



Effect of Collagen Concentrate and Plant Extracts on the Quality and Stability of Low-Fat Curd During Refrigerated Storage

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Data of the Article

First Received: 09 August 2025 | Last Revision Received: 01 October 2025

Accepted: 20 December 2025 | Published Online: 03 January 2026

DOI: <https://doi.org/10.5281/zenodo.17368316>

Keywords

Collagen Concentrate,
Plant Extracts,
Curd Product,
Storage Stability,
Yarrow,
Sage

This study developed and evaluated a novel low-fat soft curd enriched with 8 % collagen concentrate and 4 % plant extracts (sea-buckthorn/rose-hip or yarrow/sage). Compared with the unfortified control, enriched samples demonstrated significantly improved water-holding capacity, viscosity, and mechanical stability, alongside reduced water activity and lower yeast and mold counts ($p < 0.05$). Sensory analysis confirmed better flavor, texture, and color retention, particularly in the sea-buckthorn/rose-hip variant. Storage at 0–2 °C was identified as optimal for maintaining structural integrity and sensory quality for at least 96 h. These findings highlight the combined role of collagen and plant bioactives in enhancing the shelf life and consumer acceptability of functional curd products, with promising applications in sports and health-Oriented Nutrition.

1. Introduction

In recent years, there has been growing interest in developing functional fermented dairy products enriched with biologically active ingredients, such as collagen-containing concentrates and plant extracts. These products possess not only enhanced nutritional value but also potential health-promoting effects (Bankole et al., 2023; Cui et al., 2024; Kandyliari et al., 2023; Znamirowska-Piotrowska, Szajnar, & Pawlos, 2020). Collagen peptides can stimulate dermal fibroblasts to improve skin elasticity and hydration, alongside benefits for joint function and digestive health (Campos et al., 2023). However, the incorporation of these additional components may significantly influence the

structural, mechanical, organoleptic, physicochemical, and microbiological characteristics of dairy products during storage. Dairy product quality reflects how well a product retains its structure, flavor, and safety over time. A high-quality curd should hold moisture without leaking whey, feel pleasant to eat, and have stable acidity and pH to prevent spoilage. It must meet nutritional standards for fat and protein, while keeping microbial counts within safe limits. Lastly, taste, aroma, color, and texture should remain appealing throughout the refrigerated shelf life (Smolnikova et al., 2018).

Studies examining collagen and plant extracts individually have also revealed substantial impacts on dairy product attributes. For instance, adding

collagen to sheep and goat milk has been shown to increase post-fermentation pH, reduce lactic acid levels, and enhance desirable flavor profiles (Szopa, Pawlos, & Znamirowska-Piotrowska, 2023; Szopa et al., 2022). However, different collagen forms exhibit varied effects on the physicochemical, structural, mechanical, organoleptic, and microbiological properties of fermented dairy products. Researchers underscore the necessity for further investigations to comprehensively evaluate these impacts across diverse dairy matrices (Ayati et al., 2022; Cao et al., 2022; León-López et al., 2020; Permadi et al., 2024; Shori, Yong, & Baba, 2020; Soutelino et al., 2024).

Numerous studies have demonstrated that plant extracts can improve both the functional and sensory characteristics of fermented dairy products. For example, sea-buckthorn supplementation enhances gel strength and increases protein, fat, and polysaccharide content, while olive leaf extracts elevate antioxidant capacity—albeit at the expense of viscosity at high concentrations (Barukčić et al., 2022; Brodziak et al., 2021; Schubertová et al., 2021). Sage and related herb extracts inhibit lipid oxidation and stabilize acidity during storage (Cedeño-Pinos et al., 2023). More broadly, bioactive constituents such as polyphenols, organic acids, and polysaccharides have been shown to modulate texture, mouthfeel, and microbial stability, thereby extending shelf life (Abd El-Aziz, Salama, & Sayed, 2023; El-Sayed & Youssef, 2019; Iriundo-DeHond, Miguel, & Del Castillo, 2018; Iztileuov et al., 2024; Kandyliari et al., 2023). Recent work confirms that sea-buckthorn inclusion significantly sharpens acidity profiles, improves rheology, and boosts sensory appeal in fermented milks (Ge et al., 2022; Tang et al., 2026), and that plant-based biopreparations can increase protein content, enhance texture, and raise yield in low-fat curd products (Ryzhkova et al., 2023).

Several investigations have shown that adding collagen and plant extracts can enhance the functional and sensory properties of fermented dairy products. For instance, *Lycium barbarum* extract combined with fish-derived collagen improved protein proteolysis, acidity control, and ACE-inhibitory activity in yogurt without compromising flavor over 21 days (Shori, Ming, & Baba, 2021). Similarly, açai pulp, whey, and hydrolyzed collagen increased viscosity, nutrient content, and microbial stability in probiotic beverages over 28 days (da Mata Rigoto et al., 2019), while collagen–calcium enrichment yielded softer curd with higher protein and improved texture (Shchekotova et al., 2018; Tang et al., 2022) further highlighted collagen’s compatibility

with milk proteins and its role in enhancing product stability and texture for extended-shelf applications (Tang et al., 2022).

Accordingly, this research tests the hypothesis that enriching non-fat curd with 8 % collagen concentrate and 4 % plant extracts (either sea-buckthorn with rose-hip or yarrow with sage) will improve its structural–mechanical stability, sensory attributes, and microbiological safety compared to the unfortified control. Collagen and selected plant extracts were combined to strengthen curd structure and slow spoilage. Collagen peptides form a network within the casein gel that holds water, buffers acidity, and resists breakdown during storage. Sea-buckthorn (*Hippophae rhamnoides*) and rose-hip (*Rósa majális*) extracts are rich in pectins and flavonoids (e.g., quercetin, vitamin C), which increase serum viscosity and protect against oxidation (Sławińska et al., 2023; Wirkowska-Wojdyła et al., 2024). Yarrow (*Achillea millefolium*) and steppe sage (*Salvia stepposa*) provide terpenoids and phenolic acids (e.g., rosmarinic acid) that disrupt microbial membranes and slow acid production (Baran et al., 2025; Kurska et al., 2022). Together, these components synergistically enhance water retention, preserve textural integrity, and prolong the refrigerated shelf life of low-fat curd.

This study aimed to evaluate the impact of adding collagen-containing concentrate and plant extracts on the quality and stability of a non-fat curd product during refrigerated storage at varying temperatures and intervals. Preliminary findings on the composition of a product variant with sea buckthorn and rosehip extracts were previously reported in Zharykbasov et al. (2024), focusing primarily on formulation and bioactivity. The current investigation significantly expands upon earlier research, emphasizing storage stability, microbiological safety, and detailed analyses of physicochemical and structural-mechanical properties under different storage conditions.

2. Materials and Methods

2.1. Method of Obtaining an Extract from Rosehip and Sea Buckthorn Fruits

The plant raw materials, comprising cinnamon rose-hip (*Rósa majális*) and sea-buckthorn (*Hippophae rhamnoides*) fruits, were first cleaned to remove impurities and rinsed with cold water (≤ 20 °C) to preserve thermolabile constituents. The *Rósa majális* rose-hip and *Hippophae rhamnoides* sea-buckthorn

fruits were collected during route expeditions in the Abay region of the Republic of Kazakhstan, ensuring fresh, locally sourced material (Zharykbasov et al., 2024). The cleaned fruits were air-dried to a constant weight, then milled to a fine, homogenous powder to maximize extraction surface area.

Equal masses (100 g each) of the dried rose-hip and sea-buckthorn powders were combined and transferred into a batch extractor equipped with a mechanical stirrer. Extraction was carried out at 25 °C for three hours with continuous agitation at 175 rpm, using 75 % (v/v) ethanol in a 1:5 (w/v) solvent-to-berry ratio. Upon completion, the resulting suspension was allowed to settle in settling cylinders for 12–15 hours to promote sedimentation of coarse particulates and ballast substances. The clarified supernatant was then passed through a pleated filter paper to remove remaining solid residues.

Concentration of the filtrate was performed in a rotary evaporator at 76 °C for two hours to remove the majority of ethanol. Subsequent evaporation at 80 °C eliminated residual water, yielding an orange-red, plastisol-like viscous mass with a characteristic rose-hip aroma. The final extract was weighed, packaged, and stored under refrigeration until further use.

2.2. Method of Obtaining an Extract from Yarrow and Sage Plants

Plant raw materials, common yarrow (*Achillea millefolium*) and steppe sage (*Salvia stepposa*), were first cleaned to remove impurities, rinsed with cold water (≤ 20 °C) to preserve thermolabile components, and air-dried to constant weight. The dried materials were milled to a fine, homogeneous powder to increase extraction surface area.

Equal masses of the yarrow and sage powders were combined and loaded into a batch extractor equipped with a mechanical stirrer. Extraction was performed at 25 °C for three hours with continuous agitation at 175 rpm, using 96 % (v/v) ethanol in a 1:10 (w/v) plant-to-solvent ratio. Following extraction, the suspension was allowed to settle in standing cylinders for 12–15 hours to enable sedimentation of coarse particulates and ballast substances. The clear supernatant was then filtered through pleated filter paper to remove remaining solids.

