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# QUALITY CHARACTERISTICS OF YOGURT FROM BUFFALO MILK SUPPLEMENTED WITH ARONIA (*Aronia melanocarpa*) JUICE

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***Abstract***

*Yogurt was prepared from buffalo milk supplemented with 3% and 5% Aronia (Aronia melanocarpa) juice. The mineral and fatty acid composition, free amino acid composition, vit. B1, B2, B6 and antioxidant activity were investigated. Buffalo yogurt produced with 3% aronia coagulated in a shorter time (135 min) compared to natural (control) yogurt and the one produced with 5% aronia (158 min). Buffalo yogurt produced with 5% aronia juice has the highest content of potassium (1004 mg/kg) and zinc (5.28 mg/kg) and the lowest of calcium, magnesium and manganese compared to the control yogurt and yogurt with 3% aronia addition. Aronia supplementation increased the amount of unsaturated fatty acids in buffalo yogurt by 5.7% (3% aronia) and 7.3% (5% aronia), respectively. Polyunsaturated fatty acids increased by 15.7% in 3% aronia yogurt and 22.6% in 5% aronia yogurt respectively, compared to natural buffalo yogurt. Yogurt produced with 5% aronia juice has the highest antioxidant activity and also has a higher content of vitamins B1, B2 and B6 compared to the control yogurt and yogurt with 3% aronia.*

***Key words****: antioxidant activity, aronia, buffalo milk, fatty acid composition, yogurt.*

## INTRODUCTION

Yogurt is a wide-spread fermented dairy product and has long been recognized as a functional food with desirable health effects (Rul, 2017). It is rich of nutrients with high biological value as well as essential micronutrients and beneficial microorganisms. Although dairy products have their obvious health benefits, however, fermented dairy products, including yogurt, are not documented as a rich source of bioactive compounds like antioxidants and polyphenols (Sheikh et al., 2022)

On the other hand, people are becoming more aware of their nutrition and demand of novel functional foods shows a steady rise in the last few decades. Yogurt is considered to provide a perfect matrix where different substances can be introduced and in recent years numerous experiments has been conducted in attempts to increase its biological activities. The most common are the supplementations of yogurt with different fruits, herbs and fibers because of their natural origin and potential advantages for human health.

Fruits of aronia (*Aronia melanocarpa*) are known to be a great source of polyphenols, including anthocyanins, flavonols, flavanols, proanthocyanidins, and phenolic acids (Oszmiański & Lachowicz, 2016; Tolić et al., 2017) and has also been used to enhance the benefits of yogurt. Several studies have reported that fortification of cow and goat yogurt with fruits of aronia (black chokeberry) significantly increased its total phenolic content, antioxidant activity (Dimitrellou et al., 2020) and percentage of polyunsaturated fatty acids in it (Boycheva et al., 2011). The abundance of bioactive components in aronia is linked with preventative and healing capacity against many chronic diseases. Its fruits display antidiabetic (Qin & Anderson, 2012; Lipińska & Jóźwik, 2017), anti-inflammatory (Zapolska-Downar et al., 2012) and anticancer activity (Cvetanović et al., 2018). Aronia is also known to reduce oxidative stress by cleaning free radicals (Dietrich-Muszalska et al., 2014) and can exhibit anti-toxic effects against numerous harmful substances (Borowska & Brzóska, 2016).

Although the yogurt production worldwide is predominated by cow milk, over the last years there is an increased interest in using buffalo milk as a raw material. Compared with bovine milk, buffalo milk is characterized by higher levels of calcium, fat, lactose, protein, casein, and ash content, and is a good source of minerals, such as magnesium, potassium, and phosphorus (Abd El-Salam & El-Shibiny, 2011;

Abdel-Hamid et al., 2017; Khan et al., 2017). It also contains more tocopherols, vitamin A (Basilicata et al., 2018) and is associated with lower risk of allergies than cow`s milk (Sheehan & Phipatanakul, 2009). As a result, buffalo milk has been used with considerable success for producing of numerous dairy products, including yoghurt (Abdel-Hamid et al., 2017). The combination of buffalo yogurt high nutritional value with bioactive phenolic compounds in aronia fruits is an option for creating a natural functional food with beneficial effect on human health.

