



Proximate Composition and Microbiological Safety of Yogurts Produced by Fermentation of a Milk-Whey Mixture and Supplemented with Fruits

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ABSTRACT

Background: The study aimed to evaluate the effect of incorporating liquid whey as a functional ingredient in yogurt supplemented with strawberries or aronia berries on the proximate composition, dietary mineral content and microbiological safety of freshly prepared yogurts.

Methods: Yogurt was prepared using pasteurized milk, with 25% liquid whey added to the milk. Control products were simultaneously prepared without liquid whey and both were fermented for 3 hours before being supplemented with 12% strawberries or aronia berries. The analysis included pH, proximate composition (protein, fat, carbohydrates and ash) and dietary mineral content [iron (Fe), zinc (Zn), magnesium (Mg), calcium (Ca), phosphorus (P), sodium (Na) and potassium (K)]. Microbiological analysis was performed to assess the presence of *Enterobacteriaceae*, *Listeria monocytogenes*, *Escherichia coli*, yeast and mold.

Result: The average pH of control and functional yogurts was 4.2 and 4.11 for aronia berries and 4.17 and 4.15 for strawberries. Functional yogurts had lower fat (2.08% for strawberry, 2.43% for aronia) and protein content (2.21% for strawberry, 2.28% for aronia) than control yogurts. Control yogurts had higher levels of Fe, Zn, Ca, P, Na and K, but differences were not significant. The Mg content in control yogurt with aronia berries (107.71 mg kg⁻¹) differed from functional yogurt (84.29 mg kg⁻¹). *Enterobacteriaceae*, *E. coli* and *L. monocytogenes* were not detected in the yogurt samples. Functional yogurts underscore the potential of liquid whey and fruit supplementation as functional ingredients, had a lower fat content, making them more appealing to consumers and remained safe for consumption throughout the storage period.

Key words: Dietary minerals, Liquid whey, Nutrients, Safety, Yogurt.

INTRODUCTION

Yogurt is one of the most popular among different types of fermented milk products (Rahman *et al.*, 2020). Consumers are increasingly interested into the nutritional value of foods and the popularity of yogurt derives primarily from its excellent nutritional and health-promoting properties (Cais-Sokolińska and Walkowiak-Tomczak, 2021). Yogurt is a valuable source of high-quality protein that can be easily digested and enhances satiety. It is safe to recommend and may support weight management, muscle growth and bone health (Bankole *et al.*, 2023). Milk and dairy products are rich sources of essential elements like calcium, magnesium and zinc, crucial for metabolism, growth and development, particularly in children (Almášiová *et al.*, 2023). The rising consumer demand for dairy products possessing functional attributes is a crucial driver contributing to the growth of sales value in developed markets (Oladipo *et al.*, 2014).

Liquid whey, a major by-product of cheese manufacturing, represents both an environmental burden and a valuable source of nutrients if properly utilized. It is used in various food applications, including recycling in cheese factories to produce other dairy products and creating non-conventional products like unfermented and fermented whey-derived beverages (Lavelli and Beccalli, 2022).

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Pushpa *et al.* (2018) developed hypotonic electrolyte rehydration drinks using paneer and cheese whey. Repurposing whey in dairy product development not only contributes to waste reduction and sustainability but also enhances the nutritional and functional value of foods. Given the rising consumer demand for healthier and more

functional dairy products, incorporating whey into yogurt formulations is of particular interest. Bioactive food components possess multiple metabolic activities that contribute to beneficial effects across various diseases and target tissues (Deepa *et al.*, 2016). Functional yogurt is favored more and more by consumers due to its enrichment with various health-promoting additives.

