

## Evolution of Physico-chemical Indices and Functional Properties of Fruit Yogurt during Storage

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### Abstract

**Background:** In the context of the current trend of consumers to consume foods as natural as possible, increasing the nutritional and biological value of yogurt by using raw materials (goat's milk and fruit) with a well-balanced chemical composition is of great interest in the dairy products manufacture.

**General Objective:** The study and analysis of fruit nutritional and biological value impact on quality indices and functional properties of goat's milk and cow's milk yogurt during the storage.

**Methods:** The yogurt was obtained from a mixture of 50% goat's milk and 50% cow's milk with the addition of aronia (*Aronia melanocarpa*), raspberries (*Rubus idaeus*), strawberry (*Fragaria xanassa*) fruits. Physico-chemical indices and biological value were determined according to standard methods in 1, 5, 10, 15 days of storage.

**Results:** Sample's titratable acidity shows increasing values, being influenced by the addition of fruits, that have a higher acidity compared to classic yogurt but remaining in the range of allowable values. The pH of the yogurt samples decreases with increasing storage period, as the metabolic activity of the starter culture persists. Yogurt with added aronia (P2) has the best values of total dry matter ( $18.45 \pm 0.31\%$ ), water activity ( $0.875 \pm 0.025$ ), viscosity ( $2500 \pm 0.023$  mPa·s) and the highest content of anthocyanins of 66.03 mg/100g, the highest polyphenol content of 268.97 mg GAE · 100g<sup>-1</sup> and the best values of antioxidant property of  $2.2 \pm 0.025$  mg AA/g SH. Strawberry yogurt (P4) has the highest content of vitamin C (47.87 mg/100g) and carotenoids 0.292 mg/100g.

**Conclusion:** Fruit addition had a positive impact on the quality indices of yogurt during storage.

**Keywords:** Goat Milk; Cow Milk; Fruits Puree; Fermentation; Yogurt; Antioxidant Activity Hydrosoluble Antioxidants

### Introduction

The composition of goat's milk is superior to cow's milk due to its nutritional, toning, antirachitic, antianemic and anti-infective effects [1,2]. Goat's milk is absorbed more easily than cow's milk [1], leaving behind less undigested residues in the colon to ferment and cause uncomfortable symptoms of lactose intolerance

[3]. Goat's milk has technological advantages over cow's milk, it has a smaller size of fat globules that provide a finer texture in dairy products [4-8]. Goat's milk due to a higher  $\beta$ -casein content forms a gel with a weaker network compared to cow's milk where the  $\alpha$ -casein content prevails, a higher water retention capacity and a lower viscosity [4,5,9].

Fermented dairy products are foods with high functionality due to their pleasant sensory characteristics and their potential to maintain and improve the consumer’s health, but for a greater impact, a possible change in taste and consequently the requirements of all categories of consumers - a wide range of fortified dairy products with fruits, cereals, fiber, and other high biological value additives is proposed [10].

Among the first chemical compounds in food, which have been examined to determine their functional properties, are proteins and polysaccharides [11]. The functional properties of proteins and polysaccharides have served as the scientific basis of technology for the animal and vegetable origin products manufacture with determined texture, nutritional value and sensory properties [12]. Fruits are especially valuable for the content of carbohydrates, vitamins, minerals, pigments and other biologically active substances [13]. The fruits of aronia, raspberry, strawberry have an alkalizing effect in the body and are constituted as foods that favorably influence the health of the body and reduce the risk of chronic high-risk diseases [14]. Their antimicrobial activity is determined by a high content of phenolic compounds - substances that contain aromatic rings with a hydroxyl group and their functional derivatives. These include tannins, flavonoids, glycosides, phenol carboxylic acids, phenolic alcohols, anthocyanins and simple phenols (Table 1) [15].

