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TEXTURAL AND SENSORY PROPERTIES OF YOGURT OBTAINED BY BIOCONVERSION OF MILK-LIQUID WHEY MIXTURE AND ARONIA (ARONIA MELANOCARPA) SUPPLEMENTATION

SUMMARY

Yogurt is a fermented dairy product of great importance worldwide because of its nutritional and health benefits. The objective of this study was the bioconversion of a milk-liquid whey mixture into yogurt and Aronia (*Aronia melanocarpa*) supplementation. *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* were used as microorganisms for control and functional yogurt production. Samples were analyzed in terms of syneresis index and textural and sensory properties throughout cold storage. The syneresis index was determined using the drainage method, and the textural parameters were obtained from the texture profile analysis of samples. Sensory evaluation was used to assess consumer acceptance and responses to the sensory properties of functional yogurt. Functional yogurt expressed differences in syneresis index during storage and was significantly higher than control yogurt on day 21. The textural properties of the samples were similar during storage. Higher values of cohesiveness and springiness and lower values for hardness, adhesiveness, and gumminess were observed in functional yogurt. The results showed that color and taste had no statistically significant effect on yogurts. Only the level of whey separation differed considerably on the last day of storage, according to an evaluation of yogurt qualities. The bioconversion of liquid whey into functional yogurt provides a technology for whey valorization that promotes human health and environmental sustainability.

Keywords: bioconversion, liquid whey, yogurt, Aronia, sensory properties

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INTRODUCTION

Cheese whey is the yellow-green colored liquid portion of milk, also called serum, obtained after separation of curd (Minj and Anand, 2020). The main nutrients found in whey are mineral salts, lipids, soluble proteins, and lactose (León-López *et al.*, 2022). The manufacturing of cheese and casein yields whey, a byproduct that is quite important in the dairy sector because of its high yield and nutritious contents (Mollea *et al.*, 2013). The improper disposal of whey by the dairy industry is seen as an environmentally damaging practice due to its significant potential for pollution (Schaefer *et al.*, 2023). This clarifies why the disposal of whey is highly regulated and why large-scale dairy enterprises must find other ways to dispose of it. (Flinois *et al.*, 2019). The dairy industry is very concerned about managing dairy waste to reduce pollution to the environment by using whey as the main waste material in various food and non-food items (Hameed *et al.*, 2023). North Macedonia's dairy industry faces ongoing whey discharge concerns. Wasted components can be removed from dairy products utilizing several extraction processes, such as traditional homogenization, ultra-high-pressure homogenization, ultrafiltration, reverse osmosis, and nanofiltration (Hameed *et al.*, 2023). However, bioconversion of whey in liquid form without further processing has numerous advantages. Dairy products are at the top of consumers' choices for functional foods, as they are considered the best host for functional ingredients (Dimitrellou *et al.*, 2020). Yogurt is a semi-solid, fermented dairy product that is rich in numerous nutrients and minerals. Its consumption has expanded globally given its nutritional benefits and ease of digestion (Plessas *et al.*, 2023). As yogurt is an excellent source of proteins, vitamins and minerals, it plays a significant part in a healthy diet (Gómez *et al.*, 2020). Using liquid whey (LW) as an ingredient in yogurt production, along with Aronia, gives a novel functional yogurt with increased nutritional content. However, it can increase the risk of whey separation, as indicated by the syneresis index (SI). The primary causes of whey separation are (i) a higher whey protein concentration than casein, (ii) lower total solids concentrations, and (iii) changes in the organic acids produced by living lactic acid bacteria (LAB) during storage (Plessas *et al.*, 2023). According to Cais-Sokolińska and Walkowiak-Tomczak (2021), adding fruit (juice, pulp, or pureè) to yogurt improves its functional and nutritional properties, shelf life, and consumer appeal. Aronia is high in anthocyanins, minerals, antioxidants, and vitamin C, all of which assist to boost the immune system (Cușmenco and Bulgaru, 2020). Aronia melanocarpa's polyphenolic components have numerous health benefits, including anti-inflammatory, anticancer, antibacterial, antiviral, antidiabetic, antiatherosclerotic, hypotensive, antiplatelet, and antioxidant properties (Jurendić and Ščetar, 2021). Buffalo yogurt's high nutritional value, mixed with the bioactive phenolic compounds present in Aronia fruits, provides a way to create naturally functional food that is beneficial to people's health (Zheleva *et al.*, 2023). According to Cușmenco and Bulgaru (2020), adding Aronia fruits, strawberries, raspberries, and peaches greatly increases the biological value and quality indexes of goat milk yogurt with fruits. More and more importantly, in today's climate, individuals are increasingly willing to experiment with new flavor combinations, especially those that contain chemicals with possible health advantages (Plessas *et al.*, 2023).