The purpose of this study is to investigate the effects of aronia juice on antioxidant activity, fatty acid composition, content of free amino acids, minerals, vitamins B1, B2, B6 of buffalo yogurt.

## MATERIALS AND METHODS

***Fresh milk and starter cultures***. Fresh raw buffalo milk and a starter culture, containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* (Lactina 17, Bankya, Sofia, Bulgaria) ready for direct vat inoculation were used for yogurt preparation. The buffaloes' yogurt was prepared in laboratory conditions.

***Yogurt preparation***. The milk was pasteurized (95°C/30 min), cooled to 45°C, and inoculated with 1.5% yogurt culture consisting of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* (Lactina 17, Bankya, Sofia, Bulgaria). The raw milk was divided into three lots - control and 2 experimental. Prior to adding the starter, 3 and 5 g/kg fruit juice from aronia was added to the experimental milk samples. The samples were then cultivated at 42°C until coagulation, then cooled and stored in a refrigerator at 4-6°C.

***Fatty acid composition***

The extraction of milk fat was done by the method of Rose-Gottlieb using diethyl ether and petroleum ether (Methodenbuch, Bd. VI VDLUFA-Verlag, Darmstadt, 1985). After that the solvents were evaporated on a vacuum-rotary evaporator. Sodium methylate ($CH_3ONa$) was used for obtaining methyl esters of the fatty acids. The fatty acid composition of raw milk and yoghurt was determined by gas chromatography "Clarus 500" with flame ionization detector and column Thermo Scientific, 60 m, ID 0.25 mm, Film: 0,25 μm.

***Vitamins determination***

The determination of vitamins B1, B2 and B6 was carried out using VitaFast® by extracting the respective vitamin from a homogenized sample and diluting the extract. The diluted extract and standards were applied to the wells of a microtiter plate coated with *Lactobacillus fermentum* (B1), *Lactobacillus rhamnosus* (B2) and *Saccharomyces cerevisiae* (B6), respectively.

***Free amino acid analysis and mineral composition***

The free amino acid was done by EZ: fast Amino Acid Analyis Kit from Phenomenex Inc for GC/FID.

The macro- and microelement composition were determined after dry incineration of the sample, its mineralization in a muffle furnace at 550°C and preparation of a hydrochloric acid solution. Mineral composition was determined by atomic absorption spectrophotometer "Perkin-Elmer 380".

***Determination of antioxidant activity***

*Determination of Total Flavonoid Content (TFC)*

TFC was determined via the aluminium trichloride method, using catechin as reference material (Dinev et al., 2021). In brief, 1.0 mL sample (of skimmed milk with protein removed or juice diluted by deionized water 1:4) with, 0.3 mL 5% $NaNO_3$, and after 5 min, 0.3 mL 10% $AlCl_3$ were added in a 10 mL volumetric flask containing 4.0 mL deionized water in this order. After 6 min, 2.0 mL of 1 M NaOH solution was added and the total volume was adjusted up to 10 mL using deionized water. The suspension obtained was homogenized and centrifuged (4000 x g) at room temperature. The absorbance of the supernatant was measured against a prepared reagent blank at λ = 510 nm on a Thermo Scientific Evolution 300 spectrophotometer. Standard solutions of (+)-catechin hydrate (Sigma Aldrich, St. Louis, MO, USA) in the concentration range from 10 to 100 mg/L were used to plot the calibration curve. The total flavonoid content was expressed as μg catechin equivalent (CE) in 1 L (μg CE/L). Each sample was analysed in triplicate.

