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Development and study of the nutritional value and storage stability of a soft cottage cheese product enriched with collagen and antioxidant-rich plant extracts

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ABSTRACT

This study investigates the development of a novel soft cottage cheese product enriched with collagen concentrate from poultry processing by-products and antioxidant-rich plant extracts for the adaptive nutrition of athletes. Collagen concentrate was obtained from chicken skin, bone tissue, and feet through enzymatic hydrolysis and freeze-drying. Antioxidant-rich extracts were prepared from sea buckthorn and cinnamon rosehip using ethanol extraction. The plant extract demonstrated high antioxidant potential, containing 1.98% phenolic compounds, 29.8 mg/100g vitamin A, 48.9 mg/100g vitamin E, and 756.4 mg/100g vitamin C. The antioxidant extract demonstrated significant immune-boosting effects in experimental rats by enhancing lymphocyte and T-cell counts. Various ratios of collagen concentrate and plant extract were tested in the cottage cheese product. Optimal water-holding capacity and effective viscosity were achieved with a 6:4 or 8:4 collagen-to-extract ratio, balancing collagen's gelation properties with the antioxidant benefits. The addition of 8% dry collagen concentrate and 4% sea buckthorn and rosehip extract resulted in an enhanced nutritional profile, particularly through increased polyunsaturated fatty acids (Omega-3 and Omega-6), vitamins A, C, E, and essential minerals like calcium, phosphorus, and magnesium. Storage stability studies indicated optimal preservation of product structure at 0-2°C for up to 96 hours, maintaining a viscosity loss coefficient between 15.0-15.8%. This enhanced soft cottage cheese product demonstrates improved nutritional profiles and antioxidant properties while maintaining structural stability, making it a promising functional food for athletes and health-conscious consumers.

Keywords: collagen concentrate, plant extracts, functional dairy product, polyunsaturated fatty acids, antioxidant, texture stability

INTRODUCTION

In recent years, globally and in Kazakhstan, significant attention has been directed toward developing innovative, nutritionally balanced products, particularly for athletes. Manufacturing functional foods enriched with complex additives from animal and plant-based sources is gaining momentum as a promising approach to meet the growing demand for optimal nutrition. Dairy products, a staple in many diets, are an ideal platform for incorporating plant-based bioactive compounds, which enhance their health-promoting properties. These fortified products not only contribute to overall health by supporting physiological functions and preventing diseases but also play a crucial role in restoring the body's functional indicators after intense physical activity and aiding the recovery of the musculoskeletal system during rehabilitation **[1]**, **[2]**, **[3]**, **[4]**.

One key functional ingredient that plays a significant role in this recovery process is collagen. This animal-derived protein has been shown to enhance connective tissue repair and alleviate pain by boosting collagen production. Athletes who consume collagen-containing foods can maximise their physical performance **[5]**, **[6]**.
Collagen extracted from secondary products of the meat, fish and poultry processing industries is widely used in producing food additives. Collagen from secondary protein raw materials of the poultry processing industry, including chicken feet, feathers, scallops, bones and skin, is in particular demand. It was found that collagen from the skin contains the largest amount of proline and oxyproline (about 20%) and nearly none of the aromatic and sulfur-containing amino acids, which makes it especially valuable for further research **[7]**, **[8]**, **[9]**, **[10]**.

Integrating plant-derived bioactive compounds and extracts into dairy products offers multiple benefits. These include extending shelf life through antioxidant and antimicrobial activities, imparting positive health effects, and improving physicochemical, textural, and organoleptic properties **[11]**, **[12]**. Various plant sources, such as fruit peels, herbs, and byproducts, have been successfully utilised to fortify yoghurt, cheese, and kefir, enhancing their nutritional profiles and potential health benefits **[13]**, **[14]**, **[15]**.

As consumer interest in functional foods continues to rise, incorporating plant-based additives into dairy products represents a valuable strategy for producing nutritious, health-promoting food products that align with modern dietary trends.

While incorporating plant-based additives into dairy products offers numerous benefits, it is crucial to understand the specific functional ingredients that make these fortified products particularly valuable for athletes and their unique nutritional needs. Plant-based antioxidants-rich supplements offer targeted advantages, particularly for athletes **[16]**, **[17]**. These antioxidants, including phenolic compounds, carotenoids, and vitamins, are critical in combating oxidative stress and muscle damage caused by intense physical activity. Antioxidants are necessary to reduce the level of free radicals, so it is recommended that athletes include antioxidant-rich plant foods in their diet **[18]**. Using foods containing antioxidants in athletes' diets helps reduce tissue damage and accelerate their recovery after high physical loads during training and competition **[19]**, **[20]**.

This work plans to investigate fruit and berry plants to obtain biologically active additives (BAA) with pronounced antioxidant properties since they are characterised by an increased content of antioxidants **[21]**, **[22]**.

Taking into account the relevance of the production of specialised milk products for the adaptive nutrition of athletes, in this paper, we aimed to develop a formulation of a soft cottage cheese product with the application of collagen concentrate from secondary raw materials of the poultry processing industry and dietary supplements from plant raw materials with a high content of antioxidants. This combination of functional ingredients in cottage cheese products is unique. It simultaneously strengthens the musculoskeletal system due to collagen and protects the body from oxidative stress due to antioxidants, which is especially important for athletes.

Scientific hypothesis

Incorporating specific proportions of dry collagen concentrate derived from secondary poultry processing by-products and antioxidant-rich extracts from sea buckthorn and cinnamon rosehip into soft cottage cheese will significantly enhance the product's nutritional and biological value—particularly benefiting athletes by supporting musculoskeletal health and providing antioxidant protection—without adversely affecting its organoleptic properties or shelf life.

MATERIAL AND METHODOLOGY

Samples

Fresh cow's milk was purchased from farmers' markets in Semey. Fruit and berry plants (sea buckthorn and cinnamon rosehip) were collected during route expeditions in the Abay region of the Republic of Kazakhstan. Chicken skin, bone tissue, and chicken feet were obtained after deboning of bird carcasses purchased in a specialized store of the poultry processing enterprise “Ardager” (Semey, Kazakhstan).

Chemicals and biological material

Hydrochloric acid (mass fraction of hydrochloric acid (HCl), 35-38%, pure for analysis, Snabservice Astana LLP, Astana, Kazakhstan).
Sodium hydroxide, NaOH, (mass fraction of sodium hydroxide (NaOH), 99.3%, Snabservice Astana LLP, Astana, Kazakhstan).
Ethyl alcohol (70%, Snabservice Astana LLP, Astana, Kazakhstan)
Sodium chloride (40%, Kelun-Kazfarm LLP, Almaty, Kazakhstan)
Papain enzyme (Sigma-Aldrich, Burlington, United States).