The filtrate was concentrated in a rotary evaporator at 76 °C for two hours to remove the bulk of the ethanol.

Residual water was evaporated at 80 °C until a viscous, plastisol-like mass was obtained. The final extract, characterized by a yellowish-brown hue and distinctive herbal aroma, was weighed, packaged, and stored under refrigeration for subsequent analyses.

2.3. Method of Collagen-containing Concentrate Preparation

Collagen concentrate was prepared from chicken skin and chicken bone-and-foot mixture. Chicken skin was first cut into 2–3 cm pieces and combined with water at a 1:8 (w/w) ratio. This mixture was heated to 65 °C and simmered for three hours, then cooled to 36 °C. Papain enzyme was added at a 1:10 (w/w) enzyme-to-skin ratio, and the mixture was incubated at 36 °C for 24 h to effect enzymatic hydrolysis. Following fermentation, the mass was cooled to 20–25 °C and allowed to settle for one hour. The resulting gel phase was separated from solids and centrifuged at 1,000 rpm to remove fat. The purified gel was chilled to 5–6 °C.

Separately, chicken bone tissue and feet were combined in a 1:1 (w/w) ratio, heated at 65 °C for 3–4 h, and then cooled to 25 °C. The softened mixture was finely ground before adding papain at a 1:10 (w/w) ratio. Fermentation proceeded at 36 °C for 24 h. The hydrolyzed bone-and-foot concentrate was then cooled to 5–6 °C.

Equal portions of the skin-derived gel and the bone-and-foot concentrate were blended to form a homogeneous collagen-containing composition. This mixture was lyophilized at -44 °C until moisture content fell below 8–10 %. The dried concentrate was packaged in laminated foil-film pouches and stored at ≤ 8 °C and ≤ 70 % relative humidity for up to 48 h prior to use (Zharykbasov et al., 2024).

2.4. Preparation of Curd Products

Three variants of a non-fat curd product were prepared under laboratory conditions: an untreated control and two experimental formulations enriched with bioactive components. Experimental Sample 1 (denoted as Coll+SB+RH) received 8 % (w/w) collagen-containing concentrate and 4 % (w/w) plant extract from a mixture of sea-buckthorn (*Hippophae rhamnoides*) and rose-hip (*Rósa majális*), while Experimental Sample 2 (denoted as Coll+Y+S) was similarly supplemented with 8 % collagen concentrate and 4 % extract of common yarrow (*Achillea millefolium*) and steppe sage (*Salvia stepposa*). The 8 % collagen and 4 % extract levels were chosen based on

prior formulation work (Zharykbasov et al., 2024), which employed dose–response trials to balance functional performance with sensory acceptance. In that study, collagen additions below 5 % failed to produce significant improvements in gel strength and water retention, while concentrations above 10 % adversely affected mouthfeel. Similarly, plant extracts at 2–5 % were required to achieve measurable antioxidant and antimicrobial activity without imparting excessive bitterness or astringency. Consequently, an 8:4 (collagen:extract) ratio was identified as optimal for reinforcing the protein network, enhancing water-binding capacity, and delaying spoilage—while preserving high consumer acceptability in sensory evaluations.

Fresh cow’s milk (acidity ≤ 18 °T, Thorner units) was separated at 30–35 °C to yield skim milk, which was then pasteurized at 76–78 °C for 30–40 s. The milk was cooled to 30–32 °C and inoculated with 5 % (v/v) of a mixed starter culture comprising mesophilic and thermophilic lactic streptococci. A 40 % NaCl solution

(0.4 g·kg⁻¹ milk) and 1 % rennet were added, and fermentation proceeded at 30–32 °C for 4–6 h until titratable acidity reached 71–73 °T.

The coagulum was cut into 1–2 cm cubes, slowly heated to 35–40 °C, and held for 20–30 min to enhance whey expulsion. Whey was removed by gentle self-pressing at 25–28 °C for 30–40 min, followed by continued pressing for 1–2 h until the curd’s moisture content reached 65–70 %.

The resulting curd was passed through a colloid mill to obtain a homogeneous mass, heated to 35 °C, and thoroughly stirred while adding 8 % dry collagen concentrate and 4 % plant extract. The finished product was then cooled to storage temperature. Samples were packaged in 330 mL polypropylene containers (type KN-15) with peel-seal lids to ensure minimal oxygen permeability and stored in a refrigerated chamber (Biryusa 114, Russia) at controlled temperatures between 0 °C and +6 °C.



Figure 1: Samples of Curd Products.

The following analytical methods were employed to characterize the physicochemical properties of the curd products:

2.5. Determination of Chemical Composition

Fat content was determined by the acid hydrolysis method by GOST 5867-2023 (“Milk and Dairy Products. Methods for Determination of Fat”). In this procedure, proteins are decomposed by treatment with concentrated sulfuric acid, and the liberated fat is separated by centrifugation (Russian Institute of Standardization, 2024).

Protein content was measured by the Kjeldahl method following GOST 34454-2018 (“Dairy Products. Determination of Protein Content by Kjeldahl Method”) (Standartinform, 2018).

Moisture content was assessed gravimetrically in compliance with GOST 3626-73 (“Milk and Dairy

Products. Methods for Determination of Moisture and Dry Matter”) (Standartinform, 1973).

2.6. Determination of pH and Titratable Acidity

pH was recorded using a potentiometric technique as prescribed by GOST 32892-2014 (“Milk and Dairy Products. Method for Measurement of Active Acidity”) (Standartinform, 2015).

Titratable acidity was evaluated by titrimetry according to GOST 3624-92 (“Milk and Dairy Products. Titrimetric Methods for Determination of Acidity”) (Standartinform, 2009).

2.7. Sensory Evaluation of Curd Products

A structured five-point scoring system was developed to conduct a standardized sensory evaluation of the control and enriched non-fat curd samples (Suppl. File Table S13-S14). Seven trained panelists conducted the

sensory evaluations. We used tailored questionnaires—one for the unfortified control and separate ones for each plant-enriched variant—while maintaining the same five-point scale for taste, aroma, consistency, and color. On the control form, any unexpected herbal notes were marked as defects, whereas on the enriched forms, those same notes were treated as deliberate, recipe-specific attributes. This ensured that plant-derived flavors in the fortified samples were not mistaken for spoilage and allowed each product to be judged against its appropriate sensory standard, while still enabling direct statistical comparison of scores across all three groups. Assessments were performed on four key attributes—taste, aroma, consistency, and color—using a scale where 5 denotes optimal quality and 1 signifies unacceptable quality. Descriptor definitions were adapted from (Standartinform, 2014).

2.8. Microbiological Analyses

Lactic acid bacteria were enumerated on MRS agar according to GOST 33951-2016 (“Milk and Dairy Products. Methods for Determination of Lactic Acid Microorganisms”) (Standartinform, 2016). Yeasts and molds were quantified on Sabouraud Dextrose Agar supplemented with chloramphenicol, in accordance with GOST 33566-2015 (“Milk and Dairy Products. Determination of Yeasts and Molds”) (Standartinform, 2019).

2.9. Determination of Structural-Mechanical Parameters

Effective viscosity and yield stress of the curd products were measured using a Brookfield LVDV-2T rotational viscometer (Brookfield, USA) at 20 ± 1 °C. Tests were performed across a series of fixed shear rates to generate rheological flow curves and evaluate the products’ deformation behavior under applied shear.

The yield stress (τ_0) was calculated based on the Bingham model using the formula:

$$\tau = \tau_0 + \eta \times D \quad (1)$$

where:

- τ – shear stress, Pa;
- τ_0 - yield stress, Pa;
- η - plastic viscosity, Pa-s;
- D - shear rate, s^{-1} .

Viscosity loss coefficient (VLC) was calculated according to the formula:

$$VLC (\%) = \left(\frac{\eta_b - \eta_a}{\eta_b} \right) \times 100 \quad (2)$$

where:

- η_b - effective viscosity before exposure (Pa-s);
- η_a - viscosity after exposure (Pa-s).

The mechanical stability coefficient (MSC) was calculated according to the formula:

$$MSC = \frac{\eta_a}{\eta_b} \quad (3)$$

2.10. Determination of the Water-binding Capacity of Curd Product

Samples of curd product (10.00 ± 0.01 g) are placed in pre-weighed centrifuge tubes. The weight of the empty tube and the weight of the tube with product (M_1) are recorded. Centrifugation is carried out at 20 ± 2 °C for 10 minutes at 3000-5000 rpm, depending on the density of the product. After centrifugation is complete, the separated serum is removed without disturbing the sample and the tube with the precipitate is reweighed (M_2).

The mass of separated moisture is determined by the difference:

$$m = M_1 - M_2 \quad (4)$$

where:

- M_1 – mass of the test tube with sample, g
- M_2 – mass of the test tube with precipitate, g

Water-binding capacity is calculated according to the formula:

$$WBC (\%) = \left(\frac{m}{M_1 - M_{tube}} \right) \times 100 \quad (5)$$

where:

- m – mass of separated moisture, g
- M_1 - mass of the test tube with sample, g
- M_{tube} – mass of empty test tube, g

Measurements were performed in triplicate.