To the best of our knowledge, this is the first study to make yogurt using both liquid whey and Aronia (*Aronia melanocarpa*) supplements. As a result, the study focused on the bioconversion of the milk-liquid whey mixture into yogurt and Aronia (*Aronia melanocarpa*) supplementation for improved health benefits, as well as the syneresis index, textural, and sensory qualities under refrigeration.

MATERIAL AND METHODS

Raw materials and yogurt samples

Yogurt was produced with milk, whey, and a starter culture containing *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* YoFlex ® Premium 3.0, Chr. Hansen (Copenhagen, Denmark) for DVS inoculation (provided by Vemilk, a milk processing company in Republic of North Macedonia). White sugar and frozen strawberries (stored at –18°C) were acquired from a local market in Bitola, Republic of North Macedonia.

The milk was pasteurized for 5 minutes at 90 ± 2℃ and cooled to 4°C. Whey (0.15% fat) was pasteurized at 90 ± 1℃ for 25 minutes before cooling to 45 ± 2℃. Frozen Aronia berries were melted and pasteurized at 65 to 70 °C for 15 minutes. The fruits were then cooled to between 25 and 30 °C. Later, milk (3.05% fat) was heated to 43.8 °C and 4% sugar was added. Milk and milk with 25% whey were inoculated with 0.04% and 0.05% active cultures, respectively (Hiseni, 2023). The pH of the milk and milk with whey were 6.54 and 6.39, respectively, prior to fermentation. After three hours of fermentation at 43 °C, the pH of conventional and functional yogurt was 4.64. Yogurt samples were cooled to an ambient temperature of 25 to 30 °C before being blended with Aronia berries (12%) for 5 minutes. Finally, (CYA) conventional yogurt with Aronia and (FYA) functional yogurt with Aronia samples were stored at 4 to 8℃ for 21 days.

Syneresis index

The yogurt syneresis index (SI) was determined using the drainage method (Isanga and Zhang, 2007). SI was measured in one replicate.

Textural profile analysis

Textural parameters were extracted using texture profile analysis (TPA) (CT3-10kg, Amtek Brookfield, USA) using a cylindrical-shaped probe (38.1mm diameter). The tests were performed at temperatures ranging from 20 to 25°C, whereas the samples were collected at 4 to 8°C. Yogurt samples were kept between 10 and 12°C. TPA was applied to yogurt samples in their original 110-mL container, which was placed underneath the probe with a 70% deformation test target at 1 mm/s (Hiseni, 2023). Hardness, adhesiveness, cohesiveness, gumminess, and springiness were determined. The analyzer runs on a PC using TexturePro CT V1.8 software.

Sensory evaluation

The sensory analysis of the yogurts was conducted using the corrected five-point scoring system (scoring with 25 points), a commonly used ranking approach for dairy samples (Radovanović and Popov-Raljić, 2000/2001; Makarijoski, 2018). It was used to assess consumer acceptance of products. According to the international standards (ISO 22935-1:2009; ISO 22935-2:2009; ISO 22935-3:2009), sensory descriptive analysis of yogurts was conducted on 14 attributes: the distribution of the ingredients (Aronia); the presence of mold; the whey separation; the aroma of the milk; the fermentation aroma; the intensity of the flavor; and the taste of the yogurt—sweet, bitter, sour, Aronia, milky, whey, yogurt, and aftertaste (Hiseni, 2023). Each quality was scored using an ascending scale of 1 (not present), 2 (very weak), 3 (moderate), 4 (intense), and 5 (extremely intense).

Statistical analysis

Mean comparison was done by student's t-test where statistical significance was shown at $P<0.05$, using Minitab 18 (Minitab Inc, USA). All figures were drawn using OriginPro 2021b (OriginLab Corporation, Northampton, MA).

