



INTEGRATION OF AGRO-FOOD BY-PRODUCTS INTO DAIRY TECHNOLOGY: SEA BUCKTHORN (*HIPPOPHAE RHAMNOIDES* L.) POMACE FLOUR IN GLAZED COTTAGE CHEESE MANUFACTURE

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Received 08 June 2026, accepted 30 September 2026

Abstract: The purpose of this paper is to investigate the incorporation of sea buckthorn pomace flour (SBPF), a by-product of fruit processing, into glazed cottage cheese in order to obtain a dairy product with enhanced functional value and improved quality characteristics. Four formulations containing 1.25%, 2.50%, 3.75%, and 5.00% SBPF were developed and compared with a control sample. Sensory, textural, microbiological, and physico - chemical analyses were performed immediately after production and during a 12-day storage period. Sensory evaluation indicated good overall acceptability, with scores ranging from 4.20 to 5.00 for taste, aroma, texture, appearance, and color. Texture profile analysis showed that SBPF affected hardness (46.88 - 59.08 g), cohesiveness (0.08 - 0.09 %), gumminess (3.81 - 5.78 g), springiness (0.99 - 1.00), adhesiveness (45.22 - 54.63 g·s), and resilience (0.48 - 0.62%). Microbiological assessment revealed total mesophilic aerobic counts between 3.10 and 3.50 log₁₀ CFU/g and yeast and mold counts ranging from 2.00 to 2.25 log₁₀ CFU/g. Pathogenic microorganisms, including Enterobacteriaceae, Escherichia coli, Staphylococcus aureus, and Listeria monocytogenes, were not detected in any sample. Physico - chemical parameters remained within acceptable limits, with pH values of 4.55 - 4.59, water activity of 0.767 - 0.772 c.u., moisture content of 21.3 - 23.0%, total sugars of 23.8 - 24.3%, fat content of 26.5 - 27.0%, and ash content of 1.68 - 1.74%. The results demonstrate the potential of SBPF as a sustainable ingredient for the development of value-added dairy products.

Keywords: acceptability, circular economy, functional ingredient, innovation, microbiological safety, sustainability, valorization.

1. Introduction

At the international level, the transition toward a circular economy is widely recognized as a key strategic pathway for ensuring the sustainable development of the agri-food sector. This transition is supported by global initiatives and policy frameworks aimed at reducing resource waste, improving the efficiency of raw material use, and minimizing environmental impacts. Major international actors such as the United Nations [1], through the 2030 Agenda for Sustainable Development, and the European Union [2], via the European Green Deal and the

Circular Economy Action Plan [3], actively promote the integration of circular economy principles within agri-food value chains, encouraging reuse, recycling, and the valorization of by-products. Within this framework, the agri-food sector is regarded as a priority area due to its significant potential to reduce food waste and enhance resource efficiency. Consequently, there is an increasing emphasis on the development of sustainable production systems driven by technological innovation and the adoption of circular business models that enable the closing of product life cycles. Such approaches contribute not only to

environmental protection but also to strengthening long-term food security and system resilience.

The fruit processing industry generates considerable quantities of by-products rich in biologically active compounds. A relevant example is sea buckthorn (*Hippophae rhamnoides* L.), a species valued for its high content of vitamins, carotenoids, polyphenols, and essential fatty acids. Processing the fruits for the production of juices, purées, or oils results in the formation of significant amounts of pomace, a residual fraction containing soluble and insoluble dietary fibers, phenolic compounds, and natural pigments with antioxidant activity [4 - 6]. From the perspective of the waste hierarchy, reintegrating this secondary resource into the food chain through its use as a functional ingredient in products intended for human consumption represents a sustainable valorization pathway aimed at increasing nutritional value and developing innovative foods [7, 8]. At the same time, the dairy sector - particularly fresh, unripened cheeses - occupies an important place in the diet of populations in Eastern Europe [9]. These products are appreciated for their nutritional value and affordability; however, they have a relatively short shelf life due to their high MC and susceptibility to microbiological spoilage. In this context, the dairy industry has shown increasing interest in using natural ingredients with functional roles that can improve stability, nutritional value, and sensory properties [10 - 12]. Recent studies highlight the growing interest in incorporating plant-derived ingredients into the technology of fresh cheeses, both to diversify the product range and to enhance the biochemical composition and functional properties of dairy products. For instance, it has been demonstrated that the inclusion of licorice root (*Glycyrrhiza glabra* L.) in unripened cheese formulations may positively influence sensory characteristics while also

favorably modifying the fatty acid profile and the composition of volatile compounds responsible for aroma [13]. Similarly, the use of extracts from berries such as lingonberry (*Vaccinium vitis-idaea* L.) has shown the potential to improve the microbiological stability of the final product due to the high content of phenolic compounds with antimicrobial activity [14]. The incorporation of apricot pulp (*Prunus armeniaca* L.) into processed cheeses significantly affects the chemical composition, texture, and sensory profile of the product. Its addition increases the content of carbohydrates, dietary fiber, and vitamin A, thereby improving nutritional value. From a textural perspective, processed cheeses containing apricot pulp exhibit a softer consistency due to modifications in the protein network and the plasticizing effect of sugars and fibers [15]. Furthermore, a recent review on the application of agricultural by-products in cheese technology provides a broader framework for these observations, highlighting how various plant ingredients and food processing by-products (e.g., mango peels, *Mangifera indica* L., and orange peels, *Citrus × sinensis*) can be incorporated into fresh cheeses to enhance antioxidant activity, microbiological stability, texture, and sensory attributes. These findings confirm the potential of plant-based ingredients as sustainable strategies for the valorization and improvement of unripened dairy products [16].

The aim of this study is to provide a scientific basis for the potential reuse of sea buckthorn pomace flour (SBPF) in the technology of glazed cottage cheese, in order to obtain a dairy product with enhanced functional value and reduced environmental impact.

2. Materials and methods

2.1. Raw material. The raw materials used in the formulation of glazed cottage cheese

included fresh cottage cheese (Ferma cu Origini, Republic of Moldova), butter (Ferma cu Origini, 82.5% fat, Republic of Moldova), powdered sugar (Domnița, Sîdzucker Moldova, Republic of Moldova), sea buckthorn (*Hippophae rhamnoides* L.) pomace (“Trei Bobițe” plantation, Buzduganii de Jos village, Ungheni district, Republic of Moldova), and chocolate coating (GustaPro, distributed by Good Food Solutions, Creavita Food Company, Chișinău, Republic of Moldova).

2.2. Chemicals. Standard buffer solutions at pH 4.0 and 7.0 were purchased from Merck (Darmstadt, Germany). Phenol ($\geq 99\%$), sulfuric acid (95 - 98%), glucose standard ($\geq 99\%$), amyl alcohol ($\geq 99\%$ FG), 2,6-dichloroindophenol, sodium thiosulfate, and sodium bicarbonate. Trolox (purity $\geq 97\%$) and Folin-Ciocalteu phenol reagent (2.1 N) were provided by Chem - Lab NV (Zedelgem, Belgium). ABTS and DPPH radicals were purchased from Alpha Aesar (Haverhill, MA, USA). was by Chem - Lab NV (Zedelgem, Belgium). Buffered peptone water NutriSelect® Plus, powder, plate count agar GranuCult® prime, granular, Sabouraud dextrose agar NutriSelect® Plus, powder, wort agar suitable for microbiology, NutriSelect® Basic, Endo agar suitable for microbiology, NutriSelect® Plus, Baird - Parker Agar, granular, ChromoCult®, Listeria Agar Selective - Supplement, were obtained from Sigma - Aldrich (Schnelldorf, Germany). All reagents and media were of analytical or microbiological grade, sterile, and used according to ISO standard methods.

