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THE ROLE OF BERRIES IN QUALITY AND SAFETY ENSURING OF GOAT'S AND COW'S MILK YOGHURT

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Abstract. The yogurt was obtained from a combination of 50% goat's milk and 50% cow's milk with the inclusion of scald fruits of aronia (*Aronia melanocarpa*), raspberries (*Rubus idaeus*), strawberry (*Fragaria xanassa*). Physico-chemical and microbiological indices were determined, according to standard methods, after manufacture and storage, after 1, 5, 10, 15 days. Compared to other samples, yogurt with aronia showed the best values of the dynamics specific to the development of microorganisms: $2.93.10^7$ cfu/ml; the growth rate of lactic acid bacteria at fermentation 0.95 μ; physico-chemical indices: titratable acidity 85 ± 0.078ºT, pH 4.28 ± 0.002, water activity 0.875 ± 0.025; total dry matter 18.45 ± 0.31%, viscosity 2500 ± 0.023 mPa s, ash content 0.89 ± 0.10% and the optical density 2.531 ± 0.054 nm. Yeasts and molds were not detected in any of the samples. From a physico-chemical point of view, in storage, in all fruit yogurt samples the titratable acidity showed increasing values, pH remaining in the range of permissible values. In storage fruits formed an association to control the microbiological risk and stability of yogurt. Fruit yogurt shows a synergism with *Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus, Lactococcus lactis subsp lactis biovar diacetilactis*. The overall Pearson coefficient (Pc = f(pH and MC) for all fruit yogurt samples is -0.95066.

Keywords: *fermentation, growth curve, lactic acid, lactic acid bacteria, metabolic process, microbial counts (MC), starter culture, synergism.*

Introduction

Nutritionists call yogurt a food product that has a high nutritional value, especially due to its low lactose content and high calcium content, as well as positive bioactive effects, due to prebiotic ingredients and probiotic bacteria contained [1].

Yogurt is obtained by lactic acid fermentation of milk under the action of lactic acid bacteria, which has a significant impact on health because in the fermentation process bioactive peptides are released [2].

Consumption of yogurt reduces blood cholesterol levels [3], has an antihypertensive and protective effect on the bone system [4]. The balance of lactic acid bacteria contained in yogurt are important in maintaining intestinal health and could help protect against cancer and coronary heart disease [5]. Yogurt has higher antioxidant properties compared to milk, by releasing biopeptides that monitor the hydrolysis of α-casein, α-lactalbumin and β-lactoglobulin [6].

The fermentation process helps to break down large organic molecules into simpler ones by the action of microorganisms [7, 8] and obtaining a safer yogurt [9, 10]. The activity of microorganisms plays a significant role in fermentation, showing changes in physicochemical properties [11]. Fermenting microorganisms are the main factors influencing the quality of yogurt [12]. Lactic bacteria are the dominant microbiota, responsible for the beneficial effects of yogurt [13, 14].

In order to extend the shelf life of yoghurt [15] and to give it a more pleasant taste [16], various stabilizers, preservatives and synthetic flavors are added to its composition, which often affect human health and inhibit the nutritional properties of yoghurt [17]. The use of berries (aronia, raspberry and strawberry) could be a healthy alternative to replacing synthetic products as preservatives. At the same time, the chemical composition of the fruits [18, 19, 20] has a direct impact on the quality of the yogurt-finished product [21, 22].

Current research has been made in search of a bactericidal substance and the lactic acid production for a potential bio-preservative. The proteins present in milk act as antimicrobial peptides precursors, improving the natural defense capacity by eliminating pathogenic microorganisms. The study demonstrates the possibility of using berries in yogurt as natural preservatives, as they can inhibit the growth of pathogenic bacteria by using biologically active substances as components for decomposition and oxidation reactions.

The aim of the research is to evaluate the physico-chemical and microbiological characteristics of goat's and cow's milk yogurt with berries. In order to achieve the goal, the following objectives were proposed:

1. Research on the physico-chemical indices of yogurt with selected cultures of *Lactobacillus lactis* and *Streptococcus thermophilus* during fermentation and during its storage.
2. Study of the scald berries addition influence on the quality of the fermentation process and the samples storage time investigated by microbiological aspects.

Materials and methods

Materials

Preparation of fruits pulp

The fruit puree was obtained according to the following manufacture stages: sorting by removing non-conforming fruits and inedible parts, washing by removing all impurities, drying, cutting, heating the pulp of hard fruits, and passing through a sieve to remove inedible parts, mixing until a homogeneous mass, heating at 95-98°C for 5 minutes, immediately pouring the fruit puree into sterile containers, cooling the packaged product, storing.

Preparation of fruit yogurt

To prepare fermented yogurt the goat and cow milk sample was received from the local farm. Before fermentation, the goat's milk was pasteurized at 85°C for 10 minutes and cow´s milk was pasteurized at 95°C for 15 minutes after which it was cooled to the inoculation temperature 42 °C. For the yogurt manufacture the Lyofast YAB 205 starter culture was used for inoculation. Inoculation was done by direct inoculation of the culture in the required amount of milk calculated depending on the milk volume. The contents were mixed for 5 minutes with sterile blender for better dispersion of the culture in the medium. In the inoculated milk mixture, the fruit puree was added, then transferred to the fermentation chamber for 6 hours. The end of the coagulation process was determined by the pH value and coagulum firmness. When the fermentation process was completed, all samples were taken to the refrigerator for storage at a temperature of 4 ± 2 ° C until the next control measurement.