RESULTS AND DISCUSSION

SI of yogurt samples during storage is plotted in Figure 1. The use of LW in yogurt with Aronia resulted in a lower SI only on day 1. SI in CYA and FYA was 45.35%, respectively, 36.46% on day 1, 55.06%, and 78% on day 21. The products with LW showed higher stability during the first week of storage, and then the increase in SI in FYA was more evident compared to CYA.

The negative impact on the SI in FYA on day 21 was due to the addition of LW. SI may become significantly higher in the case of low-fat yogurts or yogurts produced with the addition of fruit juices (Dimitrellou *et al.*, 2020). Literature shows a range of SI values depending on the evaluation method. The SI in our study for CYA and FYA was higher except for day 1 than those reported by Dimitrellou *et al.* (2020) in which the SI of yogurts with Aronia juice on day 1 and 21 were 38% and 37%, respectively. Predescu *et al.* (2022) analyzed control yogurt (0%) and yogurt with 5% black chokeberry purée and reported a continuous increase of syneresis during storage from 33.31% on day 1 to 37.81% on day 15. The SI in our study for CYA and FYA is significantly higher compared to these results, which is due to the higher fruit percentage and also the whey addition in the last product. Other authors reported that the addition of other types of fruit juices, like concentrated grape juice or carrot juice, to yogurt samples with a 0.5% stabilizer resulted in a significant ($P<0.05$) increase in syneresis (Öztürk and Öner, 1999; Kiros *et al.*, 2016).

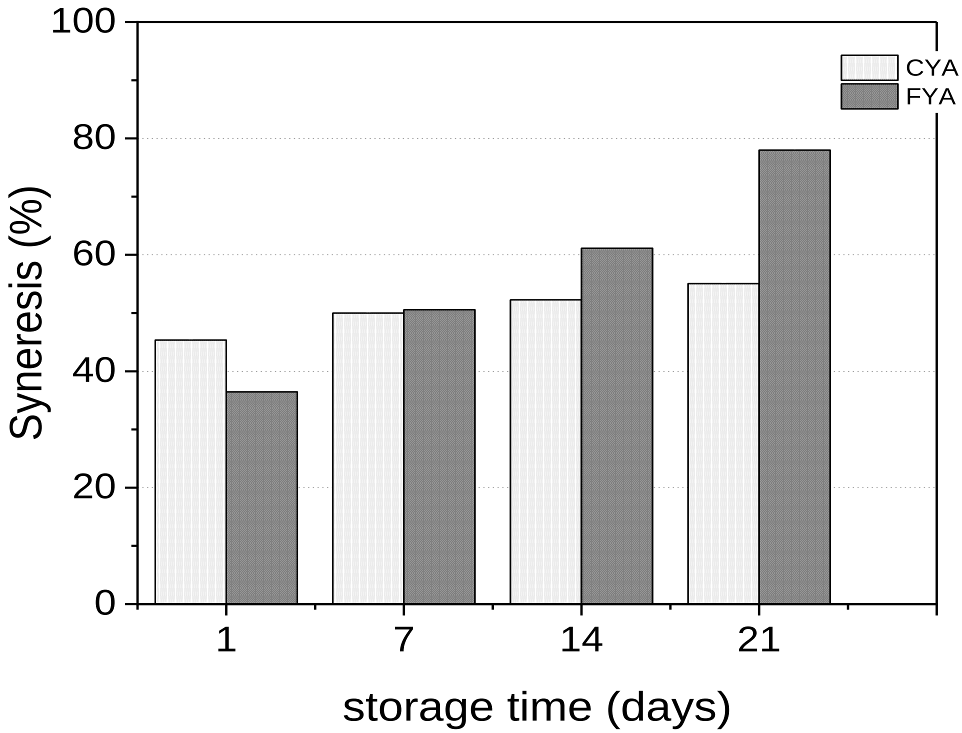

Figure 1. Comparison of syneresis index of yogurt samples. CYA = conventional yogurt with Aronia (control); FYA = functional yogurt with Aronia