*Determination of Radical Scavenging Activity by DPPH Method*

DPPH (1,10-diphenyl-2-picrylhydrazil-radical) was purchased from Sigma-Aldrich (St. Louis, MO, USA). This substance has a single electron on the nitrogen atom and its solution in methanol has an absorption maximum at λ = 517 nm. The mechanism of the DPPH method is based on the reaction between the test compound and DPPH-radical, wherein the potential free radical scavengers reduce DPPH-radical (violet solution) to a yellow coloured 1,10-diphenyl-2-(2,4,6-trinitrorhenyl) hydrazine by donating a hydrogen atom. The method described by Dinev et al. (2021) was applied to measure the radical scavenging potential of the samples of skimmed milk with protein removed, and juice diluted by deionized water 1:10 In brief, 0.1 mL of each sample was added to 3.9 mL of 100 mM solution of DPPH in methanol. The absorption of the supernatant obtained by centrifugation (4000 x g) at room temperature at λ = 517 nm was measured 30 min later on a Thermo Scientific Evolution 300 spectrophotometer. The results were calculated using regression analysis from the linear dependence between the concentration of Trolox and the absorption at 517 nm. The results were expressed as μmol Trolox equivalent (TE) in 1 L (μmol TE/L). Each sample was analysed in triplicate.

***Statistical analysis***. For statistical analysis, statistical software IBM SPSS-Inc., 2019, (SPSS Reference Guide 26 SPSS, Chicago, USA) was used.

## RESULTS AND DISCUSSIONS

***Free amino acid***

In the process of fermentation of yogurt proteins are partially hydrolysed into peptides and free amino acids due to the proteolytic activities of lactic acid bacteria (Germani et al., 2014). The total amount of amino acids in experimental samples raised significantly compared to the control, being 2.20 times higher in 5% aronia samples and more than 3 times higher in these with 3% aronia (Table 1). The same trend was observed with the quantity of essential amino acids - 4.78 and 3 times higher in 3 and 5% supplemented yogurt respectively. In our opinion the added aronia juice provided lactic acid bacteria (LAB) with beneficial substances and enhanced their growth, which resulted in bigger quantities of free amino acids in enriched yogurt. The studies of Nguyen & Hwang (2016) and of Boycheva et al. (2011) showed that yogurt containing aronia juice showed higher LAB counts than plain one. Another possibility for higher levels of amino acids in fruit samples is the ability of polyphenol compounds to interact with milk proteins (O'Connell & Fox, 2001). The main representative of essential amino acids in three samples was lysine with its amount increasing from 30.32 mg% in control to 74.39 mg% and 140.01 mg% in 5% and 3% aronia enriched yogurt.

Table 1. Free amino acid content in plain and aronia supplemented buffalo yogurt, mg %

| Free amino acids | Control | Aronia 3% | Aronia 5% |
|---|---|---|---|
| | Mean±SEM | Mean±SEM | Mean±SEM |
| Alanine | $12.53^{a*}\pm0.10$ | $26.70^{a}\pm0.07$ | $21.53^{a}\pm0.27$ |
| Glycine | $2.57^{a}\pm0.18$ | $6.63\pm0.32$ | $6.91^{a}\pm0.12$ |
| Valine | $6.98^{a}\pm0.04$ | $19.19^{a}\pm0.06$ | $17.02^{a}\pm0.03$ |
| Leucine | $21.65^{a}\pm0.18$ | $57.91^{a}\pm0.06$ | $45.32^{a}\pm0.67$ |
| Isoleucine | $6.34^{ab}\pm0.03$ | $17.36^{a}\pm0.28$ | $14.84^{b}\pm0.07$ |
| Threonine | $2.34^{a}\pm0.06$ | $59.45^{a}\pm0.27$ | $48.07^{a}\pm0.37$ |
| Serine | $41.46^{a}\pm0.26$ | $38.80^{a}\pm0.06$ | $30.61^{a}\pm0.17$ |
| Proline | $11.84^{ab}\pm0.09$ | $18.60^{a}\pm0.16$ | $18.91^{b}\pm0.03$ |
| Asparagic acid | $8.07^{a}\pm0.03$ | $20.47^{a}\pm0.41$ | $21.88^{a}\pm0.05$ |
| Asparagine | $9.68^{a}\pm0.11$ | $18.51^{a}\pm0.20$ | $11.86^{a}\pm0.10$ |
| Methionine | $0.28^{a}\pm0.01$ | $0.89^{a}\pm0.00$ | $0.25^{a}\pm0.07$ |
| Glutamic acid | $4.22^{a}\pm0.05$ | $6.65^{a}\pm0.07$ | $3.89^{a}\pm0.06$ |
| Phenylalanine | $5.65^{a}\pm0.03$ | $24.70^{a}\pm0.05$ | $16.95^{a}\pm0.04$ |
| Glutamine | $2.62^{a}\pm0.05$ | $12.70^{a}\pm0.07$ | $7.82^{a}\pm0.18$ |
| Histidine | $3.76^{ab}\pm0.11$ | $9.09^{a}\pm0.06$ | $9.57^{b}\pm0.12$ |
| Lysine | $30.32^{a}\pm0.04$ | $140.01^{a}\pm0.36$ | $74.39^{a}\pm0.13$ |
| Tyrosine | $5.99^{a}\pm0.08$ | $46.14^{a}\pm0.58$ | $20.11^{a}\pm0.01$ |
| Tryptophan | $9.47^{a}\pm0.02$ | $74.99^{a}\pm0.09$ | $31.82^{a}\pm0.09$ |
| Cysteine | - | $0.77^{ab}\pm0.01$ | $0.29^{a}\pm0.01$ |
| Arginine | $7.64^{a}\pm0.05$ | $28.62^{a}\pm0.25$ | $21.34^{a}\pm0.25$ |
| **Total** | 193.48 | 628.18 | 425.79 |

