ANNALS OF THE UNIVERSITY OF CRAIOVA

Series: ✔*Biology*
✔*Horticulture*
✔*Food products processing technology*
✔*Environmental engineering*

Vol. XXXI (LXVII) - 2026

STORAGE-RELATED CHANGES IN THE PHYSICOCHEMICAL INDICATORS OF GLAZED COTTAGE CHEESE WITH SEA BUCKTHORN POMACE

Cușmenco Tatiana[1]*, Dianu Irina[1], Sandu Iuliana[1], Reștica Vladislav[1], Netreba Natalia[1], Macari Artur[1]
[1*] Technical University of Moldova
** Correspondence author. E-mail:* tatiana.cusmenco@sa.utm.md

Keywords*: valorization, enrichment, cheese, pomace, storage*

ABSTRACT

This study investigated glazed cottage cheese containing 0, 1.25, 2.50, 3.75 and 5.00 % sea buckthorn pomace during 12 days of storage at 4 ± 1 °C. The samples were analyzed on days 1, 3, 6, 9 and 12 for pH, moisture, total sugar, ash and fat contents. Increasing sea buckthorn pomace levels decreased moisture and pH, while increasing total sugar, ash and fat contents. During storage, moisture and pH decreased, whereas total sugar and fat contents increased. Ash content showed only minor changes throughout storage. A strong positive relationship was found between moisture and pH, with a coefficient of determination of 0.986. The results demonstrate that sea buckthorn pomace modified the physicochemical indicators of glazed cottage cheese and influenced their changes during refrigerated storage.

INTRODUCTION

The stability of dairy products throughout storage is a key factor determining the preservation of their quality and technological characteristics until the time of consumption. In cottage cheese-based products, this issue is particularly relevant because of their relatively high water content, the sensitivity of the protein matrix to environmental changes, and the residual metabolic activity within the dairy matrix. During storage, changes in acidity, water redistribution among product components, and the relative concentration of non-aqueous constituents may occur, resulting in progressive changes in product composition and potentially affecting its stability. Therefore, evaluating the product only at the time of manufacture does not provide sufficient information about its behavior during storage, making the monitoring of quality parameters throughout the storage period necessary. (Oliveira et al., 2023; Duarte et al., 2023; Rajani et al., 2024).

The importance of such an approach increases when plant-derived ingredients obtained from agro-food by-products are incorporated into dairy products. Sea buckthorn pomace (*Hippophae rhamnoides* L.), generated during fruit processing, represents a secondary raw material with a complex composition, including dietary fibre, mineral substances, sugars, and other components of plant tissues. Its incorporation into a cottage cheese matrix may modify the balance between the different phases of the product and influence water transfer and binding,

acidity development, and the apparent concentration of analytically determined components. Consequently, the effect of such an ingredient should not be assessed solely on the characteristics of the freshly manufactured product, but also on how these characteristics are maintained or modified throughout storage (Yu et al., 2024; Wu et al., 2024).

Monitoring physicochemical parameters during storage makes it possible to determine the direction and magnitude of changes occurring within the food matrix and provides relevant information for assessing the compositional stability of the product. At the same time, comparing samples containing different levels of sea buckthorn pomace allows storage-related changes to be distinguished from those associated with the initial composition of the product. Such an assessment is important for supporting the use of sea buckthorn pomace as an ingredient in dairy products and for identifying conditions under which their physicochemical characteristics can be maintained throughout storage (Airouyuwa et al., 2025; Dimitrova-Dicheva et al., 2026).

In this context, the aim of the present study was to evaluate the changes in the physicochemical characteristics of glazed cottage cheese containing different levels of sea buckthorn pomace during storage, by monitoring pH, moisture content, total sugar content, ash content, and fat content at different storage intervals.