Texture profile of yogurts during storage

The TPA test, a double compression test, evaluates perceived sensory qualities by imitating the first two bites of a complex oral processing action (Jonkers, 2021). The TPA test employed in this investigation yielded the following texture parameters: hardness, adhesiveness, cohesiveness, gumminess, and springiness, as shown in Figure 2. The hardness of a product is determined by the amount of force required to compress it (Jonkers, 2021). According to Figure 2, adding whey to the yogurt formulation did not affect the hardness when compared to the control sample. The hardness of CYA and FYA during storage was similar, with a slight increase during storage. The highest level of hardness in samples with whey FYA was (484 ± 127 g) and the lowest was (230 ± 159 g). CYA results on day 21 showed a very high SD. This was due to Aronia`s presence. Hardness results were not statistically significant during storage (P>0.05). Cais-Sokolińska and Walkowiak-Tomczak (2021) observed lower outcomes on day one for yogurt with restructured elderberry juice. Adhesiveness is defined as the effort required to remove food that adheres to the mouth (generally the palate) during the normal swallowing process, which is the force required to separate the material that sticks to the teeth during eating and has an inverse relationship with yogurt eating quality (Park *et al.,* 2020; Mousavi *et al.,* 2019). FYA had lower adhesion values than CYA. When compared to the control samples, the inclusion of whey significantly reduced the adhesiveness of FYA. There was a significant difference between CYA and FYA on days 1 and 21 ($P<0.05$). On day 21, CYA had the highest levels of adhesiveness (11.15 ± 0.21 mJ), while FYA had the lowest level on day 7 (0.8 ± 0.14 mJ). Ziarno and Zareba (2020) found higher adhesiveness values (15.1 mJ on day 28) in yogurt made with skim milk powder. Cohesiveness refers to the strength of internal links that comprise the food's body (Park *et al.*, 2020). Yogurt samples with the addition of whey (FYA) demonstrate higher levels of cohesiveness than CYA (Figure 2). However, a significant difference between CYA and FYA was observed only on day 21 ($P<0.05$). The highest cohesiveness level was FYA on day 14 (0.7 ± 0.07) and the lowest level was CYA on day 21 (0.35 ± 0.007).

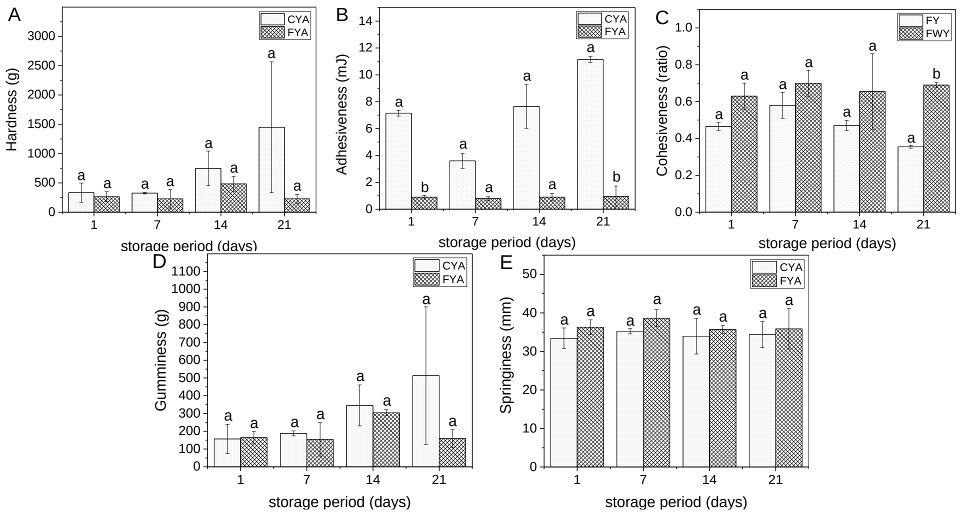

Figure 2. Comparison of yogurt samples for hardness (A), adhesiveness (B), cohesiveness (C), gumminess (D) and springiness (E). CYA = conventional yogurt with Aronia (control); FSY = functional yogurt with Aronia. The mean in the graph indicated by different letters (a–b) is significantly different ($P<0.05$) from the other product. Error bars represent the standard deviation (SD) of the mean of duplicate experiments