$^{*a,b}$ - $p<0.05$

The branched chain amino acids (BCAAs)- valine, leucine and isoleucine belong to the essential ones and are reported to play a key role in metabolism of glucose, lipids, protein synthesis and intestinal health (Nie et al., 2018). Their levels in the organism can also serve as early signals for development of some chronic diseases (Newgard et al., 2009). The biggest amounts of BCAAs, with main representative leucin was recorded in 3% aronia yogurt as every particular amino acid increased its quantity more than twice compared with the control sample content.

Among the sulfur amino acids (SAAs) methionine and cysteine are considered to be principal ones as the former is classified as essential and the latter as semi-essential one. Recently, numerous studies have investigated SAAs` significance for protein metabolism and synthesis and their role as a precursor of important molecules (Métayer et al., 2008). The content of methionine decreased slightly in 5% aronia yogurt compared with the control, while there was considerable raise in 3% yogurt - from 0.280 mg% to 0.890 mg%. Cystein wasn`t detected in the plain yogurt, however in the supplemented ones it`a amount varied from 0.290 mg% in 5% to 0.770 mg% in 3% aronia yogurt.

The greatest increase of quantity compared to the plain yogurt was observed with threonine – more than 20 times in 5% supplemented and 25 times in 3% one. As an essential amino acid threonine is important for lipid metabolism in liver and for protein synthesis in human body (Tang et al., 2021).

As to non-essential amino acids the main representative in control sample and in 5% supplemented yogurt was serine - 41.46 mg % and 30.61 mg % respectively, while in 3% yogurt it was tyrosine - 46.14 mg %. The quantity of both essential and non-essential amino acids increased in aronia supplemented yogurt compared to the plain one, more significantly in 3% enriched samples (Figure 1).

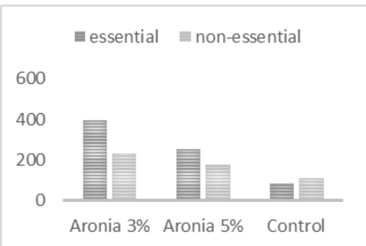


Figure 1. Essential and non-essential amino acids in yoghurt, mg%

***Fatty acid profile of yogurt***

The results showed that the amount of saturated fatty acids (SFA) in experimental samples was slightly lower than in control (Table 2). Similar data were reported by Boycheva et al. (2011) about goat yogurt, supplemented with 5%aronia juice. As expected, predominant in SFA was palmitic acid with a maximum concentration in 5% yogurt - 29.54%, while smallest percentage was reported by tridecyl acid - 0.21% in both enriched yogurts.