MATERIALS AND METHODS

Fresh cottage cheese, butter, powdered sugar, sea buckthorn pomace (SBP) and chocolate coating were used for the preparation of glazed cottage cheese (GCC). The SBP was obtained as a by-product of juice extraction from berries of the 'Clara' cultivar, harvested in September 2025 from the "Trei Bobițe" plantation, Buzduganii de Jos village, Ungheni district, Republic of Moldova. The SBP was dried at 50 ± 2 °C for 15 h, milled and sieved before use. The base formulation consisted of 78% fresh cottage cheese, 15 % butter and 7 % powdered sugar. These proportions were kept constant in all formulations. Five formulations were prepared: a control sample (S0), without SBP, and four experimental samples containing 1.25 % (S1), 2.50 % (S2), 3.75 % (S3) and 5.00 % (S4) SBP. The SBP levels were calculated based on the total mass of the base formulation and were added additionally, without replacing any component of the base formulation. The ingredients of the base formulation were homogenized, after which the corresponding amount of SBP was incorporated into the experimental formulations. The resulting mass was portioned, coated with chocolate and cooled. The samples were packaged and stored at 4 ± 1 °C for 12 days. Analyses were performed exclusively on the cottage-cheese filling after removal of the chocolate coating.

The physicochemical evolution of the samples was monitored on days 1, 3, 6, 9 and 12 of storage. The following parameters were determined: pH, moisture content (MC), total sugar content (TSC), ash content (AC) and fat content (FC).

The pH was determined using a calibrated digital pH meter according to AOAC Official Method 981.12 (AOAC INTERNATIONAL, 2023). The MC was determined gravimetrically by drying at 102 ± 2 °C to constant mass (ISO 6731:2010). The TSC was determined by the phenol–sulfuric acid method using glucose as the standard, after extraction of the sample, with absorbance measured at 490 nm (Yue et al., 2022). The results were expressed as glucose equivalents per 100 g of sample (%, m/m). The AC was determined by incineration at 550 ± 25 °C

until constant mass (ISO 9877:2026). The FC was determined by the Gerber method (ISO 2446:2008).

All analyses were performed in triplicate; the three measurements represented analytical replicates of the same sample, reflecting measurement precision rather than independent production replicates. The results were expressed as mean ± standard deviation (SD; n = 3). Statistical differences among storage periods were evaluated separately for each formulation using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) post-hoc test for pairwise comparisons, at a significance level of $p < 0.05$. The effects of formulation and storage time, as well as their interaction, were not assessed by factorial ANOVA, as the study design with analytical replicates did not allow independent estimation of between-formulation variance.

RESULTS AND DISCUSSIONS

The relationship between MC and pH of the glazed cottage cheese, depending on the formulation and storage duration, is presented in Figure 1.