The yogurt assortment obtained is presented in table 1:

Table 1

Notify the probe

Sample code	Sample description
P1	50% goat's milk + 50% cow's milk, control sample
P2	45% goat's milk + 45% cow's milk + 10% aronia.
P3	45% goat's milk + 45% cow's milk + 10% raspberries.
P4	45% goat's milk + 45% cow's milk + 10% strawberry.
Sample code	Sample description
P1	goat's milk and cow's milk (50%:50%), control sample
P2	goat's milk and cow's milk (45%:45%) + 10% aronia.
P3	goat's milk and cow's milk (45%:45%) + 10% raspberries.
P4	goat's milk and cow's milk (45%:45%) + 10% strawberry.

Method

Physico-chemical methods

Titratable acidity determination consists in the neutralization of acidic substances in milk with 0.1 n NaOH (KOH) solution and phenolphthalein as indicator [23]. The calculation formula is:

$$\text{Acidity } (^{\circ}T) = 10 \cdot V \tag{1}$$

*where:*V- is the volume of 0.1 N NaOH used in the titration.

Active acidity determination consists in determining the milk pH value using glass electrodes [23].

Water activity. To determine the activity of the water was used LabSwift, it is a portable equipment of high precision, designed by Novasin to measure the activity of the product. Novasin combines modern technology, speed and measurement accuracy. With an SD card for data storage [24].

Total dry matter content. Standard method of oven drying at 102±2°C until a constant mass of the dry residue is obtained [25].

Viscosity was determined using the "Brookfield DV - III" rheometer, with indicator no. 04, 250 rotations / min, data were read after 30 seconds of rotations [26].

Ash content was determined by the direct heating method (standard method), [27]. The ash content was calculated according to the formula.

$$\%\ \text{Ash} = \frac{Z-X}{Y-X} \times 100 \tag{2}$$

where: X = weight of the empty crucible;
Y = crucible weight + sample;
Z = crucible weight + ash.

Determination of the total protein content. The total protein contents of the were measured using the Kjeldahl method [28].

Microbiological methods.

Growth rate of lactic acid bacteria The methodology is based on the work of Lambert and others [29, 30]. The most common way to assess microbial growth in solution is the measurement of the optical density at 600 nm, or short OD_{600}. The method is described according [31 - 34].

To monitor the development of bacterial cells in the fermentation medium, a colorimetric determination is made on a Heidolf spectrophotometer (λ = 600 mm) after prior dilution with distilled water. For this purpose, 1 ml of homogeneous fermentation medium is diluted with 9 ml of distilled water, shaken and the extinction is read on a spectrophotometer in a 1 cm cuvette from the control represented by distilled water. The optical density is calculated according to the following formula:

$$DO = A_{600} \times 10, \quad (3)$$

where: A_{600} – extinction cited at 600 nm;
10 – sample dilution.

It is noted that during the fermentation process other dilutions may be used, depending on the growth capacity, in order to be able to perform the measurements [32].

Monitoring of growth. The growth of lactobacilli was studied twice by measuring optical density (OD) at λ = 600 nm and pH value. The method is described according [35] and the formula:

$$\mu = \frac{\ln X - \ln X_0}{\Delta t}, \quad (4)$$

where: X — optical density in the end of the exponential growth phase,
X_0 — optical density in the beginning of the exponential growth phase,
Δt — the time interval between observations

Determination of lactic acid by titration. The amount of lactic acid was calculated depending on the amount of NaOH used to determine the acidity, taking into account that 1 ml of 0.1 N NaOH corresponds to 0.009008g lactic acid [36].

Determination of the total number of microorganisms. The method is described according [37].

Determination of the number of yeasts and molds. AOAC Official Method 2014.05 Enumeration of Yeast and Mold in Food. 2015 AOAC INTERNATIONAL [38].

Determination of lactic acid bacteria using d eManRogosa Sharpe (MRS) agar. The lactic acid bacteria (LAB) in the yogurt were determined using deMan Rogosa Sharpe (MRS) Agar as described by Oxoid Manual [32] and the formula:

$$C_{fu/ml} = average\ count \times diluation\ factor\ (DF), \quad (5)$$

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.

Results and discussions

The technology of making yogurt, used in research, is based on the addition of the selected culture, consisting of two lactic acid bacteria: *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in a ratio of 1: 1 [39]. The inoculum was used in the form of a mixed culture, which has biotechnological properties corresponding to obtaining a quality yogurt. By using pure culture, a double effect was obtained - technological and hygienic. The technological effect consists in the fact that by the optimal concentration of specific microorganisms in milk the desired acidity and curd for yogurt was obtained. The hygienic effect was manifested by the creation of a favourable microflora dominance over resistant milk contaminants and in the yogurt manufacturing process contaminants.

The evolution of milk fermentation to obtain fruit yogurt

Milk fermentation is one of the most important phases of the yogurt manufacturing process that depends on creating the right conditions (temperature, time) for the development the specific microflora and plays an essential role in the transformation of milk into yogurt [40]. *Lactobacillus bulgaricus* has a strong tolerance to oxygen, so the lack of oxygen or the presence of oxygen in small quantities leads to its slow growth. *Streptococcus thermophilus*, being an active lactic acid producer, quickly performs the fermentation process at the optimum growth temperature (40 - 42°C) [41].