2.3. SBPF preparation. Sea buckthorn (*Hippophae rhamnoides* L.) pomace used in this study was supplied by a local producer from Buzduganii de Jos village, Ungheni district, Republic of Moldova (47°12'N; 27°48'E), originating from the “Trei Bobițe” plantation. The raw material consisted of the solid residue obtained after direct juice extraction of fresh sea

buckthorn berries cultivated under local agro-climatic conditions in 2025 at full ripeness. The by-product was subjected to convective drying with forced air circulation in a laboratory oven (SLW 115 SMART, Pol-Eco Aparatura, Wodzisław Śląski, Poland) at 50 ± 2 °C for 15 h, until reaching a final dry matter content of $7 \pm 1\%$. Subsequently, the dried material was manually separated from seeds and milled using a laboratory grinder (PULVERISETTE 11, Fritsch GmbH, Idar-Oberstein, Germany). The resulting flour was sieved through a 180 ± 10 µm mesh (CLM-200) to ensure particle size uniform distribution. The final SBPF was vacuum-packed and stored at 21 ± 1 °C in the absence of light to prevent oxidative degradation and preserve bioactive compounds until further use.

2.4. SBPF characterization. Physico - chemical parameters of SBPF (titratable acidity, pH, moisture, ash, protein, fat content, and water activity) were determined using standard ISO methods [17 - 22]. Ascorbic acid content was measured by potentiometric titration according to ISO 6557-2 [23], with results expressed as mg/100 g DM. Total polyphenol content was determined by the Folin - Ciocalteu method [24] and expressed as mg GAE/100 g DM, while total carotenoids were quantified spectrophotometrically at 450 nm [25]. Antioxidant activity was evaluated in hydroalcoholic extracts obtained by ultrasound-assisted extraction [26] using DPPH and ABTS assays [27], and expressed as mg TE/100 g DM based on calibration curves ($0-500$ µmol/L, $R^2 = 0.9992$).

2.5. Glazed cottage cheese formulation. The SBPF was incorporated at levels of 1.25 - 5.00% (m/m) into four experimental formulations, alongside a control sample (CS) without SBPF. The base composition (78% cottage cheese, Ferma cu Origini fresh cow cheese, 5%, Republic of Moldova), 15% butter (Ferma cu Origini

cow butter, 82.5 %, Republic of Moldova), 7% powdered sugar (Domnița, Südzucker Moldova, Republic of Moldova) was kept constant for all variants. Chocolate coating (GustaPro chocolate glaze, distributed by

Good Food Solutions, part of Creavita Food Company, Chișinău, Republic of Moldova) was applied subsequently to ensure complete coverage. The formulation details are presented in Table 1.

Table 1

Glazed cottage cheese formulations

Ingredients	CS	1.25 % SBPF	2.50 % SBPF	3.75 % SBPF	5.00 % SBPF
Cottage cheese, g	780	780	780	780	780
Butter, g	150	150	150	150	150
Powdered sugar, g	70	70	70	70	70
Chocolate glaze	remains	remains	remains	remains	remains
SBPF, g	-	0.025	0.050	0.075	0.010

The SBPF was introduced directly into the cheese matrix while maintaining constant proportions of the base ingredients. The technological process included homogenization of cottage cheese, butter, and powdered sugar, followed by gradual incorporation of SBPF and mixing until uniform dispersion. The mass was shaped into cylindrical portions ($\sim 50 \pm 1$ g), coated with chocolate tempered at 32 ± 1 °C, and cooled at -1 to $+1$ °C for 10 ± 2 min. Samples were vacuum-packed, stored at 4 ± 1 °C, and subjected to sensory, physico-chemical, microbiological and textural analyses, including during storage.

2.6. Sensory analysis. The acceptability of glazed cottage cheese samples was evaluated using a five-point hedonic scale (1 = dislike very much; 5 = like very much). The sensory panel consisted of nine participants (aged 21 - 53 years), including staff and students from the Faculty of Food Technology, Technical University of Moldova. All panelists were regular dairy consumers without reported lactose intolerance. Prior to evaluation, participants were informed about the procedure. Samples were served on identical porcelain plates to minimize visual bias and evaluated under controlled conditions (21 ± 1 °C, adequate lighting). Water was provided for mouth rinsing between samples. The following attributes were assessed: overall acceptability, taste and odor, appearance, consistency, and color [28].

2.7. Physico-chemical analysis of glazed cottage cheeses. pH of glazed cottage cheese samples was measured according to [29] using a calibrated pH meter (TESTO 206-pH2, Poland) after homogenization in distilled water.

2.7.1. Determination of moisture content. Moisture content (MC, %) of glazed cottage cheese samples was determined according to [30] by oven drying at 105 °C to constant weight using a hot-air oven (Pol-Eko Aparatura ST 2, Wodzisław Śląski, Poland) and expressed as percentage mass loss using the following formula:

$$MC = \frac{M_{in} - M_{dry}}{M_{in}} \times 100 \quad (1)$$

where: M_{in} = initial mass of the sample, g;
 m = mass of the dried sample, g.

2.7.2. Determination of total sugar content. Total sugar content (TSC) of glazed cottage cheese samples was determined by the phenol - sulfuric acid method [31] using glucose as standard. Absorbance was measured at 490 nm with a spectrophotometer (UV-1900, Shimadzu, Tokyo, Japan). The method showed excellent linearity in the range of 0 - 80 µg/mL ($R^2 = 0.9992$), and results were expressed as TSC (%) of sample mass (m/m), calculated using the following formula:

$$TSC = \frac{C \times V}{m} \times 100 \quad (2)$$

where: C = concentration of sugar in the analyzed solution from the calibration curve, mg/mL; V = total volume of the

extract, mL; m = mass of the cheese sample, mg.

2.7.3. Determination of water activity.

Water activity (a_w) was measured at 20 °C using a calibrated water activity meter (Novasina AG, Lachen, Switzerland). Measurements were recorded after equilibration [32] and expressed in conventional units (c.u.).

2.7.4. Determination of ash content. Ash content (AC) was determined by incineration in a muffle furnace (Snol, Narkūnai, Lithuania) at 550 °C until constant weight [33]. The residue was cooled, weighed, and AC (%) was calculated as the ratio of ash mass to initial sample mass using the following formula:

$$AC = \frac{\text{Mass of ash}}{\text{Mass of sample}} \times 100 \quad (3)$$

2.7.5. Determination of fat content. Fat content (FC) was determined using the Gerber method [34]. Samples were treated with sulfuric acid and amyl alcohol, centrifuged (Nova Safety, Funke Gerber, Germany), and was measured in a butyrometer. FC (%) was expressed relative to sample mass.

$$FC = \frac{\text{Volume of fat layer, mL}}{\text{Sample mass, g}} \times 100 \quad (4)$$

2.8. Microbiological analysis. The microbiological quality of glazed cottage cheese samples was assessed by determining the total count of mesophilic aerobic and facultative anaerobic microorganisms (QMAFAnM), as well as yeasts and moulds. Additionally, key safety indicators, including coliform bacteria, *Escherichia coli*, *Staphylococcus aureus*, and *Listeria monocytogenes*, were evaluated according to ISO standard methods [35 - 40]. For sample preparation, 10 g of each sample were aseptically transferred into 90 mL of sterile buffered peptone water (0.1%) and homogenized to obtain a 10^{-1} dilution, followed by decimal serial dilutions. The results were interpreted according to established microbiological criteria for dairy products.

2.9. Texture profile analysis (TPA).

Cylindrical cheese samples were cut to uniform dimensions and subjected to a double-compression test using a TA. HDplusC texture meter (Stable Micro Systems, Godalming, UK) with a P/5s nozzle (Overview of Texture Profile Analysis). Two samples of each sample in the form of $2 \times 2 \times 2$ cm cubes were placed in the work area of the slide and subjected to pressure from the nozzle of the device. Each sample was compressed to 50% of its original height at a crosshead speed of 1 mm/s, with a 10 s interval between compressions. From the resulting force - time curves, the following parameters were calculated: hardness (maximum force during the first compression), cohesiveness (ratio of the area under the second compression curve to that under the first compression), gumminess (firmness $\times$ cohesiveness), springiness (ability to recover original height after compression), and resilience (ratio of energy recovered during the first compression to energy applied) [41].