Instruments

Scanning electron microscope "JSM-639 LV" (JEOL Co., Ltd., Tokyo, Japan).
Vacuum freezing dryer TOPT-10C (TOPTION Co., Ltd., Xi'an, China).
LVDV-2T rotary viscometer (Dongguan Lonroy Equipment Co., Ltd, China).
Spectrophotometer Shimadzu UV-1800 (Shimadzu Corporation, Kyoto, Japan).

Laboratory Methods

1) Physico-chemical methods of analysis to study the composition of the extract:

- determination of flavonoids by spectrophotometry method according to GOST R 55312-2012 **[23]**;
- determination of vitamin A by spectrophotometry according to GOST 12823-1-2014 **[24]**;
- determination of vitamin C by titrimetric method according to GOST 34151-2017 **[25]**;
- determination of vitamin E by colorimetric method according to GOST 12822-2014 **[26]**.

2) Immunological methods of research

Forty white mongrel rats weighing 180-200 grams at three ages were used to study the effect of dietary supplements from plant raw materials with pronounced antioxidant properties on the state and metabolic processes in the immune system under in vivo conditions of laboratory animals. The use of animals in the experiment is carried out in compliance with the norms and rules regulated by the legislation of the Republic of Kazakhstan, and international recommendations of the European Convention for the Protection of Vertebrate Animals used for experiments for scientific or other purposes. During the experiment, all animals are in the same standard vivarium conditions.

Total leukocyte and lymphocyte counts were determined using a haematology analyser. The content of T-helper (CD4+) and T-suppressor (CD8+) cells was determined by flow cytometry using monoclonal antibodies labelled with fluorescent dyes. IgA, IgM and IgG immunoglobulin concentrations were determined by enzyme-linked immunosorbent assay (ELISA). The latex particle uptake test by macrophages was used to evaluate phagocytic activity. CIC levels were determined using the immune complex precipitation test. The mitogen-assisted lymphocyte proliferation test was used to determine PTML. All data are presented as median (Q1-Q3). For statistical analysis, the Mann-Whitney test was used to compare control and experimental groups. The level of significance was set at $p < 0.05$.

3) Determination of the mass fraction of collagen

To conduct the study in a conical flask with a capacity of 250 cm^3, 1 g of sample was put into it, and 100 ml of hydrochloric acid was added, with further hydrolysis for 8 hours. The resulting hydrolysate is subjected to filtration and placed in a measuring flask with a capacity of 250 cm^3. The flask's contents are cooled, and distilled water is poured to the mark on the flask with further stirring. In a 100 cm^3 measuring flask, 1 ml of hydrolysate and 60 ml of distilled water were neutralised with NaOH solution to pH 6.0 using indicator paper **[27]**.

The flask's contents are topped up to the mark with distilled water and stirred. 4 ml of the hydrolysate solution is poured into a test tube, and 2 ml of the prepared oxidation reagent is stirred and held for 20 minutes at room temperature. Then, 2 ml of the prepared colour reagent is added to the test tube with the hydrolysate solution; the test tube is closed with aluminium foil and then kept in a water bath at 60 ºC for 15 minutes. At the same time, two control solutions are prepared using distilled water instead of hydrolysate. Test tubes with experimental and control samples are cooled, and after 30 minutes, the optical density is measured on a spectrophotometer.

From the plotted calibrated graph, the concentration of oxyproline is determined.

A) The mass fraction of oxyproline in % is calculated according to formula (1):

$$X = \frac{C \times 250 \times 100 \times 100}{m \times V \times 10^6}, \quad (1)$$

Where:

C – concentration of oxyproline in the sample solution found from the calibration graph, µg/cm^3;
250 – volume of hydrolysate, cm^3;
100 – the volume of the solution obtained after dilution of the hydrolysate, cm^3;
100 – percentage conversion factor;
m – sample mass, g;
V – the volume of hydrolysate sampled for neutralization, cm^3;
10^6 – µg to g conversion factor.

B) Mass fraction of collagen (X_1) in % is calculated according to formula (2):

$$X_1 = K - X, \quad (2)$$

Where:

K – conversion factor of oxyproline to collagen (8.07);

X – a mass fraction of oxyproline calculated by formula 1.

4) Methodology for determining the pore size of dry collagen on a scanning electron microscope (SEM) - "JSM-639 LV JEOL" (Japan).

Dry collagen samples were ground into powder and then applied to copper plates for electron microscope. The surface of the collagen concentrate is coated with a thin layer of platinum with a layer thickness of 5-10 nm. The surface of the sample is scanned by electron beam, then the obtained images are fixed with different magnifications for detailed analysis of collagen structure. Pore sizes (distances between collagen fibrils) are measured on the obtained SEM-images using image analysis software.

Calculate the mean value of pore size, standard deviation, coefficient of variation.

Mean value (χ_m):

The mean value of pores is defined as the sum of all values divided by the number of these pores (3):

$$\chi_m = \frac{1}{N}\sum_{i=1}^{N}\chi_i , \quad (3)$$

Where:

χ_i – pore diameter, μm;

N – number of measurements.

Standard deviation (σ)

Standard deviation measures the average deviation of each value of a data set from the mean and is calculated using the formula (4):

$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(\chi_i - \chi_{cp})^2} , \quad (4)$$

Where:

χ_m – mean pore size, μm;

χ_i – pore size, μm;

N – number of measurements.

Coefficient of variation (CV):

CV shows the magnitude of the standard deviation of the relative mean and is calculated using the formula (5):

$$CV = \frac{\sigma}{\chi_{cp}} \times 100\% , \quad (5)$$

Where:

σ – standard deviation, μm;

χ_m – mean pore size, μm.

5) Methodology for determining water-holding capacity (WHC) according to **[28]**.

To determine the water-holding capacity 100 mg sample is taken, put it on filter paper, pressed with a device planimeter to release moisture. Then the area of the total spot and the area of the spot left by the product are measured.

The WHC (%) is calculated according to the formula (6):

$$WHC = \frac{m_w - 8.4\,(S_1 - S_2)}{m} \times 100 , \quad (6)$$

Where:

m_w – moisture content in the sample, mg;

S_1 – area of the total spot, cm^2;

S_2 – sample spot area, cm^2;

m – sample weight, mg.

6) The effective viscosity was determined on an LVDV-2T rotary viscometer (Dongguan Lonroy Equipment Co., Ltd, China).

7) The composition of non-fat soft curd product is determined based on interstate standards:

- mass fraction of fat by acid method according to GOST 5867-2023 **[29]**;
- mass fraction of protein by the Kjeldahl method according to GOST 31957-2012 **[30]**;
- mass fraction of carbohydrates by potentiometric method according to GOST 34304-2017 **[31]**;
- determination of fatty acid composition using gas chromatography according to GOST 32915-2014 **[32]**;
- determination of amino acid composition according to MVI MN 1363-2000 "Method for determination of amino acids in food products by high-performance liquid chromatography" **[33]**;
- Determination of vitamin A content according to GOST EN 12823-1-2014 by high-performance liquid chromatography **[24]**;
- Determination of vitamin E content according to GOST EN 12822-2014 by high-performance liquid chromatography **[26]**;
- determination of vitamin C content according to GOST 34151-2017 by high-performance liquid chromatography **[25]**;
- determination of vitamin D content according to GOST 12821-2014 by high-performance liquid chromatography **[34]**;
- determination of calcium, magnesium and potassium content by the spectrometric method according to GOST ISO8070/IDF 119-2014 **[35]**;
- determination of phosphorus content according to GOST 30615-99 using a spectrophotometer **[36]**.