During fermentation, the fruit yogurt samples pH was determined at certain time periods (initially, 2, 4, 6 hours), in order to follow the evolution of the fermentation process. The addition influence of aronia, raspberry and strawberry fruits on the yoghurt fermentation process was evaluated, indicating different pH values depending on the type of fruit added.

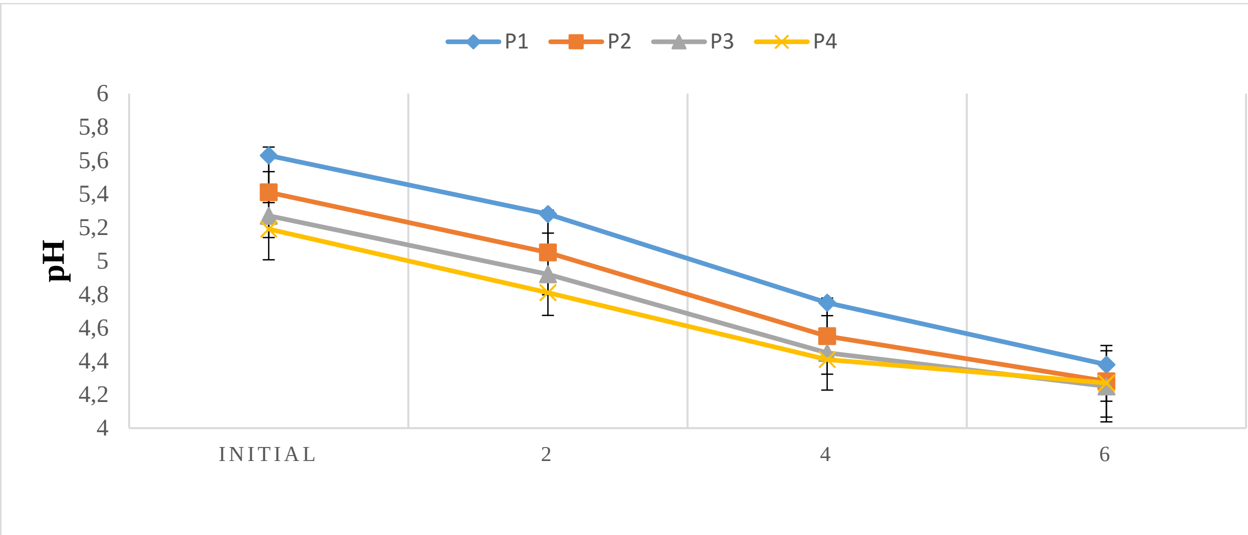

Figure 1. Fermentation time of yogurt samples, h.

From the data presented in figure 1, a specific dynamics of the microorganisms development according to the growth curve is observed. At the stopping time of the fermentation process, the pH value indicates results that the process is finished, the number of lactic microorganisms is at the peak of development, because they consume lactose as an energy source, and as a result the pH value decreases. The results obtained for the yogurt samples during fermentation varied in each sample, P2 (5.31 ± 0.002 - 4.28 ± 0.003), P3 (5.27 ± 0.003 - 4.25 ± 0.001), and P4 (5, 19 ± 0.002 - 4.27 ± 0.003) relative to P1 (5.63 ± 0.003 - 4.38 ± 0.002).

Due to its complex chemical composition [42], goat's or cow's milk [43] used in the yogurt manufacture is an excellent environment for the development of many microorganisms, lactic acid bacteria having favorable conditions. Goat's milk compared to cow's milk, in addition to a rich nutritional value, has better antimicrobial properties to pathogenic microorganisms. The antimicrobial properties of goat's milk are exerted by the lactoperoxidase system, which acts as a magnet for iron ions, thus depriving food pathogenic bacteria [44].