Similar results have been obtained for the contribution of liquid milk whey to the yogurt formulation (without fruits), which increased the cohesiveness of yogurt samples (Gauche *et al.*, 2009). Gumminess is the amount of energy required to break up a semisolid food before it is swallowable, and it is determined by firmness and cohesiveness (Mousavi *et al.*, 2019; Khorshidi *et al.*, 2021). The gumminess of yogurt with whey FYA was comparable to that of CYA. Adding whey did not result in a statistically significant difference in gumminess compared to control samples after storage. Gumminess levels demonstrated a correlation with hardness values. Overall, the FYA mean outcomes were lower. Similarly, a previous study found that adding liquid milk whey to the yogurt (without fruits) formulation reduced the gumminess in yogurt samples (Gauche *et al.,* 2009). Springiness is the rate and extent to which a deformed material returns to its original condition after the force is withdrawn, and it is determined by elements such as heat treatment, protein interaction, flexibility, and protein unfolding (Delikanli and Ozcan, 2017). Figure 2 shows that there is no substantial difference between CYA and FYA in terms of springiness. FYA has higher mean values than CYA. However, the lowest springiness level was associated with CYA (36.41 ± 2.68mm). Variable interactions between milk protein particles, as well as variances in the mineral makeup of different types of milk powders, might alter the textural features of fat-free set-type yogurt with diverse types of milk proteins (Peng *et al.,* 2009).

Sensory evaluation

Figure 3 shows the average scores for sensory qualities that are assessed for customer acceptance. FYA results were compared to CYA results during storage. The score of sensory attributes (color and taste) based on the five-point scoring system method of CYA and FYA was not statistically significant during cold storage ($P>0.05$). FYA appearance and aroma score differences were statistically significant on day 21, whereas consistency was consistent on days 14 and 21 ($P<0.05$). FYA was evaluated as having better aroma and taste on day 7 and less consistency during storage, especially on day 21.

Kim *et al.* (2019) reported that the sensory attributes of yogurt decreased with the addition of *Aronia melanocarpa* (black chokeberry) powder (0.5%, 1.0%, 1.5%, and 2.0%). Overall acceptability is calculated by giving each sensory attribute a coefficient of importance, as shown in Figure 4. Taste, aroma, and consistency received the greatest scores (8/20 points, 6/20 points, and 3/20 points, respectively). CYA and FYA had higher overall weighted acceptance during the first week ($P>0.05$). In the following weeks, CYA improved its score, but FYA decreased with a statistically significant difference ($P<0.05$). On the final day of storage, the overall acceptability of CYA and FYA on a five-point scale was 4.61 ± 0.38 points and 3.94 ± 0.51 points, respectively. Kim *et al.* (2019) found that the overall acceptability scores for yogurt with *Aronia melanocarpa* (black chokeberry) powder (0.5%, 1.0%, 1.5%, and 2.0%) varied from 4.2 to 3.7 points, whereas the control (0%) scored 4.3. Nguyen and Hwang (2016) demonstrated that the addition of 3% Aronia juice to yogurt had no harmful impacts on flavor, mouthfeel, thickness, or overall acceptance, despite the fact that Aronia has a bitter or astringent taste.

The addition of Aronia up to 12% in CYA and FYA had no adverse effect on the taste of yogurt, whereas in CYA during storage, it increased the overall acceptability.