Description of the Experiment

The method of obtaining an extract: From cruciferous sea buckthorn and cinnamon rosehip consists of the following stages: purification of plant raw materials, washing at a temperature not exceeding 20 ºC, drying and weighing of the plant raw materials, grinding, extraction in a batch extractor with a stirrer, purification of extract from ballast substances by sedimentation for 12-15 hours with further filtration of alcoholic extract and evaporation of extractant in the extraction apparatus at a temperature of 76 °C for 2 hours, evaporation of water at a temperature of 80 °C and obtaining a viscous plastic-like mass of yellow-brown colour. For the extraction of berry plants, 75 % ethyl alcohol in the ratio 1:5 (berry : alcohol) was used.

Method of obtaining dry collagen concentrate: The method of processing chicken skin consists of the following stages: cutting chicken skin 2-3 cm in size; cooking chicken skin at a temperature of 65 ºC for 3 hours; cooling the mixture with chicken skin to a temperature of 36 ºC; adding papain enzyme to the mixture with chicken skin in a ratio of 1: 10, respectively; thermostatic the mixture at 36 ºC for 24 hours; cooling the mixture to 20-25 ºC; settling the mixture at 20-25 ºC for 60 minutes; separating the solid phase from the gel; centrifugation to separate the fat from the gel at 1000 rpm; and cooling the chicken skin gel to 5-6 ºC;

The method of processing chicken bone tissue and feet consists of the following stages: selection and cleaning of chicken bone tissue and feet, washing of raw materials, chopping into pieces of 2-3 cm; and making a mixture of chicken bone tissue and feet (the ratio of 40: 60, respectively); heat treatment of raw materials for 3-4 hours at a temperature of 65 ºC; cooling of the mixture of chicken bone tissue and feet to a temperature of 25 ºC; fine grinding of the mixture; introduction of papain enzyme into the mixture of chicken bone tissue and feet at a ratio of 1:10, respectively; thermostating of the mixture at a temperature of 36 ºC for 24 hours; cooling of the obtained collagen concentrate to 5-6 ºC.

Figure 1 Dry collagen concentrate.

Gel and paste collagen concentrate are blended in a 1:1 ratio and thoroughly mixed. The obtained composition was dried in a vacuum-freezer TOPT-10C (TOPTION Co., Ltd., Xi'an, China) at minus 44 °C until the humidity reached 8 - 10%. After drying, the powder contained 46.5% of collagen. Figure 1 shows dry collagen concentrate in powder form. The powder has a light, slightly beige colour and uniform texture. The consistency of the concentrate is finely dispersed, indicating that it is finely milled, which may contribute to better dissolution and assimilation when used in the production of cottage cheese products. A flowchart of collagen concentrate preparation is present in Figure 2.

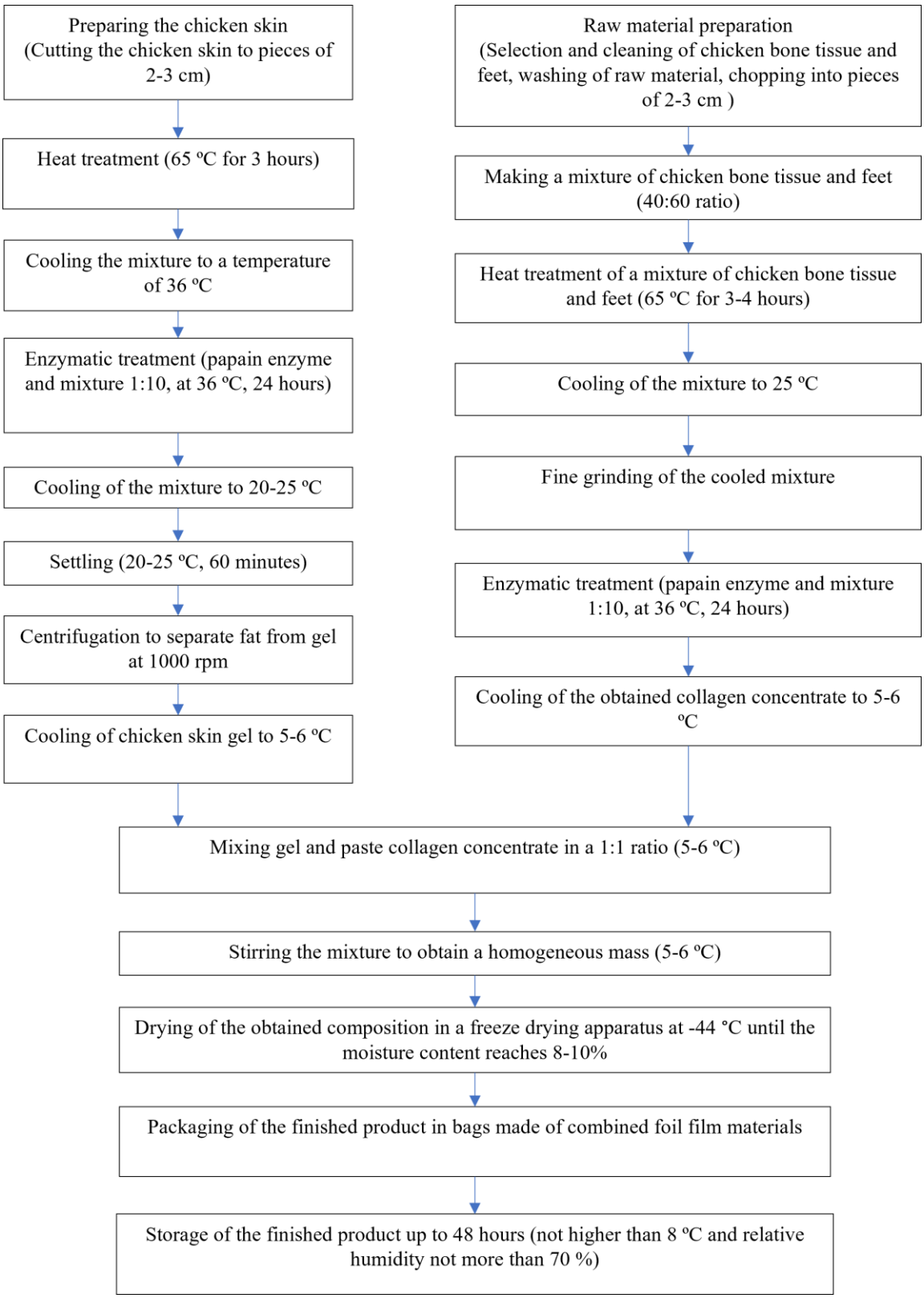

Figure 2. Flowchart of collagen concentrate preparation.