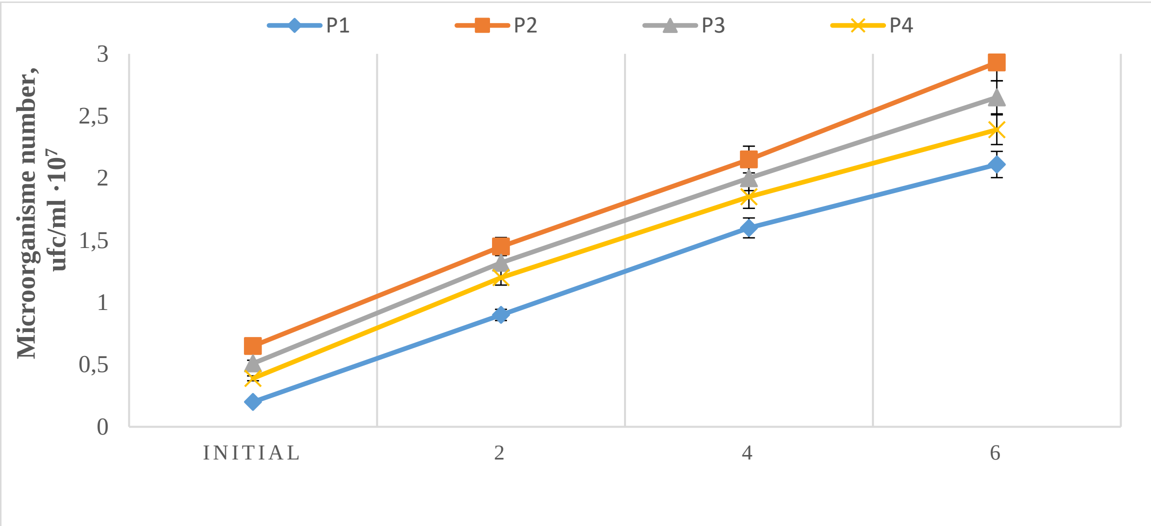

Figure 2. The growth curve of lactic acid bacteria in yogurt.

During fermentation the number of microorganisms increased exponentially and reached a maximum number at 6 hours (figure 2). The results obtained for P2 constitute 0.65 · 10^7 - 2.93 · 10^7 in relation to the control sample P1-0.20 · 10^7 - 2.11 · 10^7, P3 has values of 0.51 · 10^7 - 2.65 · 10^7, and P4 values of 0.39 · 10^7 - 2.99 · 10^7. These results can be explained by the fact that the production of hydrogen peroxide by *Lactobacillus bulgaricus* partially damaged *Streptococcus thermophilus* cells and by the symbiotic relationship of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* stimulates the increase in the number of lactic acid bacteria [45].

Accumulation of lactic acid bacteria in fruit yogurt

The yield of lactic acid production is the main indicator of the starter cultures activity, which is dependent on the biotechnological properties, but also on the physico-chemical and biological conditions [46]. The lactic acid accumulation reduces the ionization of the acidic functions of casein. The caseins average isoelectric point leads to the neutralization of electric charges, the sequestering power of α_s and β caseins against minerals decreases and the solubilisation of calcium and micellar phosphate takes place [47].

The difference in the lactic acid bacteria growth rate in the researched samples is probably due to the fact that the addition of berries constitutes an improvement of the bacterial growth medium. It was found that, in the control sample, the growth rate of lactic acid bacteria during the fermentation period for 6 h was P1 - 0.83 μ and for P2 - 0.95 μ, P3 - 0.93 μ, respectively P4 - 0,90 μ.

Table 2

Accumulation of lactic acid bacteria in fruit yogurt

Fermentation time / Evaluated parameters	P1	P2	P3	P4
		Initial (0h)		
Lactic acid quantity, $g.dm^{-3}$	2.53±0.02	3.36±0.02	2.79±0.03	2.49±0.02
A_{opt}, λ_{600nm}	0.084±0.007	0.058±0.005	0.047±0.006	0.092±0.005
		2h		
Lactic acid quantity, $g.dm^{-3}$	14.13±0.03	16.90±0.02	15.12±0.03	14.60±0.01
A_{opt}, λ_{600nm}	0.161±0.007	0.215±0.005	0.175±0.003	0.136±0.006
Growth monitoring, μ	0.21	0.35	0.29	0.30
		4h		
Lactic acid quantity, $g.dm^{-3}$	35.15±0.02	37.56±0.01	36.85±0.02	35.86±0.03
A_{opt}, λ_{600nm}	0.450±0.035	0.821±0.031	0.758±0.030	0.652±0.032
Growth monitoring, μ	0.51	0.78	0.76	0.72
		6h		
Lactic acid quantity, $g.dm^{-3}$	68.18±0.02	81.56±0.03	80.94±0.02	79.64±0.02
A_{opt}, λ_{600nm}	0.961±0.025	1.526±0.028	1.428±0.030	1.400±0.026
Growth monitoring, μ	0.83	0.95	0.93	0.90

This share that lactic acid bacteria growth is due to both: the relevant berries chemical composition and the pH reduction of the fermentation medium [48, 49].

The Pearson correlation [50] between the pH and the lactic acid bacteria growth in the fermentation process in the classic yogurt samples and with the berries addition was calculated.

A high correlation was found - close relationship between variables, inversely proportional, because the values were obtained negative.

The results are presented in Table 3 and Figure 3.

Table 3

Pearson correlation between fruit yogurt fermentation parameters

Fermentation time, h	P1		P2		P3		P4	
	MC·10^7	pH	MC·10^7	pH	MC·10^7	pH	MC·10^7	pH
0 (initial)	0.2	5.63	0.65	5.41	0.51	5.27	0.39	5.19
2	0.9	5.28	1.45	5.05	1.2	4.92	1.2	4.81
4	1.6	4.75	2.15	4.55	2	4.41	1.85	4.41
6	2.31	4.38	2.85	4.27	2.79	4.27	2.79	4.27
Pearson coefficient Pc = f(pH and MC)	-0.99672		-0.99436		-0.9782		-0.96873	
General Pearson coefficient for all fruit yogurt samples -0.95066								

*Microbial counts (x10^7 CFU/ml)

