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REDUCING THE RISK OF SPOILAGE CAUSED BY *BACILLUS CEREUS* IN COW'S AND GOAT'S MILK YOGURT WITH BERRIES PUREE

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Abstract. The objective of this study was to investigate the impact of adding aronia (*Aronia melenocarpa*), raspberry (*Rubus idaeus*), and strawberry (*Fragaria xananassa*) in the form of 10% puree to a mixture of cow's and goat's milk in order to reduce the risk of spoilage caused by activity of *Bacillus cereus* in yoghurt made from a mixture of cow's and goat's milk. During the fermentation process (360 min.), changes in water activity, pH, moisture content, bacterial growth curve were monitored. The results showed a decrease in water activity, pH, and moisture content. Adding aronia puree to yoghurt yielded the most significant results for water activity modification (0.971-0.868), pH (5.31-4.28), moisture content (85.12-81.55 %), and optical density (0.19-0.34). Also, the investigation of the kinetics of the *Bacillus cereus* grows indicated that the stationary phase was reached at 360 minutes, resulting in a total population of $5.5 \cdot 10^{13}$ c.f.u./g. Microscopic examination revealed Gram-positive strains of lactic acid bacteria, arranged in chains of varying lengths, in an amount of $139 \cdot 10^{2}$ c.f.u./g. Antimicrobial activity showed a zone of inhibition with a diameter of 18.5±0.1 mm in aronia yoghurt, a zone of inhibition with a diameter of 16.2±0.2 mm in raspberry yoghurt and a zone of inhibition with a diameter of 15.2±0.1 mm in strawberry yoghurt compared to classic yoghurt which showed a zone of inhibition with a diameter of 12.1±0.2 mm.

Keywords: *lactic acid bacteria, quality, contamination, fermentation, spoilage, inhibition.*

Rezumat. Scopul lucrării a fost de a studia influiența adaosului de pomușoare de aronia (*Aronia melenocarpa*), zmeură (*Rubus idaeus*) și căpșună (*Fragaria xananassa*) sub formă de piureu în concentrație de 10% în vederea diminuării riscului de alterare produse de *Bacillus cereus* în iaurtul din amestec de lapte de vacă și de capră. În timpul procesului de fermentare (360 min.) au fost monitorizate modificările activității apei, pH-ului, conținutului de umiditate și a curbei de creștere a bacteriei. Rezultatele au arătat o scădere a activității apei, a pH-ului și a conținutului de umiditate. Adaosul piureului de aronia în iaurt a dat cele mai semnificative rezultate ale modificării activității apei (0.971-0.868), pH (5.31–4.28), conținutului de umiditate (85.12-81.55 %) și densității optice (0.19-0.34). De asemenea, rezultatele studiului cineticii creșterii bacteriilor *Bacillus cereus* au arătat că creșterea a ajuns la faza staționară la 360 min., dezvoltând o populație totală de $5.5 \cdot 10^{13}$ u.f.c./g. Examinarea microscopică a evidențiat tulpini gram-pozitive de bacterii lactice poziționate în lanțuri de diferite lungimi în număr de $139 \cdot 10^{2}$ u.f.c./g. Activitatea antimicrobiană a evidențiat o zonă de inhibiție cu diametrul de 18.5±0.1 mm în iaurtul cu aronia, o zonă de inhibiție cu diametrul de 16.2±0.2 mm în iaurtul cu zmeură și o zonă de inhibiție cu diametrul de 15.2±0.1 mm în iaurtul cu căpșună în raport cu iaurtul clasic care a evidențiat o zonă de inhibiție cu diametrul de 12.1±0.2 mm.

Cuvinte-cheie: *bacterii lactice, calitate, contaminare, fermentare, alterare, inhibare.*

1. Introduction

The quality of yogurt is a top priority in the Moldovan economy, and the dairy industry is facing increasing pressure to maintain impeccable sanitary standards in order to prevent economic losses [1]. A high-quality yogurt must be made from milk and starter cultures of exceptional quality, with strict adherence to technological conditions, as milk is a perishable product. Milk, which is rich in proteins, carbohydrates, fats, minerals, and vitamins, provides an ideal environment for the growth and development of microorganisms and can be preferentially altered by lipase-producing microorganisms such as *Pseudomonas, Bacillus, Candida, Geotrichum, Aspergillus, Rhizopus*, among others [2].

Bacteria are known for their metabolic complexity and adaptability, which allows them to thrive in diverse environments by producing enzymes that enable them to use macromolecular organic compounds as a source of nutrition. Bacterial growth is dependent on temperature and can occur over a wide range between 10°C and 90°C, with most food spoilage caused by mesophilic bacteria that can grow at room temperature. Bacteria in their vegetative form can be thermally inactivated at pasteurization temperatures, while endospores can be inactivated at sterilization temperatures [3].