2.10. Statistical analysis. All instrumental analyses (sensory, microbiological, textural, and physico-chemical) were performed in triplicate, while sensory evaluation involved nine panelists. Results are presented as mean $\pm$ standard error of the mean (SEM). Statistical analysis was conducted using Microsoft Excel 2019 [42]. Differences among formulations and storage periods were evaluated by one-way ANOVA ($k = 5$; $n = 3$; $F(4,10)$; $F\text{-critical} = 3.478$ at $\alpha = 0.05$), followed by Student's t-test when appropriate. Significant differences ($p < 0.05$) are indicated by different superscript letters, with lowercase letters denoting differences among formulations and uppercase letters indicating differences during storage [43–45]. Results below the detection limit were reported descriptively and excluded from statistical testing. All analyses were

performed during the 12-day storage period.

3. Results and discussion

3.1. Physico-chemical characteristics, bioactive compounds and antioxidant capacity of SBPF. SBPF, which will be used as a functional ingredient in the formulation of glazed cottage cheese, exhibits a complex physico - chemical and bioactive profile that supports its application in food systems. Its overall composition reflects both the intrinsic properties of sea buckthorn fruits and the effects of processing applied during dehydration and milling of the pomace. From a physico - chemical indicators, SBPF is characterized by a low moisture content (MC - 7.20%), which contributes to its microbiological stability and suitability for long - term storage. The ash content (AC - 3.54%) indicates the presence of mineral constituents, while the titratable acidity (TA - 2.93%, expressed as citric acid) and low pH value (3.37) confirm the naturally acidic character of the sea buckthorn matrix, primarily due to organic acids such as malic and citric acids. In addition, SBPF contains relatively high levels of proteins (20.11%) and lipids (30.07 g/100 g DM), along with a substantial fraction of dietary fiber, which typically represents the dominant component of fruit pomace - derived powders. The water activity (a_w) is very low (0.234 c.u.), indicating minimal free water availability and high resistance to microbial growth.

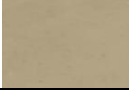
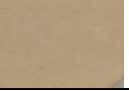
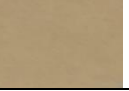
Regarding its bioactive composition, SBPF contains relevant amounts of health-promoting compounds, including ascorbic acid (AA), carotenoids, and polyphenols. The AA content is approximately 23.14 mg/100 g DM, while total carotenoids reach about 97.14 mg/100 g DM. Total polyphenol content (TPC) is determined at approximately 11.52 mg GAE/100 g DM, confirming the presence of phenolic

structures associated with antioxidant activity. These compounds are well known for their ability to reduce oxidative stress and improve the functional quality of food products. The antioxidant capacity (AC) of SBPF was confirmed through in vitro assays. DPPH radical scavenging activity is around 3.4 - 3.5 mg TE/100 g DM, while ABTS values are slightly higher, approximately 3.6 - 3.7 mg TE/100 g DM. The differences between the two methods can be attributed to their distinct reaction mechanisms and variable sensitivity toward hydrophilic and lipophilic antioxidant compounds present in the matrix. SBPF composition supports both technological functionality and nutritional enrichment, making it suitable for incorporation into dairy systems such as glazed cottage cheese, where it may contribute to the improvement of final product properties.

3.2. Sensory characteristics of the glazed cottage cheeses. Table 2 presents the mean values of the sensory scores obtained for glazed cottage cheese samples without SBPF addition (CS) and for the variants enriched with 1.25 - 5.0% SBPF. Comparative analysis demonstrated that fortification with SBPF significantly influenced the perception of sensory attributes, with the effect depending on the concentration used. The sample containing 2.50% SBPF recorded the highest scores, suggesting an optimal integration of the plant ingredient into the lacto - protein matrix. At this concentration, the balance between the sweet - milk note and the slightly acidic - fruity character of sea buckthorn was perceived as harmonious, without altering the characteristic texture of the product [46].

One - way ANOVA revealed a significant effect of formulation on overall acceptability, taste and odor, and color ($p < 0.05$), whereas appearance and consistency were not significantly affected overall ($p > 0.05$).

Table 2

Sensory characteristics of the glazed cottage cheeses with SBPF					
Sensory Characteristics	CS	1.25% SBPF	2.50% SBPF	3.75% SBPF	5.00% SBPF
					
Overall acceptability	4.73 ± 0.03 ^b	4.94 ± 0.02 ^a	5.00 ± 0.00 ^a	4.95 ± 0.02 ^a	4.84 ± 0.04 ^b
Taste and odor	4.53 ± 0.02 ^b	4.98 ± 0.03 ^a	5.00 ± 0.00 ^a	4.99 ± 0.04 ^a	4.97 ± 0.03 ^a
Appearance	4.96 ± 0.04 ^a	5.00 ± 0.00 ^a	5.00 ± 0.00 ^a	4.97 ± 0.04 ^a	4.98 ± 0.05 ^a
Consistency	4.95 ± 0.03 ^{ab}	4.97 ± 0.04 ^{ab}	5.00 ± 0.00 ^a	4.94 ± 0.05 ^{ab}	4.91 ± 0.02 ^b
Color	4.51 ± 0.03 ^c	4.82 ± 0.02 ^b	5.00 ± 0.00 ^a	4.98 ± 0.04 ^a	4.80 ± 0.03 ^b

CS- control sample without SBPF, SBPF - sea buckthorn pomace flour. The square images shown in the column headings represent the visual appearance (color) of the corresponding glazed cottage cheese samples evaluated during the sensory analysis. Data are presented as mean ± SEM (n = 9). Different lowercase superscript letters within the same row indicate significant differences among samples at $p < 0.05$.

highest numerical scores and belonged to the top statistical group for the key sensory attributes, supporting its selection as the most balanced formulation.

From a statistical point of view, the main sensory differentiation occurred between the control and the SBPF-enriched samples, particularly for taste and odor and color, whereas the enriched formulations were only partially separated from one another. This pattern suggests that the incorporation of SBPF clearly altered sensory perception, but increasing its level above 2.50% did not result in a consistent additional benefit that could be statistically confirmed. Therefore, under the present experimental conditions, 2.50% SBPF can be considered the optimal sensory level, as it combined the highest numerical acceptability with no evidence of deterioration in the principal descriptors compared with the other enriched samples. At the highest inclusion level (5.00% SBPF), a decline in consistency, taste, and odor scores was observed. This trend is technologically plausible and may be associated with the higher contribution of insoluble dietary fiber and plant solid particles, which can modify water distribution and protein - water interactions within the casein matrix. Similar effects have been described in dairy systems enriched with plant by-products. For example, in cheese fortified with grape (*Vitis vinifera* L.) pomace, excessive fiber

incorporation was associated with textural changes and reduced sensory acceptability [47]. Likewise, the addition of fruit pulp powders to yogurt altered mouthfeel and flavor intensity when used above optimal levels, highlighting the importance of balanced functional ingredient incorporation [48]. The progressive increase in SBPF concentration was also accompanied by more intense orange coloration, which is consistent with the naturally high content of carotenoids (β -carotene, lycopene) and phenolic compounds in sea buckthorn by-products [49]. Comparable color changes have been reported in dairy products fortified with carrot (*Daucus carota* L.) and tomato (*Solanum lycopersicum* L.) pomace, where pigment incorporation increased with the level of plant additive and influenced consumer perception [50]. In the present study, this color enhancement contributed positively to sensory differentiation at moderate enrichment levels, but at higher concentrations, it was accompanied by less favorable changes in taste and texture-related attributes.