Highest concentration of unsaturated fatty acids (UFA) was observed in 5% aronia yogurt. The quantity of monounsaturated fatty acids (MUFA) with main representative oleic acid raised insignificantly compared to the plain yogurt. Largest amount of polyunsaturated fatty acids (PUFA), which are considered to have prophylactic effect against some chronic diseases (de Caterina & Zampolli, 2001) was also obtained in 5% aronia yogurt with a significant increase in the percentage of linolenic acid, that was 2.2 times higher than in control. The linolenic acid is known to have cardiovascular - protective, neuroprotective, anti – inflammatory and anti - oxidative effects (Kim et al., 2014) The recent study of Tasinov et al. (2022) showed that main fatty acids in aronia juices on Bulgaria market were the nonessential stearic and essential linoleic and linolenic acids. Therefore, we presumed that the highest amount of linolenic acid in 5% aronia samples was due to the added juice. However, our results differed from these of Boycheva et al. (2011) where quantities of linolenic acid in plain goat yogurt and in 5% aronia supplemented one were quite lower and almost the same - 0.63% and 0.65% respectively. On the other hand, the concentration of conjugated linoleic acid (CLA) decreased in fortified samples, more significantly in 5% one. Compared to the results of Kowaleski et al. (2020) about 10% strawberry enriched cow yogurt, containing 1.29% CLA, the aronia supplemented buffalo samples showed much lower numbers - 0.50% in 3% and 0.38% in 5% ones.

The ratio C18:2/C18:3 varied from 1.89 in control to 2.04 in 3% and 0.78 in 5% fortified yogurts and corresponds with contemporary recommendation for healthy nutrition (Oscarsson & Hurt-Camejo, 2017).

Table 2. Fatty acid content in plain and aronia supplemented yogurt, %

| Fatty acids, % | Control | Aronia 3% | Aronia 5% |
|---|---|---|---|
| | **Mean±SEM** | **Mean±SEM** | **Mean±SEM** |
| C4:0 | 1.90±0.089 | 2.40±0.075 | 1.89±0.015 |
| C6:0 | 1.53±0.037 | 2.41±0.095 | 1.20±0.033 |
| C8:0 | 1.05±0.007 | 1.23±0.055 | 0.77±0.058 |
| C10:0 | 2.86±0.012 | 3.19±0.005 | 2.43±0.069 |
| C12:0 | 3.00±0.017 | 3.55±0.075 | 2.99±0.011 |
| C13:0 | 0.17±0.015 | 0.21±0.003 | 0.21±0.015 |
| C14:0 | 10.83±0.033 | 10.72±0.705 | 11.05±0.298 |
| C14:1 | 0.62±0.005 | 0.87±0.005 | 0.72±0.057 |
| C14:2 | 0.30±0.004 | 0.37±0.005 | 0.36±0.001 |
| C15 iso | 0.60±0.009 | 0.72±0.005 | 0.71±0.044 |
| C15:0 | 1.29±0. 002 | 1.49±0.020 | 1.35±0.073 |
| C16:0 | 29.46±0.110 | 27.70±0.645 | 29.54±0.529 |
| C16:1 | 1.36±0.009 | 1.56±0.090 | 1.77±0.098 |
| C16:2 | 0.51±0.001 | 0.36±0.005 | 0.47±0.001 |
| C17:0 | 1.24±0.011 | 1.20±0.030 | 0.87±0.005 |
| C17:1 | 0.25±0.006 | 0.25±0.005 | 0.29±0.001 |
| C18iso | 3.19±0.062 | 2.83±0.025 | 3.52±0.010 |
| C18:0 | 9.60±0. 089 | 7.25±0.095 | 9.33±0.362 |
| C18:1 | 26.53±0.752 | 27.17±1.025 | 27.29±0.346 |
| C18:2 | 2.18±0.042 | 2.75±0.020 | 1.99±0.038 |
| C18:3 | 1.15±0.062 | 1.35±0.015 | 1.04±0.009 |
| CLA | 0.57±0.002 | 0.50±0.008 | 0.38±0.009 |
| SFA | 66.73±0.971 | 64.85±1.220 | 65.81±0.199 |
| UFA | 33.27±0.971 | 35.15±1.220 | 34.19±0.199 |
| MUFA | 28.68±0.673 | 29.84±1.385 | 30.08±0.366 |
| PUFA | 4.60±0.011 | 5.32±0.065 | 4.12±0.049 |