Technology of soft non-fat cottage cheese product preparation.

The main raw material for the production of cottage cheese products is cow's milk, which has an acidity not exceeding 18 °T. The technological production of non-fat cottage cheese products includes cooling to 8-10 ° C and, cleaning milk from mechanical impurities on the separator milk purifier, heating milk to 30-35 ° C with further separation to obtain skim milk. Skim milk is pasteurised at a temperature of 76-78 ° C with a holding time of 30-40 seconds, cooling of milk to the leavening temperature to 30-32 ° C.

During fermentation of milk 5% starter, prepared on pure cultures of mesophilic and thermophilic lactic acid streptococcus, 40% sodium chloride solution (at the rate of 400 g of anhydrous salt per 1 ton of milk) and 1% rennet enzyme are added to the milk. Fermentation of skim milk is carried out at a temperature of 30-32 ° C for 4-6 hours to reach a titratable acidity of 71-73 °T. After the formation of the clot, it is cut into 1-2 cm cubes, and slowly heated to 35-40 ° C with exposure for 20-30 minutes to improve the separation of whey.

Whey is removed by self-pressing at 25-28°C for 30-40 minutes. Pressing of the clot continues under the same conditions for 1-2 hours until the moisture content of the product reaches 65-70%. The curd is ground on a colloid mill to obtain a homogeneous mass, heated to 35 °C, and added with constant mixing dry collagen concentrate (8%) and extract composition of sea buckthorn and rosehip cinnamon (4%). The finished product is cooled to 4-6 ° C, packed in airtight containers, and stored at 0-2 °C for no more than 96 hours.

Number of samples analysed: 30 samples of cottage cheese were analysed.
Number of repeated analyses: Each study was carried out 3 times.
Number of experiment replications: The study was repeated three times, with the experimental data processed using mathematical statistics methods.

Statistical Analysis

Each experiment was conducted in triplicate to ensure data reliability and statistical power. Standard deviation was calculated to assess the variability of the data, providing insight into the consistency of measurements. One-way Analysis of Variance (ANOVA) was used to determine significant differences among the experimental groups for parameters such as water-holding capacity, viscosity, and mechanical stability. Tukey's Honest Significant Difference (HSD) test was applied post-hoc to identify specific groups that showed statistically significant differences ($p < 0.05$). The Mann-Whitney test was used to compare immune response parameters between control and experimental groups due to the non-parametric distribution of the data. The results were graphically represented using Microsoft Excel for clarity and interpretation.

RESULTS AND DISCUSSION

Study of the microstructure of collagen concentrate

The highest collagen content characterises selected secondary products of the poultry processing industry. The method of obtaining dry collagen concentrate is based on the enzymatic process using the enzyme papain for hydrolysis of raw materials. This method allows for preserving the biological value of collagen due to the soft processing of raw materials. A comparative study by Munasinghe et al. shows that combining acetic acid and pepsin increases collagen production. However, the results of these studies showed that using papain enzyme for processing is preferable as it minimises the possible changes in protein structure associated with acid exposure and provides a more stable result **[37]**.

Studies were carried out to determine the pore size of dry collagen concentrate. When developing curd products enriched with dry collagen concentrate, it is important to consider their structure and pore size. Collagen pores, which are the spaces between collagen fibrils, can significantly affect the curd product's texture uniformity and water-holding capacity **[38]**. Figure 3 shows a micrograph of dry collagen concentrate at 100, 200 and 300x magnification.

As can be seen from Figure 3, the dry collagen concentrate is characterised by non-uniformity in pore size (6.32 μm; 11.33 μm; 14.00 μm; 14.8 μm; 16.12 μm). The average pore size of the obtained dry collagen concentrate is 12.514 μm, with a standard deviation of about 4.08 μm. The coefficient of variation of 32.6% indicates heterogeneity in pore size, which may affect the consistency and water-holding capacity of the curd product.

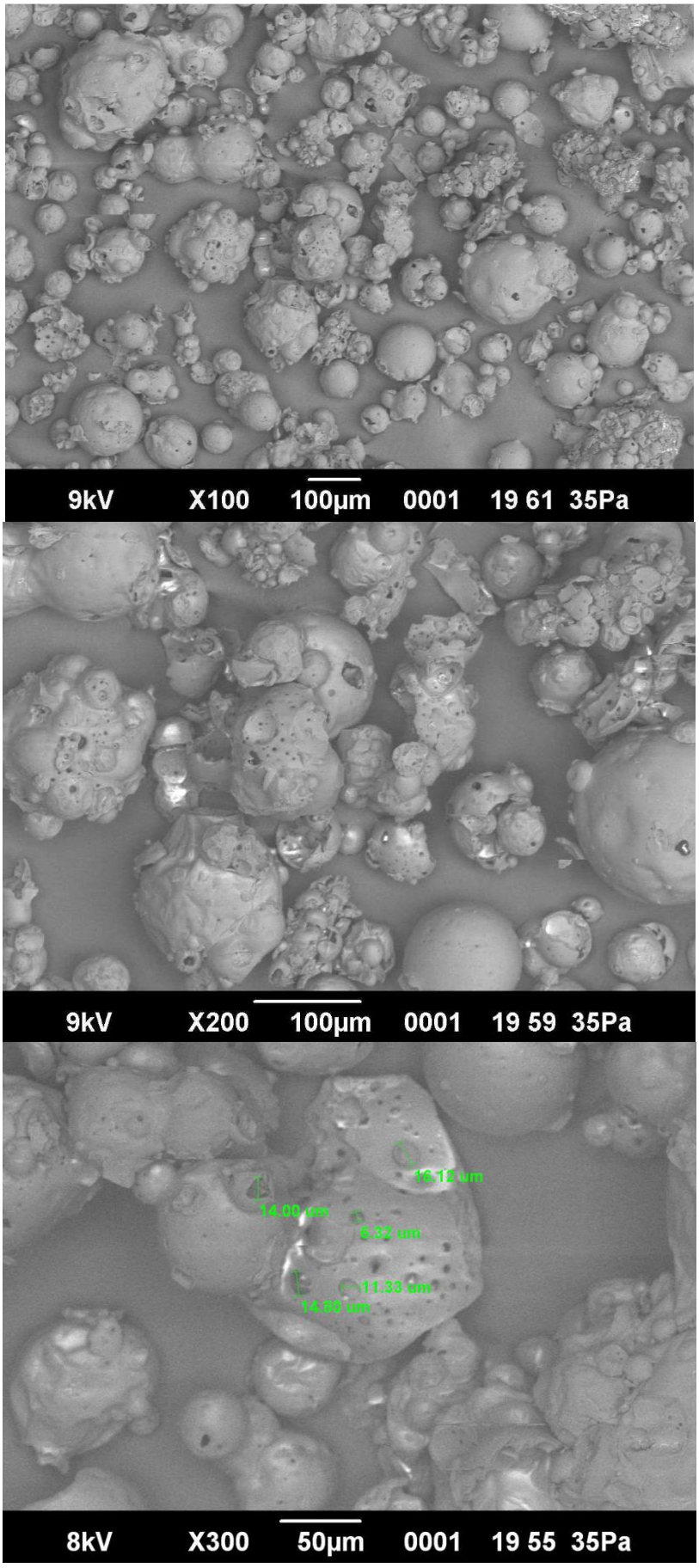

Figure 3. Microstructure of dry collagen concentrate.