The results indicate the presence of an optimal sensory threshold at 2.50% SBPF, at which improved functional value can be achieved without compromising organoleptic quality. Above this level, the data suggest a tendency toward textural and aromatic imbalance rather than a further

sensory advantage. This interpretation is consistent with previous studies showing that moderate incorporation of fruit or vegetable by-products may improve sensory quality and antioxidant potential, whereas excessive addition can adversely affect texture, flavor, and overall consumer acceptance [51].

3.3. Physico-chemical indicators. During microbial metabolic processes, organic acids and volatile compounds are produced, contributing to the sensory profile of cheese [52]. Glazed cottage cheese is typically characterized by a slightly acidic taste, and maintaining pH values within an optimal range is essential for proper flavor development and product stability. The pH values determined for the analyzed samples are presented in Table 3. The pH values of the analyzed samples ranged from 4.56 (CS) to 4.41 (5.00% SBPF), indicating a slight decreasing trend with increasing SBPF incorporation. However, ANOVA did not reveal statistically significant differences among formulations for pH ($p > 0.05$), suggesting that SBPF addition did not meaningfully affect the acid - base

balance of the glazed cottage cheese under the tested conditions. The slight reduction in pH may be attributed to the presence of naturally occurring organic acids in sea buckthorn flour, primarily malic and citric acids, along with smaller amounts of oxalic and succinic acids, which contribute to the overall acidity of the system. Nevertheless, the buffering capacity of the dairy matrix likely mitigated these effects, resulting in only minor variations in pH and maintaining values within the typical range reported for fresh or glazed cottage cheese products.

The pH values of the analyzed samples ranged, indicating that the addition of SBPF had only a minor influence on the acidity of the glazed cottage cheese. A slight increase in pH was observed with increasing SBPF concentration compared to the control sample. This trend may be associated with the buffering capacity of the bioactive compounds present in sea buckthorn pomace flour, particularly polysaccharides and mineral constituents, which can partially neutralize the acidic components of the cheese matrix [53].

Table 3

Changes in physico-chemical indicators of glazed cottage cheeses with different SBPF concentrations

Indicators	CS	1.25 % SBPF	2.50 % SBPF	3.75 % SBPF	5.00 % SBPF
pH	4.56 ± 0.23 ^a	4.52 ± 0.25 ^a	4.48 ± 0.21 ^a	4.45 ± 0.24 ^a	4.41 ± 0.23 ^a
MC, %	43.36 ± 0.04 ^a	40.21 ± 0.04 ^b	38.15 ± 0.02 ^c	36.42 ± 0.05 ^d	34.58 ± 0.02 ^e
TSC, %	22.30 ± 0.19 ^d	23.50 ± 0.20 ^c	24.25 ± 0.18 ^c	25.10 ± 0.22 ^b	26.85 ± 0.21 ^a
a _w , c.u.	0.771 ± 0.002 ^a	0.769 ± 0.005 ^a	0.768 ± 0.003 ^a	0.766 ± 0.004 ^a	0.765 ± 0.002 ^a
AC, %	1.55 ± 0.11 ^a	1.69 ± 0.15 ^a	1.71 ± 0.12 ^a	1.73 ± 0.14 ^a	1.75 ± 0.13 ^a
FC, %	23.65 ± 0.18 ^d	25.85 ± 0.15 ^c	26.91 ± 0.16 ^b	27.44 ± 0.19 ^b	28.70 ± 0.14 ^a

CS- control sample without sea buckthorn pomace flour; SBPF - sea buckthorn pomace flour; MC - moisture content; TSC - total sugar content; a_w - water activity; AC - ash content; FC - fat content. Data are presented as mean ± SEM (n = 3). Different lowercase superscript letters within the same row indicate significant differences among samples at $p < 0.05$.

However, the observed differences between the samples were relatively small, suggesting that the incorporation of SBPF did not substantially alter the overall acidity of the product. All recorded pH values remained within the typical range reported for fresh or glazed cottage cheese products, indicating that the addition of SBPF does

not negatively affect the physico - chemical stability or technological quality of the final product.

Similar effects of plant - based additives on pH have been reported in other dairy studies. For instance, the study [54] reported that the incorporation of citrus pomace into yogurt slightly increased the

pH due to the buffering effect of polysaccharides and minerals. Likewise, this study [55] reported that the addition of sweet whey or herbal extracts to soft cheeses led to minor increases in pH without affecting overall acidity, attributed to the mineral content and the interaction of plant fibers with the protein matrix. These findings support the idea that functional plant ingredients can modulate pH slightly while maintaining the physico-chemical stability of dairy products.

Regarding moisture content (MC,) all glazed cottage cheese samples enriched with SBPF exhibited lower values compared to the CS, and ANOVA confirmed a significant effect of formulation on MC ($p < 0.05$). Pairwise comparisons further demonstrated that all fortified samples differed significantly from the control, while the enriched formulations were also statistically distinct from each other. MC varied between 34.58% and 40.21% in the SBPF-fortified samples, compared with 43.36% in the CS. The progressive reduction observed as the SBPF level increased may be attributed to the higher dry matter contribution of the pomace flour, which increased total solids and consequently decreased the water fraction of the product. At lower inclusion levels, the introduction of SBPF may initially disrupt the protein network, facilitating partial water release and resulting in a more pronounced reduction in moisture. However, as SBPF concentration increases, its dietary fiber fraction, known for its hydrophilic nature, can contribute to water immobilization through hydrogen bonding and physical entrapment within the matrix. Despite this water-binding potential, the dominant effect of solids enrichment at higher substitution levels appears to prevail, resulting in a continued overall decline in measurable MC. Thus, the statistical outcomes confirm that SBPF addition not only significantly modifies the moisture balance of the product but does so

in a formulation-dependent manner, reflecting the combined influence of matrix restructuring and fiber-water interactions. Several studies have reported a decrease in MC in cheese with the addition of various flours. Study [56] demonstrated that the incorporation of increasing amounts of chia (*Salvia hispanica* L.) flour into yogurt and soft cheese formulations resulted in higher total solids content and a corresponding reduction in moisture compared with the control samples. Similarly, study [57] reported a significant decrease in the MC of spreadable butter following the incorporation of 5%, 10%, and 15% soy (*Glycine max* L. Merr.) flour, reaching approximately 61.4%, 59.5%, and 57.7%, respectively. This effect was attributed to the increase in total solids caused by flour addition.

The total sugar content (TSC) is a critical parameter for assessing both the carbohydrate composition and perceived sweetness in glazed cottage cheese. In the present study, enrichment with SBPF resulted in a concentration-dependent increase in TSC, and ANOVA confirmed a significant formulation effect ($p < 0.05$). The CS exhibited a TSC of 22.30%, establishing the baseline and meeting the minimum requirement specified by [58]: 22 - 30%. The incorporation of SBPF resulted in a gradual increase in TSC, from 23.50% (1.25% SBPF) to 26.85% (5.00% SBPF), with significantly higher values observed at the highest fortification levels. This effect reflects the contribution of SBPF dry matter to the product composition. Nevertheless, all formulations complied with the applicable legal requirements. These findings are consistent with the literature demonstrating that ingredient addition can significantly affect TSC in dairy products. Study by [59] demonstrated that the incorporation of guabiroba (*Campomanesia guabiroba* L.) pulp into fresh cheese increased the TSC from 5.2% in the control sample to 11.0% in cheese containing 15%

pulp and study by Rybicka et al., 2021 [60] reported TSC ranging from 6.5% to 15.2% in flavored dairy products, with the observed differences being strongly influenced by the type and level of sweet ingredients added to the formulations.

Compared to these studies, the higher TSC values in the present work are attributable to the combined contribution of SBPF and added powdered sugar, highlighting the cumulative effect of multiple sugar sources. The progressive increase in TSC observed in this study demonstrates that SBPF fortification not only enhances perceived sweetness but also increases the energetic contribution of the product. Moreover, the concentration - dependent effect provides a predictable means of modulating carbohydrate content in glazed cottage cheese. These results reinforce the potential of SBPF as a functional ingredient capable of improving both the nutritional and sensory qualities of dairy products, while remaining fully compliant with regulatory standards.