To accurately identify a bacterial species, a variety of tests and criteria must be considered. These may include factors such as dye affinity, family as a taxonomic unit, and content of the same type of bacteria (Gram-positive or Gram-negative). Additionally, physiological characteristics such as assimilation or fermentation of carbohydrates, relationship to oxygen, temperature, pH, and resistance to inhibitors must also be evaluated. These criteria collectively provide a comprehensive understanding of the bacterium being identified and allow for accurate classification within the bacterial taxonomy system.

Representatives of certain genus of Gram-positive bacteria, such as *Bacillus cereus*, have the ability to form highly resistant, dormant structures named endospores. These spore-forming species are referred to as sporulated bacteria that develop within the cell cytoplasm [4]. Bacillus cereus is a Gram-positive, facultatively anaerobic, toxin-producing bacterium that can grow in a moderate temperature range. Most strains are considered mesophilic, with optimal growth temperature at 37°C and survival below 10°C, which makes them easily isolated from food even in cold temperatures. *Bacillus cereus* is associated with gastrointestinal syndrome (GS), which can cause diarrheal disease without significant upper intestinal symptoms, and a predominantly upper GS with nausea and vomiting states without diarrhea [5].

It is well known that pH and water activity (a_w) are important parameters that influence to the replication of bacteria and low-pH foods guarantee bacterial stability. Water activity influences the rates of chemical spoilage reactions because water acts as a solvent, which can change mobility in the environment. Microorganisms can grow in a wide a_w range between 0.62-0.99 depending on their adaptation to the a_w limits that allow them to grow. Thus, for the development of xerophytic microorganisms, the water activity must be within 0.68±0.5, with the development of g. *Xeromyces* and *Aspergillus* moulds, osmotolerant yeasts and halotolerant bacteria; for mesophytes - 0.80±0.4, with the development of fungi and bacteria; for hydrophytes - 0.92±0.5, with the development of bacteria, including *Bacillus cereus*, whose optimum value of water activity for development is 0.93. It is known the behavior of cells in relation to a_w as an intrinsic factor which is conditioned by the amount of free water accessible to carry out vital processes [6].

Lactic acid bacteria are known for their ability to synthesize organic acids and bacteriocins, but the potential of these compounds against them spans a wide variety of genera, including a considerable number of species. The composition of starter cultures for obtaining fermented dairy products includes both lactic acid bacteria in the form of bacilli, represented by the genus *Lactobacillus*, and in the form of cocci represented by the genus *Streptococcus* [7,8].

In the study by Radmehr B. et al [9], the potential growth capacity of *Bacillus cereus* strains in dairy products was evaluated, where it was observed that pasteurized milk is an optimal substrate for its growth due to the inactivation of microorganisms and enzymes. After 5 days of storage, no growth of *Bacillus cereus* was detected in raw milk (<0.5 c.f.u./g) due to the action of natural microflora (mainly formed by lactic acid bacteria), which together with the acidification of the substrate made a remarkable contribution. A similar trend was observed in classic yoghurt, where after 5 days of storage, a very limited growth of *Bacillus cereus* was observed (<1 c.f.u /g) due to the low pH. However, as the authors of the study note, the growth in pasteurised milk after 5 days of storage was 3.08-3.11 c.f.u /g was observed [9].

Effective control of microbial growth in food products is of utmost importance in the agri-food sector. Following studies by Cowan M. [10] and Savoia D. [11], berries were found to possess antimicrobial activity due to the presence of various compounds such as terpenoids, essential oils, alkaloids, phenolic acids, quinones, flavones, flavonoids, tannins and coumarins. Phenolic compounds are the most abundant antimicrobial compounds in berries and each plant species has its own phenolic complex [12]. The effectiveness of phenolic compounds in inhibiting microbial growth is pH-dependent [13] and is caused by organic acids. Aronia [14], raspberry and strawberry [15] fruits are particularly rich in antimicrobial compounds due to their high content of vitamin C, anthocyanins, polyphenols, etc., forming a high antioxidant potential. In this context, aronia shows a polyphenol content of 719±5.71 mg/100 g, while strawberry and raspberry have an almost identical polyphenol content between them, but much lower than aronia: 295.0±8.50 and 288.9±8.84 mg/100 g. Talking about vitamin C content, strawberry contains 46.6±0.30 mg/100 g, raspberry 34.2±0.64 and aronia 40.2±0.61 mg/100 g. Concerning anthocyanin content, aronia is superior: 357±22 mg/100 g, while raspberry and strawberry contain 33.3±0.5 and 35.7±0.5 mg/100 g. These compounds form an antioxidant potential with maximum value in aronia 26.4±0.45, followed by strawberry 15.4±0.45 and raspberry 3.78±0.17 mg AA/g [16].

Biologically active substances are concentrated in various plants, including leaves, fruits, seeds or roots, which provide wellness benefits when consumed continuously. Some research on the incorporation of biologically active substances from fruits, juices or extracts into dairy matrices offers positive effects, as milk is a good medium for their incorporation, thus reducing the use of synthetic additives [17-19]. In yoghurt, the addition of bioactive compounds from fruits has the potential to improve the structure, in addition to being appreciated by consumers [20-24].