Study of antioxidant activity of extract from fruit-berry plants

To conduct experimental studies, 5 species of fruit and berry plants with pronounced antioxidant properties were selected based on the analysis of literature sources: sea buckthorn, red haw hawthorn, black chokeberry, cinnamon rosehip, and Russian olive. Route expeditions were carried out to select fruit and berry plants, taking into account their distribution area and frequency of occurrence in the Abai region of the Republic of Kazakhstan. During the route expeditions, the territories of different zones were covered: mountainous, steppe, forest-steppe, and forest. Based on the route expeditions, the frequency of occurrence and area of distribution of fruit and berry plants with pronounced antioxidant properties in the Abai region were determined (Table 1, Figure 4).

Table 1 Frequency of occurrence and area of distribution of fruit and berry plants in the Abay region of the Republic of Kazakhstan.

#	Family	Plant genus and species	Frequency of occurrence	Sampling place	Geographic coordinates of the sampling location
1	*Elaeagnaceae*	Sea buckthorn (Hippophae rhamnoides)	Soc	Pine forest of Semey city	Suburbs of Semey city, Abay region Kazakhstan 50°55'07.9"N 79°34'22.5"E
2	*Rosaceae*	Red haw hawthorn (*Crataegus sanguinea*)	Cop	Borodulikha, Beskaragai, Abai districts	1) Borodulikha district, Novopokrovka village, Abay region Kazakhstan 50°40'10.2" N 80°27'49.7" E 2) Beskaragai district, Sosnovka village, Abay region Kazakhstan 51°27'07.5" N 79°30'03.7" E 3) Abai district, foothills of the Chingiztau mountain range, Abai region, Kazakhstan 48°38'00" N 79°10'00" E
3	*Rosacae*	Black chokeberry (*Aronia melanocarp*)	Cop	Summer houses plots of Semey city	Summer houses in eastern settlement of semey city, Abay Region Kazakhstan 50°23'30.1"N 80°21'14.7"E
4	*Rosacae*	Cinnamon rosehip (*Roza cinnamomea*)	Cop	Beskaragai district, Pine forest of Semey city	Pine forest of Semey city, Beskaragai district, Glukhovka village, Abay region Kazakhstan 50°29'43.8" N 79°52'09.8" E Beskaragai district, Kanonerka village, Abay region, Kazakhstan 50°43'24.6" N 79°41'26.2" E
5	*Elaeagnaceae*	Russian olive *Elaeagnus oxycarpa*	Sol	Beskaragai district	Beskaragai district, Glukhovka village, Abay region Kazakhstan 50°29'43.8" N 79°52'09.8" E

Note: Symbols in the table: Socialis (Soc) - «very much»; Copiosa (Cop) - «average»; Solitaries (Sol)- «very rarely». (latitude): 50.4955 N, (longitude): 79.8694 E.

Figure 4 Photo of fruit and berry plants grown in the Abay region of the Republic of Kazakhstan.
Note: a) Sea buckthorn (*Hippophae rhamnoides*); b) Red haw hawthorn (*Crataegus sanguinea*); c) Black chokeberry (*Aronia melanocarp*); d) Cinnamon rosehip (*Roza cinnamomea*); e) Russian olive (*Elaeagnus oxycarpa*)

As can be seen from Table 1, taking into account the density of growth and constancy of species composition of the studied plants, 4 species of plants necessary for the experimental work were collected: sea buckthorn, red haw hawthorn, black chokeberry, cinnamon rosehip. The population density of Russian olives was very rare.
As a result of a comparative analysis of the chemical composition of 4 species of fruit and berry plants, a composition of two berries was used to obtain the extract: sea buckthorn and cinnamon rosehip. To extract berry plants, it is necessary to use 75% ethyl alcohol in a ratio of 1:5 (berry : alcohol). The results of the study of the composition of the obtained extract are presented in Table 2.

Table 2 Chemical composition of fruit and berry plant extract.

Name	Content			
	phenolic compounds, %	**vitamin A, mg/100 g**	**vitamin E, mg/100 g**	**vitamin C, mg/100 g**
Sea buckthorn and cinnamon rosehip composition extract	1.98	29.8	48.9	756.4

Based on these data, it can be concluded that the extract of sea buckthorn and rosehip composition has high antioxidant potential due to its significant content of phenolic compounds (1.98%) and vitamins A (29.8 mg/100g), E (48.9 mg/100g) and C (756.4 mg/100g). This extract can effectively neutralise free radicals and protect cells from oxidative stress, making it useful for various food products to improve their antioxidant properties **[39]**.

To determine the antioxidant potential of the obtained extract, studies of its effect on the condition and metabolic processes in the organs of the immune system of experimental animals in laboratory conditions were carried out. The studies were carried out on white mongrel rats. The animals were randomly divided into control and experimental groups. The results of the study are presented in Table 3.

Table 3 Comparative analysis of cellular and humoral immunity indicators.

Indicator	First control group of animals Me (Q1-Q3)	Second experimental group of animals Me (Q1-Q3)	U	Z	P
Leukocytes	8.500 (7.700-9.100)	8.650 (7.900-9.125)	69.5	-0.306	0.760
Lymphocytes	3.600 (3.200-3.800)	4.400 (3.725-4.450)	28.5	-2.587	0.01*
T-helpers	0.790 (0.700-0.850)	1.015 (0.885-1.152)	23.0	-2.886	0.004*
T-suppressors	0.47 (0.43-0.61)	0.67 (0.49-0.78)	37.0	-2.111	0.03*
T-lymphocytes	1.40 (1.33-1.52)	1.66 (1.51-1.83)	31.0	-2.444	0.015*
B-lymphocytes	0.6 (0.5-0.7)	0.7 (0.6-0.9)	34.5	-2.310	0.021*
Phagocytic count	2.6 (2.0-3.0)	2.2 (1.9-2.5)	52.0	-1.280	0.201
Phagocytic index	52 (49-56)	47 (43-52)	42.5	-1.810	0.07
Circulating immune complex	21 (19-24)	22.5 (18.75-22.50)	62.5	-0.694	0.488
Reaction of leukocyte migration inhibition	21 (19-24)	22.5 (17.75-26.75)	60.0	-0.836	0.403
HCT (Phagocyte activity)	3 (2-5)	4 (2-7)	97.0	-0.451	0.652
IgA	7.129 (5.624-8.610)	6.122 (4.853-6.804)	43.0	-1.775	0.07
IgM	7.406 (6.992-8.308)	6.485 (5.902-7.734)	46.0	-1.610	0.107
IgG	5.896 (5.337-6.179)	6.698 (5.018-7.926)	65.0	-0.555	0.579

Note: Mann-Whitney U Criterion was used for two independent samples. * level of statistical significance $p < 0.05$.