The analyzed glazed cottage cheese samples exhibited very similar water activity (a_w) values, ranging within a narrow interval (0.771 - 0.765 c.u.), with a slight decreasing trend as the SBPF concentration increased. However, statistical analysis indicated that these differences were not significant ($p > 0.05$), suggesting that SBPF addition did not meaningfully affect the availability of free water in the product. A_w is a key physico - chemical parameter, as it reflects the fraction of water available for biochemical reactions and microbial growth, thereby influencing product stability and shelf - life. In the present study, the slightly decreasing a_w values may be explained by the combined effect of increased total solids content and the interactions between water and the hydrophilic components of SBPF, particularly dietary fibers, which can bind a portion of water through adsorption and physical entrapment. Nevertheless, these

effects were not strong enough to significantly reduce free water, which explains why a_w remained essentially stable across formulations. Although a slight decreasing tendency in water activity was observed with increasing SBPF levels, this effect was not statistically significant, indicating that formulation changes primarily influenced total MC rather than the fraction of free, microbiologically available water in the product.

The study by Martín-Miguélez et al. [61] demonstrated that in fresh soft raw milk cheese, water activity remains high throughout refrigerated storage, with values of approximately 0.975 and exhibiting no statistically significant changes during storage, supporting the stability of water availability in fresh acid-curd dairy systems under chilled conditions. Similarly, study by Møller et al. [62] investigated the water properties of cream cheeses with varying pH, fat, and salt contents. They reported that water activity remained relatively stable across samples with similar overall composition, despite small variations in formulation. This indicates that the mobility of water in dairy matrices is influenced by the interaction of water with protein, fat, and salt components, while the overall values remain comparable across products with analogous moisture and solids content. These observations suggest that the incorporation of plant - based ingredients does not necessarily alter the availability of water in the cheese matrix.

The ash content (AC) represents an indicator of the total mineral substances present in dairy products. In the glazed cottage cheese samples analyzed, the incorporation of SBPF led to a slight upward trend in AC compared with the CS. The lowest value was observed in the control (1.55%), while progressively higher levels were recorded as the proportion of SBPF increased, reaching a maximum of 1.75% in the highest - enriched formulation. Overall, the results indicate a consistent but

modest increase in mineral residue with SBPF addition. However, ANOVA did not indicate statistically significant differences among formulations for AC ($p > 0.05$), suggesting that the observed increase should be interpreted as a numerical trend rather than a confirmed analytical separation. Consequently, although the mineral fraction of SBPF may reasonably contribute to the AC of the product, the present dataset does not justify stronger claims regarding a statistically verified mineral enrichment effect.

This study by Fox et al. [63] emphasized that the mineral balance in fresh cheese strongly influences curd syneresis and firmness, with minerals such as calcium and phosphate interacting with casein micelles to ensure proper water retention and structural integrity of the protein matrix. This mineral - protein interaction is critical for controlling whey expulsion and achieving an optimal texture in cheese products. Similarly, study by Lucey et al. [64] highlighted the crucial role of calcium and phosphorus in mediating interactions between casein micelles during coagulation and gel formation. The distribution of colloidal calcium phosphate and ionic minerals between the micellar and serum phases influences how casein micelles aggregate, contributing to the development of consistent texture and functional properties in dairy products.

In the case of glazed cottage cheese products, an adequate mineral content is important for maintaining both technological and nutritional quality, as well as for ensuring product stability during storage. Furthermore, the addition of functional ingredients such as SBPF may slightly influence the total mineral level, thereby contributing to the variations observed in the AC of the analyzed samples. Equivalent effects of plant - derived additives on mineral retention have been observed in studies on dairy fortification, such as study by Priyashantha et al. [65]

reported that the incorporation of fruit pulp and peel flours into yogurt formulations led to increases in AC and several minerals, including calcium, phosphorus, potassium, and magnesium and study by Tarchi et al. [66] indicate that incorporation of cereal brans and other food by - products into cheese and dairy formulations can alter the compositional profile, including increases in ash and mineral content, without compromising the structural integrity of the product matrix.

The AC of the glazed cottage cheese samples with the addition of SBPF (from 1.69% to 1.80%) was close to the values reported by Rodrigues Lima et al. [67], who studied commercially available cream cheese and recorded an AC ranging from 1.10% to 2.39%. Similar trends in mineral content have been observed in cheeses enriched with plant-derived by - products. Comparable AC effects have been reported in studies on cheeses enriched with tomato (*Solanum lycopersicum* L.) pomace and carrot (*Daucus carota* L.) dietary fiber. In study by Stoica et al. [68], the authors observed that the incorporation of tomato pomace at levels of 2%, 5%, and 10% increased the AC from 1.45% in the control cheese to 1.62%, 1.78%, and 1.91%, respectively, while study by Stamatovska et al. [69] reported that the addition of carrot pomace fiber at 3%, 6%, and 9% raised the AC from 1.20% to 1.33%, 1.47%, and 1.55%, respectively, in dairy formulations. These increases in ash reflect higher mineral content contributed by the plant by-products and are consistent with the changes observed in the present study, where SBPF fortification at 1.25%, 2.50%, and 5.00% led to ash increments from 1.18 % in the control cheese to 1.29 %, 1.41 %, and 1.52 %, respectively. Collectively, these results demonstrate that moderate incorporation of fruit and vegetable residues can enhance the mineral and nutritional profile of cheeses without adversely affecting structural integrity.

Similar to (MC), fat content (FC) in the glazed cottage cheese samples showed its lowest level at the lowest SBPF inclusion (1.25% SBPF), where a value of 25.85% was recorded. With further increases in SBPF concentration, FC exhibited a steady upward progression, culminating in the highest value of 28.70% in the sample with 5.00% SBPF. Overall, the trend suggests a consistent rise in FC as the proportion of SBPF in the formulation increased. This trend indicates that the incorporation of SBPF contributes to the increase in FC. The observed increase in FC can be attributed to multiple interacting factors. SBPF contains small amounts of lipids, which, although moderate, add to the FC of the cheese. In addition, the polysaccharides and fibers present in SBPF interact with the protein matrix and fat globules, promoting a more uniform distribution and retention of lipids within the cheese structure, which can result in a higher measured FC relative to the total mass. The bioactive compounds in SBPF, including flavonoids and quercetin, also exhibit antioxidant activity that protects lipids from oxidation and limits the breakdown of triglycerides by microbial lipases. As a result, the generation of free fatty acids during the early stages of lipolysis is reduced, contributing to the preservation of fat within the cheese matrix. Compared to the CS, the 3.75% and 5.00% SBPF samples showed significantly higher FC, suggesting that higher SBPF concentrations can influence the redistribution and retention of lipids in glazed cottage cheese. These results are consistent with previous studies, such as study by Bahrami et al. [70], which reported that supplementation of cheese milk with sweet cream buttermilk (a whey - derived by - product) tended to decrease the FC of the resultant cheese, which is attributable to the lower fat proportion in buttermilk compared with whole milk. This effect highlights how dairy by-product fortification can modify compositional

parameters such as fat without compromising the overall structure of the cheese matrix, and study by Ningtyas et al. [71], who found lower FC (0.50 - 25.17%) in cream cheese compared to those observed in the present study. Similar effects have been reported for the inclusion of date by-products in cheese matrices. For instance, study by Alqahtani et al. [72] on block-type processed cheeses fortified with date seed fiber (*Phoenix dactylifera* L.) found that the incorporation of this plant-derived ingredient influenced the measured FC and distribution within the protein matrix, likely due to enhanced structural integration and water- and fat-binding interactions of the dietary fibers. Another study by Qu et al. [73] reported that inclusion of oat β -glucan enhanced the water - holding capacity and modified the microstructure of set - type yogurt, supporting improved retention of the dispersed phases, including fat, due to enhanced water - fat - protein interactions in the gel network.