Utilizing different technologies to valorize whey and integrating it into products like functional yogurt is a key aspect of advancing sustainability across environmental, economic and societal dimensions (Soumati *et al.*, 2023). Gauche *et al.* (2009) reported physical properties of yoghurt manufactured with 30% milk whey and transglutaminase. Janiaski *et al.* (2016) reported physico-chemical analysis and sensory profile of strawberry-flavored dairy products with liquid or reconstituted whey that were sold in Brazil. The textural and sensory acceptance of yogurt fermented with whey and supplemented with strawberry (Hyseni *et al.*, 2024a) and aronia (Hyseni *et al.*, 2024b) has also been reported. No previous research is known to have examined the proximate composition, dietary mineral content and microbiological safety of yogurts produced by fermenting a milk–liquid whey mixture with fruit incorporation.

Therefore, the aim of this study was to evaluate the potential of liquid whey as a functional ingredient in yogurt supplemented with strawberries or aronia berries. The specific objectives were to investigate its impact on the proximate composition, dietary mineral content and microbiological safety of yogurt. Hence, this research addresses the dual need of sustainable whey valorization and the development of nutritionally enriched dairy products.

MATERIALS AND METHODS

Materials

Milk and whey were supplied by Vemilk (North Macedonia). The yogurt starter culture (YO-FLEX® Premium 3.0, Chr. Hansen, Denmark) contained *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Hyseni *et al.*, 2024). White sugar, frozen strawberries and aronia berries (*Aronia melanocarpa*), stored at -18°C, were sourced locally (Bitola, North Macedonia). Table 1 presents the physicochemical properties of milk, whey and fruits. The yeast and mold count in strawberries and aronia berries were 245±77.8 CFU g⁻¹ and 2,400,000±565,685 CFU g⁻¹, respectively (Hyseni, 2023).

Yogurt preparation

The milk was pasteurized at 90±2°C for 5 min and cooled to 4°C, while whey was pasteurized at 90±1°C for 25 min and cooled to 45±2°C. Milk 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% starter culture, respectively. After 3 hours of fermentation at 43°C, pH values were 4.64 (control), 4.55 (strawberries) and 4.64 (aronia berries). Thawed strawberries and aronia berries were pasteurized at 65-70°C for 15 min and cooled to 25-30°C. Yogurt was also cooled to 25-30°C, supplemented with 12% fruit and mixed for 5 min. Control (CYS, CYA) and functional (FYS, FYA) yogurts were stored at 4-8°C for 21 days (Hyseni *et al.*, 2024).

Assessment of yogurt pH

The pH value of yogurt samples was determined using a pH meter (HI 2210-02, Hanna Instruments).

Proximate analysis

The nutritional composition of yogurt samples was analyzed for macronutrients and dietary minerals at Anima-Vet laboratory (Bitola, North Macedonia). Protein content was determined by the Kjeldahl method using MKC EN ISO 8968-1:2014, with a nitrogen-to-protein conversion factor of 6.38. Total solids were measured gravimetrically according to MKC EN ISO 6731:2012 by oven-drying at 102±2°C until constant weight. Fat content was determined using ISO 11870 (Gerber method) with a butyrometer and sulfuric acid digestion. Moisture content was calculated as the difference between sample weight and total solids, while carbohydrate content was estimated by difference (100 - [moisture protein + fat + ash]). Ash content was determined by dry ashing in a muffle furnace at 550°C according to AOAC Method 942.05 Revisited (Hyseni, 2023).

Analysis of dietary minerals

Mineral analysis of Fe, Zn, Mg, Ca, P, Na and K was performed on 500±200 mg of sample in duplicate on day 14 of cold storage. The samples were mixed with 5 mL of water, 5 mL of 65% nitric acid (HNO₃) and 2 mL of 32% hydrochloric acid (HCl) in a digestion vessel. After 48 hours of pre-digestion, the samples were heated in a microwave (Speedwave™ MWS-3+, Begerhof, Germany) at the Agroviet

Table 1: pH and chemical composition (g 100 g⁻¹) of milk, whey and fruits.