Physico-chemical indices	Strawberry	Raspberry	Aronia
Dry matter, %	10.0	9.75	12.1
pH	3.5	3.5	3.5
Titrate acidity, %	1.03	1.10	0.96
a <sub>w</sub>	0,988	0.982	0.985
L-hidroxiacorbic acid, mg/10g	46.6 ± 0.30	34.2 ± 0.64	40.2 ± 0.61
Polifenols, mg/100g	295.0 ± 8.50	288.9 ± 8.84	719 ± 571
Antocyanins, mg/100g	33.3 ± 0.5	35.7 ± 0.5	357 ± 22
Antioxidant potential, K, mg AA/g SH	15.4 ± 0.45	3.78 ± 0.17	26,4 ± 0,45

**Table 1:** Physico-chemical indices of strawberry, raspberry and aronia fruits [16,17].

The stability of dairy products over time is troubling for the dairy industry, as some processes (oxidation) can lead to decrease the yogurt nutritional quality. The milk and dairy products oxidative stability depends on the concentration of vitamins (C and carotenoids), polyphenols, anthocyanins, etc. Processing, packaging, conditions and storage period have a pronounced effect on the expansion of natural antioxidants use.

**Materials and Methods**

**Materials**

**Preparation of fruits puree**

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, which contain *Streptococcus thermophilus*, *Lactobacillus delbrueckii subsp. bulgaricus*, *Lactococcus lactis subsp lactis biovar diacetylactis*, sucrose and maltodextrin. 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 2.

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.

**Table 2:** Notify the probe.

## Methods

Determination of titrable acidity (TA). Method consists in neutralizing the acidic milk substances with 0.1n NaOH (KOH) solution using phenolphthalein as an indicator [18].

Determination of pH. Was determined using pH metre (glass electrodes).

Determination of total dry matter content. Standard method of oven drying at  $102 \pm 2^\circ\text{C}$  until a constant mass of the dry residue is obtained [19].

Determination of water activity. It is measured the vapor pressure of the water around the food and divided by the vapor pressure of the pure water to give a value between 0.0 and 1.0 [20].

Determination viscosity of acid dairy products was determined using the „Brookfield DV - III” rheometer, with indicator no. 04, 250 rotations/min, data were read after 30 seconds of rotations [21].

Determination of vitamin C content - prepared extract titration with 0.001 N indicator solution of 2,6 diclorfenolindofenol [22].

Determination of carotenoids content - the method is based on the photometric determination of the mass carotene concentration in the solution obtained after the carotenoids extraction with an organic solvent and purified of the accompanying substances using dye by column chromatography [23].

Determination of anthocyanin content was measured by spectrophotometric method at 540 nm, extracted with a solution of 95% ethyl alcohol and 1.5 n HCl to discoloration [24].

Determination of total phenolic compounds- estimated according Folin-Ciocalteu method. A volume of 1 ml of methanolic extract of each sample was added to 1 ml of Folin-Ciocalteu's solution in a test tube. After 3 minutes, 1 ml of 20% sodium carbonate solution was added to the mixture and adjusted to 10 ml with distilled water. The mixture was allowed to stand at room temperature in a dark environment for 30 min. Absorbance was measured against the blank reagent at 725 nm. Gallic acid was used for the calibration curve with a concentration range of 50 - 1000  $\mu\text{g}\cdot\text{mL}^{-1}$ . Results were expressed as mg GAE $\cdot 100\text{g}^{-1}$  [25].

Determination of the redox state [26], before to determination, 10 - 15g of yogurt was diluted 1:1 using distilled water and placed in measuring cell. In cell II was introduced buffer solution with pH of the analyzed product - the reference solution, electrodes of platinum and silver chloride are introduced in cells. We register the difference between the redox potentials of cells, and in cell II we dose the etalon solution (ascorbic acid) until there is equality between the potentials of cell I and II. The potentiometer shows  $E = 0$ . The redox state of the tested products is expressed by the K coefficient as ascorbic acid equivalent mg per g product hydrosoluble solids (HS). The K coefficient is calculated using the following relation:

$$K = \frac{C_1 \times V_1 \times m_1}{m_2 \times C_2 \times m_3} \quad (2)$$

Where:

$C_1$  - Concentration of the etalon solution, mg/ml;

$C_2$  - The mass fraction of hydrosoluble solids, g/g;

$V_1$  - The volume of the etalon solution of ascorbic acid that is equivalent to the redox potential of the tested system, ml;

$m_1$  - The mass of the analyzed sample after dilution, g;

$m_2$  - The mass of reference solution, g;

$m_3$  - The mass of the analyzed sample, g.