3.4. Microbiological analysis.

Microbiological quality represents a key indicator for evaluating the safety and shelf stability of dairy products. Previous studies have demonstrated that the incorporation of plant - derived powders rich in dietary fiber and phenolic compounds can influence microbial growth in cheese matrices. Several authors reported that fruit and vegetable by - products may exert mild antimicrobial effects due to the presence of polyphenols, organic acids, and antioxidant compounds, which can inhibit the development of spoilage microorganisms and improve product stability during storage. In addition, plant fibers may reduce water activity and modify the cheese microstructure, limiting microbial proliferation [74]. The microbiological indicators of glazed cottage cheese samples enriched with different concentrations of SBPF are presented in Table 4. One - way ANOVA revealed a significant effect of

formulation on total aerobic mesophilic microorganisms and on yeasts and moulds ($p < 0.05$). At the same time, pairwise grouping showed partial overlap among

neighboring formulations, indicating that the antimicrobial effect associated with SBPF incorporation was gradual rather than abrupt.

Table 4

Microbiological quality of glazed cottage cheeses samples enriched with SBPF

Indicators	CS	1.25% SBPF	2.50% SBPF	3.75% SBPF	5.00% SBPF
QMAFAnM ($\log_{10}$ CFU/g)	3.25 ± 0.05	3.18 ± 0.04	3.10 ± 0.06	3.05 ± 0.05	2.98 ± 0.04
Yeasts and moulds ($\log_{10}$ CFU/g)	2.10 ± 0.03	2.05 ± 0.04	2.00 ± 0.05	1.95 ± 0.04	1.90 ± 0.03
Enterobacteriaceae ($\log_{10}$ CFU/g)	<1.0				
<i>Escherichia coli</i> ($\log_{10}$ CFU/g)	ND				
<i>Staphylococcus aureus</i> ($\log_{10}$ CFU/g)	<1.0				
<i>Listeria monocytogenes</i> (presence/absence in 25 g)	Absent				

CS - control sample without SBPF; SBPF - sea buckthorn pomace flour; ND - not detected; QMAFAnM- quantity of mesophilic aerobic and facultative anaerobic microorganisms; CFU- colony forming unit. Data are presented as mean $\pm$ SEM ($n = 3$). Different lowercase superscript letters within the same row indicate significant differences among samples at $p < 0.05$. Values <1.0 $\log_{10}$ CFU/g were below the detection limit.

The CS presented the highest initial counts, with total aerobic mesophilic microorganisms at $3.25 \log_{10}$ CFU/g and yeasts and moulds at $2.10 \log_{10}$ CFU/g, whereas the sample containing 5.00% SBPF showed the lowest values for both indicators, namely 2.98 and 1.90 $\log_{10}$ CFU/g, respectively. Thus, the most statistically defensible interpretation is that the addition of SBPF was associated with lower initial spoilage-related microbial counts, while all formulations remained within the microbiological safety limits evaluated in this study. Enterobacteriaceae and *Staphylococcus aureus* remained below the detection limit, *Escherichia coli* was not detected, and *Listeria monocytogenes* was absent in 25 g in all samples, confirming that SBPF incorporation did not compromise product safety.

The observed gradual reduction in microbial counts may be related to the presence of phenolic compounds and other bioactive constituents in sea buckthorn pomace, as well as to structural modifications of the cheese matrix that can limit the availability of nutrients and free water for microbial development [75].

Although the numerical decrease was moderate, the lowest microbial counts were

consistently recorded in the formulation with 5.00% SBPF, suggesting a tendency toward improved microbiological stability as the level of enrichment increased. However, this effect should be interpreted as a formulation - related trend supported by the statistical pattern rather than as direct mechanistic proof of antimicrobial action. This interpretation is consistent with previous reports emphasizing the importance of plant-derived bioactive ingredients for the microbiological quality and shelf stability of dairy products. Study by Roila et al. [76] demonstrated that polyphenolic extracts obtained from olive (*Olea europaea*) oil processing by-products, when added to “Fior di latte” cheese at 250 and 500 $\mu\text{g/mL}$, reduced the growth of *Pseudomonas fluorescens* and Enterobacteriaceae and delayed the attainment of microbiological limits by 2 - 4 days during storage, thereby extending shelf life. Similarly, study by Ferguous et al. [77] reported that olive pomace extract significantly influenced the microbiological quality of pasteurized white cheese: aerobic mesophilic counts decreased to $3.62 \log_{10}$ CFU/g after 30 days at 5 °C compared with $4.04 \log_{10}$ CFU/g in the control, while yeasts and moulds were completely

inhibited for 14 days at all tested concentrations. Comparable effects have also been described for berry - derived powders. Study by Sturza et al. [78] showed that the addition of rosehip (*Rosa canina*) and aronia (*Aronia melanocarpa*) powders to cream cheese significantly reduced *Salmonella* Abony counts and exerted marked effects against *Escherichia coli* and *Staphylococcus aureus*, supporting the use of such natural ingredients to improve microbial safety in dairy products. In addition to direct effects on microbial development, plant-based fortification may also enhance the phenolic profile and antioxidant potential of dairy matrices, which can indirectly contribute to improved product stability. In this regard, study by Koczka et al. [79] reported that aqueous and ethanolic extracts from rosehip (*Rosa canina* and other species) exhibited substantial total phenolic content - up to 299.2 mg GAE/100 g DW - and significant antioxidant capacity (FRAP values up to 314.4 mmol AAE/g), supporting the

potential use of rosehip by - products as multifunctional ingredients in fortified foods.

3.5. Texture Profile Analysis (TPA). The TPA of glazed cottage cheese enriched with different concentrations of SBPF revealed a consistent formulation-dependent effect across all measured parameters (Table 5). According to one-way ANOVA, most texture attributes were significantly influenced by SBPF incorporation ($p < 0.05$), indicating that the addition of pomace systematically modified the mechanical behavior of the cheese matrix. The CS exhibited baseline values characteristic of standard glazed cottage cheese, including hardness (46.88 g), cohesiveness (0.08 %), springiness (1.00 %), gumminess (3.81 g), adhesiveness (45.22 g·s), and resilience (0.48 %).

However, based on the statistical grouping, these changes were only partially significant, indicating that low-level enrichment produced a limited modification of the protein network.

Table 5
Influence of SBPF incorporation on the texture profile analysis of glazed cottage cheeses with different SBPF concentrations

Properties	CS	1.25 % SBPF	2.50 % SBPF	3.75 % SBPF	5.00 % SBPF
Hardness, g	46.88 ± 0.75 ^a	47.46 ± 0.56 ^a	54.38 ± 0.25 ^b	56.38 ± 0.34 ^c	59.08 ± 0.42 ^d
Cohesiveness, %	0.08 ± 0.02 ^a	0.08 ± 0.04 ^{ab}	0.08 ± 0.07 ^b	0.09 ± 0.05 ^c	0.09 ± 0.05 ^d
Springiness, %	1.00 ± 0.01 ^a	0.99 ± 0.02 ^a	0.99 ± 0.01 ^a	0.99 ± 0.03 ^a	0.99 ± 0.02 ^a
Gumminess, g	3.81 ± 0.25 ^a	3.93 ± 0.41 ^a	4.70 ± 0.54 ^b	5.16 ± 0.35 ^c	5.78 ± 0.25 ^d
Adhesiveness, g·s	45.22 ± 0.58 ^a	45.94 ± 0.45 ^a	51.02 ± 0.61 ^b	51.70 ± 0.55 ^{bc}	54.63 ± 0.48 ^c
Resilience, %	0.48 ± 0.03 ^a	0.55 ± 0.03 ^b	0.58 ± 0.03 ^c	0.61 ± 0.02 ^d	0.62 ± 0.03 ^d

CS- control sample without SBPF, SBPF - sea buckthorn pomace flour. Data are presented as mean ± SEM (n = 3). Different lowercase superscript letters within the same row indicate significant differences among samples at $p < 0.05$.