The Food and Agriculture Organization (FAO) [25] has issued a report stating that the most common cause of body ailments is the consumption of is contaminated food, and it is also an important cause of the economic decline. The possibility of using berries as a natural bactericide in yoghurt allows the shelf-life to be extended due to their chemical composition, more specifically their content of compounds with enhanced biological value [26]. It is known that compounds with enhanced biological value in an optimal concentration have a strong impact on pathogenic bacteria and their use is a complete option due to their antimicrobial properties. Thus, on the antimicrobial properties of berries influences the diameter of the growth inhibition zones of *Bacillus cereus*, which in aronia can be 17.4...18.6 mm, in raspberry 15.6...16.4 mm and in strawberry 14.5...15.05 mm [27].

Taking into consideration the aspects described, this research aims to investigate the impact of adding aronia (*Aronia melenocarpa*), raspberry (*Rubus idaeus*), and strawberry (*Fragaria xananassa*) in the form of puree to yoghurt made from a mixture of cow's and goat's milk for reduce the risk of spoilage produced by *Bacillus cereus*. In order to achieve this, a number of indicators have to be studied during the fermentation process: changes in water activity, pH, moisture content, bacterial growth dynamics as well as antimicrobial activity.

2. Materials and methods

2.1. Materials

The yoghurt was obtained in the laboratory of the Food Technology Department of the Technical University of Moldova, where the following ingredients were used: cow's milk purchased from the farm ”Ferma cu Origini” SRL from the village of Horăști, district of Ialoveni [28], goat's milk purchased from the farm ”Vilador” SRL from the village of Slobozia-Măgura, district of Sângerei [29], starter culture Lyofast YAB 205 *(Streptococcus thermophillus*, *Lactobacillus delb. Bulgaricus*) purchased from ”Ingreda” SRL [30], aronia berries (collected from plantations in the village of Cuizăuca, Rezina district), raspberry (collected from plantations in the village of Elizavetovca, Donduseni district), strawberry (collected from plantations in the village of Sadova, district of Călărași) [31], sugar (purchased from the ”Suedzucker Moldova” company) [32], nutrient agar medium M001 (HiMedia Laboratories) and the bacterial culture *Bacillus cereus* M091 (HiMedia Laboratories) [33]. During the yogurt production, the consecutiveness of all steps, the technological process regimes (by thermostat method) and hygienic conditions were respected [34].

2.2. Preparation of berries puree

The berries puree was obtained according to the technology described by [16].

2.3. Preparation of yogurt with addition of berries puree

The yoghurt with added berries puree was obtained according to the technology described by [16]. Coding of yogurt is: P1 (control sample), P2 (yogurt with aronia puree), P3 (yogurt with raspberry puree), and P4 (yogurt with strawberry puree).

2.4. Methods

The physico-chemical parameters of the yoghurt were determined during fermentation for 360 min at 120 min intervals. The growth dynamics of *Bacillus cereus* in the yoghurt were examined for 900 min to determine the number of cells during the different growth periods in order to determine the optimum growth point of the culture.

2.4.1. Determination of physico-chemical indices of berries yogurt

pH. The pH of yoghurt samples was determined by the method described by [35] with a TESTO 205 portable pH meter.

Water activity. The water activity was determined according to the method described by [36] using the Novasin LabSwift.

Moisture content. This parameter was determined by the method of AOAC [37].

***2.4.2. Determination of microbiological indices* yogurt with addition of berries puree**

Number of bacteria. Inoculation of the diluted samples of the finished product on a nutrient agar medium was performed to determine the number of bacteria. The dilution method was used in order to count more clearly the colonies that will grow after noculation. The results were expressed in colony-forming units per gram (c.f.u./g) [38].

Monitoring of lactobacilli growth. For the determination of growth of the population of lactic acid bacteria, diluted (10^{-2}) yogurt samples were inoculated on a nutrient agar medium. To monitor this growth in the fermentation medium, a colorimetric determination was made on a Heidolf spectrophotometer (Λ = 600 nm) after prior dilution with distilled water. For this purpose, 1 ml of yogurt is diluted with 9 ml of distilled water, shaken and the extinction read on the spectrophotometer in a 1 cm cuvette with distilled water. The growth of lactobacilli was studied twice by measuring optical density (OD) at λ= 600 nm. The method is described according to [39].

Growth Rate of bacteria *Bacillus cereus species*. The methodology for this experiment was based on the work of Lambert et al [40]. The sequence of growth phases of a periodic culture of *Bacillus cereus* was investigated to study the growth and die-off patterns and to determine the duration of each phase: lag, exponential, stationary and die-off phases. The studies were carried out over a 24-hour period.

Antimicrobial activity of yogurt samples was performed using the disk diffusion method [41]. 15 mL of nutrient agar medium was added to the Petri plate. After solidification, 3 indentations were created in each Petri plate using a sterile tube of 5 mm diameter. The medium was intentionally contaminated with *Bacillus cereus* culture. 1 g of each sample was introduced under sterile conditions into the recesses, with subsequent thermostatted at 37^{o} C for 72 h. The zone of inhibition that appeared as a transparent area around the disk was measured using the vernier caliper.