## Statistical analysis

The variance analysis of the results was carried out by least square method with application of Student test and Microsoft Office Excel program version 2010. The differences were considered statistically significant if probability was greater than 95% (p-val-

ue < 0.05). All assays were performed in triplicate. The experimental results are expressed as average  $\pm$  SD.

## Results and Discussion

### Analysis of the fruit yogurt physico-chemical indices on storage

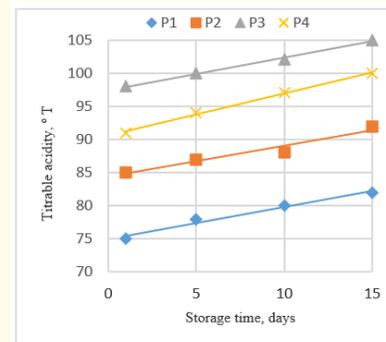
The physico-chemical properties and the biological value of the fruits yogurt were analysis during storage in first day, the 5<sup>th</sup>, the 10<sup>th</sup> and the 15<sup>th</sup> day, when yogurt became more compact and the taste, smell are better highlighted.

Fermentation is a method used for thousands of years to provide a longer shelf life for easily perishable foods and to increase the aroma and smell of final foods [27]. Fermentation temperature [28] plays a very important role in the development of acidity, the recommended temperature being 38 - 42°C. Titrable acidity is the basic quality indices and the criterion of freshness of fermented dairy products, the increase in acidity is due to the transformation process of reducing carbohydrates, a process that begins immediately after the addition of starter cultures and continues during the fermentation process [29].

Figure 1 shows the evolution of the fruit yogurt titratable acidity on stored. During the storage period of the yogurt samples, the following data were recorded (minimum and maximum):  $75 \pm 0.080 - 82 \pm 0.085$  °T for P1 (titratable acidity increases by 9.3%),  $85 \pm 0.078 - 92 \pm 0.080$  °T for P2 (acidity titratable acidity increases by 13.3%),  $98 \pm 0.082 - 105 \pm 0.083$  °T P3 (titratable acidity increases by 30.7%) and  $91 \pm 0.079 - 100 \pm 0.081$  ° T P4 (titratable acidity increases by 21.4%). These results indicate that yogurt is a favorable environment for the development of lactic acid bacteria [30]. The aronia, strawberry and raspberry fruits introduced in yogurt have a high acidity (Table 1), thus leading to an increase in titratable acidity compared to the control sample but maintaining it in the range of permissible values, according to [31]. During storage due to the ascending fermentation process, specific to the accumulation of lactic acid, the titratable acidity of the product increases (Figure 1).

pH measurement is considered a sensitive instrument to detect change in yogurt active acidity. The pH, which corresponds to 4.0 - 4.5, is specific to inhibiting the development of foreign microflora [32]. In yogurt manufacturing technology a pH of less than 4.0 is not accepted, as the excess of lactic acid accumulation negatively

influences the vital activity of beneficial microorganisms in the end product [33], thus reducing the benefit of the product on consumer health [34].



**Figure 1:** The evolution of the fruit yogurt titratable acidity on stored.

**Figure 2:** The evolution of the fruit yogurt pH on stored.

The yogurt samples pH decreases with increasing storage period, as the metabolic activity of the starter culture persists. Refrigeration temperature is responsible for lowering the pH of yogurt samples during storage in the presence of yogurt thermophilic microorganisms [35]. Analyzing the results presented in figure 2, is observed differences in the pH values of fruit yogurt and the classic one after the first day of storage. The initial yogurt samples pH values vary within  $4.28 \pm 0.02$  for P2 (aronia yoghurt),  $4.27 \pm 0.03$  for P4 (strawberry yoghurt),  $4.25 \pm 0.02$  for P3 (raspberries yogurt) compared to  $4.30 \pm 0.03$  in P1 (control sample). The change in pH during storage showed slightly decreasing values within 1

day  $4.28 \pm 0.03$  and on day 15  $4.22 \pm 0.04$  in P2, for P3 in 1 day the pH indicates results of  $4.25 \pm 0.03$  and on the 15<sup>th</sup> day  $4.19 \pm 0.028$ , for P4 in 1 day the pH indicates values of  $4.27 \pm 0.03$  and on the 15<sup>th</sup> day  $4.20 \pm 0.028$  compared to sample P1 with values on 1 day  $4.30 \pm 0.02$  and on the 15<sup>th</sup> day  $4.24 \pm 0.03$ . Similar results, decreasing pH values and increasing titratable acidity values for yogurt

samples during storage were also obtained by [36]. A pH value in fruit yogurt samples may also be influenced by the increase in dry matter content. Increasing milk total solids had significant effect on decreasing rate of pH during fermentation [37] and total solids content had no adverse effect on starter activity or coagulation time [38].