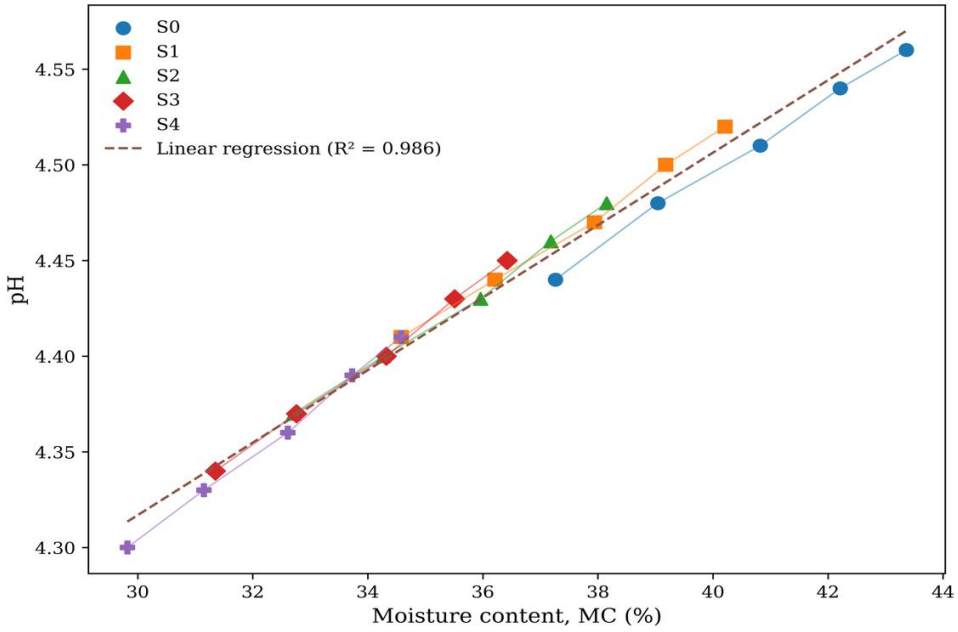


Figure 1. Relationship between MC and pH of GCC during refrigerated storage

Figure 1 demonstrates a very pronounced positive linear relationship between MC and pH of the GCC, with a coefficient of determination of $R^2 = 0.986$. The high value of this coefficient indicates a very strong association between the two parameters over the analyzed period, with higher MC corresponding to higher pH values, while a decrease in MC is accompanied by a reduction in pH. The distribution of the data points also highlights a clear differentiation among the investigated formulations, indicating that the positioning of the samples in the graph is determined both by the product composition and by the changes occurring during storage.

Differences among the formulations were evident from the beginning of the storage period. The S0 sample exhibited 43.36 % MC and pH 4.56, whereas S4 sample, containing the highest level of SBP, recorded 34.58 % MC and pH 4.41. Thus, increasing the SBP level was associated with a concomitant reduction in both MC and pH. This trend was maintained throughout storage, with differences among the formulations remaining evident until day 12. At this stage, S0 sample exhibited 37.26 % MC and pH 4.44, S1 - 34.58 % and 4.41, S2 - 32.71 % and 4.37, S3 - 31.35 % and 4.34, and S4 - 29.82 % and 4.30, respectively. Therefore, formulations containing higher levels of SBP were characterized by lower values of both indicators throughout the storage period.

Refrigerated storage resulted, regardless of formulation, in a decreasing trend in both MC and pH. In S0 sample, the MC decreased from 43.36 % on day 1 to 37.26 % on day 12, accompanied by a decrease in pH from 4.56 to 4.44. For S4 sample, MC decreased from 34.58 % to 29.82 %, while pH decreased from 4.41 to 4.30. These changes indicate that the storage period was accompanied by a progressive decrease in the water fraction and a concomitant shift in the product's reaction toward more acidic values. The decrease in MC was more pronounced than the change in pH, indicating different sensitivities of the two indicators to storage conditions.

The decrease in MC may be associated with water redistribution within the product, water migration between components, and the gradual loss of water from the product during storage. In the complex structure of GCC, these changes may affect the ratio between the aqueous and non-aqueous phases and may modify interactions among proteins, lipids, sugars, and plant-derived components. At the same time, the reduction in pH may reflect changes in the acid-base equilibrium of the dairy product and, possibly, residual biochemical processes occurring to a limited extent under refrigerated conditions. One possible explanation for the observed decrease in pH is the residual activity of lactic acid bacteria and the associated formation of lactic and other organic acids; however, these mechanisms were not directly assessed in the present study. Therefore, the association between the decrease in MC and the reduction in pH should not be interpreted as a direct causal relationship, but rather as an expression of the concurrent evolution of the physicochemical indicators of the product during storage (Duarte et al., 2023).

Differences among the formulations may also be explained by the specific contribution of SBP. The incorporation of this plant-derived ingredient modifies the product composition through the contribution of organic acids, mineral substances, and other soluble compounds, which may contribute to the initially lower pH values observed in samples containing higher levels of SBP. At the same time, the presence of pomace particles may influence water distribution and the water-retention properties of the food system, as reflected by the lower MC values observed in formulations with higher levels of incorporation. The persistence of differences among the samples throughout storage suggests that the effect of the initial composition is superimposed on the changes induced by storage.

The pooled-data analysis revealed a very strong positive linear association between MC and pH ($R^2 = 0.986$), indicating that variations in moisture content were closely accompanied by corresponding changes in pH across the investigated formulations and storage periods. However, this association should be interpreted descriptively, as the pooled dataset combines observations differing in SBP level and storage duration, both of which may contribute to the observed pattern. Thus, the progressive displacement of the data points toward lower MC values was

accompanied by a concurrent reduction in pH, while the separation of the formulations reflects the combined influence of their initial composition and storage-related changes. Overall, the observed MC-pH relationship provides additional evidence of the coordinated evolution of these physicochemical indicators during refrigerated storage, without implying a direct causal effect of MC on pH.