*p>0.05 for all samples; SFA – saturated fatty acids; USA - unsaturated fatty acids; MUFA - monounsaturated fatty acids; polyunsaturated fatty acids (PUFA); CLA - conjugated linoleic acid

### *Mineral content*

The highest amounts of calcium, zink and copper were measured in raw milk. The percentage of phosphorus and potassium moved slightly down in control and in 3% aronia yogurt compared to the raw milk and in 5% samples raised, reaching the numbers of raw milk again (Table 3).

Table 3. Average mineral content in raw milk and in plain and aronia supplemented yogurts

| Elements | Raw milk | Control yogurt | Aronia 3% | Aronia 5% |
|---|---|---|---|---|
| | **Mean±SEM** | **Mean±SEM** | **Mean±SEM** | **Mean±SEM** |
| Ca, % | 0.200±0.000 | 0.199±0.000 | 0.190±0.003 | 0.188±0.001 |
| P, % | 0.156±0.000 | 0.151±0.001 | 0.138±0.002 | 0.151±0.003 |
| K, mg/kg | 1000±1.11 | 982±2.84 | 962±1.17 | 1004±10.31 |
| Mg, mg/kg | 192.9±1.13 | 189.2±9.81 | 192.3±8.65 | 177.2±6.27 |
| Zn, mg/kg | 5.66±0.040 | 5.25±0.045 | 5.20±0.318 | 5.28±0.044 |
| Mn, mg/kg | 0.118±0.002 | 0.122±0.001 | 0.129±0.002 | 0.104±0.003 |
| Cu, mg/kg | 0.416±0.006 | 0.388±0.130 | 0.382±0.012 | 0.351±0.008 |
| Fe, mg/kg | 1.73±0.020 | 1.85±0.012 | 1.80±0.010 | 1.71±0.021 |

Iron increased its amount in control and 3% samples and in 5% one decreased to the levels of raw milk. Significantly higher amount of iron was reported by Belyaev et al. (2022) in goat milk, supplemented with 5% aronia - 21.43 mg/kg, but lower of phosphorus - 0.109%. The quantity of calcium, magnesium manganese, zinc and copper gradually reduced and were lowest in 5% fortified yogurt. Our results differ from those of Paszczyk & Tonska (2022), where yogurts had higher contents of copper and manganese compared to the raw milk. Despite the fact, that aronia juice is rich source of potassium and zinc (Kulling & Rawel, 2008), there wasn`t considerable rise in their levels in fortified yogurts - on the contrary the percentage of zinc decreased with 0.38% in supplemented samples compared to the raw milk. The same was observed with the amounts of copper and manganese, even though, according to Pavlovic et al. (2015), aronia juice contains big amount of copper, and manganese. Decreased levels of these minerals can be explained with the metabolism of lactobacteria in the process of fermentation.

### *Vitamins*

During our study, the levels of vitamin B1 (thiamine), B2 (riboflavin) and B6 (pyridoxin) were measured. All three vitamins showed in increase in their levels, most prominent in B6, which amount raised more than 3 times in 5% aronia samples compared to the control (Table 4).