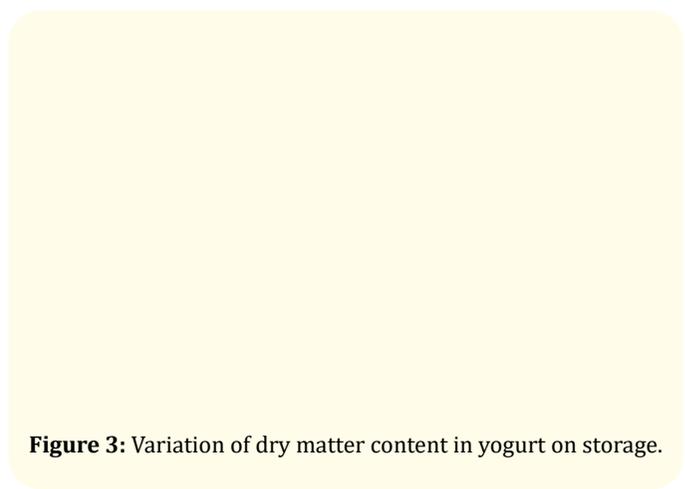
Yogurt sample	Regression equations		Correlation coefficients	
	Titrable acidity	pH	Titrable acidity	pH
P1	$y = 0.4853x + 74.989$	$y = -0.0038x + 4.3047$	$R^2 = 0.9752$	$R^2 = 0.8584$
P2	$y = 0.4695x + 84.361$	$y = -0.0042x + 4.2529$	$R^2 = 0.9391$	$R^2 = 0.9973$
P3	$y = 0.6366x + 90.577$	$y = -0.0042x + 4.2829$	$R^2 = 0.9973$	$R^2 = 0.9973$
P4	$y = 0.4898x + 97.454$	$y = -0.0042x + 4.2675$	$R^2 = 0.9934$	$R^2 = 0.9752$

**Table 3:** Regression equations and correlation coefficients R2 for the determination the titratable acidity and pH of yogurt samples with fruit during storage.

The analysis of the regression equations and the correlation coefficient  $R^2$  (the square of the Pearson coefficient) during storage of the yogurt samples (Table 2) shows the credibility of the obtained results ( $R^2 = 0.957 \dots 0.9985$ ).

The dry matter plays an important role in forming the end product texture. It is recommended to follow 3 important steps in the technological process, to obtain a yogurt with a perfect structure: heat treatment of milk, fermentation temperature, cooling process [39,40]. The sugars from fruits together with milk proteins (cow milk caseine), influences the dry matter of the product [41], respectively the quality of the formed curd. Fruits added to yogurt have had a positive impact on texture indices, possibly also due to the hydrocolloids contained that confer thickening, stabilizing, gelling and emulsifying properties to food products [42,43], and polyphenols due to their binding with proteins [4,44], hypothesis also supported by the results obtained for yogurt samples in storage, especially for yoghurt with aronia, which is highlighted by a high polyphenol content (Figure 9) as well as a higher total dry matter content (Figure 3).

The total dry matter results shows higher values for P2 (aronia yoghurt) the maximum value is  $18.45 \pm 0.31\%$  on the first day and an increase, due to the decrease in syneresis, to  $19.15 \pm 0.28\%$  on the 15th day, in compared to P1 (classic yogurt) with a value of  $17.57 \pm 0.22\%$ , (Figure 3).