The TSC showed a progressive increase with increasing SBP incorporation and throughout refrigerated storage (Table 1).

Table 1

Total sugar, ash and fat contents of GCC formulated with SBP during storage

Indicator / Storage, day	S0	S1	S2	S3	S4
TSC, %					
1st day	22.30 ± 0.19^{e}	23.50 ± 0.20^{e}	24.25 ± 0.18^{e}	25.10 ± 0.22^{d}	26.85 ± 0.21^{d}
3rd day	22.84 ± 0.17^{d}	24.04 ± 0.18^{d}	24.76 ± 0.17^{d}	25.61 ± 0.19^{d}	27.34 ± 0.18^{d}
6th day	23.48 ± 0.18^{c}	24.68 ± 0.17^{c}	25.38 ± 0.19^{c}	26.22 ± 0.18^{c}	27.92 ± 0.20^{c}
9th day	24.17 ± 0.16^{b}	25.35 ± 0.19^{b}	26.02 ± 0.18^{b}	26.86 ± 0.21^{b}	28.51 ± 0.19^{b}
12th day	24.91 ± 0.19^{a}	26.02 ± 0.18^{a}	26.71 ± 0.20^{a}	27.52 ± 0.19^{a}	29.13 ± 0.21^{a}
AC, %					
1st day	1.55 ± 0.11^{a}	1.57 ± 0.10^{a}	1.61 ± 0.09^{a}	1.66 ± 0.11^{a}	1.71 ± 0.12^{a}
3rd day	1.69 ± 0.15^{a}	1.71 ± 0.12^{a}	1.75 ± 0.11^{a}	1.79 ± 0.13^{a}	1.84 ± 0.12^{a}
6th day	1.71 ± 0.12^{a}	1.73 ± 0.11^{a}	1.77 ± 0.10^{a}	1.81 ± 0.12^{a}	1.86 ± 0.11^{a}
9th day	1.73 ± 0.14^{a}	1.75 ± 0.12^{a}	1.79 ± 0.13^{a}	1.83 ± 0.11^{a}	1.88 ± 0.12^{a}
12th day	1.75 ± 0.13^{a}	1.77 ± 0.11^{a}	1.81 ± 0.12^{a}	1.85 ± 0.10^{a}	1.90 ± 0.12^{a}
FC, %					
1st day	23.65 ± 0.18^{e}	25.85 ± 0.15^{e}	26.91 ± 0.16^{e}	27.44 ± 0.19^{e}	28.70 ± 0.14^{e}
3rd day	24.28 ± 0.17^{d}	26.42 ± 0.16^{d}	27.47 ± 0.17^{d}	28.01 ± 0.18^{d}	29.26 ± 0.16^{d}
6th day	25.14 ± 0.16^{c}	27.21 ± 0.18^{c}	28.19 ± 0.15^{c}	28.73 ± 0.17^{c}	29.98 ± 0.17^{c}
9th day	26.02 ± 0.18^{b}	28.02 ± 0.17^{b}	28.94 ± 0.17^{b}	29.48 ± 0.16^{b}	30.71 ± 0.16^{b}
12th day	26.81 ± 0.17^{a}	28.76 ± 0.16^{a}	29.72 ± 0.16^{a}	30.24 ± 0.18^{a}	31.43 ± 0.17^{a}

Note: Values are expressed as mean ± standard deviation (n = 3; analytical replicates). Different lowercase superscript letters within the same column indicate significant differences among storage periods for that formulation (one-way ANOVA, Tukey's HSD test, $p < 0.05$). Superscript letters apply within each column only and do not indicate statistical comparisons between formulations.

On day 1, TSC increased from 22.30 % in S0 to 26.85 % in S4, corresponding to a difference of 4.55 percentage points between the control and the formulation containing 5.00 % SBP. By day 12, the respective values reached 24.91 % and 29.13 %. The higher TSC values observed in the SBP-containing formulations are consistent with the contribution of soluble carbohydrates naturally present in the plant material.