Parameter	Milk				Whey		Strawberries	Aronia berries
	CYS	FYS	CYA	FYA	FYS	FYA		
pH	6.50	6.38	6.60	6.63	5.48	5.47	-	-
Protein	2.98	2.98	2.96	2.97	0.62	0.58	0.9	0.002
Total solids	8.44	8.56	8.40	8.37	6.22	6.11	-	-
Fat	3.13	2.92	3.07	3.05	0.13	0.15	<0.5	<0.5
Carbohydrates	4.39	4.40	4.37	4.34	-	-	5.8	9.6
Fiber	-	-	-	-	-	-	2.1	1.3

laboratory (Fushë Kosovë, Republic of Kosovo). Dietary minerals were analyzed using ICP-OES (Thermo Scientific iCAP 7000 Series) following EPA Method 6010C at the University of Beograd, Republic of Serbia (Hiseni, 2023).

Microbiological analysis

Microbiological analyses of yogurt samples were carried out in the Anima-Vet laboratory (Bitola, North Macedonia) following standardized ISO methods. *Enterobacteriaceae* were determined according to MKC EN ISO 21528-2:2017 by surface plating on Violet Red Bile Glucose Agar (VRBG) after sample homogenization and incubation at 37°C for 24 h. *Listeria monocytogenes* and *Listeria spp.* were examined using the enrichment and selective plating procedure described in MKC EN ISO 11290-1:2018, with Fraser broth and selective agar media, followed by confirmation of typical colonies. *Escherichia coli* was quantified using MKC ISO 16649-2:2008 on Tryptone Bile X-Glucuronide (TBX) agar, incubated at 44°C for 24 h. Yeasts and molds were determined according to MKC EN ISO 21527-1:2008 by spread-plating on Dichloran Rose Bengal Chloramphenicol (DRBC) agar, with incubation at 25°C for 5 days. Results were expressed as colony-forming units per gram of sample (CFU/g).

Statistical analysis

Statistical analyses were conducted using OriginPro2021b (OriginLab, Northampton, MA). Normality was assessed via the Shapiro-Wilk test. ANOVA with Fisher's LSD test was used for normal data, while the Kruskal-Wallis and Dunn's tests were applied for non-normal data. Significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

pH and proximate composition of yogurts

The average pH of control and functional yogurts with aronia berries was 4.2 ± 0.01 and 4.11 ± 0.01 , respectively, showing a significant difference ($P < 0.05$) as seen in Table 2. No significant difference was found between control and functional yogurts with strawberries ($P > 0.05$), with pH values of 4.17 ± 0.08 and 4.15 ± 0.06 , respectively. Gauche *et al.* (2009) reported a pH of 4.34 ± 0.01 for yogurt made

with 30% milk whey. Janiaski *et al.* (2016) also found a comparable pH of 4.2 ± 0.0 in low-fat yogurts with whey and strawberry pulp, aligning with our results. The moisture content of control yogurts with strawberries and aronia berries was $85.35 \pm 0.12\%$ and $83.92 \pm 0.21\%$, respectively, with no significant difference ($P > 0.05$). Functional yogurts showed slight increases to $86.36 \pm 0.07\%$ and $84.24 \pm 1.24\%$, but remained statistically similar. Variations may stem from formulation and whey percentages. Total solids in functional yogurts with aronia and strawberries were $15.69 \pm 0.86\%$ and $13.61 \pm 0.09\%$, respectively, lower than controls ($16.18 \pm 0.25\%$ and $14.93 \pm 0.69\%$), though only functional yogurt with strawberries and yogurt with aronia differed significantly ($P < 0.05$). Ash content in control yogurts with aronia ($0.64 \pm 0.01\%$) and strawberries ($0.60 \pm 0.06\%$) showed no significant difference from functional variants ($0.62 \pm 0.01\%$ and $0.59 \pm 0.01\%$) ($P > 0.05$). This is likely due to the higher ash content in aronia berries compared to strawberries and the replacement of milk with whey before fermentation. Similar findings on whey's effect on ash content were reported by Janiaski *et al.* (2016), though Gauche *et al.* (2009) found 34.33% less ash in yogurt made with 30% milk whey. The fat content of control yogurts with aronia berries and strawberries was $2.93 \pm 0.34\%$ and $2.82 \pm 0.26\%$, respectively and significantly differed from functional yogurts ($2.43 \pm 0.21\%$ and $2.08 \pm 0.15\%$) ($P < 0.05$). The decrease in fat content with whey addition is similar to Janiaski *et al.* (2016), where low-fat yogurts with whey and strawberry pulp showed a 29.7% decrease in fat. Functional yogurt with aronia berries showed a 17.06% decrease in fat. This variation in fat content is attributed to the addition of whey, which aligns with current trends favoring healthier, low-fat options. The average protein content of functional yogurts with strawberries ($2.21 \pm 0.06\%$) and aronia berries ($2.28 \pm 0.19\%$) was significantly lower than that of control yogurts ($2.63 \pm 0.12\%$ and $2.77 \pm 0.13\%$) ($P < 0.05$). Gauche *et al.* (2009) reported 29.93% less protein in yogurt made with 30% milk whey, while our study found 15.96% and 17.68% less protein in functional yogurts with whey and fruits. This could be due to differences in whey protein concentration,