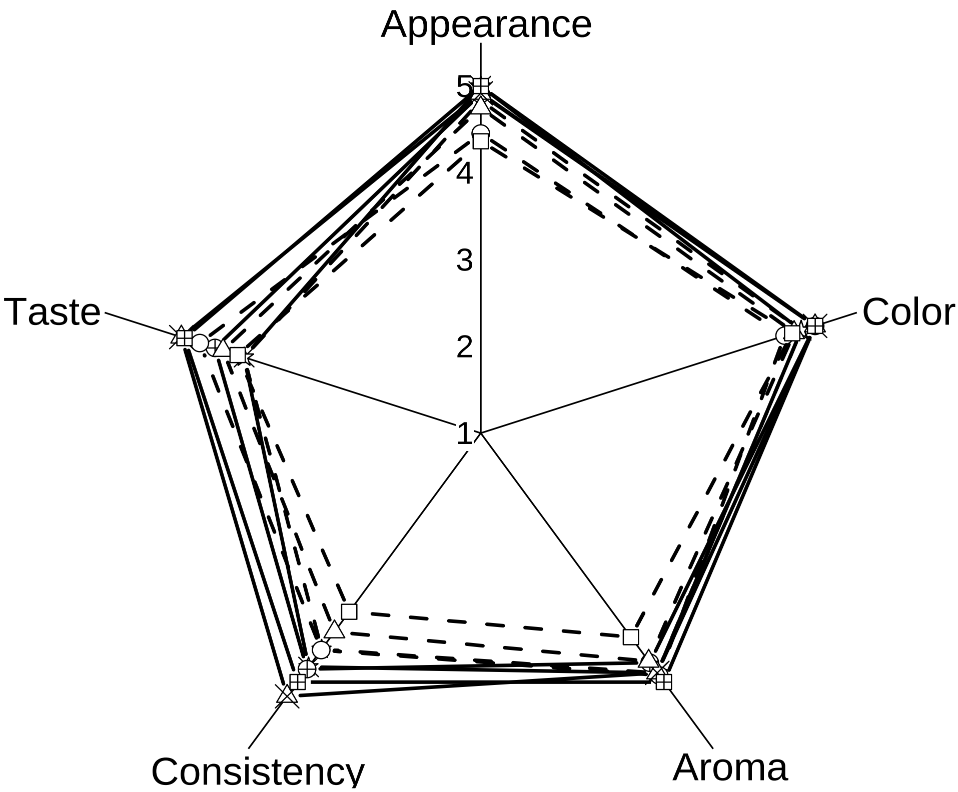

Figure 3. Sensory attributes of yogurts during storage: CYA = conventional yogurt with Aronia (, day 1; , day 7; , day 14; , day 21); FYA = functional yogurt with Aronia (, day 1; , day 7; , day 14, , day 21)

Figure 5 shows descriptive sensory evaluation. Distribution of ingredients scored higher on FYA on days 7 and 14 (P>0.05). Aronia contains anthocyanins, which contribute to the purple color of yogurt (Nguyen and Hwang, 2016).

It remained stored without developing any mold. Whey separation in FYA was more obvious on day 21 and there was a significant difference (P<0.05) compared to CYA. This demonstrates that the sensory panel showed strong syneresis, indicating a deteriorated structure on the last day of storage. Milk odor was present at a very weak intensity during storage because the Aronia has a strong flavor (P>0.05). Fermentation odor was very weak to moderate intensity in CYA and FYA (P>0.05). During storage, flavor intensity, sweetness taste, bitter taste, sour taste, milky taste, and Aronia taste obtained identical scores for CYA and FYA samples (P>0.05). Whey was tasted only on day 7 by one assessor (P>0.05).

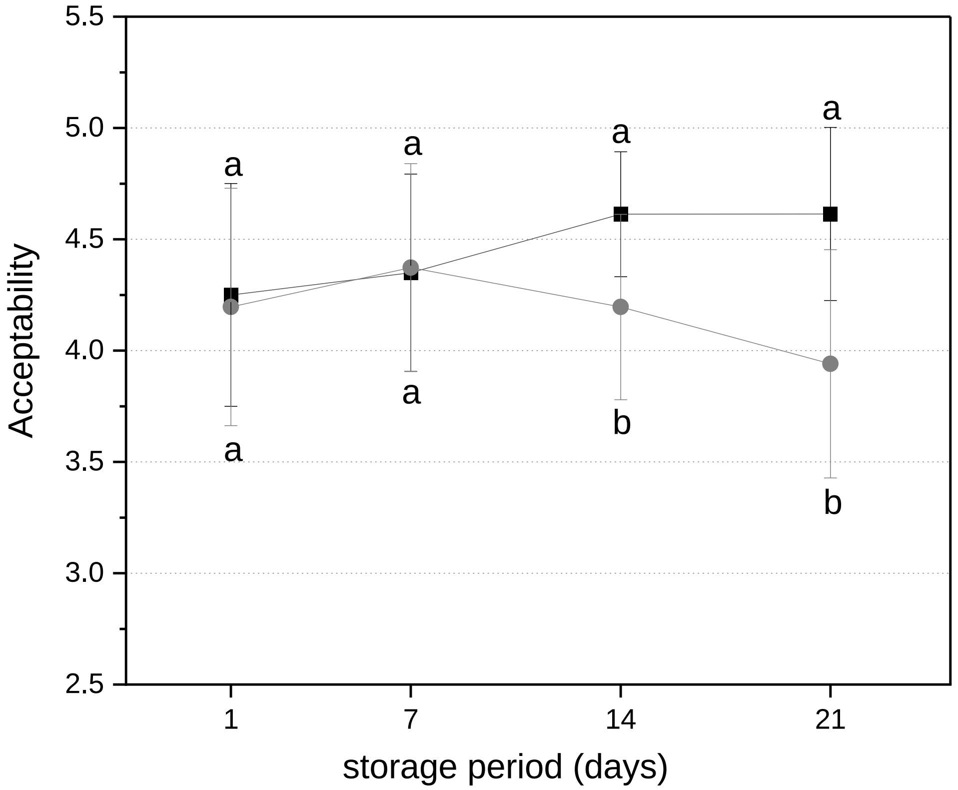

Figure 4. The overall weighted acceptability of yogurts (means ± SD). CYA = conventional yogurt with Aronia (■); FYA = functional yogurt with Aronia (●). The mean values in the graph indicated by different letters indicate significant differences (P<0.05). Error bars represent the standard deviation (SD) of the mean of twelve assessors (N = 12)