A more pronounced effect was observed at 2.50% SBPF, where hardness, gumminess and adhesiveness increased compared to the control. At this level, several parameters showed statistically significant differences, suggesting the formation of a denser and more cohesive matrix. At the same time, springiness remained practically

unchanged, indicating that the elastic recovery of the structure was preserved despite the increase in structural density. This balance between reinforcement and elasticity is consistent with the sensory results, where the same formulation achieved the highest acceptability [80]. Further increases in SBPF concentration

(3.75% and 5.00%) resulted in a continued rise in most TPA parameters. Hardness, cohesiveness, gumminess and resilience all reached their highest numerical values at 5.00% SBPF. However, the statistical grouping indicates that several of these parameters showed partial overlap between adjacent formulations, suggesting that the increase was progressive rather than characterized by clearly separated levels. Therefore, although the highest SBPF concentration produced the most rigid and dense structure, the differences between the upper concentration levels were not always statistically distinct.

From a structural perspective, the observed changes can be associated with interactions between milk proteins and fiber-rich components of SBPF.

Insoluble polysaccharides and plant particles likely act as fillers within the casein network, promoting the formation of a more compact matrix and increasing resistance to deformation. In addition, the presence of polyphenolic compounds may contribute to protein - polyphenol interactions, further reinforcing the structure [81]. However, these mechanisms should be interpreted as technologically plausible explanations rather than directly proven effects in the present study.

Overall, the TPA results demonstrate that increasing SBPF concentration leads to progressive reinforcement of the cheese matrix, expressed by higher hardness, cohesiveness and gumminess. At the same time, the relatively stable springiness values indicate that the elastic properties of the product were largely preserved. When considered together with the sensory results, these findings suggest that moderate enrichment (2.50% SBPF) provides a balanced textural profile, whereas higher concentrations tend to produce a denser and more rigid structure without a proportional improvement in consumer-relevant quality. Recent literature provides clear evidence on the influence of plant - based ingredients on

the textural properties of cheeses. Study by Ahmed Farahat et al., 2021 [82] reported that processed cheeses fortified with vegetable powders - carrot (*Daucus carota* L.), bean (*Phaseolus vulgaris* L.), pea (*Pisum sativum* L.), and celery (*Apium graveolens* L.) - exhibited an increase in hardness from 5.803 g in control samples to 6.254 - 6.457 g in fortified samples. Cohesiveness increased from 0.487 % to 0.518 - 0.532 %, while springiness reached 0.875 - 0.893 % compared with 0.851 % in the control, indicating a reinforcement of the protein network through interactions with dietary fibers. In another study [83] demonstrated that processed cheeses supplemented with chia seeds (*Salvia hispanica* L.) and other plant - based ingredients - white mulberry (*Morus alba* L.), green barley (*Hordeum vulgare* L.), and spirulina (*Spirulina platensis*) - showed increased hardness, from 5.954 g in the control to 6.308 - 6.602 g in fortified samples. Gumminess increased proportionally, while springiness rose from 0.842 % to 0.885 - 0.902 %, reflecting a denser and more coherent matrix. Furthermore, the review by Ghani et al. [84] indicated that cheeses enriched with fruit and vegetable pomace powders exhibited typical hardness values between 6.105 and 6.751 g, cohesiveness between 0.504 and 0.545 %, and springiness between 0.872 and 0.906 %, confirming the stabilizing effect of fibers on texture and water retention.

3.6. Assessment of microbiological safety and storage stability. According to the data presented in Table 6, the water activity and microbiological characteristics of the glazed cheese samples remained generally stable throughout and refrigerated storage. In the present work, statistical evaluation during storage was performed within each formulation in order to determine whether quality indicators changed significantly over time. For this reason, superscript letters were not assigned in Table 6, since

the table combines two experimental dimensions simultaneously, namely formulation and storage time, and the main interpretation of significance is provided in

the text. Based on the analysis of Table 6, water activity remained stable during storage, with a_w values ranging between 0.762 and 0.773 c.u., and no statistically

Table 6

Water activity and microbiological safety of glazed cheese samples with SBPF during storage					
Indicator / Storage, day	CS	1.25 % SBPF	2.50 % SBPF	3.75 % SBPF	5.00 % SBPF
a_w (c.u.)					
1 st day	0.771 ± 0.001 ^{aA}	0.773 ± 0.003 ^{aA}	0.772 ± 0.003 ^{aA}	0.773 ± 0.002 ^{aA}	0.773 ± 0.003 ^{aA}
3 rd day	0.768 ± 0.003 ^{aA}	0.771 ± 0.002 ^{aA}	0.770 ± 0.001 ^{aA}	0.771 ± 0.001 ^{aA}	0.772 ± 0.001 ^{aA}
6 th day	0.767 ± 0.002 ^{aA}	0.771 ± 0.003 ^{aA}	0.768 ± 0.002 ^{aA}	0.770 ± 0.002 ^{aA}	0.770 ± 0.003 ^{aA}
9 th day	0.766 ± 0.002 ^{aA}	0.769 ± 0.001 ^{aA}	0.768 ± 0.001 ^{aA}	0.769 ± 0.003 ^{aA}	0.768 ± 0.001 ^{aA}
12 th day	0.762 ± 0.001 ^{aA}	0.769 ± 0.002 ^{aA}	0.767 ± 0.003 ^{aA}	0.769 ± 0.00 ^{aA}	0.768 ± 0.002 ^{aA}
QMAFAnM (log₁₀ CFU/g)					
1 st day	3.25 ± 0.05 ^{aA}	3.18 ± 0.04 ^{abA}	3.10 ± 0.06 ^{abcA}	3.05 ± 0.05 ^{bcA}	2.98 ± 0.04 ^{cA}
3 rd day	3.24 ± 0.04 ^{aA}	3.17 ± 0.05 ^{abA}	3.09 ± 0.05 ^{abcA}	3.04 ± 0.06 ^{bcA}	2.97 ± 0.04 ^{cA}
6 th day	3.23 ± 0.05 ^{aA}	3.16 ± 0.04 ^{abA}	3.08 ± 0.06 ^{abcA}	3.03 ± 0.05 ^{bcA}	2.96 ± 0.03 ^{cA}
9 th day	3.22 ± 0.06 ^{aA}	3.15 ± 0.04 ^{abA}	3.07 ± 0.05 ^{abcA}	3.02 ± 0.05 ^{bcA}	2.95 ± 0.04 ^{cA}
12 th day	3.20 ± 0.05 ^{aA}	3.14 ± 0.04 ^{abA}	3.06 ± 0.05 ^{abcA}	3.01 ± 0.05 ^{bcA}	2.94 ± 0.04 ^{cA}
Yeasts and moulds (log₁₀ CFU/g)					
1 st day	2.10 ± 0.03 ^{aA}	2.05 ± 0.04 ^{abA}	2.00 ± 0.05 ^{abcA}	1.95 ± 0.04 ^{bcA}	1.90 ± 0.03 ^{cA}
3 rd day	2.09 ± 0.04 ^{aA}	2.04 ± 0.03 ^{abA}	1.99 ± 0.04 ^{abcA}	1.94 ± 0.03 ^{bcA}	1.89 ± 0.03 ^{cA}
6 th day	2.08 ± 0.03 ^{aA}	2.03 ± 0.03 ^{abA}	1.98 ± 0.04 ^{abcA}	1.93 ± 0.03 ^{bcA}	1.88 ± 0.03 ^{cA}
9 th day	2.07 ± 0.03 ^{aA}	2.02 ± 0.03 ^{abA}	1.97 ± 0.04 ^{abcA}	1.92 ± 0.03 ^{bcA}	1.87 ± 0.03 ^{cA}
12 th day	2.06 ± 0.03 ^{aA}	2.01 ± 0.03 ^{abA}	1.96 ± 0.04 ^{abcA}	1.91 ± 0.03 ^{bcA}	1.86 ± 0.03 ^{cA}