2.11. Determination of water activity

Water activity was measured at 25 ± 0.2 °C using an HD-4B Smart Water Activity Meter (NADE, China). The measurements were carried out in triplicate.

2.12. Statistical Analysis

All results are expressed as mean \pm standard deviation (M \pm SD) based on three to five independent replicates (n = 3-5). Data normality was assessed by the Shapiro–Wilk test. Between-group differences were evaluated using one-way analysis of variance (ANOVA) followed by Tukey’s post hoc test. Differences were considered statistically significant at $p < 0.05$. Analyses were performed using IBM SPSS Statistics v.27 and Microsoft Excel 2019.

3. Results

3.1. Study of Physical and Chemical Parameters During Storage of the Curd Products

Physicochemical parameters of curd products during storage determine their consumer quality and shelf life, which is critically important for the industrial manufacture of functional foods. The choice of storage temperature range (0°C, +2°C, +4°C, +6 °C) is due to the need to conduct a comprehensive assessment of the stability of the curd product enriched with collagen-containing raw materials and plant extracts within the regulatory values defined by GOST and technical regulations, according to which the storage temperature of curd is 4 ± 2 °C. The temperature of 0 °C was taken into the study as the lowest temperature condition, allowing the stability of the product to be assessed under the slowest microbiological and physicochemical processes corresponding to the lowest permissible cooling threshold. This approach allows for the simulation of various storage modes, including optimal and extreme conditions characteristic of the stages of storage, transportation, and sale, thereby identifying critical thresholds for the product’s resistance to physical-chemical, microbiological, and structural changes. Using a temperature range instead of a single stable point provides a more complete and reliable analysis of the effect of temperature conditions on the quality and shelf life of the product under study (Karlsson et al., 2019; Putina & Chernikova, 2020).

The results indicated that temperature markedly influenced product stability. The control sample (without additives) exhibited pronounced moisture loss from 80.0% to 72.0% at +6 °C over five days, coupled with increased titratable acidity from 220 to 240 °T and pH decline from 4.6 to 4.3, reflecting accelerated lactic acid fermentation (Table 1, 2). At lower temperatures (0 and +2 °C), these changes were minimal, demonstrating

better physicochemical stability, with moisture maintained at 76.5–79.0%, acidity increasing only by 5–6 °T, and pH remaining stable.

Fortification significantly enhanced product stability. Experimental samples initially possessed higher moisture content (~81%) due to the hydrating properties of collagen peptides (Table 1). At 0 and +2 °C, fortified samples showed reduced moisture loss (0.5–2.5% compared to 3.5% in control) and slower acidity increases (229–235 °T versus 240 °T in control), indicating improved moisture binding and reduced microbial activity (Table 2). However, at higher storage temperatures (+4 and +6 °C), experimental samples exhibited intensified moisture loss (up to 10%) despite lower acidity increments, suggesting thermal thresholds where microbial and enzymatic activities overshadow the stabilizing effects.

Table 1: Change in Mass Fraction of Moisture Depending on Storage Time and Temperature, %.

Storage Time	Control	Experimental 1	Experimental 2
At Storage Temperature 0 °C			
1 day	80.0 \pm 1.5 ^{Aa}	81.0 \pm 1.2 ^{Aa}	81.0 \pm 1.3 ^{Aa}
2 days	79.8 \pm 1.0 ^{Aa}	80.9 \pm 1.4 ^{Aa}	80.9 \pm 1.2 ^{Aa}
3 days	79.5 \pm 1.1 ^{Aa}	80.7 \pm 1.0 ^{Aa}	80.8 \pm 1.1 ^{Aa}
4 days	79.2 \pm 1.0 ^{Aa}	80.5 \pm 1.4 ^{Aa}	80.7 \pm 1.2 ^{Aa}
5 days	79.0 \pm 1.5 ^{Aa}	80.3 \pm 1.3 ^{Aa}	80.5 \pm 1.1 ^{Aa}
At Storage Temperature +2 °C			
1 day	80.0 \pm 1.5 ^{Aa}	81.0 \pm 1.3 ^{Aa}	81.0 \pm 1.1 ^{Aa}
2 days	79.5 \pm 0.7 ^{Aa}	80.8 \pm 1.9 ^{Aa}	80.9 \pm 1.3 ^{Aa}
3 days	78.5 \pm 1.3 ^{Aa}	80.5 \pm 1.1 ^{Aa}	80.7 \pm 1.6 ^{Aa}
4 days	77.5 \pm 0.8 ^{Aa}	79.5 \pm 1.3 ^{Aa}	79.8 \pm 0.9 ^{Aa}
5 days	76.5 \pm 1.2 ^{Ab}	78.5 \pm 0.9 ^{Aa}	78.5 \pm 0.9 ^{Aa}
At Storage Temperature +4 °C			
1 day	80.0 \pm 1.2 ^{Aa}	81.0 \pm 1.3 ^{Aa}	81.0 \pm 1.5 ^{Aa}
2 days	79.0 \pm 1.4 ^{Aa}	80.5 \pm 1.4 ^{Aa}	80.8 \pm 1.5 ^{Aa}
3 days	77.5 \pm 0.9 ^{Aa}	79.5 \pm 1.0 ^{Aa}	79.8 \pm 0.6 ^{Aa}
4 days	76.0 \pm 1.2 ^{Ab}	77.5 \pm 1.2 ^{Aa}	77.5 \pm 0.9 ^a
5 days	74.5 \pm 1.3 ^{Ab}	76.0 \pm 0.8 ^{Ab}	76.0 \pm 1.1 ^{Ab}
At Storage Temperature +6 °C			
1 day	80.0 \pm 1.3 ^{Aa}	81.0 \pm 1.4 ^{Aa}	81.0 \pm 1.3 ^{Aa}
2 days	75.5 \pm 1.3 ^{Ab}	80.5 \pm 1.5 ^{Ba}	80.5 \pm 1.1 ^{Ba}
3 days	75.0 \pm 1.0 ^{Ab}	79.0 \pm 1.2 ^{Aa}	79.5 \pm 1.2 ^{Aa}
4 days	74.0 \pm 0.9 ^{Abc}	74.0 \pm 0.9 ^{Ab}	74.5 \pm 1.3 ^{Abc}
5 days	72.0 \pm 1.2 ^{Abc}	71.0 \pm 0.9 ^{Ac}	72.0 \pm 1.2 ^{Ac}

^{A-B} Different uppercase letters indicate a significant difference between the different samples within the same row ($p < 0.05$)
^{a-c} Different lowercase letters indicate statistically significant differences within the same column for the same sample, depending on the storage time ($p < 0.05$).
 Values are expressed as the mean \pm SD.

Protein content increased from 18.0% in the control to 18.4–18.6% in experimental samples, attributed to the protein-rich collagen concentrate (Supp. File Table S1-S4). This increase improved the structural network, enhancing resistance to syneresis and structural degradation during

storage. Comparative analysis between botanical extracts revealed that the sea buckthorn-rosehip combination slightly outperformed the yarrow-sage blend regarding moisture retention, possibly due to rosehip's higher pectin content enhancing serum viscosity and delaying

why drainage. Conversely, the yarrow-sage extract demonstrated slightly greater effectiveness in moderating acidity, likely due to the antimicrobial properties of lipophilic monoterpenoids in sage.

Table 2: Change of Titratable Acidity Depending on Storage Time and Temperature, %.

Storage Time	Control	Experimental 1	Experimental 2
At Storage Temperature 0 °C			
1 day	220±2 ^{Aa}	225±4 ^{Aa}	225±3 ^{Aa}
2 days	220±4 ^{Aa}	225±3 ^{Aa}	225±3 ^{Aa}
3 days	220±3 ^{Aa}	225±3 ^{Aa}	225±4 ^{Aa}
4 days	221±3 ^{Aa}	226±4 ^{Aa}	226±4 ^{Aa}
5 days	221±3 ^{Aa}	226±5 ^{Aa}	226±4 ^{Aa}
At Storage Temperature +2 °C			
1 day	220±2 ^{Aa}	225±3 ^{Aa}	225±4 ^{Aa}
2 days	220±2 ^{Aa}	225±4 ^{Aa}	225±4 ^{Aa}
3 days	221±3 ^{Aa}	226±4 ^{Aa}	226±4 ^{Aa}
4 days	223±4 ^{Aa}	227±4 ^{Aa}	227±4 ^{Aa}
5 days	225±4 ^{Aa}	229±5 ^{Aa}	229±5 ^{Aa}
At Storage Temperature +4 °C			
1 day	220±2 ^{Aa}	225±3 ^{Aa}	225±3 ^{Aa}
2 days	221±2 ^{Aa}	226±4 ^{Aa}	226±4 ^{Aa}
3 days	224±3 ^{Aa}	228±4 ^{Aa}	228±4 ^{Aa}
4 days	227±4 ^{Ab}	231±5 ^{Aa}	230±4 ^{Aa}
5 days	231±5 ^{Ab}	234±5 ^{Ab}	232±4 ^{Ab}
At Storage Temperature +6 °C			
1 day	220±2 ^{Aa}	225±4 ^{Aa}	225±3 ^{Aa}
2 days	222±3 ^{Aa}	228±4 ^{Aa}	227±4 ^{Aa}
3 days	225±3 ^{Aa}	230±5 ^{Aa}	230±4 ^{Aa}
4 days	230±4 ^{Ab}	234±5 ^{Ab}	231±4 ^{Aa}
5 days	240±6 ^{Ab}	238±5 ^{Ab}	235±5 ^{Ab}

^A Similar uppercase letters indicate a non-significant difference between the different samples within the same row (p>0.05)
^{a-b} Different lowercase letters indicate statistically significant differences within the same column for the same sample, depending on the storage time (p < 0.05).
 Values are expressed as the mean ± SD.