The study results showed that the obtained extract significantly affects experimental rats' immune system by increasing the number of lymphocytes, T-helper cells, T-suppressors, T-lymphocytes and B-lymphocytes. The extract significantly increases the number of lymphocytes, indicating its stimulating effect on cellular immunity and may increase the body's overall ability to mount an immune response. An increase in T-helper cells indicates an enhanced coordination of the immune response, as T-helper cells play a key role in activating other immune cells **[40]**. Increased T suppressors may indicate better control of the immune response and prevention of over-activation of the immune system. Increased levels of T-lymphocytes indicate an overall activation of cellular immunity. An increase in the number of B-lymphocytes indicates a stimulating effect of the extract on humoral immunity **[41]**, **[42]**.

Study of the influence of different doses of dry collagen concentrate and extract from fruit and berry plants on the quality indicators of soft curd product

In the next stage, the effect of the obtained functional ingredients on the quality indicators of soft curd products was investigated. The research was carried out to prepare a formulation of cottage cheese products for sports nutrition. The influence of different doses of a mixture of dry collagen concentrate and extract of sea buckthorn and rosehip composition on the properties of soft curd product was investigated. For the study, 9 experimental samples of soft curd products were selected, in which the above components were added in different ratios (Table 4).

Table 4 Ratio of components of the experimental sample.

Sample	Ratio, %	
	collagen concentrate	sea buckthorn and rosehip composite extract
Sample 1	4	2
Sample 2	4	4
Sample 3	4	6
Sample 4	6	2
Sample 5	6	4
Sample 6	6	6
Sample 7	8	2
Sample 8	8	4
Sample 9	8	6

These ratios (Table 4) are selected for a comprehensive study of the influence of the doses of the ingredients on the quality of soft curd products. This approach will allow identifying the optimal proportions that will provide the product's physicochemical, organoleptic and functional properties. The mixture of dry collagen-containing concentrate and extract of sea buckthorn and rosehip composition was added to the ready curd mass, which is made according to traditional technology. It is known that the stability and structural characteristics of collagen are affected by temperature **[43]**. At temperatures above 40°C, collagen loses its structural stability, which may destroy its gel-forming properties, which are important for creating structure in food products **[44]**. Therefore, the studies were conducted at temperatures of 20 °C; 25 °C; 30 °C; 35 °C; and 40 °C.

The study results of the effect of different doses of the mixture of dry collagen concentrate and extract of sea buckthorn and rosehip composition on the water-holding capacity of cottage cheese products at different temperatures of their incorporation are presented in Figure 5.

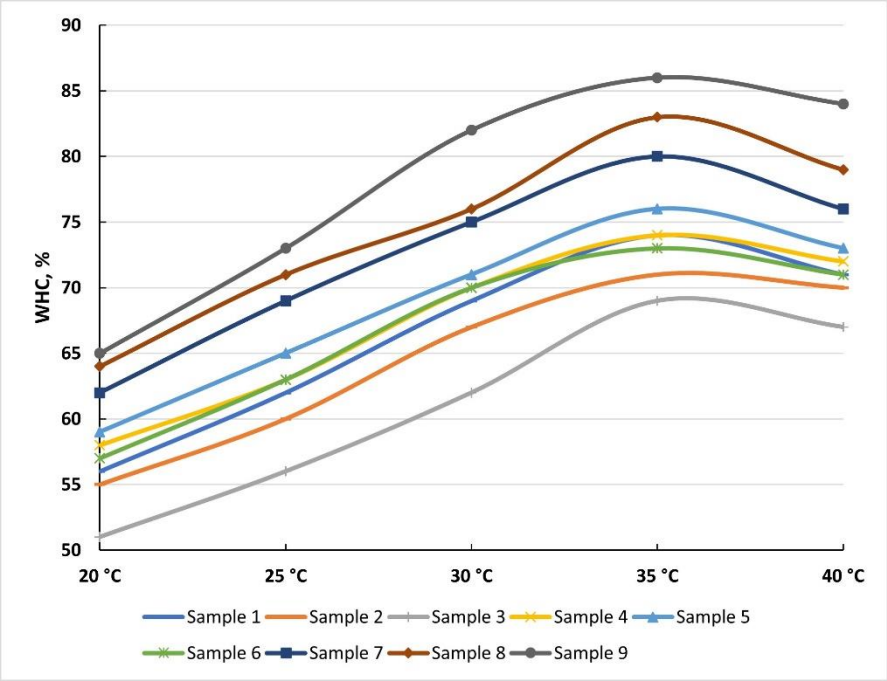

Figure 5 Dynamics of change in water-holding capacity of cottage cheese product.

As seen in Figure 5, all the experimental samples show an increase in the water-holding capacity of the curd product with an increase in temperature up to 35°C. This is probably due to the improvement of the collagen gelation process at this temperature, which increases the water-holding capacity of the product **[45]**. At 40°C, a decrease in water-holding capacity was observed. This may be due to partial denaturation of collagen and deterioration of its gelation properties.

The samples (1st, 2nd, 3rd, 6th) show the lowest values of water-holding capacity at all investigated temperatures. Reduced content of collagen and extract characterises the first experimental sample. The second and sixth experimental samples contain dry collagen concentrate and extract of sea buckthorn and rosehip composition in equal ratios. In the third experimental sample, the extract of sea buckthorn and rosehip composition

is contained more. The higher content of the extract reduces the hydrophilic properties of the product, which leads to a decrease in the ability to retain moisture despite the presence of collagen.
With the increase in the content of dry collagen concentrate in the experimental samples, an increase in the water-holding capacity of the soft curd product is observed. Balanced results with good water-holding capacity are observed in the fourth and fifth experimental samples, in which collagen concentrate and extract content is 6:2 and 6:4, respectively.

The samples with a high collagen-containing concentration (7th, 8th and 9th) show the highest water-holding capacity.

For a comprehensive understanding of the rheological properties of the curd product after determining the dynamics of changes in water-holding capacity, studies of the effect of different doses of a mixture of dry collagen concentrate and extract of sea buckthorn and rosehip composition on the change in the effective viscosity of the curd product at a temperature of 35°C were carried out. This temperature was chosen as the optimum temperature based on the study of the dynamics of change in the water-holding capacity of the curd product. The results of the study are presented in Figure 6. Some studies have reported that the gelation properties of collagen are impaired at temperatures above 50°C to 70°C **[46]**, **[47]**. Still, the results of these studies show a decrease in water-holding capacity at temperatures above 35°C, which is probably due to the deterioration of its gelation properties.