Volatile phenols generated through the metabolic processes of probiotic or yogurt cultures can contribute to an enriched flavor and aromatic profile (Plessas *et al.*, 2023).

The yogurt taste score was higher for CYA (P>0.05). FYA's aftertaste score was higher in the first week and decreased in the last week. These findings suggest that liquid whey addition is appropriate for producing functional yogurt with Aronia without the user being aware of the whey inclusion.

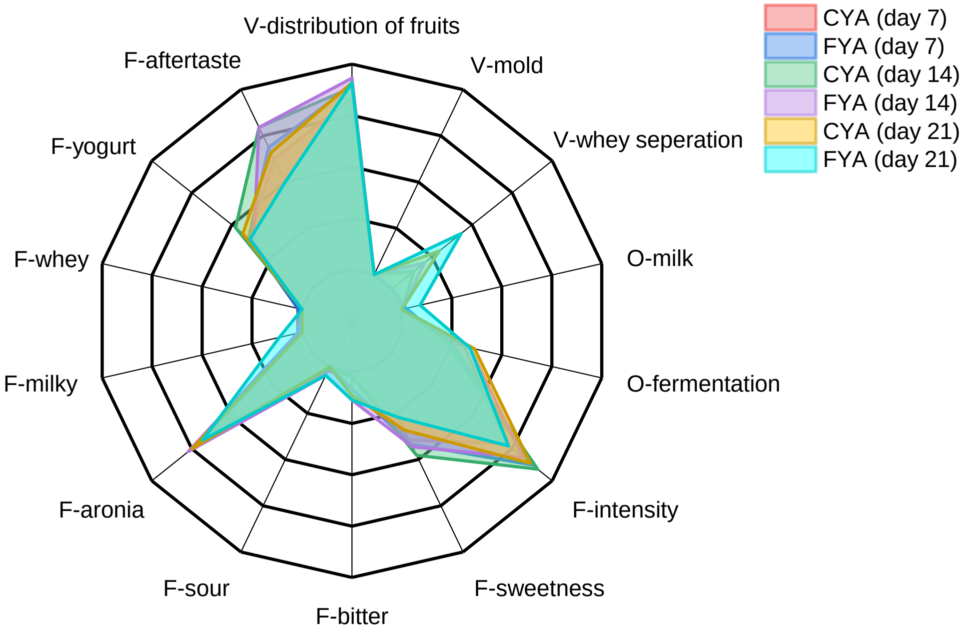

Figure 5. Sensory results from the quantitative descriptive analysis of yogurt samples: CYA = conventional yogurt with Aronia; FYA = functional yogurt with Aronia of 14 major attributes. V = visual; O = odor; F = flavor

CONCLUSIONS

Yogurt produced through the bioconversion of milk-liquid whey mixture and Aronia (*Aronia melanocarpa*) supplementation is a promising beneficial dairy product. On the final day of storage, whey supplementation had an impact on the syneresis index. Except for adhesiveness on days 1 and 21, and cohesiveness on day 21, the textural parameters of functional yogurt samples did not differ significantly from ordinary yogurt during storage. The addition of whey did not affect the flavor or color of the functional yogurt. Other qualities, such as appearance, aroma, and consistency, differed from ordinary yogurt, particularly on the final day of storage. On the last day of storage, functional yogurt with Aronia received consumption acceptance ratings of 3.94 points on a five-point scale. The findings provide valuable insights for the utilization of whey into functional yogurt with Aronia, which can offer health benefits beyond its nutritional value. In addition, in vivo studies would also be helpful to delineate the potential health benefits.

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The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Универзитет „Св. Климент

Охридски"—Битола, Технолошко—технички факултет Велес (protocol code 10-428/2 approved on 2 December 2020).

Informed consent was obtained from all subjects involved in the study.

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