Table 2: pH and chemical composition ($\text{g } 100\text{g}^{-1}$) of the yogurt samples.

	CYS	FYS	CYA	FYA
pH ¹	4.17 ± 0.08^{ab}	4.15 ± 0.06^{ab}	4.2 ± 0.01^a	4.11 ± 0.01^b
Moisture	85.35 ± 0.12^{abc}	86.36 ± 0.07^a	83.92 ± 0.21^c	84.24 ± 1.24^{bc}
Total solids	14.93 ± 0.69^{abc}	13.61 ± 0.09^c	16.18 ± 0.25^a	15.69 ± 0.86^{ab}
Ash	0.60 ± 0.06^{ab}	0.59 ± 0.01^b	0.64 ± 0.01^a	0.62 ± 0.01^{ab}
Fat	2.82 ± 0.26^a	2.08 ± 0.15^c	2.93 ± 0.34^a	2.43 ± 0.21^b
Proteins	2.63 ± 0.12^b	2.21 ± 0.06^c	2.77 ± 0.13^a	2.28 ± 0.19^c
Carbohydrates	8.58 ± 0.33^{cd}	8.73 ± 0.13^c	9.72 ± 0.5^{ab}	10.18 ± 1.39^a

^{*}Results are mean $\pm$ SD of measurements over 21 days ($n = 8$). Different letters (a-d) in a row indicate significant differences ($P < 0.05$) between control and functional yogurts.

¹Mean values are based on evaluations from days 14 and 21.

fruit usage and fermentation duration. Janiaski *et al.* (2016) found similar protein content in low-fat yogurts with whey and strawberry pulp. This study indicates that cheese whey is a valuable whey protein source compared to milk whey and can be used in functional yogurts. While adding whey affects protein composition, it remains a high-quality protein source with all essential amino acids. Additionally, whey proteins offer therapeutic benefits, including anti-cancer, antiviral, antimicrobial, immunomodulatory effects and improved brain function (Chawla *et al.*, 2023). The average carbohydrate content of functional yogurts with aronia berries ($10.18\pm1.39\%$) and strawberries ($8.73\pm0.13\%$) was similar to that of control yogurts ($9.72\pm0.5\%$ and $8.58\pm0.33\%$). The carbohydrate content of control yogurt was lower than in functional yogurt, possibly due to enhanced fermentation during cold storage or differences in composition of the functional yogurt with whey. These results are lower than those in Janiaski *et al.* (2016), where low-fat yogurt with whey and strawberry pulp contained $18.2\pm0.3\%$.