Incorporating collagen concentrate and plant-derived antioxidants significantly improves low-fat curd products' physicochemical, microbiological, and sensory stability during storage, especially at optimal temperatures (0–2 °C). This approach effectively extends shelf life and maintains product quality, highlighting its potential for developing functional dairy products suitable for specialized nutrition markets.

3.2. Study of Microbiological Indicators During the Storage of Curd Products

The assessment of microbiological parameters enables the determination of the effects of storage conditions on the safety and quality of the product under study. The results of the physicochemical analyses of the curd product samples during storage at different temperatures are presented in Tables 3, 4, 5, and 6.

Table 3: Microbiological Parameters of Curd Product Samples During Storage at 0 °C.

Storage Time	Indicator	Control Sample	Experimental Sample 1	Experimental Sample 2
1 day	Quantity of lactic acid microorganisms, log ₁₀ CFU/g	7.000	6.954	6.929
	Yeasts and molds, log ₁₀ CFU/g	0.477	0.477	0.301
2 days	Quantity of lactic acid microorganisms, log ₁₀ CFU/g	7.004	6.959	6.929
	Yeasts and molds, log ₁₀ CFU/g	0.602	0.301	0.301
3 days	Quantity of lactic acid microorganisms, log ₁₀ CFU/g	7.009	6.964	6.940
	Yeasts and molds, log ₁₀ CFU/g	0.699	0.477	0.477
4 days	Quantity of lactic acid microorganisms, log ₁₀ CFU/g	7.013	6.944	6.968
	Yeasts and molds, log ₁₀ CFU/g	0.778	0.602	0.602
5 days	Quantity of lactic acid microorganisms, log ₁₀ CFU/g	7.021	6.949	6.973
	Yeasts and molds, log ₁₀ CFU/g	0.845	0.699	0.699



Table 4: Microbiological Parameters of Curd Product Samples During Storage at +2 °C.

Storage Time	Indicator	Control Sample	Experimental Sample 1	Experimental Sample 2
1 day	Quantity of lactic acid microorganisms, log ₁₀ CFU/g	7.000	6.954	6.929
	Yeasts and molds, log ₁₀ CFU/g	0.699	0.477	0.477
2 days	Quantity of lactic acid microorganisms, log ₁₀ CFU/g	7.021	6.964	6.944
	Yeasts and molds, log ₁₀ CFU/g	0.778	0.602	0.602
3 days	Quantity of lactic acid microorganisms, log ₁₀ CFU/g	7.041	6.978	6.954
	Yeasts and molds, log ₁₀ CFU/g	0.903	0.699	0.778
4 days	Quantity of lactic acid microorganisms, log ₁₀ CFU/g	7.079	6.991	6.978
	Yeasts and molds, log ₁₀ CFU/g	1.079	0.845	0.954
5 days	Quantity of lactic acid microorganisms, log ₁₀ CFU/g	7.114	7.000	7.000
	Yeasts and molds, log ₁₀ CFU/g	1.255	1.000	1.114

Table 5: Microbiological Parameters of Curd Product Samples During Storage at +4 °C.

Storage Time	Indicator	Control Sample	Experimental Sample 1	Experimental Sample 2
1 day	Quantity of lactic acid microorganisms, log ₁₀ CFU/g	7.000	6.954	6.929
	Yeasts and molds, log ₁₀ CFU/g	0.845	0.602	0.602
2 days	Quantity of lactic acid microorganisms, log ₁₀ CFU/g	7.041	6.964	6.944
	Yeasts and molds, log ₁₀ CFU/g	1.000	0.699	0.778
3 days	Quantity of lactic acid microorganisms, log ₁₀ CFU/g	7.079	6.978	6.954
	Yeasts and molds, log ₁₀ CFU/g	1.176	0.845	0.954
4 days	Quantity of lactic acid microorganisms, log ₁₀ CFU/g	7.114	6.991	9.678
	Yeasts and molds, log ₁₀ CFU/g	1.398	1.000	1.114
5 days	Quantity of lactic acid microorganisms, log ₁₀ CFU/g	7.146	7.000	7.000
	Yeasts and molds, log ₁₀ CFU/g	1.544	1.176	1.255

Table 6: Microbiological Parameters of Curd Product Samples During Storage at +6 °C.

Storage Time	Indicator	Control Sample	Experimental Sample 1	Experimental Sample 2
1 day	Quantity of lactic acid microorganisms, log ₁₀ CFU/g	7.000	6.954	6.929
	Yeasts and molds, log ₁₀ CFU/g	1.000	0.699	0.699
2 days	Quantity of lactic acid microorganisms, log ₁₀ CFU/g	7.079	6.978	6.954
	Yeasts and molds, log ₁₀ CFU/g	1.176	0.845	0.903
3 days	Quantity of lactic acid microorganisms, log ₁₀ CFU/g	7.146	7.041	7.000
	Yeasts and molds, log ₁₀ CFU/g	1.398	1.000	1.079
4 days	Quantity of lactic acid microorganisms, log ₁₀ CFU/g	7.204	7.114	7.079
	Yeasts and molds, log ₁₀ CFU/g	1.544	1.146	1.255
5 days	Quantity of lactic acid microorganisms, log ₁₀ CFU/g	7.255	7.176	7.146
	Yeasts and molds, log ₁₀ CFU/g	1.699	1.301	1.398

The study demonstrated that the proliferation of lactic acid bacteria, as well as the development of yeast and mold flora during storage, proceeded with differing intensities in the control and experimental samples depending on the temperature regimen. The control sample, without additives, exhibited the greatest increase in both lactic acid bacteria and yeast/mold counts, particularly at +4 °C and +6 °C, where by day 5 yeast and mold populations reached 35 and 50 CFU/g, respectively. The experimental samples enriched with collagen concentrate and plant extracts (sea-buckthorn with rose-hip or yarrow with sage) exhibited slightly slower microbial growth compared with the control. However, these differences were not statistically significant ($p > 0.05$) and therefore should be interpreted as indicative trends rather than confirmed effects.

A notably slower rise in yeast and mold counts was observed in the fortified samples: at +6 °C on day 5,

these counts did not exceed 20–25 CFU/g, compared to 50 CFU/g in the control. A similar trend was seen at +4 °C, where fungal counts in the experimental samples were 1.5–2 times lower than in the control. The most stable microbiological parameters were recorded at 0 °C and +2 °C, indicating the high efficacy of combining low-temperature storage with bioactive fortification in delaying microbial spoilage.

3.3. Study of Change in Effective Viscosity During Storage of Curd Product Samples

The investigation of changes in effective viscosity during storage of the curd product samples was undertaken to objectively assess the structural stability of low-fat curds under various storage conditions. Effective viscosity, which reflects the product’s resistance to deformation under applied stress, serves as a key indicator of integrity in the protein network. Alterations in viscosity over

time may reveal disruptions to the system's structural-mechanical bonds, arising from syneresis, proteolysis, or other physicochemical transformations occurring within the product. Accordingly, this study measured the effective viscosity of the control and of the experimental samples, each fortified with collagen concentrate and

plant extracts, to elucidate the dynamics of structural change during storage at different temperatures. The results detailing viscosity profiles over time are presented in Figures 2, 3, 4, and 5. The data obtained for storage at 0, +2, +4, +6 °C are specifically illustrated in Figure 2.