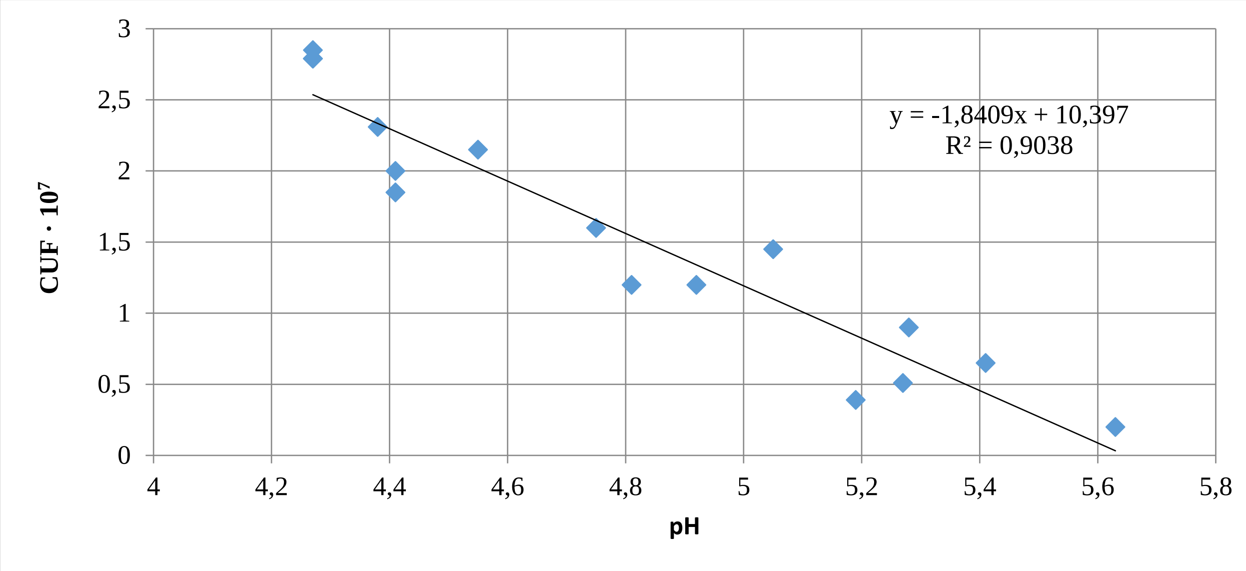

Figure 3. The interdependence between pH and the lactic acid bacteria number in fruit yogurt.

Analysis of the fruit yogurt physico-chemical indices.

In this research were studied the fruit yogurt physico-chemical indices. Measuring the yogurt titratable acidity (ºT) is a valuable determining practice that must correlate with the additions introduced [51]. The added scald fruits had a great impact on the acidity values. Higher values were obtained for P3 - 98 ± 0.082 ºT for P4 - 91 ± 0.079 ºT and lower acidity for P2 - 85 ± 0.078 ºT, as fruits contain more acid than milk, compared to P1 - 75 ± 0.080 ºT (control sample). After fermentation, the maximum pH value was detected in P2 - 4.28 ± 0.002, this pH may be caused by the buffering action of higher proteins and minerals present in yogurt. The lower value was obtained for sample P4 - 4.27 ± 0.003 and P3 - 4.25 ± 0.002, possibly determined by the metabolic activity that persists during refrigeration [52], responsible for this decrease in yogurt pH values. The pH of sample P1 is 4.30 ± 0.003 which fits perfectly into the usual pH range of classic yogurt after fermentation (4.0-4.4).

The amount of water available to microorganisms is characterized by a_w. The water activity (a_w) measurement forms the product basis and provides information on the microorganisms growing possibility or on the product. As the water activity value is between 0.8 - 1, then we have a perishable product with a risk of rapid microorganisms development. The water activity differs in each sample, respectively showing the following values: P2 (0.875 ± 0.025), P3 (0.873 ± 0.028) and P4 (0.872 ± 0.023) compared to P1 (0.869 ± 0.021). The results obtained are due to the potential acting as a solvent and participating in chemical / biochemical reactions and the microorganisms growth [53].

Table 4

Physico-chemical indices of fruit yogurt

Indices	Yogurt Samples			
	P1	P2	P3	P4
Titrable acidity, °T	75±0,080	85±0,078	98±0,082	91±0,079
pH	4.30±0,003	4.28±0,002	4.25±0,002	4.27±0,03
A_w	0.869±0,021	0.875±0,025	0.873±0,028	0.872±0,023
Total dry matter, %	17.57±0,22	18.45±0,31	18.28±0,26	18.11±0,28
Viscozity, mPa·s	1250±0,027	2500±0,023	1906±0,022	1829±0,026
Ash content, %	0.65±0,12	0.89±0,10	0.79±0,12	0.69±0,10
Protein content, %	3.98±0.052	3.96±0.05	3.94±0.04	3.93±0,04

Total dry matter plays a significant role in developing the desired yogurt consistency. There were differences in the total solids content of the yogurt samples. The highest value was recorded in the case of sample P2 (18.45 ± 0.31%). This result indicated that dry matter increased compared to classic yogurt (17.57 ± 0.22%) with the addition of scald aronia fruit, and casein was in the isoelectric state in which the activity of water particles decreased, not affecting the hydrolysis effectiveness [54]. For the sample P3 and P4 were obtained 18.28 ± 0.26% and 18.11 ± 0.28% respectively.