Dietary minerals in yogurts

A one-way ANOVA with Fisher's LSD test showed no significant difference in Ca content between control and functional yogurts ($P>0.05$) (Table 3). The average Ca content in control yogurts with aronia berries and strawberries was 933 ± 202 mg kg⁻¹ and 821.3 ± 16.3 mg kg⁻¹, respectively, both lower than levels reported by Sanchez-Segarra *et al.* (2000) and Luis *et al.* (2015) for marketed yogurts in Spain. Functional yogurts had lower Ca than those in Brazilian whey beverages (Souza *et al.*, 2018). Variations in Ca content may be due to milk composition, fruit type and whey addition. K content in control yogurts was 1064 ± 33 mg kg⁻¹ (strawberries) and 933 ± 316 mg kg⁻¹ (aronia), with no significant difference from functional yogurts ($P>0.05$). Yogurts with aronia had lower K content than those with strawberries. The results were lower than Souza *et al.* (2018) for whey beverages (1410 ± 210 mg kg⁻¹) and similar to Luis *et al.* (2015), where K was the most abundant mineral in flavored yogurts. Control yogurt with aronia had 107.71 ± 1.16 mg kg⁻¹ Mg, significantly higher than functional yogurt (84.29 ± 3.52 mg kg⁻¹, $P<0.05$). Mg content did not differ significantly between control and functional yogurt with strawberries ($P>0.05$). These results align with Luis *et al.* (2015) (109 ± 20 mg kg⁻¹ in flavored yogurts) and Souza *et al.*

(2018) (160 ± 40 mg kg⁻¹ in Brazilian yogurts, 110 ± 10 mg kg⁻¹ in whey beverages). Yogurts with aronia had higher Mg content, while functional yogurts with whey had lower Mg levels. Functional yogurts with strawberries and aronia had Na contents of 237 ± 17.3 mg kg⁻¹ and 218 ± 12.46 mg kg⁻¹, respectively, lower than control yogurts (265.6 ± 57.2 mg kg⁻¹ and 260.2 ± 50.5 mg kg⁻¹), with no significant difference ($P>0.05$). The levels were much lower than those reported by Luis *et al.* (2015) for flavored yogurts (447 ± 54 mg kg⁻¹) and Souza *et al.* (2018) for Brazilian yogurts and whey beverages (740 ± 150 mg kg⁻¹ and 680 ± 90 mg kg⁻¹). The addition of whey resulted in lower Na content in functional yogurts. The P content of control yogurts with aronia and strawberries was 775 ± 141.2 mg kg⁻¹ and 660.7 ± 26.7 mg kg⁻¹, respectively, with no significant difference from functional yogurts (623.53 ± 2.18 mg kg⁻¹ and 561.9 ± 67.4 mg kg⁻¹, $P>0.05$). The highest P content was found in control yogurt with aronia, likely due to higher P content in aronia compared to strawberries. Tarakci and Dag (2013) reported higher P content in traditional Turkish yogurts, with a value of 983.1 ± 8.63 mg kg⁻¹. This study found higher P in control than functional yogurts, possibly due to whey addition, though differences were not significant. Control and functional yogurt with strawberries had Fe contents of 17.21 ± 7.44 mg kg⁻¹ and 7.02 ± 3.92 mg kg⁻¹, respectively. For aronia, control and functional yogurt had 6.14 ± 2.98 mg kg⁻¹ and 16.5 ± 14.4 mg kg⁻¹, respectively ($P>0.05$). These levels were higher than those reported by Sanchez-Segarra *et al.* (2000) for strawberry yogurts (1.18 ± 0.28 mg kg⁻¹) but comparable to Souza *et al.* (2018), who reported 10 ± 0 mg kg⁻¹ in Brazilian yogurts and whey beverages. The Zn content in control yogurts with aronia and strawberries was 2.65 ± 0.148 mg kg⁻¹ and 2.26 ± 0.12 mg kg⁻¹, respectively, with no significant difference from functional yogurts (3.34 ± 2.23 mg kg⁻¹ and 1.6 ± 0.07 mg kg⁻¹, $P>0.05$). Functional yogurt with aronia had the highest Zn content. Tarakci and Dag (2013) reported higher Zn in Turkish yogurts (4.51 ± 0.53 mg kg⁻¹), while Luis *et al.* (2015) found 2.47 ± 0.21 mg kg⁻¹ in flavored yogurts and Sanchez-Segarra *et al.* (2000) reported 3.2 ± 0.19 mg kg⁻¹ in strawberry yogurts, aligning with this study. Variations in Fe and Zn may result from milk composition, fruit type and whey addition. Further studies with more samples are needed to reduce deviations.