Table 4. The content of vitamins B1, B2 and B6 in plain and aronia supplemented yogurts, mg/100 g

| Sample | Vit. B1 | Vit. B2 | Vit.B6 |
|---|---|---|---|
| | **Mean±SEM** | **Mean±SEM** | **Mean±SEM** |
| Control | 0.061±0.001 | 0.098±0.002 | 0.051±0.001 |
| Aronia 3% | 0.058±0.001 | 0.146±0.004 | 0.057±0.001 |
| Aronia 5% | 0.069±0.003 | 0.162±0.003 | 0.166±0.003 |

Vitamin B1 is an essential cofactor in the metabolism of carbohydrates and amino acids (Smith et al., 2021). Its levels changed most insignificantly in the three types of yogurts. Vitamin B2 acts as an antioxidant (Cheung et al., 2014), plays an important role in regulation of cellular functions of and its derivatives are cofactors in essential enzyme-catalyzed reactions (Mosegaard et al., 2020). The quantity of riboflavin raised from 0.098 mg/100 g in control to 0.162 mg/100 g in 5% supplemented yogurt. Vitamin B6 has an important role in the function of the human nervous system and coenzyme forms of pyridoxine are involved in numerous biochemical reactions

(Dakshinamurti et al., 1990). Its amount was more than 3 times higher in 5% aronia yogurt compared to the plain one. The fruits of aronia are not a rich source of B1, B2, and B6 vitamins (Blinnikova, 2013) and this considerable raise of riboflavin and pyridoxine in 5% enriched samples may occur because of aronia`s juice bioactive components, that support the growth of LAB. Although some strains in starters are able to produce B vitamins (Gahriue et al., 2015), *Streptococcus thermophilus* and/or *Lactobacillus delbrueckii* subsp. *Bulgaricus* are not documented to have high capability to biosynthesize vitamins B2 and B6 (Patel et al., 2013). Presumably, the cooperation between mentioned ingredients resulted in increased formation of these vitamins in aronia yogurt.

***Antioxidant activity***

Yogurt in combination with fruits has a functional role in the human body due to the supply of fibre, vitamins, minerals, phytonutrients, polyphenols, anthocyanins (Limei et al., 2007). Fruit supplementing in yogurt is a popular approach to increase phenolic content and improve antioxidant profile. Also, yogurt fortified by natural antioxidants meets consumer requirements for "clean label" foods (Tolic, 2015; Gao et al., 2015).

Some fruits, e.g. Aronia are good source of phenolic compounds, especially of anthocyanins. Polyphenols are known to interact with milk proteins and form insoluble complexes. This reduces the total content of free polyphenols (Chouchouli et al., 2013).

In Table 5 are presented the results total flavonoid content (TFC) and the radical scavenging capacity obtained in yoghurt fortified by different amounts of Aronia juice. Yoghurt with 5% Aronia juice had the highest TFC (0.369 μg CE/L), and the control yoghurt - the lowest (0.075 μg CE/L). The total flavonoid content increased 2,3-, and 4,9-fold in the yoghurt with 3% and 5% aronia juice, respectively. The results in the present study confirmed those obtained by Nguyen & Hwang (2016), and Cuşmenco & Bulgaru (2020). The cited researchers concluded a proportional relationship between the TFC and the amount of juice added.

Table 5. The antioxidant activity of plain and aronia supplemented yogurt

| Sample | Antioxidant activity | |
|---|---|---|
| | μmol TE/L | μg CE/L |
| Control | 5.5 | 0.075 |
| Aronia 3% | 9.6 | 0.173 |
| Aronia 5% | 13.15 | 0.369 |
| Aronia juice | 508.2 | 220.9 |

Our research team found the same correlation between the radical scavenging capacity and the amount of aronia juice added. (Table 5): the lowest results are 5.5 μmol TE/L (control yoghurt sample) and the highest - 13.15 μmol TE/L (yoghurt with 5% aronia juice). This is expected, because the flavonoids are known with their antioxidant properties and especially with the ability to scavenge free radicals. Also, the higher antioxidant activity in yogurt containing aronia may result from the phytochemical content of the juice and microbial metabolic activity (Nguyen & Hwang, 2016).

## CONCLUSIONS

The present study demonstrated that supplementation of buffalo yogurt with aronia juice effectively increased total amount of amino acids, including essential ones, the percentage of linolenic acid, of vitamins B2 and B6, the total flavonoid content and antioxidant activity in experimental samples. This contributed to buffalo`s yogurt high nutritional value and mineral content and opens up a possibility to add extra functionality to this type of product.

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