Different technological factors influence the rheological properties of yogurt, such as: heat treatment of milk, incubation temperature, the type of culture used and the cooling process. The structure of yogurt gel is a proteins network formed during acid gelation [55]. The gel formation during the yogurt manufacture occurred due to the unstable casein complex that coagulated easily [56]. The viscosity of all fruit yogurt samples showed satisfactory values. Sample P2 recorded the highest values 2500 ± 0.023 mPa·s, because it has a firmer and well-formed curd than the other samples, due to the higher total dry matter content and the increase of the consistency index, process explained by the ratio of casein fractions to the ratio of casein: serum protein in raw materials. This confirms that the aronia addition contributes more to the whey retention in the gel structure and to the stable gel formation over time as a result of its arrangement in the protein network [57]. As a result of these findings, sample P3 indicates values of 1906 ± 0.022 mPa·s, sample P4 values of 1829 ± 0.026 mPa·s compared to values of sample P1 - 1250 ± 0.027 mPa·s.

Mineral salts have a high nutritional value and have an important influence in the technological processes where the milk coagulation phases take place. The addition of scald aronia, raspberry and strawberry has led to an increase in the yoghurt ash content [58]. The ash content changed in each sample, maximum values were obtained for P2 - 0.89 ± 0.10% and lower values for P3 - 0.79 ± 0.12%, P4 - 0.69 ± 0, 10% in relation to P1 - 0.65 ± 0.12%.

Protein [59] plays a key role in the yogurt nutritional and technological value. The values obtained for the protein content in yogurt samples were slightly influenced by the addition of scald fruit (Table 3).

Microbiological indices analysis of fruit yogurt

Yogurt production raises a large number of questions for microbiological safety technologies: how to prevent contamination with microorganisms, what to do with microorganisms that cannot be eliminated by various processing operations, how to preserve taste, texture and ensure durability of acceptable validity. In most "problematic" cases these are yeasts, fungi, heterofermentative lactobacilli [60].

Yeast [61] and mold [62] are agents of yogurt microbial spoilage. They may be present in yoghurt due to contamination during manufaturing, including added prepared fruit, from packaging materials. Overall, yogurt should contain 10^7 cfu / ml of viable bacteria [63].

Table 5

Microbiological indices of fruit yogurt

	P1	P2	P3	P4
MC, ufc/ml·10^7	2,11± 0.17	2,93± 0.30	2,65± 0.25	2,39± 0.28
Yeast, ufc/1 g, max	Absent	Absent	Absent	Absent
Mold, ufc/1 g, max	Absent	Absent	Absent	Absent

Previous research [64 - 67], indicates that aronia, raspberry and strawberry have bactericidal and bacteriostatic effects on pathogenic and conditionally pathogenic microorganisms such as: *Salmonella, L monocytogenes, E. coli, Klebsiella, Bacillus sp*. From the data presented in table 4 it results that the berries form a synergism in the control of the microbiological risk and the stability of the yogurt in storage.

The results of research [68] showed that the combined use of lactic acid bacteria with the berries addition had a synergistic effect, as expected, on the risk posed by *Bacillus bacteria* in food. Aronia was the most effective natural preservative, from the tested fruits, to prevent the damage of yogurt from cow's and goat's milk by *Bacillus*.

The effect of storage on the fruit yogurt physico-chemical indices

Titratable acidity

Titratable acidity describes the freshness of the yogurt, especially during storage. Milk acidification leads to the destruction of the internal structure of casein mycelium due to the solubilization of k-casein. During storage, due to the upward fermentation process, the titratable acidity of fruit yogurt increases (figure 4). During the yogurt samples storage, the following data were recorded (minimum and maximum): 75 ± 0.080 - 82 ± 0.085 °T for P1, 85 ± 0.078 - 92 ± 0.080 °T for P2, 98 ± 0.082 - 105 ± 0.083 °T P3 and 91 ± 0.079 - 100 ± 0.081 ° T P4. These results indicate that yogurt is a favorable environment for the lactic acid bacteria development [69]. The aronia, strawberry and raspberry fruits introduced in yogurt have a high acidity, thus leading to an increase in titratable acidity compared to the control sample but maintaining it in the range of permissible values according to [70].

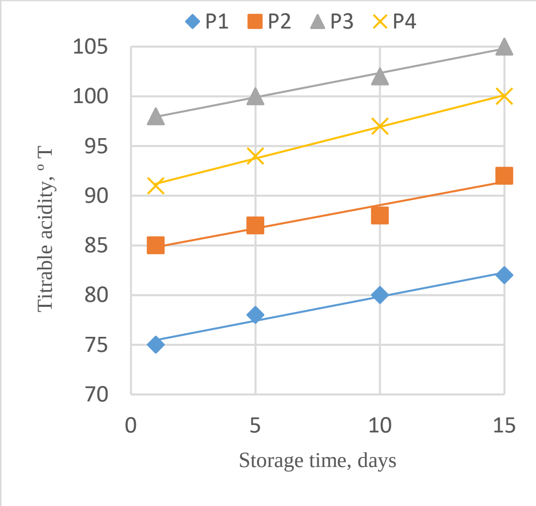

Figure 4. The evolution of the fruit yogurt titratable acidity on stored.