2.5. Statistical analysis

The analysis of the variance of the results was performed by applying the Student test and the version of the Microsoft Office Excel 2010 program. All tests were performed in triplicate. Experimental results are expressed as mean ± SD.

3. Results and Discussion

3.1. Modification of some physicochemical indices during the fermentation of yogurt with addition of berries puree

Amplification of the microbiota is due to the presence of free water, which acts as a solvent, as a reaction medium for cellular enzymes, and for the bidirectional transport of metabolic products. Water activity (a_w) has a direct influence on the growth of the microorganisms, as it accelerates the growth of yeasts, molds, and bacteria; it favors their production of toxins and participates in various chemical and biochemical reactions that can destroy: texture, color, odor, appearance and nutritional value of yogurt on storage [42]. Figure 1 represents the aw variation during fermentation, where a decrease is observed in all samples, and the values that were recorded in P1 (0.971-0.868), P2 (0.975-0.875), P3 (0.974-0.873), P4 (0.973-0, 871).

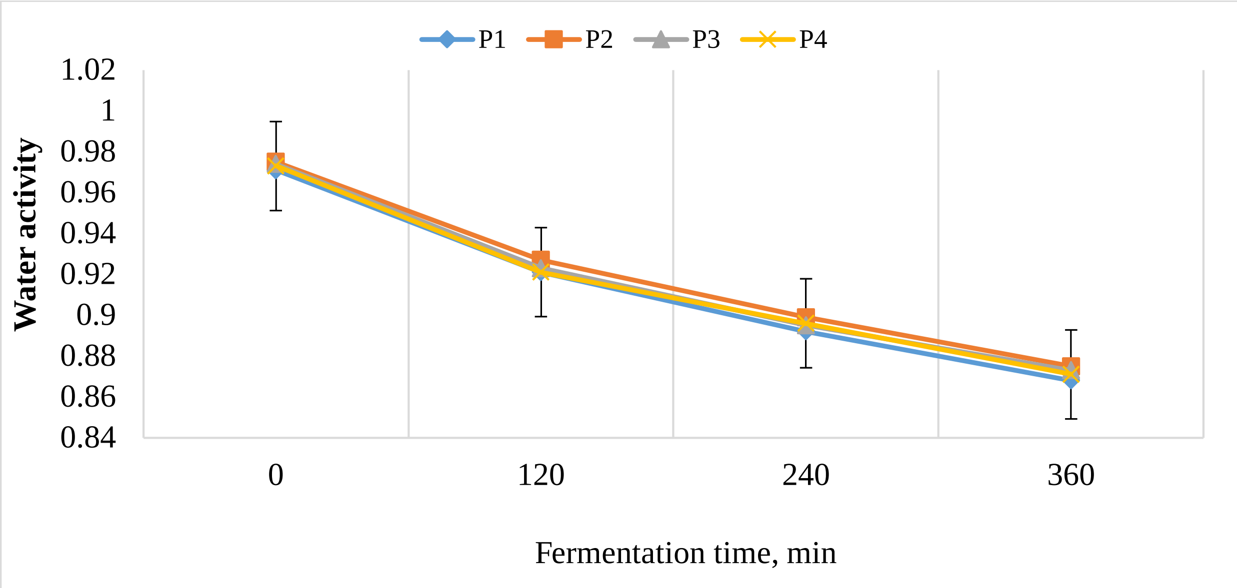

Figure 1. Modification of water activity of yogurt samples during fermentation: P1-control sample, P2-aronia yoghurt, P3-raspberry yoghurt and P4-strawberry yoghurt.

The obtained data support the hypothesis of [43] that water was so tightly bound to a protein molecule that it could not participate in the hydrolysis reaction, thus the amount of water was reduced and the fermentation process contributed to the preservation of the yoghurt.

Table 1 represents the results of the pH evolution of the yoghurt sample during fermentation, where it can be seen that the pH decreasing trend was attributed to the activity of the microorganisms which was due to sugar consumption and organic acid production. Berries contain several acidic compounds, such as citric, malic, acetic, tartaric, succinic and phosphoric, hence these acids contributed to lower pH values in samples with berries [44].

Table 1

pH evolution of yoghurt samples during fermentation

Sample	Time, min.				Correlation coefficients pH-time
	0	120	240	360	
P1	5.63±0.03	5.22±0.02	5.09±0.01	4.38±0.02	R^2= 0.8554
P2	5.31±0.02	5.18±0.01	5.01±0.02	4.28±0.03	R^2= 0.9964
P3	5.27±0.03	5.16±0.03	4.98±0.02	4.25±0.01	R^2= 0.9938
P4	5.19±0.02	5.10±0.01	4.95±0.03	4.27±0.03	R^2= 0.9725

Note: P1-control sample, P2-aronia yogurt, P3-raspberry yogurt, and P4-strawberry yogurt. Results are expressed as mean ± standard deviation, insignificant ($p > 0.05$).