**Figure 3:** Variation of dry matter content in yogurt on storage.

In order to assess the stability of manufactured yogurt samples for storage, the activity of water was determined, being an indicator that characterizes the food quality and stability of both physically, chemically and microbiologically [45]. The stability and safety of food depends directly on the water activity in the product. Water activity can be applied in very useful ways: predicting the growth of bacteria, yeasts or molds [46] in order to have a product with a long shelf life. Water activity is believed to be partially responsible for minimizing browning reactions of non-enzymatic origin and lipid autocatalytic oxidation reactions, prolonging the enzymes and vitamins activity, optimizing the yogurt physical properties such as texture, flavor, and shelf life.

**Figure 4:** Variation of yogurt water activity on storage.

As the value of water activity is between 0.8 - 1, then we can say that have a perishable product with a risk of microorganisms rapid development. In the case of the researched samples, the value of the water activity increases during yogurt storage, respectively the number of lactic bacteria also increases. P2 shows good results for water activity values on storage compared to the other samples, at the 15<sup>th</sup> day of storage  $0.877 \pm 0.025$  (Figure 4).

The yogurt rheological properties are determined by the manufacturing conditions and the raw materials composition. Disturbance of the balance between milk components has a direct impact on the rheological yogurt properties [47]. FAO and WHO recommend in the fortified yogurts manufacture (with added value) to use a concentration of 5-15% fruit [48,49]. Fruit pectin and fructose improve the consistency and viscosity of yogurt and therefore the mouthfeel is improved. Pectins are added to acidified dairy products to avoid syneresis [50]. Pectin is absorbed irreversibly on casein leading to an increase in steric repulsion, and thus decreasing its aggregation power [51,52].

**Figure 5:** Variation of yogurt viscosity on storage.

Yogurt gels are a type of soft solids, and these networks are relatively dynamic systems, which are prone to structural rearrangements. The physical properties of yogurt gels can be qualitatively explained using a model for casein interactions that emphasizes a balance between attractive forces (e.g. hydrophobic attractions, cross-links of casein supported by calcium phosphate nanoclusters, and covalent cross-links denatured whey proteins) and repulsive (e.g. electrostatic or charging repulsions, being stronger at the beginning of the fermentation process) [53].

Changes in the fruit yogurt viscosity values are shown in figure 5. The viscosity of all fruit yogurt samples decreases with increasing shelf life. The highest values on storage,  $2500 \pm 0.023$  mPa·s first day and  $1935 \pm 0.025$  mPa·s 15<sup>th</sup> day were recorded in P2 (aronia yogurt) because it has a firmer and well-formed curd than the other sample, a process explained by the ratio of casein fractions to the ratio of casein: serum proteins in raw materials and the binding of milk proteins to aronia polyphenols. This confirms that the addition of aronia contributes more, compared to strawberry and raspberries, to the whey retention in the gel structure and stable gel formation over time as a result of its arrangement in the protein network [54].

#### Analysis of the fruit yogurt functional properties during the storage

The addition of vegetable materials in yogurt allows the regulation of the vitamins, carbohydrates, minerals and dietary fiber content. Also, it give dairy products a pronounced taste and smell of added fruit, as well as an attractive appearance. The use of biologically active compounds obtained from plant materials, is a promising direction in the production of functional products.

Ascorbic acid is one of the most powerful natural antioxidants and the least toxic. It is the main water-soluble antioxidant present in milk and the free radical scavenging activity of ascorbic acid is due to its low redox potential (330 mV) [55]. Regular fruit intake is linked to the reduced diseases risk such as cancer and cardiovascular disease, as they include natural antioxidants. Vitamin C contributes to the normal functioning of the immune system, stimulates the production of leukocytes and improves their function [56].

The evolution of the vitamin C content of all yogurt samples during storage is shown in figure 6.

**Figure 6:** Variation of yogurt content in vitamin C on storage.

The vitamin C content decreases in all yogurt samples during storage, because the ascorbic acid oxidation depends on temperature, light, oxygen, the amount of catalysts and its main task being the protection of polyunsaturated fatty acids and biochemical compounds.

The variation in vitamin C content shows decreasing values. From the fruits category used to fortify yogurt, P4 (strawberry yogurt) has the highest content of vitamin C. On the first day of storage P4 has a value of 47.87 mg/100g, on the 15<sup>th</sup> day of storage - 25.18 mg/100g, decreasing during storage by 32.45%. The vitamin C content of P3 (raspberry yogurt) decreased by 18.70%, P2 (aronia yogurt) decreased by 25.10% during storage. Because of vitamin C is unstable to an alkaline environment, oxygen, light and heat, losses are substantial in all samples, including the control sample.