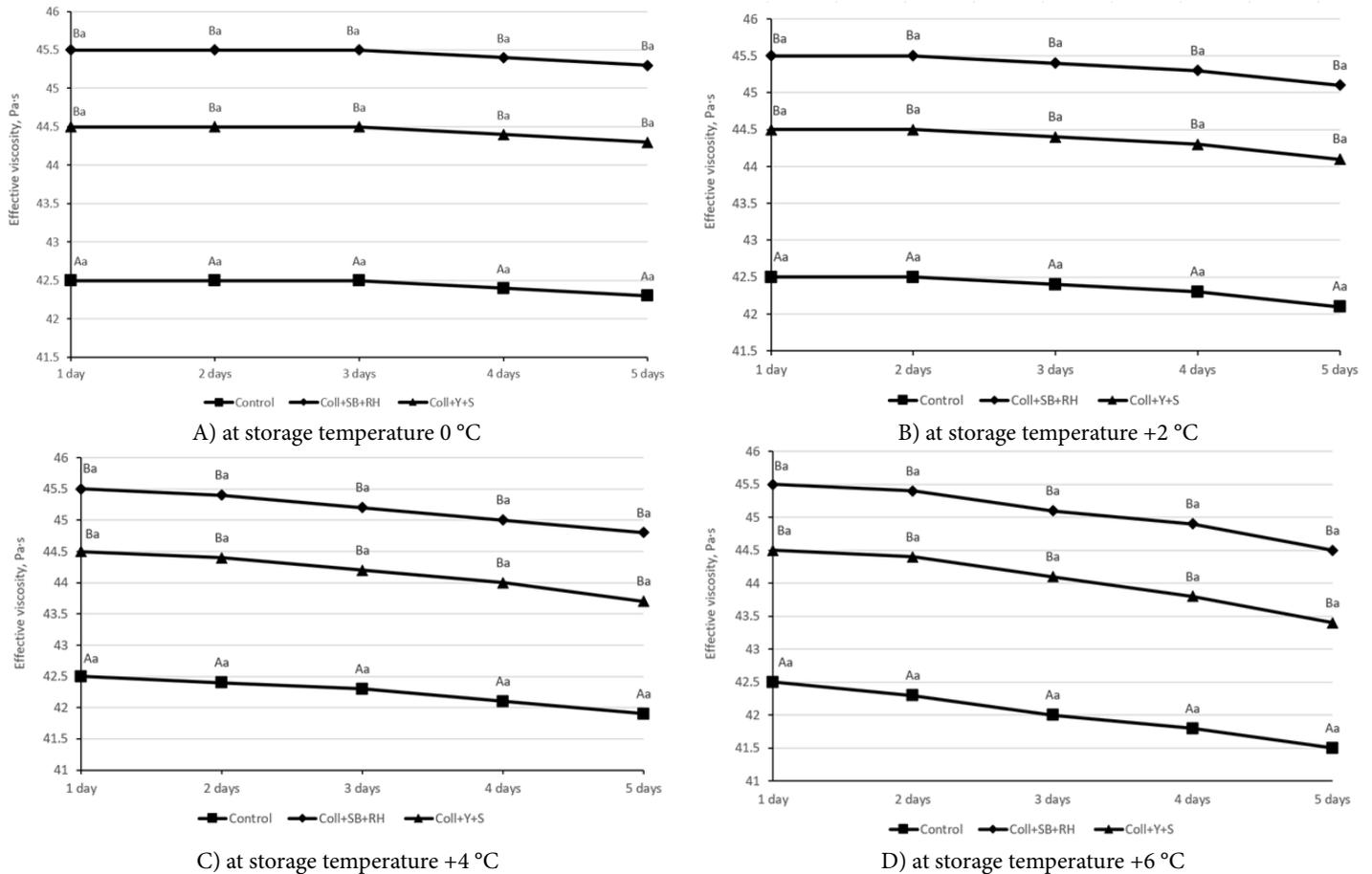


Figure 2: Change in Effective Viscosity of Curd Products at Different Storage Temperatures.

At 0 °C and +2 °C, all three variants exhibited a similar loss of viscosity (−0.2 and −0.4 Pa·s, respectively; $p > 0.05$), indicating that neither collagen nor plant extracts affected the minimal thinning that occurs under optimal refrigeration conditions. Under mild thermal stress (+4 °C, +6 °C), the enriched products exhibited only marginally different losses (0.1–0.2 Pa·s) relative to the control, and these differences were not statistically significant ($p > 0.05$). Thus, while the experimental formulations start with higher initial viscosity, their rate of decline over five days mirrors that of the unfortified curd.

When expressed as the change in effective viscosity from Day 1 to Day 5, all three formulations showed only minor declines at 0 °C and +2 °C, with no statistically significant differences among groups ($p >$

0.05), indicating minimal structural relaxation under optimal refrigeration. At +4 °C and +6 °C, viscosity decreased more noticeably, but the magnitude of loss remained statistically comparable across the control and enriched samples ($p > 0.05$). Although the experimental variants started at higher absolute viscosity levels, their relative stability during storage did not differ significantly from the unfortified curd, suggesting that the functional ingredients primarily influenced the initial gel strength rather than the rate of viscosity decline. The data show that adding 8 % collagen concentrate and 4 % plant extract increases the initial viscosity but does not change the rate at which viscosity decreases over time. Consequently, the enriched curds remain firmer throughout storage, even though their gels weaken at the same pace as the control.

3.4. Investigation of the Change in Yield Stress During Storage of Curd Product Samples

An experiment was conducted to evaluate the structural integrity and mechanical properties of the non-fat curd product during storage by measuring the yield stress of shear, which reflects the strength of the internal gel matrix under applied load. This parameter serves as a critical indicator of textural stability, enabling quantitative assessment of the degree of network breakdown over time and at different storage temperatures. The study compared the control sample with two experimental formulations enriched with collagen concentrate and plant extracts, in order to determine the effect of these functional additives on deformation resistance and to verify their stabilizing impact.

Table 7: Change in Yield Stress During Storage of Curd Product Samples, Pa.

Storage Time	Control	Experimental 1	Experimental 2
At Storage Temperature 0 °C			
1 day	63±1 ^{Aa}	81±1 ^{Ab}	78±1 ^{Ab}
2 days	63±1 ^{Aa}	81±1 ^{Ab}	78±1 ^{Ab}
3 days	63±1 ^{Aa}	81±1 ^{Ab}	78±1 ^{Ab}
4 days	62±1 ^{Aa}	80±1 ^{Ab}	77±1 ^{Ab}
5 days	62±1 ^{Aa}	80±1 ^{Ab}	77±1 ^{Ab}
At Storage Temperature +2 °C			
1 day	62±1 ^{Aa}	80±1 ^{Ab}	77±1 ^{Ab}
2 days	62±1 ^{Aa}	80±1 ^{Ab}	77±1 ^{Ab}
3 days	61±1 ^{Aa}	79±1 ^{Ab}	76±1 ^{Ab}
4 days	60±1 ^{Aa}	78±1 ^{Ab}	75±1 ^{Ab}
5 days	59±1 ^{Ba}	77±1 ^{Ab}	74±1 ^{Ab}
At Storage Temperature +4 °C			
1 day	61±1 ^{Aa}	78±1 ^{Ab}	76±1 ^{Ab}
2 days	60±1 ^{Aa}	77±1 ^{Ab}	75±1 ^{Ab}
3 days	59±1 ^{Aa}	76±1 ^{Ab}	74±1 ^{Ab}
4 days	58±1 ^{ABa}	75±1 ^{ABb}	73±1 ^{ABb}
5 days	56±1 ^{Ba}	73±1 ^{Bb}	71±1 ^{Bb}
At Storage Temperature +6 °C			
1 day	61±1 ^{Aa}	78±1 ^{Ab}	76±1 ^{Ab}
2 days	60±1 ^{Aa}	77±1 ^{Ab}	75±1 ^{Ab}
3 days	58±1 ^{Ba}	75±1 ^{ABb}	73±1 ^{ABb}
4 days	56±1 ^{Ba}	73±1 ^{Bb}	71±1 ^{Bb}
5 days	53±1 ^{Ca}	70±1 ^{Bb}	68±1 ^{Bb}

^{A-C} Different uppercase letters indicate a significant difference within the same column between the same sample, depending on the storage time and temperature ($p < 0.05$).

^{a-b} Different lowercase letters indicate statistically significant differences within the same row for the samples, depending on the storage time and temperature ($p < 0.05$).

Values are expressed as the mean ± SD.

The study demonstrated that the smallest reduction in yield stress (τ_0), which reflects the structural–mechanical stability of the soft curd product, occurred during storage at 0 °C and +2 °C. Over the five-day storage period, τ_0 decreased by only 1–3 Pa under these conditions, whereas at +4 °C and most notably at +6 °C the loss of structural strength was markedly greater (Table 7). Experimental samples fortified with 8 % collagen concentrate and 4 %

botanical extracts (either rose-hip with sea-buckthorn or yarrow with sage) consistently exhibited higher τ_0 values than the control, regardless of the storage temperature. Therefore, the optimal storage conditions for preserving textural properties and resistance to structural collapse are 0 °C and +2 °C, particularly when combined with these functional additives.

3.5. Investigation of Changes in Water-Binding Capacity and Water Activity During Storage of the Curd Product Samples

To provide a comprehensive assessment of the structural and microbiological stability of the curd products during storage, water-holding capacity and water activity were also evaluated. These parameters reflect the product's ability to retain moisture in a bound form, thereby preventing syneresis and inhibiting undesirable microbial growth, and reveal changes in the physicochemical state of the system under different temperature regimens.

The water-binding capacity (WBC) of all curd variants declined progressively during storage (Table 8), but both enriched formulations retained significantly more bound water than the control ($p < 0.05$). At 0 °C, the control's WBC fell from 79.0 % to 68.7% over five days, whereas Sample 1 (collagen + sea-buckthorn/rose-hip) dropped from 83.0% to 74.7% and Sample 2 (collagen + yarrow/sage) from 82.0% to 73.0% (all inter-sample differences at day 5 were significant, $p < 0.05$). This pattern was mirrored at +2, +4, and +6 °C, with enriched samples consistently outperforming the control by 4–9 percentage points ($p < 0.05$ for each time–temperature combination).