The recorded values of the samples are P2 (5.31-4.28), P3 (5.27-4.25), P4 (5.19-4.27) in comparison with control sample P1 (5.63-4.38). The observations of yogurt in the present study are in agreement with the results of similar studies investigated by Fitratullah et al.

[45] on the development of yogurt incorporated with red dragon fruit. A decrease in the pH of yogurt was also obtained in his study, where it is observed that the addition of fruit showed a pH value ranging from 5.21 to 4.28.

Moisture is an important aspect of food, present in various vegetal and animal products as a cellular and extracellular component, as a dispersion medium and solvent that determines texture and structure, but also affects appearance, taste, and stability [46]. Figure 2 indicates the modification of the moisture content during fermentation.

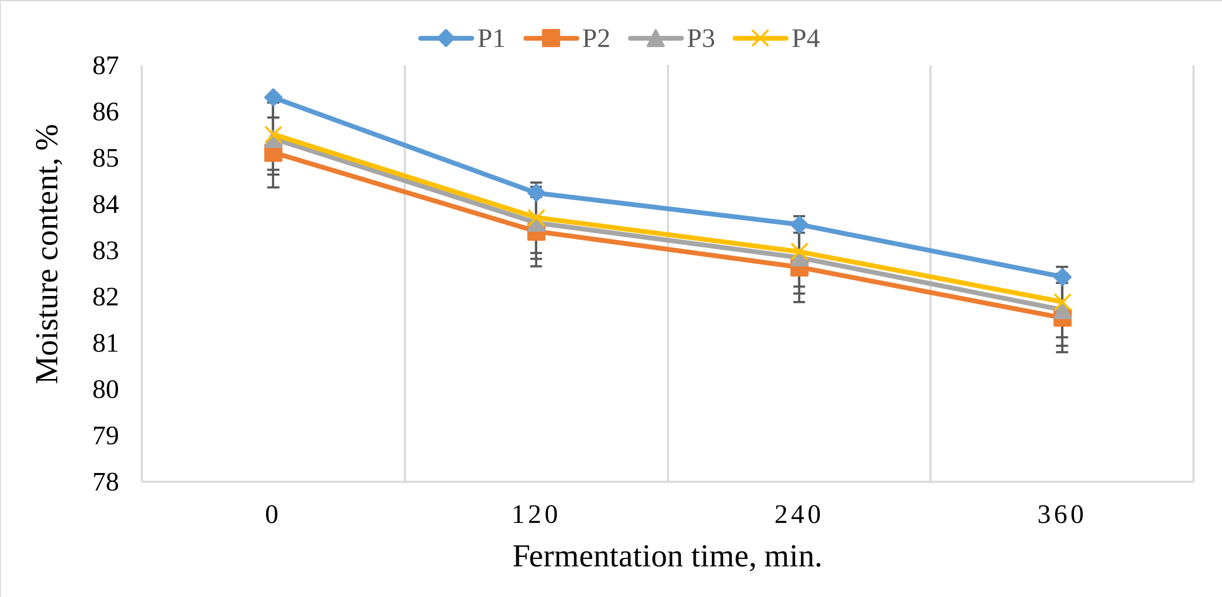

Figure 2. Reducing of moisture content or yogurt samples during fermentation: P1-control sample, P2-aronia yoghurt, P3-raspberry yoghurt and P4-strawberry yoghurt.

The results obtained for all samples show a reduction in moisture content and have the following values: P2 - 85.12-81.55%, P3 - 85.42-81.72%, P4 - 85.51-81.89% in comparison with control sample P1 - 86.31- 82.43%. This decrease in moisture content is due to casein argumentation was in the isoelectric state in which the activity of water particles decreased, not affecting the hydrolysis efficiency. These results are in agreement with those of Suriasih K. [47], who reported that the moisture content of yogurt resulted in acceleration of starter culture to fermentation. However, the results of the present study are consistent with the results of De Silva and Rathnayaka [48], where he reported that the moisture content of fruit yoghurt ranged between 79.18% and 81.15%, respectively.

3.2. Investigation of the dynamics of *Bacillus cereus* growth in yogurt with addition of berries puree

Contamination of food with *Bacillus cereus* can be attributed to the presence of persistent spores that can be transferred via soil particles or dust and can survive in extreme conditions such as heat [49]. Despite the fact that initial contamination is usually low, spores can germinate under improper storage conditions and bacteria can subsequently proliferate. The growth range of *Bacillus cereus* is between 7 and 40 °C, but *Bacillus cereus* may contain thermotolerant spores capable of growing even at higher temperatures, but at lower temperatures, microbial growth is, however, significantly slower [50]. Heat treatment, such as pasteurisation (at least 70°C for 2 minutes), is necessary to inhibit vegetative forms of *Bacillus cereus* [51,52]. Figure 3 represents the investigation of the Bacillus cereus growth curve on the number of colonies grown.