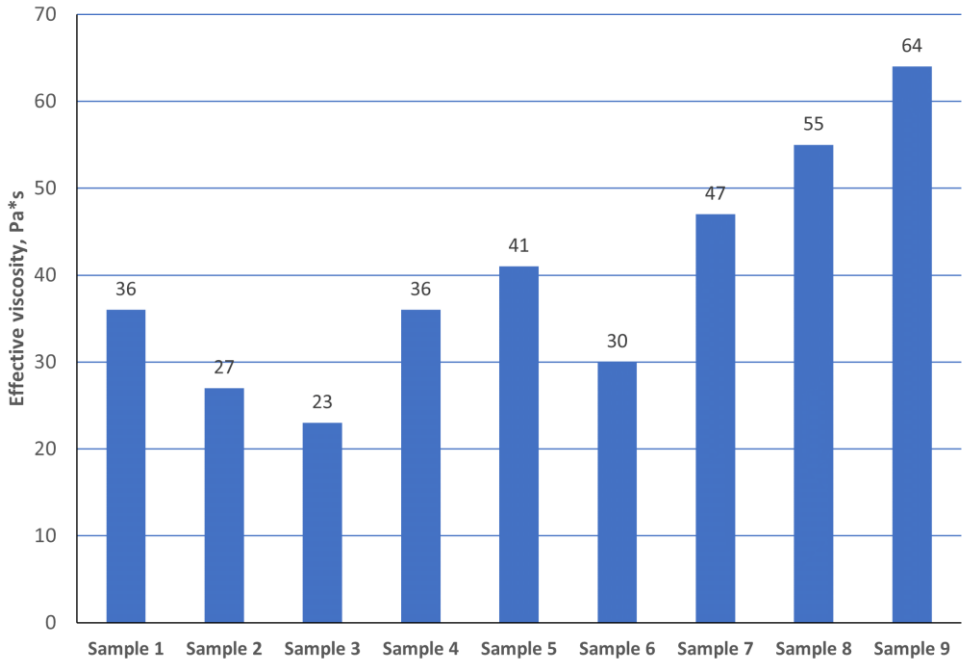

Figure 6 Effect of different doses of applied ingredients on effective viscosity.

As can be seen from Figure 5, the effective viscosity of the curd product depends on the ratio of dry collagen concentrate and sea buckthorn and rosehip extract: an increase in the proportion of collagen (especially at ratios of 8:4 and 8:6) leads to a significant increase in viscosity, indicating the formation of a denser and more stable product structure. At lower extract contents (4:2, 6:2), viscosity also remains high, indicating the ability of collagen to retain moisture and form a stable texture.

On the contrary, when the extracted content is increased to 4:6 and 6:6, viscosity decreases, which is associated with excess hydration and a decrease in product density. It should be noted that the results of the effective viscosity study correlate well with the water-holding capacity of the curd product: samples with high collagen content and optimum extract level (8:4 and 8:6), which show the highest viscosity also have the best water-holding capacity, indicating a dense and stable product structure. At the same time, samples (2nd, 3rd and 6th) with lower viscosity (4:4; 4:6 and 6:6) show decreased moisture retention capacity due to excess hydration and reduced density of the structure.

Thus, the best textural characteristics and product stability are achieved at ratios that balance collagen and extract content, as evidenced by viscosity and moisture retention properties.
In the next stage, the study of indicators that characterise the stability of the curd product structure to destruction (viscosity loss coefficient and mechanical stability coefficient) at the addition of different doses of a mixture of

dry collagen concentrate and extract of sea buckthorn and rosehip composition was carried out. The results of the study are presented in Figure 7 and Figure 8.

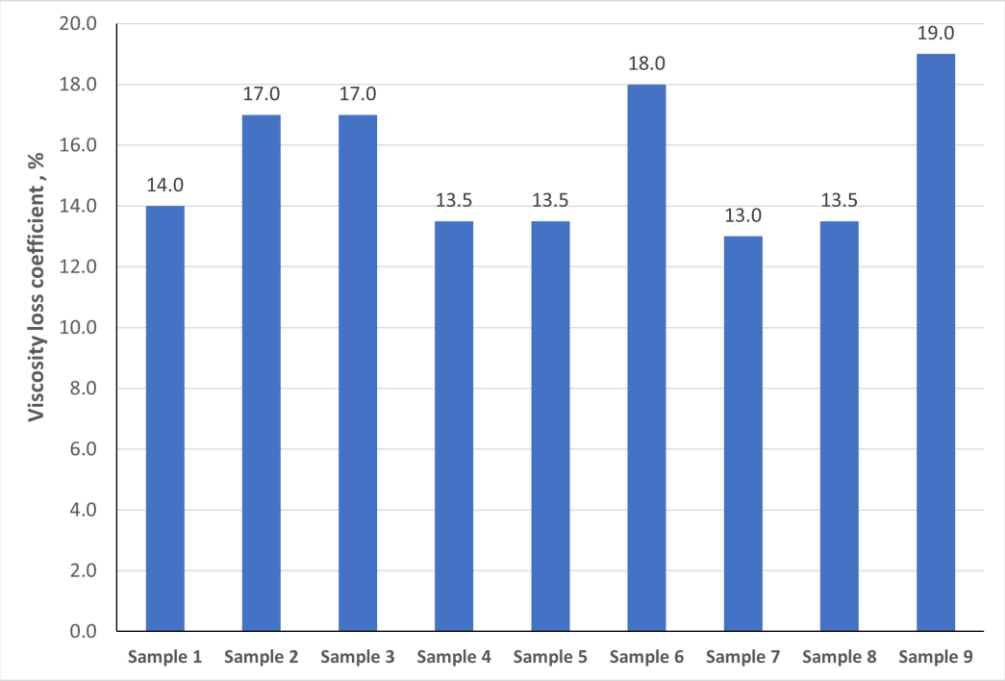

Figure 7 Viscosity loss coefficient of curd product when adding different amounts of functional ingredients.

As can be seen from Figure 7, the highest viscosity loss ratio (17-19%) was observed in the second, third, sixth and ninth samples. This result may indicate increased extract content in these ratios increases viscosity loss. This is probably because a higher concentration of extract changes the physicochemical properties of the compositions, making them less stable and increasing the fluidity of the systems.

The lowest coefficient of viscosity loss (13.5-14.0 %) is found in the first, fourth, fifth, seventh, and eighth samples. In these samples, the collagen concentrate content exceeds the extract amount by 2-4 times. This may indicate that a higher concentration of collagen component leads to an increase in the viscosity of the samples, probably due to the formation of a denser network of collagen fibers.