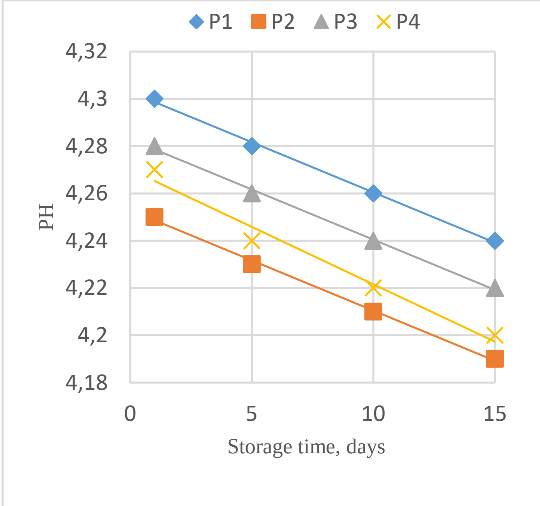

Figure 5. The evolution of the fruit yogurt pH on stored.

pH

The role of pH monitoring in the yogurt manufacture is crucial. PH measurement is considered a sensitive tool to detect changes in the yogurt active acidity. Some technological stages (heat treatment, fermentation, cooling) have a major influence in the finished product pH values during storage. Refrigeration temperature is responsible for lowering the fruit yogurt samples pH during storage. Analyzing the results presented in Figure 5, we observe differences in the pH values of fruit yogurt and the classic one after the first storage day. Initial pH values in yogurt samples vary, 4.28 ± 0.002 for P2, 4.27 ± 0.003 for P4, 4.25 ± 0.002 for P3 front of 4.30 ± 0.03 at P1. The change in pH during storage showed slightly decreasing values within 1 day 4.28 ± 0.002 and on day 15 4.22 ± 0.004 in P2, at sample P3 in 1 day the pH indicates results of 4.25 ± 0.002 and on the 15th day 4.19 ± 0.003, at sample P4 in 1 day the pH indicates values of 4.27 ± 0.003 and at 15 days 4.20 ± 0.004 compared to sample P1 with values on 1 day 4.30 ± 0.002 and on the 15th day 4.24 ± 0.003. Similar results, decreased pH values and increased titratable acidity values for yogurt samples during storage were also obtained by [71, 72, 73].

Lactic acid

In the mixed culture, between the lactic bacteria, cooperation relations are established that positively influence the growth of the other [74]. The production of lactic acid by *Lactobacillus bulgaricus* is stimulated at low concentrations of formic acid produced by *Streptococcus thermophilus* in the absence of oxygen and CO_2 released by fermentation [75]. *Streptococci* grow faster and are responsible for acidity while *lactobacilli* add flavour mainly due to the formation of acetic aldehyde. Through their activity, *lactobacilli* that have peptidase activity produce nitrogen compounds, assimilable for *streptococci*, which explains the synergistic relationship between *streptococci* and *lactobacilli* in the yogurt manufacture [76].

Optical density (λ, nm) is one of the important physico-chemical methods for evaluating the microorganism’s growth and development. In the goat's milk nutrient medium [77] all strains are characterized by higher acidification rate - 4.4-7.7 hours, compared to cow's milk medium [78] - 4.6-9.3 hours.

Table 6

Indicators of fruit yogurt lactic fermentation

Samp les nr.	Time, days	Titrable acidity , °T	pH	Lactic acid, g	Optical density, 600 nm
P 1	1	75±0.080	4.30±0.002	0.02167±0.025	1.146±0.058
	5	78±0.083	4.28±0.003	0.02908±0.026	1.232±0.055
	10	80±0.081	4.26±0.002	0.03569±0.023	1.331±0.052
	15	82±0.085	4.24±0.003	0.04980±0.027	1.466±0.053
P2	1	85±0.078	4.28±0.002	0.09782±0.027	2.531±0.054
	5	87±0.081	4.26±0.004	0.10719±0.025	2.613±0.055
	10	88±0.083	4.24±0.003	0.11563±0.031	2.873±0.053
	15	92±0.080	4.22±0.004	0.12250±0.028	2.922±0.056
P3	1	98±0.082	4.25±0.003	0.08855±0.028	2.241±0.056
	5	100±0.084	4.23±0.002	0.10599±0.025	2.519±0.059
	10	102±0.081	4.23±0.003	0.10989±0.028	2.765±0.057
	15	105±0.083	4.21±0.003	0.11512±0.026	2.868±0.051
P4	1	91±0.079	4.27±0.003	0.06215±0.028	2.162±0.058
	5	94±0.082	4.24±0.004	0.07656±0.029	2.391±0.053
	10	97±0.083	4.22±0.002	0.09818±0.025	2.692±0.055
	15	100±0.081	4.20±0.004	0.10008±0.026	2.754±0.055