Storage temperature exerted a strong effect on WBC in every variant: at +6 °C the control lost nearly 18 % of its initial bound water (79.0 to 60.8%), whereas Samples 1 and 2 lost only 13.1 % (83.0 to 69.9%) and 13.1 % (82.0 to 68.9%), respectively ($p < 0.05$ compared with control). The two-stage decline (steeper from day 0 to 2, then again after day 3) indicates that thermal stress accelerates syneresis once the protein network contracts, but the collagen–polyphenol matrix slows that progression ($p < 0.05$ between day 2 and 5 in enriched vs. control).

Mechanistically, the collagen concentrate forms a cold-set fibrillar network that increases serum entrapment, while plant-derived polysaccharides (pectin in rose-hip) and polyphenols (in yarrow/sage) further thicken the continuous phase and enhance water immobilization (Pasqui, De Cagna, & Barbucci, 2012; Yan et al., 2020).



Table 8: Changes in Water-binding Capacity During Storage of the Curd Product Samples.

Storage Time	Control	Experimental 1	Experimental 2
At Storage Temperature 0 °C			
0 day	79.0±0.9 ^{Aa}	83.0±1.1 ^{Ab}	82.0±1.2 ^{Aab}
1 day	78.2±1.3 ^{Aa}	82.2±1.5 ^{Ab}	81.2±1.0 ^{Ab}
2 days	76.6±1.0 ^{Aa}	81.3±1.1 ^{ABb}	80.8±1.1 ^{Ab}
3 days	71.9±1.0 ^{Ba}	77.2±1.3 ^{Bb}	76.7±0.9 ^{Bb}
4 days	70.3±0.8 ^{Ba}	75.5±1.2 ^{Bb}	74.2±0.8 ^{Bb}
5 days	68.7±0.9 ^{Ca}	74.7±1.1 ^{Bb}	73.0±0.8 ^{Bb}
At Storage Temperature +2 °C			
0 day	79.0±1.1 ^{Aa}	83.0±0.6 ^{Ab}	82.0±1.2 ^{Aab}
1 day	78.0±1.5 ^{Aa}	81.8±1.2 ^{Aa}	80.7±1.4 ^{Aa}
2 days	75.8±0.8 ^{Aa}	81.1±1.0 ^{Ab}	79.4±1.3 ^{Aab}
3 days	71.1±0.8 ^{Ba}	76.4±0.9 ^{Bb}	76.3±1.0 ^{Bb}
4 days	69.5±0.9 ^{BCa}	74.7±1.4 ^{Bb}	73.0±0.8 ^{BCb}
5 days	67.9±0.7 ^{Ca}	73.0±0.8 ^{Bb}	72.2±1.0 ^{Cb}
At Storage Temperature +4 °C			
0 day	79.0±0.8 ^{Aa}	83.0±0.9 ^{Ab}	82.0±1.1 ^{Aab}
1 day	77.4±1.1 ^{Aa}	81.2±1.5 ^{Aa}	79.8±1.3 ^{Aa}
2 days	74.3±0.8 ^{Ba}	80.1±0.5 ^{Ab}	78.6±1.4 ^{Aab}
3 days	69.5±1.1 ^{Ca}	74.7±1.2 ^{Bb}	73.8±1.1 ^{Bb}
4 days	67.2±1.1 ^{CDa}	73.0±0.9 ^{Bb}	72.7±1.0 ^{Bb}
5 days	64.8±0.5 ^{Da}	71.4±1.1 ^{Cb}	71.4±1.0 ^{Bb}
At Storage Temperature +6 °C			
0 day	79.0±1.1 ^{Aa}	83.0±1.6 ^{Ab}	82.0±1.0 ^{Aab}
1 day	75.8±0.8 ^{Aa}	80.5±0.9 ^{ABb}	79.0±1.1 ^{ABb}
2 days	72.7±1.0 ^{Ba}	79.4±0.9 ^{Bb}	77.7±1.1 ^{Bb}
3 days	66.4±0.6 ^{Ca}	73.9±0.9 ^{Cb}	72.5±1.0 ^{Cb}
4 days	64.8±0.6 ^{Ca}	72.2±1.2 ^{Cb}	71.8±1.1 ^{Cb}
5 days	60.8±0.9 ^{Da}	69.9±1.0 ^{Cb}	68.9±0.8 ^{Cb}

^{A-C} Different uppercase letters indicate a significant difference within the same column between the same sample, depending on the storage time and temperature ($p < 0.05$).
^{a-b} Different lowercase letters indicate statistically significant differences within the same row for the samples, depending on the storage time and temperature ($p < 0.05$).
 Values are expressed as the mean ± SD.

Sample 1’s slightly higher WBC relative to Sample 2 (e.g. 74.7 % vs. 73.0 % at 0 °C, day 5) reflects rose-hip’s richer pectin content, which can bind additional water ($p < 0.05$ for inter-sample comparison). Conversely, Sample 2 still maintained WBC superior to control across all conditions ($p < 0.05$), confirming that both botanical blends synergise with collagen to reinforce the protein network.

These findings demonstrate that adding 8% collagen concentrate and 4 % plant extracts significantly enhances the curd’s ability to retain moisture during refrigerated storage ($p < 0.05$). By reducing moisture loss by up to 30% at 0–2 °C and 25% at +6 °C relative to the control, the enriched formulations promise prolonged textural stability and shelf-life under commercial cold-chain conditions.

Subsequently, water activity (a_w) dynamics were assessed in the same curd formulations during storage. The results of these measurements are presented in Figures 6 through 9, with the data for 0, +2, +4, and +6°C depicted in Figure 3.

The study demonstrated that water activity (a_w) decreased in all curd product samples over the storage period, but

the magnitude and rate of this decline were dependent on both temperature and formulation. The most pronounced reduction occurred at +6 °C, where a_w in the control fell from 0.978 to 0.960, whereas in Experimental Samples 1 and 2 it decreased from 0.979 to 0.959 and 0.961, respectively, despite their higher initial moisture content. The difference likely stems from the higher pectin/hemicellulose content of rose hip compared with sage/yarrow, the former absorbing part of the free water that migrates during thermal abuse (Balcerak et al., 2019; Said, Olawuyi, & Lee, 2023). At +4 °C, a_w declined to 0.964 in the control and to 0.966 in the fortified samples. Even more stable a_w values were recorded at +2 °C (0.967 in the control and 0.971 in the experimental variants), and especially at 0 °C, where a_w remained virtually unchanged at 0.974–0.976 by day 5. The slower a_w decline indicates that water remains entrapped within the densified protein–polyphenol matrix rather than diffusing to the surface, supporting both rheological stability and microbial control (Maltini et al., 2003). These findings confirm that the incorporation of collagen concentrate and plant extracts enhances the retention of water in a bound state, thereby limiting its availability for microbial activity.