CS – control sample without SBPF; SBPF - sea buckthorn pomace flour; a_w - water activity. Data are presented as mean ± SEM ($n = 3$). Different lowercase superscript letters within the same row indicate significant differences among formulations at $p < 0.05$; different uppercase superscript letters within the same column indicate significant differences across storage days within each formulation at $p < 0.05$ (one-way ANOVA). *Enterobacteriaceae* and *Staphylococcus aureus* remained below the detection limit ($< 1.0 \log_{10}$ CFU/g); *Escherichia coli* was not detected and *Listeria monocytogenes* was absent in 25 g in all samples at all storage points; these indicators were excluded from inferential testing.

significant changes were observed within each formulation ($p > 0.05$). Differences among samples were also minimal, indicating that neither refrigerated storage nor the addition of SBPF materially changed the availability of free water in the product. Consequently, although formulation significantly affected moisture content in freshly prepared samples, this did not translate into a measurable change in a_w during storage. The stability of a_w supports the conclusion that the glazed cheese matrix remained physically stable during the short-refrigerated shelf - life evaluated in this study. Following the two-letter convention widely adopted in shelf-life studies of fortified dairy products [43 - 45], Table 6 reports lowercase superscript letters along

the rows (significant differences among formulations within the same storage day) and uppercase superscript letters along the columns (significant differences across storage days within the same formulation). Within-formulation one-way ANOVA across the five sampling points (1, 3, 6, 9, 12 days) yielded $F(4, 10) = 0.085 - 2.82$ with $p = 0.084 - 0.985$ for all three indicators and all five formulations - all below the critical value $F_{0.05}(4, 10) = 3.478$. Accordingly, no storage time point within a given formulation differed significantly from the others, and all column entries share the uppercase letter *A*, confirming physico - chemical and microbiological stability throughout the 12 - day refrigerated storage. The row - wise grouping reproduces the

pattern already established in Tables 4 and 5, indicating that the formulation effect persists across the entire storage period and that storage time, under the conditions of this experiment, does not introduce additional significant variation. Published studies on cheeses supplemented with plant-derived ingredients show similar trends. Furthermore, study by Tița et al. [85] reported that dairy products enriched with vegetable peel ingredients (potato (*Solanum tuberosum* L.) and carrot (*Daucus carota* L.) peels) maintained stable water activity values during cold storage, indicating that the incorporation of these by-products did not adversely affect the moisture dynamics and overall physico-chemical stability of the cheese matrix, while the study by Imankulova et al. [86] found that brine cheeses fortified with plant materials also showed only minimal water activity variation over time, with values in fortified cheese samples remaining high and essentially stable throughout storage (e.g., decreasing from 0.998 to 0.991), indicating that the addition of vegetable additives did not significantly alter the physico-chemical availability of water in the cheese matrix. Taken together, these findings support the view that the addition of fiber- and phenolic-rich plant ingredients does not necessarily alter free-water availability, even when modest compositional changes occur in the product matrix.

Microbiological indicators also remained stable during storage. For total aerobic mesophilic microorganisms, only slight numerical decreases were observed from day 1 to day 12 within each formulation, and these differences were not statistically significant ($p > 0.05$). The same tendency was recorded for yeasts and moulds. Importantly, the ranking of the formulations remained consistent during the entire storage period: samples enriched with SBPF maintained lower microbial counts than the control, with the 5.00% SBPF formulation showing the lowest values at

each storage point. Therefore, the principal analytical separation remained associated with formulation rather than with storage time. Under the conditions of this experiment, the results are better interpreted as evidence of microbiological stability during refrigeration than as proof of a strong time-dependent inhibitory effect.

This interpretation is consistent with the initial microbiological pattern observed in freshly produced samples, where SBPF-containing cheeses already showed lower counts of spoilage-related microorganisms than the control. During storage, these differences persisted but did not widen markedly, which suggests that SBPF contributed to a more favorable initial microbiological status and maintained this advantage over the 12-day period. However, because the absolute changes during storage were small, the present data do not support strong claims regarding progressive antimicrobial action over time. Instead, they support a stable formulation-related effect under refrigerated conditions. Regarding safety-related microbiological indicators, *Enterobacteriaceae* and *Staphylococcus aureus* remained below the detection limit ($<1.0 \log_{10}$ CFU/g) throughout storage, while *Escherichia coli* was not detected in any sample. In addition, *Listeria monocytogenes* was absent in 25 g of product at all storage points. These findings confirm that all formulations complied with the microbiological safety criteria assessed in the present study and that the addition of SBPF did not compromise product safety during storage. Comparable observations have been reported in the literature, where dairy products enriched with plant-derived ingredients maintained stable microbiological profiles during refrigerated storage. For example, yogurt fortified with elderberry (*Sambucus nigra* L.) extract exhibited stable LAB counts of 7.2 - 7.5 $\log_{10}$ CFU/g and mesophilic counts of 4.5 - 4.8 $\log_{10}$ CFU/g, with no detectable spoilage

organisms over a 21 - day period [87]. Similarly, fortified dairy products containing plant extracts such as citrus peel (*Citrus aurantium* L., *Citrus limon* L.) and rosehip seeds (*Rosa canina* L.) maintained microbial stability, with total aerobic mesophilic counts remaining below 5.0 log₁₀ CFU/g and yeasts and molds below detection limits (<1 log₁₀ CFU/g) throughout refrigerated storage, while preserving overall product safety and quality [87, 66]. In this context, the present results fit well within the broader evidence that plant by-products rich in dietary fiber and phenolic compounds can support the microbiological stability of dairy systems without negatively affecting their storage safety.

Overall, the storage study demonstrates that glazed cheeses enriched with SBPF remained stable from both physico-chemical and microbiological perspectives during 12 days of refrigeration. The absence of significant time-related changes in *a_w*, total aerobic mesophilic microorganisms, and yeasts and moulds indicate that storage did not adversely affect product quality within the studied period. At the same time, the consistently lower microbial counts observed in the SBPF-enriched samples suggest that the incorporation of sea buckthorn pomace flour may contribute to maintaining favorable product stability during refrigerated storage.

4. Conclusion

In conclusion, we can remark that this study demonstrated that SBPF can be successfully incorporated into glazed cottage cheese as a functional ingredient, with its effects on product quality being concentration-dependent. Among the tested formulations, the addition of 2.50% SBPF provided the most balanced overall performance and was identified as the optimal level under the experimental conditions. Moderate incorporation of SBPF improved sensory properties, particularly overall

acceptability, taste and odor, and color, while maintaining the characteristic identity of the product. In contrast, higher inclusion levels tended to reduce sensory balance, most likely due to the increased presence of insoluble plant particles and more pronounced flavor notes. The physico-chemical and textural results further confirmed the suitability of the 2.50% formulation, showing that moderate enrichment enhanced composition and structural stability without negatively affecting product quality or consumer-relevant attributes. Microbiological analysis confirmed that all formulations met the evaluated safety criteria, and SBPF-enriched samples generally exhibited lower spoilage-related microbial counts than the control sample. During refrigerated storage, the products remained stable from both physico-chemical and microbiological perspectives, indicating that SBPF incorporation did not adversely affect short-term storage stability.

Taken together, these findings indicate that 2.50% SBPF represents the most suitable enrichment level for glazed cottage cheese, ensuring an optimal balance between functional enhancement and preservation of product quality. Overall, the study highlights the potential of sea buckthorn pomace flour as a valuable by-product ingredient for the development of innovative dairy products with improved technological and functional properties.

5. Acknowledgments

The research was supported by the Institutional Project, subprogram 020405 “Optimizing food processing technologies in the context of the circular bioeconomy and climate change”, Bio-OpTehPAS, being implemented at the Technical University of Moldova.

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