According to the data recorded in Table 6, it is observed that the amount of lactic acid increased during the fruit yogurt storage period. The initial values of lactic acid vary in each sample. In the case of sample P2 the value obtained is 0.09782 ± 0.027, for sample P3 is 0.08855 ± 0.028, for sample P4 - 0.06215 ± 0.028, compared to sample P1 0.02167 ± 0.025. The change in lactic acid during the storage period showed slightly decreasing values within first day of 0.09782 ± 0.027 and on the 15th day of 0.12250 ± 0.028 in P2, for sample P3 in 1 day lactic acid indicates results of 0.08855 ± 0.028 and on the 15th day of 0.11512 ± 0.026, for sample P4 in 1 day lactic acid indicates values of 0.06215 ± 0.028 and at 15 days of 40.10008 ± 0.026 compared to P1 with values at 1 day of 0.02167 ± 0.025 and on the 15th day of 0.04980 ± 0.027. As the number of microorganisms increased, yogurt optical density increased in all samples. For sample P2 the results indicate values increasing at 1 day of 2,531 ± 0.054 nm and to the 15th day of 2.922 ± 0.056 nm, for P3 - 1 day of 2.241 ± 0.056 nm to the 15th of 2.868 ± 0.051 nm, for P4 - 1 day of 2,162 ± 0.058 nm to a15th day of 2,754 ± 0.055 nm, compared to P4 -1 day 1,146 ± 0.058 nm to the 15th day of 1,466 ± 0.053 nm. The rise in titratable acidity and the decline in pH was due to the activity of lactic acid bacteria to produce energy [79] 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 [80].

The effect of storage on the fruit yogurt microbiological quality

In the manufacture of yogurt, it is very important to facilitate the survival of the initial bacteria [81]. The viability of beneficial bacteria depends on the availability of nutrients, the presence of growth promoters and / or inhibitors, sugar concentration, oxygen, incubation temperature, fermentation time and storage temperature [82].

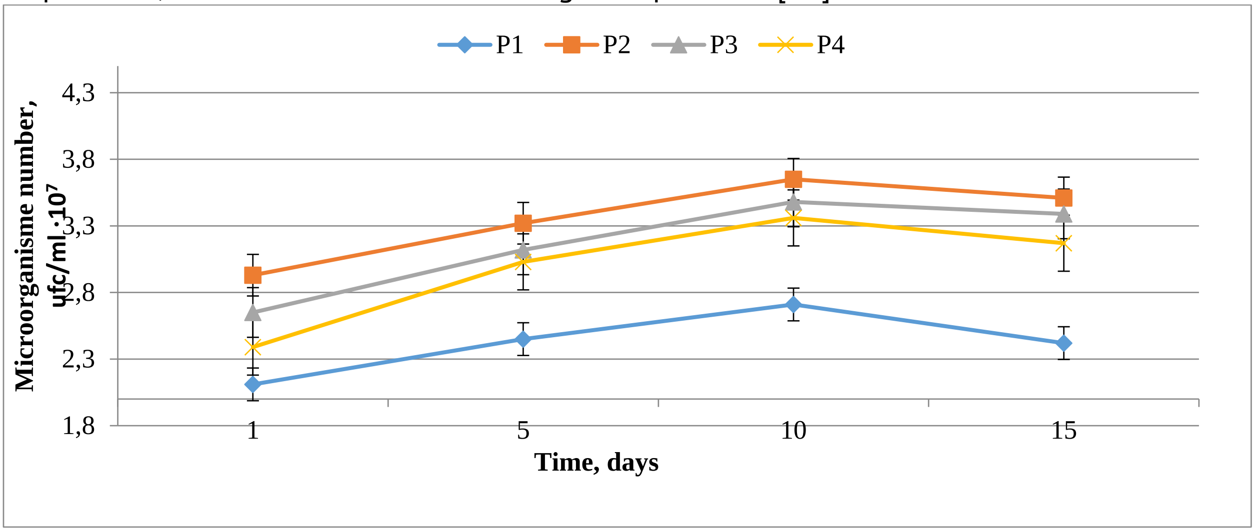

Figure 6. Yogurt lactic bacteria variation in storage.

In a standard environment with fructose or sucrose there is a microorganism's growth inhibition in the mixed culture. Moreover, such an inhibition could be partly due to the osmotic pressure and chemical composition of fruits containing fruit-oligo-saccharide (FOZ) [83].

The results obtained for the variation of fruit yogurt lactic bacteria are satisfactory during the storage period of 1 - 15 days at a temperature of 4 ° C and fall within the values stipulated in the normative documents [84]. During storage the lactic acid bacteria number increased, the values being between $2.93 \cdot 10^7 \pm 0.30$ and $3.51 \cdot 10^7 \pm 0.29$ for sample P2, between $2.65 \cdot 10^7 \pm 0.30$ and $3.39 \cdot 10^7 \pm 0.29$ for sample P3, between $2.39 \cdot 10^7 \pm 0.30$ and $3.17 \cdot 10^7 \pm 0.29$ for sample P4 compared to sample P1 $2.11 \cdot 10^7 \pm 0.30$ and $2.42 \cdot 10^7 \pm 0.29$. Such results suggested that berries have a strong effect by modulating the survival capacity of lactic acid bacteria, when simple sugars such as fructose and glucose were consumed almost entirely during fermentation [85], aronia having the greatest barrier role, in which the loss of lactic acid bacteria was the least reduced.

Conclusion

Based on the experimental study, it was shown that berries have a relevant composition in biologically active substances, have antimicrobial properties and form a synergism with starter culture in goat's and cow's milk yogurt with scald berries. The incorporation of aronia is highlighted by the best results of physico-chemical and microbiological indices in the initial phase and during storage period, compared to classic yogurt. The average Pearson correlation (Pc = f(pH and MC)) for the yogurt samples tested was -0.95066.

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