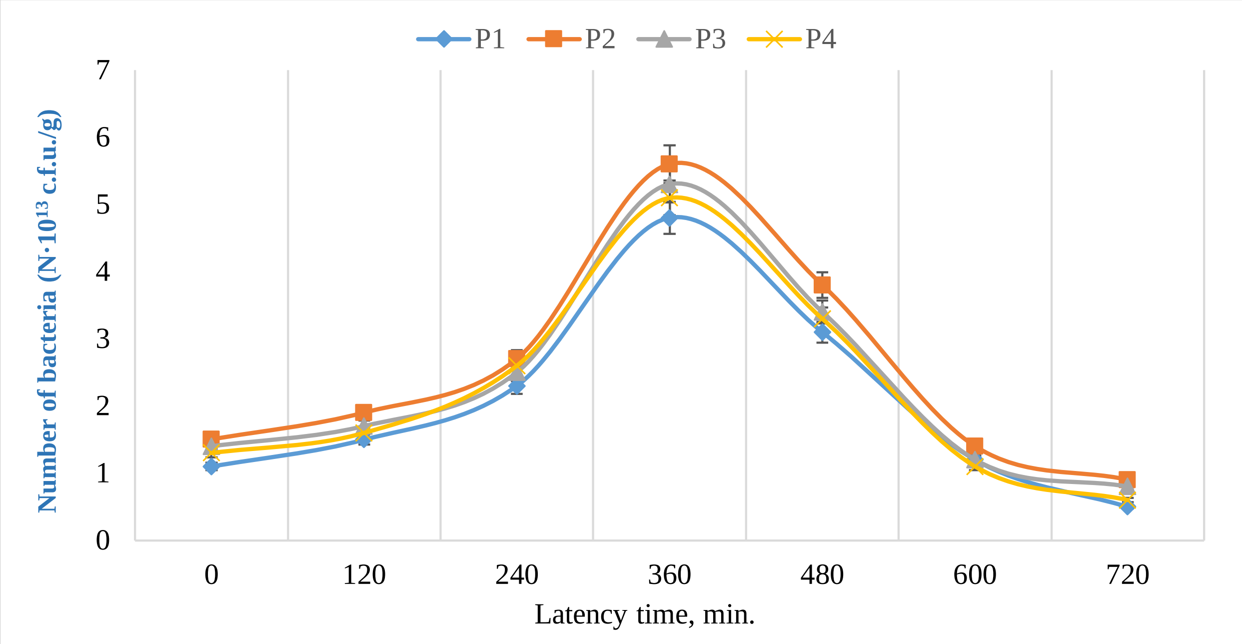

Figure 3. Growth curve of *Bacillus cereus* in yogurt samples during fermentation: P1-control sample, P2-aronia yoghurt, P3-raspberry yoghurt and P4-strawberry yoghurt.

The results showed that in all samples the growth of bacteria reached the stationary phase at 360 min of fermentation of yogurt, reaching a total population of $5.5\text{-}10^{13}$ c.f.u./g in P2; $5.3\text{-}10^{13}$ c.f.u.//g in P3; $5.1\text{-}10^{13}$ c.f.u.//g in P4, compared to the control sample P1 with a total population of $4.8\text{-}10^{13}$ c.f.u.//g.

Lactic acid bacteria are a specialized group of microorganisms that catalyze the process of acid lactic fermentation by metabolizing carbohydrates to lactic acid [53]. These bacteria have a high nutritional demand and thrive in chemically complex environments [54,55]. The findings depicted in Figure 4 demonstrate that the addition of berry puree (i.e., aronia, raspberry, and strawberry) in combination with lactic acid bacteria had a comprehensive effect on mitigating the risk of spoilage associated with *Bacillus cereus*, as anticipated.