The increase in TSC during storage may also be related to the simultaneous decrease in moisture content, which increases the relative proportion of non-water constituents in the product. Thus, the observed increase in TSC should primarily be interpreted as a change in concentration expressed on a product-mass basis rather than as evidence of an increase in the absolute amount of sugars. The slightly smaller increase observed in formulations with higher SBP levels may be related to their higher initial TSC values and to differences in water distribution and retention within the product matrix. Overall, the results indicate that both SBP incorporation and storage-related changes in moisture contributed to the observed TSC pattern.

The AC showed comparatively smaller changes during storage. On day 1, AC ranged from 1.55 % in S0 to 1.71 % in S4, whereas by day 12 the corresponding values were 1.75 % and 1.90 %. The consistently higher AC values observed in formulations containing higher levels of SBP are compatible with the contribution of mineral-containing plant material to the overall composition of the product. The differences among formulations were maintained throughout storage, with S4 showing the highest numerical values and S0 the lowest. However, the increase in AC over storage was relatively limited compared with the changes observed for TSC and FC. This comparatively small variation suggests that the mineral fraction was less affected by the storage-related changes observed for the other compositional parameters. Accordingly, the results indicate that SBP mainly affected the initial ash level, whereas storage produced only modest changes in this parameter. This behaviour is consistent with the non-volatile nature of mineral constituents and their limited mobility within the food matrix under refrigerated conditions, as reported for enriched dairy products (Rajani et al. 2024). The AC among storage periods were not statistically significant at $p < 0.05$ within any formulation, consistent with the small absolute changes observed. Differences among formulations at the same storage day were not assessed by within-row letter notation and should be interpreted from the numerical values directly.

The FC exhibited clear numerical differences among the formulations. On day 1, FC increased from 23.65 % in S0 to 28.70 % in S4, while on day 12 the corresponding values were 26.81 % and 31.43%. The order S0 < S1 < S2 < S3 < S4 was maintained throughout the storage period, indicating that the formulations retained their distinct numerical fat-content profiles. The higher FC values in the SBP-containing samples should be considered in relation to the overall formulation and, particularly, to their lower moisture content rather than interpreted as evidence that SBP caused an increase in the absolute amount of fat. During storage, FC increased numerically in all formulations, coinciding with the observed reduction in moisture content. As water represents a decreasing proportion of the product mass, the relative proportion of fat and other non-water constituents consequently increases when expressed as a percentage of total mass. Therefore, the increase in FC is most appropriately interpreted together with the changes in MC and TSC, as reflecting changes in the relative composition of the product during storage rather than an actual accumulation of fat.

Comparison of TSC and FC revealed a highly similar pattern of evolution for the two parameters. In each sample, the increase in TSC during storage was accompanied by an increase in FC, while samples with higher TSC values consistently exhibited higher FC values. This association suggests that water loss played an important role in the changes observed in the composition expressed on a percentage basis. At the same time, AC showed considerably smaller variations,

indicating that the changes observed in TSC and FC were mainly associated with alterations in the ratio between water and organic components rather than with major changes in the mineral fraction.

An important feature was the evolution of the differences among samples throughout storage. For TSC, S4 exceeded S0 by 4.55 percentage points on the first day and by 4.22 percentage points on day 12. For FC, the difference between the same samples was 5.05 percentage points at the beginning and 4.62 percentage points at the end of storage. This slight narrowing of the differences indicates that the changes occurring during storage were more pronounced in samples with a lower initial solids content. Thus, the differences introduced by formulation were maintained, although they were slightly attenuated during storage.

The extent of the quantitative changes varied depending on the storage period. Changes in TSC and FC were statistically significant between each consecutive storage period in all or most formulations, with the absolute magnitude of the differences increasing progressively during storage. This progressive trend coincided with a gradual decrease in moisture content observed throughout the storage period and is consistent with a relative increase in the concentration of non-aqueous components as the water content decreased. Thus, changes in TSC and FC became more pronounced with prolonged storage, whilst characteristic differences between formulations persisted throughout the study period. These results indicate that both the initial formulation and the duration of storage in the refrigerator contributed to the observed changes in the product's composition.