Dairy products are some of the most interesting and promising foods in terms of their potential antioxidant activity, due to their wide diversity of antioxidant molecules, such as milk casein and whey protein [57]. Furthermore, milk contains a variety of antioxidant molecules, namely low molecular weight thiols, ascorbate, tocopherol, retinol and carotenoids. Carotenoids are among the most studied phytonutrients because they are the most widespread pigments in vegetables and due to their provitamin [58] and antioxidant role. Carotenoids, present in fruits, are fat-soluble dyes, from light yellow to orange to deep red.

$\beta$ -carotene is considered a preventive antioxidant; it can neutralize simple oxygen. Carotenoid compounds play an important role in photosynthesis, and in terms of human nutrition

they intervene in the body redox processes [56]. Carotenoids are synthesized by plants, algae, fungi, yeasts and bacteria. The main carotenoids found in food are  $\beta$ -carotene,  $\alpha$ -carotene,  $\beta$ -cryptoxanthin, lutein and lycopene [59]. Carotenoses are provitamin A with a beneficial role in health: they improve the immune system, reduce the risk of cancer, cardiovascular disease, cataracts, etc. [60].

**Figure 7:** Variation of yogurt content in carotenoids on storage.

The evolution of the carotenoid content in fruit yogurt during storage is shown in figure 7. During the storage period there is a decrease in carotenoid content in all fruit yogurt samples, because carotenoids do not have good stability over time, absorbs oxygen and passes thus through an oxidation process. This is explained by the fact that they have lipophilic molecules that tend to accumulate in the yogurt fat globules membrane.

Significant amounts of carotenoids were obtained in P4 (strawberry yogurt), values at 1 day of that have a tendency to decrease during the storage period, from 0.292 mg/100g to 0.098 mg/100g, decreasing by 33.15%. P3 (raspberry yogurt) 0.292 mg/100g to 0.098 mg/100g, decreasing by 28.15%. P2 (aronia yogurt) has values from 0.182 mg/100g to 0.011 mg/100g decreasing by 30.15%. The tendency to decrease was visible for P1 (clasic yogurt) from 0.008 mg/100g to 0.002%, the decrease being of 25%.

Anthocyanins play an important role in determining the fruit yogurt quality due to the colors impregnated with the product and can be used in the yogurt manufacture as harmless natural dyes [61]. Anthocyanins are of great interest to the food industry due to their antioxidant power, attractive color and stability in acid-rich foods [62].

The anthocyanins stability during storage is affected by temperature, pH value, water activity, exposure to light, microbial activity, fat content, degree of methoxylation of pectin used in yogurt production [13,63]. Practically all the exposed conditions are specific to yogurts, which can serve as an explanation for the reduction of the anthocyanin content in the fruit yogurt samples (Figure 8) during storage. P2 (aronia yogurt) was highlighted with a maximum anthocyanins content due to the high content of the component in the aronia fruit, but decreasing during storage, from 66.03mg/100g up to 18.12 mg/100g. The decrease in storage is 65%. The initial anthocyanin content in P3 is 37.16 mg/100 g, which decreased up to 15 days -28.36 mg/100 g, the decrease constitutes 60% during storage. P4 showed initial value of 31.82 mg/100g, which decreased to 15 days -15.52 mg/100g, with a 55% decrease in storage. For P1 is observed a decrease in anthocyanin content of 70% - 0.691 mg/100 g to 0.115 mg/100g.

**Figure 8:** Variation of yogurt anthocyanin content on storage.

Phenolic compounds have received significant attention in recent years due to their antioxidant, anti-inflammatory, anti-mutagenic and anti-coagulation power, which has been correlated with a reduced risk of cardiovascular disease and cancer development [64]. The main food source of phenolic compounds are fruits [65]. It has been suggested that fruit purees, powders [66] and extracts have the potential to be used as functional ingredients in the food industry, including the dairy sector [67].