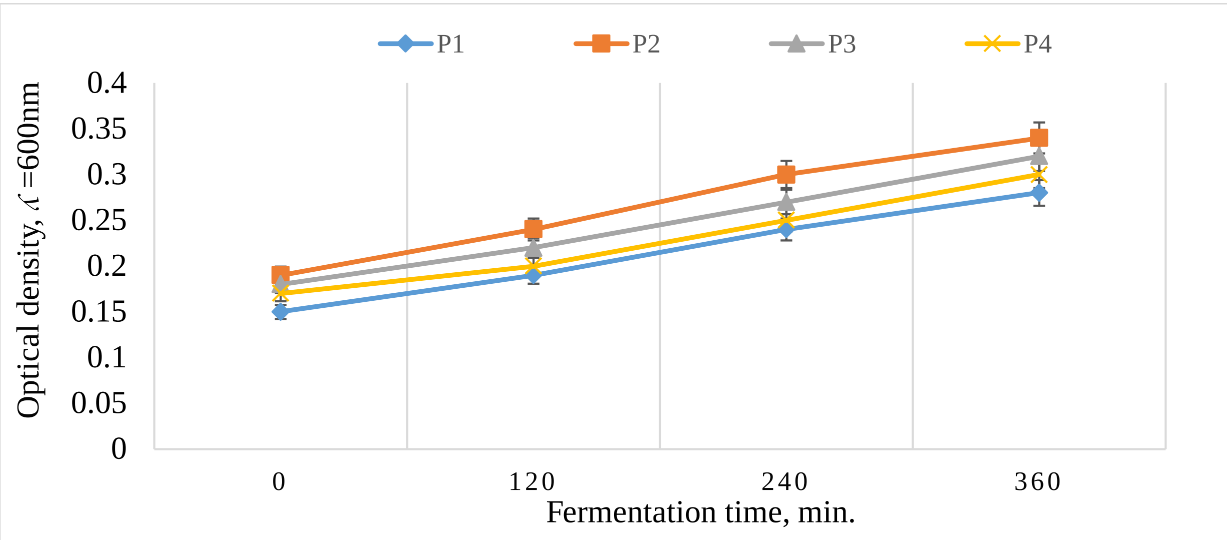

Figure 4. Variation of optical density in yogurt samples during fermentation P1-control sample, P2-aronia yoghurt, P3-raspberry yoghurt and P4-strawberry yoghurt

When fermenting yoghurt, the optical density increased in all yoghurt samples because a synergism was formed between the lactic acid bacteria and the chemical composition of the added, which kept the risk of spoilage associated with of *Bacillus cereus* under control. The decline of pH was due to the activity of lactic acid bacteria to produce energy through the fermentation process and by breaking the substrate into simpler components. At the same time, the intensive growth of bacteria did not lead to excessive lactic acid accumulation, favorably influenced the physico-chemical parameters of yogurt. The highest proportion of optical density increase was observed in P2-0.34, followed by P3-0.32, and P4-0.30, in comparison with control sample P1-0.28. These findings are also reflected in Mantzourani's research [56] suggesting that pomegranate juice is an effective natural preservative in yogurt puree because lactic acid bacteria can alter nutrient compounds and bioavailability through their metabolism and interactions with gut microbiota and the immune system. Furthermore, lactic acid fermentation can increase the antioxidant activity of phenolic derivatives beyond that of their precursors [57,58]. In Terpou [59] and Di Cagno's [60] study it is reported that lactic acid fermentation phenolic compounds provide higher antioxidant activity than its predecessors. Thus, when bioconverting phenolic compounds, lactic acid fermentation can positively transform food components that have a strong influence on consumer welfare. In this context, it has been shown that fruits have a rich content of biologically active substances, which simultaneously grant enhanced bioavailability when fermenting milk by lactic acid with *Lactobacillus spp.* [61-63].

Microscopic examination of the prepared dried and fixed Gram stained smears revealed Gram-positive strains of lactic acid bacteria arranged in different chain lengths of bacilliform (*Lactobacillus lactis*) and cocciform (*Streptococcus thermophilus*) microorganisms. The most representative sample was P2, with a colony count of $139 \cdot 10^2$ c.f.u./g. The mechanisms regulating the number, type, and metabolic activity of bacteria are highly complex. However, some bacteria (of the *Lactobacillus* and *Streptococcus* genus) act antagonistically towards pathogenic bacteria. Both bacterial species are found in the microbiota of the gastrointestinal tract, but their numbers decrease with age, alcohol consumption, antibiotic administration, stress, and other factors [64,65]. The study by Kim K. H. et al. have shown that the addition of cherry blossoms to yogurt enhances the growth of lactic acid bacteria [66], but the study by Oh H. et al. [67] revealed that the addition of acanthopanax powder to yogurt inhibits the growth of lactic acid bacteria.

3.3. Analysis of the bactericidal potential of yogurt with addition of berries puree

As microbial resistance to antibiotics continues to increase and interest in eco-friendly lifestyles grows, there is a growing focus on natural antimicrobial compounds [68]. Inhibition of microbial growth has been discovered in a wide range of food plants, including various berries and fruits [69,70]. One of the functional properties of yogurt is its antimicrobial activity, which is produced during the fermentation process. The presence of antimicrobial activity, as indicated by the diameter of the inhibition zone, indicates that the tested bacteria cannot grow around the indentations. Inhibiting the growth of pathogenic and conditionally pathogenic microorganisms is an important microbiological property for ensuring the quality and safety of yogurt [71,72]. In Nohynek L.J.'s study it is reported that there is a direct correlation between the phenolic content of fruits and their antimicrobial effects [73].

The results obtained are in agreement with those of Naidu et al. [74] which confirm that the antibacterial activity of yogurt increased with decreasing pH value, as the unbound lactic acid penetrated the cell membranes and cytoplasm. The antibacterial mechanism is mediated through several pathways, such as disruption of cell wall formation, reaction with cell membranes, and inhibiting enzyme activity that causes disrupting cellular metabolism and bioactive components that interfere with the nucleic acid formation, damaging genetic material and disrupting the cell's cleavage process. The ability of antimicrobial compounds to inhibit microbial growth in the food system can be influenced by physical factors such as: acidity, pH, oxygen availability, temperature as well as the interaction between these factors [75].