CONCLUSIONS

The incorporation of SBP at levels of 1.25–5.00 % into glazed cottage cheese was associated with lower moisture content and pH and higher total sugar, ash and fat contents compared with the control formulation. The differences in physicochemical composition among the formulations were maintained throughout 12 days of storage at 4 ± 1 °C. The S4 formulation containing 5.00 % SBP showed the lowest initial moisture content (34.58 %) and pH (4.41), compared with 43.36 % and 4.56, respectively, in S0; by day 12, these values decreased to 29.82 % and 4.30 in S4 and to 37.26 % and 4.44 in S0. Total sugar and fat contents increased during storage, consistent with the progressive reduction in moisture content, whereas ash content showed comparatively smaller numerical changes. A very strong positive linear association was observed between moisture content and pH in the pooled dataset ($R^2 = 0.986$). Overall, the results demonstrate that both SBP incorporation level and refrigerated storage influenced the physicochemical characteristics of glazed cottage cheese under the conditions investigated.

ACKNOWLEDGMENT

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.

REFERENCES

Airouyuwa J. O., Mudgil P., Maqsood S. 2025. An investigation into impact of date seed bioactive compound addition on the quality attributes, shelf life, in vitro

digestibility, and bioactive properties of cottage cheese. Food Science & Nutrition, 13(7), e70535.

AOAC INTERNATIONAL. 2023. AOAC Official Method 981.12 - pH of Acidified Foods. In: Official Methods of Analysis of AOAC INTERNATIONAL, 22nd ed. Oxford University Press, Oxford.

Dimitrova-Dicheva M., Balabanova T., Boyanova P., Ivanova I., Ivanova M., Balabanov A. 2026. Plant agro-food by-products as functional additives in dairy products: A review. Food Science and Applied Biotechnology, 9(1), 79-100.

Duarte R. V., Lopes-da-Silva J. A., Gomes A. M., Delgadillo I., Barba F. J., Saraiva J. A. 2023. Improving fresh cheese shelf-life through hyperbaric storage at variable room temperature. Journal of Food Science, 88(1), 391-402.

International Organization for Standardization (ISO). 2008. Milk - Determination of fat content. ISO 2446:2008, Geneva, Switzerland.

International Organization for Standardization (ISO). 2010. Milk, cream and evaporated milk - Determination of total solids content (Reference method). ISO 6731:2010, IDF 21:2010, Geneva, Switzerland.

International Organization for Standardization (ISO). 2026. Milk and milk products - Determination of ash. ISO 9877:2026, Geneva, Switzerland.

Oliveira C. M. S. de, Grisi C. V. B., Silva G. de S., Lopes Neto J. H. P., Medeiros L. L. de, Santos K. M. O. dos, Cardarelli H. R. 2023. Use of *Lactiplantibacillus plantarum* CNPC 003 for the manufacture of functional skimmed fresh cheese. International Dairy Journal, 141, 105628.

Rajani B., Jana A. H., Bihola A., Adil K. 2024. Changes in physico-chemical and functional properties of Pizza cheeses made using ‘dual acidification’ method during refrigerated storage. Discover Food, 4, 157.

Wu D., Yang Z., Li J., Huang H., Xia Q., Ye X., Liu D. 2024. Optimizing the solvent selection of the ultrasound-assisted extraction of Sea Buckthorn (*Hippophae rhamnoides* L.) pomace: phenolic profiles and antioxidant activity. Foods, 13(3), 482.

Yu W., Du Y., Li S., Wu L., Guo X., Qin W., Kuang X., Gao X., Wang Q., Kuang H. 2024. Sea buckthorn-nutritional composition, bioactivity, safety, and applications: A review. Journal of Food Composition and Analysis, 133, 106371.

Yue F., Zhang J., Xu J., Niu T., Lü X., Liu M. 2022. Effects of monosaccharide composition on quantitative analysis of total sugar content by phenol-sulfuric acid method. Frontiers in Nutrition, 9, 963318.