Natural polyphenols are compounds that help increase the total antioxidant activity of yogurt. Like most biological compounds, the type and amount of phenols in fruit vary depending on the origin, year of harvest, maturation degree, processing and storage [68]. Representatives of polyphenols, anthocyanins, differ from other

phenolic compounds due to their ability to form different structures depending on the pH [69]. An important aspect to mention is the fermentation effect on the phenols and phytates reduction. The phytates reduction during fermentation is well established [70], has resulted in the action of phytase produced by microorganisms of which *Lactobacillus* species. The decrease in the phenolic compound can be attributed to the action of microbial polyphenol oxidase, peroxidase [71].

The evolution of the polyphenol content results for all yogurt samples is shown in figure 9.

**Figure 9:** Variation of yogurt polyphenol content on storage.

The decrease in polyphenol content during storage is moderate. All fruits have a high content of polyphenols, P2 (aronia yogurt) having the highest content of 268.97 mg GAE · 100g<sup>-1</sup> which on storage decreases to 156.47 mg GAE · 100g<sup>-1</sup>, decrease is 27%, followed by P3 (raspberries yogurt) with a content ranging from 174.01 mg GAE · 100g<sup>-1</sup> to 122.45 mg GAE · 100g<sup>-1</sup> with a decrease in storage of 22% and P4 (strawberry yogurt) with a content between 159.89 mg GAE · 100g<sup>-1</sup> to 95.29 mg GAE · 100g<sup>-1</sup> with a decrease in storage of 32%. For P1 (classic yogurt) the amount of polyphenols varies between 0.985 mg GAE · 100g<sup>-1</sup> - 0.36 mg GAE · 100g<sup>-1</sup> with a decrease in storage of 45%.

Vegetables, rich in natural and phenolic antioxidants, are used progressively in the yogurt manufacture, to improve nutritional and therapeutic properties [72]. Natural fruit-based antioxidants can be used to control the excessive formation of free radicals and to increase antioxidant capacity, as well as to replace synthetic antioxidants.

The dairy food antioxidant activity is important both for the product shelf life and for protection against oxidative damage to the human body.

The evolution of the antioxidant properties for is shown in figure 10.

**Figure 10:** Variation of yogurt antioxidant potential on storage.

The milk and dairy products antioxidant capacity is mainly due to sulfur-containing amino acids such as cysteine, phosphate, vitamins A, E, carotenoids, zinc, selenium, enzyme systems, superoxide dismutase, catalase, glutathione peroxidase, milk oligosaccharides and peptides which are produced during fermentation [73]. The antioxidant activity of dairy products can be improved by phytochemical supplementation, while fermented dairy products have been reported with a higher capacity for antioxidants compared to unfermented dairy products [74].

The maximum value for antioxidant property was obtained in sample P2 (aronia yogurt) which increases visibly during storage from  $2.2 \pm 0.025$  mg AA/g SH to  $3.6 \pm 0.021$  mg AA/g SH, an increase of 45%. These results are due to the bioactive substances in the aronia fruits that prevent the oxidation of the reactions promoted by oxygen or peroxides accumulated during the yogurt fermentation [75]. For sample P4 (strawberry yogurt) and sample P3 (raspberry yogurt) the variation over time of antioxidant properties shows lower values in first day of  $1.49 \pm 0.021$  mg AA/g SH in P4 and  $1.30 \pm 0.022$  mg AA/g SH in P3, values that increase slightly during storage, by 15%. In the case of control sample P1 (classic yogurt) the antioxidant properties in first day has values of 0.002 mg AA/g SH g which by the end of storage increase to 0.006 mg AA/g SH, an increase of 2.5%.

The increase in antioxidant value is due to the diffusion of phenolic substances, ascorbic acid in the product from the fruit.

## Conclusion

The addition of aronia, raspberry and strawberry fruits contributed to the increase of the yogurt nutritional and biological value, positively influenced the physico-chemical indices (pH, titratable acidity, dry matter, water activity, viscosity), which showed acceptable values according to normative documents during the storage period of 15 days. The addition of aronia fruits had the greatest contribution on improving the functional properties of yogurt, of course due to the aronia chemical composition and high, well-balanced biological value, as well as the compatibility of this fruit with milk. The addition of strawberries attests to the best results in the vitamin C and carotenoids content compared to the classic yogurt during storage.

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## Conflict of Interest

There is no conflict of interest.

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