Table 3: Mineral content composition (mg kg⁻¹) in yogurts.

Mineral composition ¹	CYS	FYS	CYA	FYA
Ca	821.3 ± 16.3^a	657.9 ± 66.6^a	933 ± 202^a	707.8 ± 33.6^a
K	1064 ± 33^a	954.9 ± 19.7^a	933 ± 316^a	839.3 ± 32.6^a
Mg	81.29 ± 0.42^{bc}	74.19 ± 3.59^c	107.71 ± 1.16^a	84.29 ± 3.52^b
Na	265.6 ± 57.2^a	237 ± 17.3^a	260.2 ± 50.5^a	218 ± 12.46^a
P	660.7 ± 26.7^a	561.9 ± 67.4^a	775 ± 141.2^a	623.53 ± 2.18^a
Fe	17.21 ± 7.44^a	7.02 ± 3.92^a	6.14 ± 2.98^a	16.5 ± 14.4^a
Zn	2.26 ± 0.12^a	1.6 ± 0.07^a	2.65 ± 0.148^a	3.34 ± 2.23^a

¹Results are mean $\pm$ SD of two determinations (n = 2). Different letters (a-c) in a row indicate significant differences ($P<0.05$) between control and functional yogurts.

Microbial safety of yogurts

Enterobacteriaceae and *E. coli* were absent in the yogurt samples during storage and *L. monocytogenes* was not detected in 25 g of yogurt samples over 21 days. Yeast and mold were found in one control yogurt with aronia berries sample on day 14 (20 CFU g⁻¹). The absence of coliforms during cold storage indicates effective milk heat treatment and high hygienic standards, preventing recontamination (Awad *et al.*, 2023). Rodak and Molska (2010) reported similar results, with no *Enterobacteriaceae* or *E. coli* in 10 g of yogurt. Castro *et al.* (2013) observed no coliforms in probiotic milk drinks with strawberries. However, Prodanov *et al.* (2012) found 19.5% of yogurts in North Macedonia tested positive for *Enterobacteriaceae*. Al-Farsi *et al.* (2025) reported microbiological results for plain and flavored Greek yogurts. The microbial values in the samples showed that yeasts and molds ranged between 3.09×10^2 and 1.07×10^5 CFU g⁻¹, while no *E. coli* was detected in any of the evaluated samples. Our study showed good hygienic quality and safe yogurt samples for consumption throughout the 21-day storage period. Although this study evaluated the proximate composition, mineral content and microbiological safety of whey-supplemented yogurts, it has some limitations. Functional properties such as antioxidant activity, anti-inflammatory potential and bioactive compound retention were not assessed and the potential health effects of these yogurts remain unexplored.

CONCLUSION

The findings of this study highlight the potential of fermenting a milk-liquid whey mixture and supplementing it with fruits as a sustainable strategy for valorizing dairy industry byproducts. Incorporation of whey not only influenced the nutritional profile of the yogurts by reducing fat content in line with consumer preferences but also preserved valuable whey proteins, thereby contributing to overall nutritional quality. Both control and functional yogurts provided essential elements, particularly potassium and iron and remained microbiologically safe throughout storage, underscoring the effectiveness of hygienic processing practices. Beyond their nutritional attributes, these results demonstrate that bioprocessing liquid whey into functional yogurts enriched with fruits such as strawberries or aronia berries can contribute to reducing food waste, expanding functional food options and meeting growing consumer demand for healthier, more sustainable dairy products. Future research should further investigate the bioactive properties, stability and potential health benefits of whey-enriched yogurts, ultimately supporting innovation in functional dairy foods and circular bioeconomy practices.

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Disclaimers

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Informed consent

Not applicable.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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