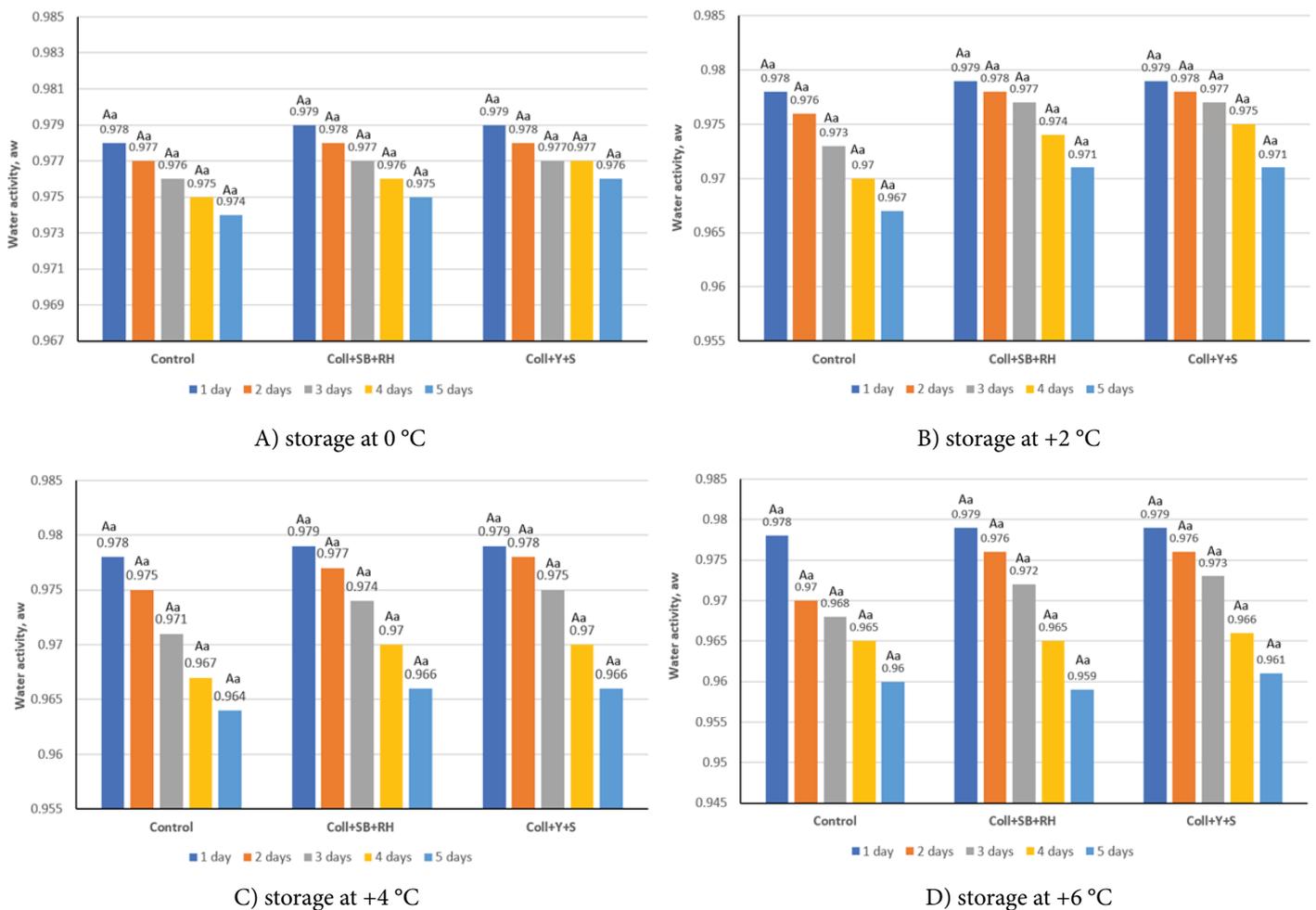


Figure 3: Change in Water Activity During Storage of Curd Products at Different Storage Temperatures (Same lowercase letter (a) indicates Statistically Nonsignificant Differences Depending on Storage Time ($p > 0.05$). Same Uppercase Letter (A) indicates Statistically Nonsignificant Differences between Samples ($p > 0.05$).

3.6. Study of Changes in Sensory Indicators During Storage

Sensory scores for all samples remained at the maximum level (5/5) during the first two days of storage at every temperature, confirming that the freshly prepared curds were indistinguishable in taste, aroma, consistency and color. However, the untreated control began to decline more rapidly than the enriched variants once storage extended beyond 48 hours, particularly at temperatures above 2 °C (Suppl. File Tables S15-S26).

At 0 °C, both experimental formulations preserved near-optimal sensory quality through day 5, with only a slight decrease to 4/5 in taste and consistency by the final day. In contrast, the control fell to 4/5 across all attributes on days 4–5, indicating the onset of perceptible textural coarsening and flavor flattening ($p < 0.05$ vs. day 1).

When stored at +2 °C, the control’s scores dropped to 3/5 by day 5, notably in taste and aroma (from 5 to 3; p

< 0.05), whereas both enriched curds maintained scores of 4/5 for consistency and aroma and only modestly declined in taste and color (to 3/5). This preservation of mouthfeel and sensory appeal mirrors the improved water-binding and rheological stability conferred by the added collagen and phenolic extracts.

Under more stressful conditions (4 °C and 6 °C), sensory degradation accelerated in all samples but remained significantly slower in the enriched products. By day 5 at +4 °C, the control scored 3/5 in taste and color and 2/5 in aroma, whereas Sample 1 and Sample 2 retained 3–4/5 across attributes ($p < 0.05$ vs. control). At +6 °C, the control’s taste and color fell to 1–2/5 by day 5, but the enriched variants sustained 2–3/5, demonstrating a clear sensory advantage ($p < 0.05$).

These results confirm that the addition of 8 % collagen concentrate and 4 % plant extracts significantly extends the period during which the curd products retain high sensory acceptability, especially under typical

refrigeration (0–2 °C). The slower decline in taste, aroma, consistency and color directly supports the hypothesis that collagen–polyphenol synergy stabilizes both structure and flavor compounds, thereby prolonging consumer-relevant quality.

4. Discussion

The results of the physical parameters and rheological study of control and enriched curd samples showed a significant effect of both temperature and functional composition on the preservation of sensory properties. The most stable indicators of consistency, taste, odor and color were recorded during storage at 0 °C and +2 °C, whereas at +4 and +6 °C a pronounced decrease in scores was observed, especially in taste and color. Similar results were obtained by Regu, Yilma, & Seifu (2016), where it was found that storing Ayib curd product at reduced temperature helps to slow down microbiological processes and increase shelf life.

The control sample without additives lost organoleptic stability by 3–4 days of storage at temperatures above 2 °C. In contrast, the experimental samples containing 8% of collagen-containing concentrate and 4% of plant extract demonstrated increased resistance to degradation. This may be due to the synergistic effect of the antioxidant components (ascorbic acid, quercetin, rosmarinic and ursolic acid et.al.) of the extracts and the gelling properties of collagen, which help to preserve the texture and sensory profile of the product. Similar results were obtained in studies where the use of plant extracts in cheese and curd products, such as *Tagetes erecta* extract and phytochemicals based on medicinal plants, contributed to an increase in shelf life by slowing down microbiological and oxidative processes without deterioration of organoleptic characteristics (Dupas et al., 2020; Gautam, Siddiqui, & Jharkharia, 2022; León-López et al., 2020; Ritota & Manzi, 2020; Szopa et al., 2022).

Experimental sample 1, enriched with sea buckthorn and rosehip extracts, demonstrated the greatest sensory stability during storage, which is probably due to the high content of vitamin C, flavonoids and carotenoids, which are biologically active antioxidants (Wirkowska-Wojdyła et al., 2024). Experimental sample 2, containing extracts of yarrow and sage, also showed improved organoleptic characteristics compared to the control, but its flavor stability was slightly lower, which may be due to the bitter phytones of these plants.

Thus, enrichment of curd products with collagen-containing concentrate in combination with plant

antioxidants contributes to the increase of sensory stability of products during storage, especially at reduced temperatures, which confirms the prospect of using this approach in the development of functional dairy products.

The established regularities in the change of organoleptic characteristics are consistent with the dynamics of physicochemical parameters, in particular, the mass fraction of moisture, titratable acidity, and pH, which requires further detailed analysis. At temperatures of 0 °C and +2 °C, minimal changes in parameters such as mass fraction of moisture, titratable acidity, and pH are ensured, indicating high physico-chemical stability of the curd product during storage. Similar regularities were established in previous studies by Mali, Karthikeyan, & Kumaresan (2020), where lowering the storage temperature contributed to the retardation of syneresis and lactic acid fermentation processes, ensuring the preservation of curd quality characteristics. The addition of collagen-containing concentrate and plant extracts contributed to the stabilization of the curd product structure, which was expressed in reduced moisture loss and lower changes in acidity compared to the control sample. This effect is probably associated with the slowing down of microflora activity and lactic acid fermentation processes due to the action of natural antimicrobial agents - bacteriocins of lactic acid bacteria and components of plant extracts, as noted by Ho, Howes, & Bhandari (2016), as well as with the improvement of the moisture-holding capacity of the product due to the formation of a stable gel-like structure with the participation of collagen-containing concentrate, according to Shchekotova et al. (2018).

The results of microbiological analysis indicate differences in the dynamics of microflora development between control and experimental samples of curd product during storage at different temperatures. All microbial counts derive from the commercial mixed mesophilic/thermophilic starter added at inoculation, not from spontaneous flora. The slowed acidification and reduced yeast–mold proliferation in curds enriched with collagen concentrate and plant extracts likely reflect synergistic effects: phenolic compounds (e.g., quercetin, cineole) disrupt microbial membranes and chelate metal cofactors essential for glycolysis; collagen-derived peptides buffer pH by binding free protons; and the combined collagen–polyphenol network lowers water activity and imposes a diffusional barrier to nutrient and metabolite exchange (Belashova et al., 2020). Together, these factors inhibit starter metabolism and spoilage microflora under refrigeration—particularly at 0–2

°C—resulting in significantly lower post-acidification rates and 40–50 % fewer yeasts/molds compared with the control ($p < 0.05$). The control sample, containing no additional components, showed more intensive growth of lactic-acid microorganisms and yeast microflora, especially at higher temperatures (+4 °C and +6 °C), where on the 5th day of storage, the number of yeasts and molds reached 35-50 CFU/g.

At the same time, experimental samples enriched with collagen-containing concentrate and plant extracts were characterized by a lower rate of microbiological changes. On the 5th day of storage at +6 °C, the number of yeasts and molds in these samples did not exceed 20-25 CFU/g, which is 40-50% lower compared to the control sample. A particularly pronounced effect was observed during storage at 0 °C and +2 °C, where the level of microbial contamination remained minimal throughout the study period.

The obtained data are consistent with the literature data on the ability of extracts to inhibit the growth of microorganisms due to the content of phenolic compounds, flavonoids and other biologically active substances with antimicrobial activity. Such components are able to disrupt the integrity of cell walls of pathogenic microorganisms, inhibit vital enzymatic processes and reduce the pH of the medium, which together limit the growth of undesirable microflora (Damani & Topi, 2022; Vitas et al., 2018). The study (Pinto et al., 2023) confirms that plant antimicrobial compounds effectively suppress the development of both bacterial and fungal pathogens through combined effects on membrane structures, metabolic pathways, and biofilm formation mechanisms, which makes them promising natural preservatives for the food industry.

