth and survival of the studied probiotic strains.
3. Natural additives such as chokeberry fiber and psyllium fiber can be used in the production of milk fermented by *Bifidobacterium* BB-12 and *L. casei*.
4. Psyllium fiber can be recommended for its syneresis-reducing properties. However, chokeberry fiber, due to its high polyphenol content, can be used as a natural colorant and taste-enhancing additive.
5. Studies on the use of various fibers in fermented milk production should be continued to determine their technological and health-promoting potential.

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WPLYW DODATKU BŁONNIKA Z BABKI PLESZNIK I BŁONNIKA Z ARONII NA JAKOŚĆ PROBIOTYCZNEGO MLEKA FERMENTOWANEGO

Streszczenie

Wprowadzenie. Błonnik z owoców, warzyw i błonnik innego pochodzenia mogą zwiększać różnorodność mikroflory jelitowej i wykazywać działanie przeciwzapalne. Zastosowanie błonnika do produkcji probiotycznego mleka fermentowanego ma ogromny potencjał, ponieważ może zapewnić szereg korzyści zarówno konsumentom, jak i producentom. Błonnik pokarmowy oprócz poznanych korzyści fizjologicznych może zmniejszać ryzyko wystąpienia chorób cywilizacyjnych. Konsument oczekuje od współczesnej żywności prozdrowotnego działania, a świadomość konsumentcka zwiększa się dzięki coraz szerszemu asortymentowi i kampaniom reklamowym promującym żywność prozdrowotną, co także wykorzystują producenci żywności. Celem badań było określenie wpływu dodatku błonnika babki płesznik i aronii w połączeniu z kulturami probiotycznymi *Bifidobacterium animalis* ssp. *lactis* BB-12 i *Lactocaseibacillus casei* 431 na jakość mleka fermentowanego. W celu zbadania wpływu błonnika na jakość mleka fermentowanego dokonano oceny kwasowości, tekstury, synerезy i barwy, a także przeprowadzono ocenę organoleptyczną i analizę mikrobiologiczną.

Wyniki i wnioski. Do produkcji mleka fermentowanego przez *Bifidobacterium* BB-12 i *L. casei* można zastosować naturalne dodatki takie jak błonnik aroniowy i błonnik babki płesznik. Błonnik babki płesznik można polecić ze względu na jego właściwości zmniejszające synerезę. Natomiast błonnik aroniowy ze względu na wysoką zawartość polifenoli może być stosowany jako naturalny barwnik i dodatek poprawiający smak. Dodatek błonnika, takiego jak aronia czy babka płesznik, pozytywnie stymulował wzrost i przeżywalność bakterii *Bifidobacterium* BB-12 i *L. casei* podczas przechowywania. Twardość żelu mlecznego fermentowanego przez *Bifidobacterium* BB-12 lub *L. casei* uległa znacznej poprawie poprzez dodatek błonnika babki płesznik, zwiększając ją aż o około 2,5 N i tendencja ta utrzymywała się również po 28 dniach przechowywania. Należy kontynuować badania nad wykorzystaniem różnych źródeł

błonnika w produkcji mleka fermentowanego w celu określenia ich potencjału prozdrowotnego i technologicznego.

Słowa kluczowe: mleko fermentowane, aronia, babka płesznik, błonnik, *Lactocaseibacillus casei*, *Bifidobacterium animalis* ☒