The different zones of inhibition (Figure 5) of samples P2 (18.5±0.1 mm), P3 (16.2±0.2 mm), and P4 (15.2±0.1 mm) in comparison with the control sample (12.1±0.2 mm) can be explained by the chemical composition of the aronia [76], raspberry or strawberry berries [77], being representative for the content of antioxidants, polyphenols, vitamins, anthocyanins, organic acids, etc. and based on their beneficial effects on cytoplasmic membrane destabilization, cell membrane permeability, enzyme inhibition, direct action on microbial metabolism or inhibition of microbial growth of the substrate, especially mineral substances.

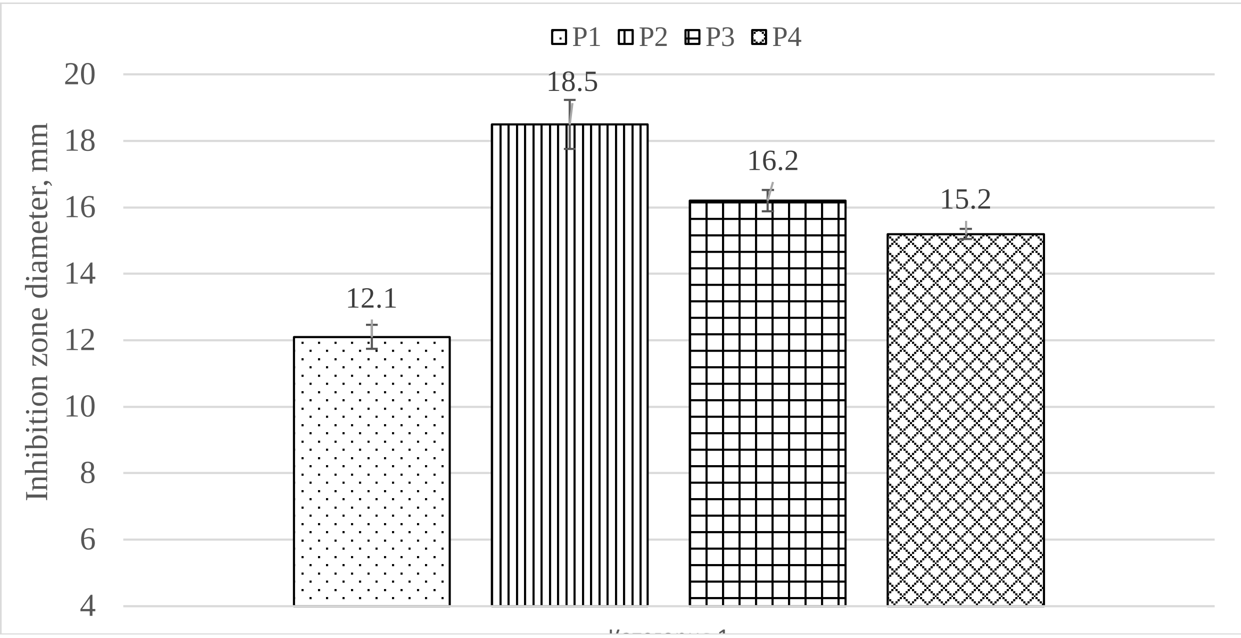

Figure 5. Antimicrobial activity of yogurt samples
P1-control sample, P2-aronia yoghurt, P3-raspberry yoghurt and P4-strawberry yoghurt.

In the study conducted by Vicenssuto et.al. [78], it was observed that the addition of fruit to yogurt resulted in an increase in its antioxidant activity. Furthermore, the presence of phenolic compounds in the fruit helped in inhibiting the oxidation of lipids, which is responsible for the development of unpleasant flavors in yogurt. This is due to the ability of phenolic compounds to donate electrons to neutralize the chain reaction initiated by free radicals, thereby extending the shelf life of yogurt. Additionally, the acidic environment of yogurt with a pH of 4.6 facilitated the interaction of phenolic compounds with yogurt, making it an excellent carrier of these compounds. Hence, the incorporation of phenolic compounds from fruits had a positive impact on the antioxidant properties of yogurt.

4. Conclusions

This study indicated that berries could be applicable in food as a source of antibacterial products. The incorporation of 10% berries puree to yogurt, derived from cow and goat milk, exhibited promising functional properties through its antimicrobial activity.

The findings suggest that pH was not the only determining factor for the antimicrobial activity observed in the puree. While organic acids are known for their strong antimicrobial effect, the whole complex of biologically active compounds present in the puree was found to play an important role in providing the observed antimicrobial properties. Aronia puree, in particular, demonstrated exceptional potential as a natural preservative due to the presence of several antimicrobial compounds that act as a barrier against harmful bacteria, thereby prolonging the shelf life of yogurt. Fermenting lactic acid bacteria with aronia and incorporating it into yoghurt increases aronia's polyphenolic and antioxidant capacity, creating a blend of prebiotic and probiotic compounds with enhanced benefits. The microbiological analysis of yogurt revealed the significant potential of berries to mitigate the risk of spoilage caused by *Bacillus cereus* activity. Overall, this study highlights the potential of Moldovan berries as a natural source of antimicrobial compounds for enhancing the safety and shelf life of dairy products.

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