Similar results were also obtained by Brodziak et al. (2021), where the introduction of sea buckthorn mouse (*Hippophae rhamnoides L.*) into the composition of probiotic yogurt contributed to a decrease in the total number of mesophilic aerobic bacteria and stabilization of the level of yeast microflora during storage without negative effects on the viability of lactic acid bacteria. This confirms that the use of plant extracts in the technology of curd products can contribute to the increase of their microbiological stability and prolongation of shelf life.

The study found that the effective viscosity of all samples decreased during storage, with the rate of change depending on the product composition and storage temperature. At 0 °C and +2 °C, the viscosity decrease was

minimal in both control and experimental samples, which indicates the stability of the structural-mechanical matrix at low temperatures. When the storage temperature was increased to +4 °C and +6 °C, a more pronounced decrease in viscosity was observed, especially in the control sample, where the decrease reached 2.4% and 2.3%, respectively, which is probably associated with the activation of syneresis and destruction of protein structures.

The experimental samples enriched with collagen-containing concentrate and plant extracts showed higher initial viscosity values and a lower degree of viscosity decrease throughout the shelf life. Incorporation of collagen-containing concentrate contributed to the increase in effective viscosity in the experimental samples compared to the control curd product sample due to strengthening of the protein network and increasing the water-holding capacity of the product. These results are consistent with the data of Cao et al. (2022), according to which the addition of collagen derived from animal by-products promotes the formation of a denser gel-like structure, improved textural characteristics and increased stability of food systems by enhancing intermolecular interactions and increasing water-holding capacity. The practical effectiveness of the use of collagen-containing hydrolysate to improve the texture and stability of fermented dairy products is also confirmed by the results of Dzyuba et al. (2017), where the use of collagen in the formulation of dairy dessert for baby food contributed to the formation of a dense and stable consistency of the product.

At the same time, the effective viscosity of the sample with rosehip and sea buckthorn extract was consistently higher than that of the sample with yarrow and sage extract. This difference may be due to the higher content of water-soluble polysaccharides, including pectins, as noted by Wu (2025), in the rosehip and sea buckthorn composite extract, which contributes to the strengthening of the protein-polysaccharide network by forming additional intermolecular interactions. While yarrow and sage composite extracts contain high concentrations of phenolic compounds, essential oils, providing pronounced antioxidant activity, but their ability to enhance the mechanical strength of protein-polysaccharide network in fermented milk products is limited due to the low content of water-soluble polysaccharides and almost complete absence of pectin (Huang et al., 2024; Jažo et al., 2023).

The obtained results show that the increase in storage temperature is conducive to the activation of syneresis processes and the destabilization of the protein structure

of curd products. At the same time, enrichment of the product with stabilizing components slows down these processes, preserving the structural integrity of the system. Thus, complex enrichment of curd products with collagen-containing concentrate and plant extracts contributes to the increase of their textural stability during storage and maintenance of high structural and mechanical characteristics.

The yield stress of the studied samples changed similarly to the dynamics of effective viscosity: all samples showed a decrease in this index during storage, and the rate of change depended on the storage temperature and product composition. The minimum decrease in the yield stress was recorded at temperatures of 0 °C and +2 °C, which indicates a high resistance of the structural matrix of the product to deformation loads. At the same time, during storage at +4 °C and +6 °C, the decrease in the index was more pronounced, especially in the control sample.

Experimental samples enriched with collagen-containing concentrate and plant extracts showed higher values of yield stress throughout the storage period and a lower rate of its decrease. At the same time, the sample with rosehip and sea buckthorn extract was characterized by the highest stability of mechanical properties, which is associated with the presence of water-soluble polysaccharides, including pectins, which contribute to the strengthening of the protein-polysaccharide network and increase water-holding capacity. The sample with yarrow and sage extract, despite high antioxidant activity, showed less pronounced resistance to deformation effects, which is probably due to the low content of structure-forming polysaccharides.

Thus, the obtained results confirm the effectiveness of collagen-containing concentrate and plant extracts for increasing the structural and mechanical stability of curd products during storage, especially at low temperatures.

Experimental samples enriched with collagen-containing concentrate and plant extracts showed better structural stability indicators in comparison with the control sample, which confirms the positive effect of collagen on strengthening the protein network and increasing the product's resistance to mechanical influences. The obtained data are consistent with the results of other studies (Cao et al., 2022; Gerhardt et al., 2013), where it is shown that the addition of hydrolyzed collagen helps to reduce the level of syneresis, reduce sedimentation and increase the stability of the structure of fermented dairy products.

Thus, complex enrichment of curd product with collagen-containing concentrate and plant extracts provides an increase in its structural stability under storage conditions, especially at temperatures of 0-2 °C.

Analysis of the dynamics of water-binding capacity change showed that in all the samples under study there was a gradual decrease in its water-binding capacity in the course of storage. At the same time, the highest values of water-binding capacity were preserved in samples enriched with 8% collagen-containing concentrate obtained from chicken skin, bones and paws, and 4% vegetable extract. Particularly pronounced moisture retention was observed at storage temperatures of 0 °C and +2 °C, which indicates the slowing down of syneresis processes and preservation of the integrity of the gel-like structure of the product.

The results of the study confirm the positive effect of the introduction of collagen-containing components on the stabilization of water-binding properties of curd product during storage. In contrast to Szopa et al. (2022), in which different types of collagen at doses of 1.5% and 3.0% were used to enrich probiotic sheep milk, in the present study, 8% collagen-containing concentrate obtained from secondary products of the poultry processing industry was used. The use of a higher dosage of functional ingredient allowed for a more pronounced increase in moisture-binding capacity and improved texture stability of the curd product during storage.

Thus, the results of the study of moisture-binding ability confirmed the feasibility of using 8% collagen-containing concentrate obtained from by-products of the poultry processing industry to improve the structural stability of the curd product during storage. In order to comprehensively assess the physicochemical stability of the samples under study, we additionally evaluated the dynamics of water activity change, which is one of the key factors determining the microbiological safety and shelf life of fermented milk products.

The analysis of water activity dynamics in the curd product samples revealed a gradual decline throughout storage, with the most pronounced decreases occurring at +4 °C and +6 °C. The control sample exhibited not only higher initial aw values but also a steeper reduction over time, whereas the experimental variants enriched with 8 % collagen-containing concentrate maintained more stable aw profiles across the entire storage period. This stability indicates an enhanced resilience of the product's aqueous phase, attributable to reinforcement of the protein network by the functional additives.

Moreover, the observed aw trends correlate directly with the microbiological stability of the curds: elevated water activity increases the fraction of free water available for microbial proliferation, whereas its reduction constrains the growth of yeast and mold populations. These findings align with the conclusions of Tapia, Alzamora, & Chirife (2020), who highlighted water activity as a principal barrier limiting microbial growth in food matrices.

It should also be noted that in dairy products, water activity profoundly influences not only microbiological stability but also the preservation of textural and functional properties through its interactions with the protein networks that form the gel matrix (Fox et al., 2015). Accordingly, controlling water activity by incorporating 8 % collagen-containing concentrate derived from poultry-processing by-products enhances both the physicochemical and microbiological stability of curd products, while also extending shelf life without compromising sensory quality.

5. Conclusions

The results show that adding 8% collagen concentrate together with 4% plant extract markedly improves the stability of low-fat curd under refrigerated storage. Both enriched formulations outperformed the control in retaining moisture, texture, and microbiological safety, but the sea-buckthorn/rose-hip blend (Sample 1) provided the greatest overall benefit: it retained up to 1 % more moisture, exhibited the smallest decline in acidity, and maintained the highest sensory scores, especially at 4–6 °C. The yarrow/sage variant (Sample 2) also slowed acidification and microbial growth but lost slightly more water under thermal stress. Optimal quality was achieved when storing at 0 °C to +2 °C: under these conditions Sample 1 maintained its gel strength, water-holding capacity, and flavor for at least five days, extending shelf life by 50 % compared to the control. These findings confirm the effectiveness of using collagen concentrate in combination with plant extracts for the development of functional curd products, including those intended for sports nutrition, with extended shelf life and high consumer acceptability.

Author Contributions: Conceptualization, A.K. and G.B.; Data curation, A.K. and G.B.; Formal analysis, Z.Y.; Investigation, G.R., Z.K. and M.J.; Methodology, G.R., K.Z., Z.K., Y.Z., M.J. and A.K.; Resources, G.R., G.B., A.K. and M.A.; Software, Y.Z. and M.A.;

Validation, K.Z.; Visualization, Y.Z.; Writing – original draft, K.Z. and Z.K.; Writing – review & editing, A.K. and Z.Y..

Funding: The article was prepared within the scientific project of grant financing of the Ministry of Science and Higher Education of the Republic of Kazakhstan on the theme AP19679638 “Scientific and practical basis for the use of collagen-containing concentrate in the production of specialized curd products for the nutrition of athletes”.

Conflicts of Interest: The authors declare no conflicts of interest.

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