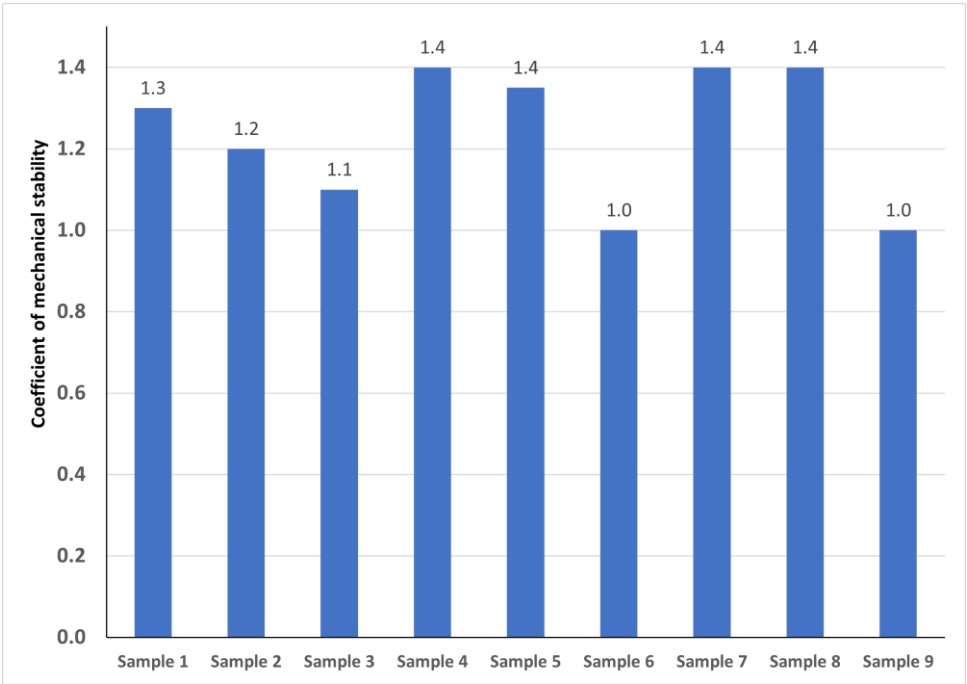

Figure 8 Coefficient of mechanical stability of cottage cheese product with the addition of different amounts of functional ingredients.

As can be seen from Figure 8, the highest coefficient of mechanical stability of the curd product is observed in the experimental samples in which the lowest viscosity loss coefficient indices (first, fourth, fifth, seventh and eighth) were established. The results show that increasing the proportion of dry collagen concentrate in the experimental samples also affects the increase in the stability of the curd product.

Study of nutritional and biological value of non-fat soft curd product

Based on the research conducted, a non-fat soft curd product was obtained. Recipe of cottage cheese product is presented in Table 5. By the recipe, after separation of whey and cooling to a temperature of 8 °C a dry collagen concentrate and extract composition of sea buckthorn and cinnamon rosehip are added to the curd mass. Nonfat soft curd product with a creamy texture and soft pink color, has a rich sweet-sour taste with a slight flavor of berry fillers (Figure 9).

Table 5. Recipe for cottage cheese product

#	Ingredient	Ingredient usage (kg per 1 t, excluding losses)
1	Low-fat soft cottage cheese product	88.0
2	Dry collagen concentrate	8.0
3	Sea buckthorn and rosehip composite extract	4.0
	Total	100

Figure 9 Appearance of low-fat soft curd product.

As can be seen in Figure 8 the curd product has fine flecks that are evenly distributed throughout the mass. These flecks result from sea buckthorn and cinnamon rosehip extract, which give the product flavour, aromatic characteristics, and visual texture. Such flecks add textural variation to the product, creating a pleasant contrast against its delicate pink hue. The nutritional and biological value of the finished product was investigated. The results of the study are presented in Table 6.

Table 6 Nutritional and biological value of experimental and control samples of soft curd product.

#	Nutrients	Control sample	Experimental sample
1.	Protein, g	16.00±0.30	16.40±0.33
2.	Fats, g	0.50±0.01	0.80±0.01*
3.	Carbohydrates, g	2.70±0.06	2.90±0.04
4.	Fatty acid composition, %:		
5.	Sum of polyunsaturated fatty acids, including	0.25±0.00	1.30±0.02*
	Sum of omega-3 polyunsaturated fatty acids	0.05±0.00	0.30±0.00*
	Sum of omega-6 polyunsaturated fatty acids	0.20±0.00	1.00±0.01*
6.	Vitamins in 100 g:		
	Vitamin A, mcg	20.00±0.30	75.00±0.79*
	Vitamin D, mcg	0.05±0.00	0.08±0.00
	Vitamin E, mg	0.10±0.00	0.21±0.01*
	Vitamin C, mg	1.00±0.01	6.00±0.10*
7.	Mineral substances mg/100 g		
	Calcium	100.00±1.26	119.00±1.32*
	Phosphorus	141.00±2.40	172.00±2.53
	Magnesium	10.00±0.16	19.00±0.28*
	Potassium	80.00±1.19	96.00±1.36*
8.	Essential amino acids, mg/100g		
	Tryptophan	0.10±0.00	0.15±0.00*
	Leucine	1.10±0.02	1.40±0.02*
	Isoleucine	0.60±0.01	0.80±0.01*
	Valine	0.70±0.01	0.80±0.01
	Threonine	0.50±0.00	0.53±0.00
	Lysine	0.90±0.01	1.20±0.02*
	Methionine + cystine	0.20±0.00	0.25±0.00*
	Phenylalanine + tyrosine	0.40±0.01	0.60±0.01*
	Energy value, kcal	78.76	83.8

Note: *indicate a significant difference from the control sample ($p<0.05$).

The data presented in Table 6 show that the experimental sample shows a slight increase in the mass fraction of proteins, fats, and carbohydrates in the soft curd product due to the addition of functional fillers. A study of a soft curd product identified omega-3 and omega-6 fatty acids. These fatty acids are crucial for maintaining cardiovascular health, reducing inflammation, and improving cognitive function and body repair **[48]**. Consequently, they are particularly important in athletes' nutrition, aiming for peak performance and rapid recovery.

The determination of vitamins A, D, E, and C is justified by their key role in maintaining immune function, antioxidant defense, skin and bone health, and ensuring normal metabolism, which is especially important for maintaining high physical activity and recovery of athletes. The determination of calcium, phosphorus, magnesium and potassium is substantiated by their critical importance in maintaining the musculoskeletal system, energy metabolism and optimal cardiovascular function, making this product particularly useful in athletes' nutrition **[49]**.

Introducing 8% collagen concentrate and 4% sea buckthorn and rosehip extract significantly increases the biological value of non-fat cottage cheese products. In particular, there is a significant increase in the content of polyunsaturated fatty acids (Omega-3 and Omega-6). The vitamin and mineral composition of the experimental sample was also significantly enriched, which is especially evident in vitamins A, C and E, as well as calcium, phosphorus and magnesium.

As a result of these changes, the prototype demonstrates a more balanced composition in terms of antioxidant protection and immune system support, which is achieved due to the increased content of polyunsaturated fatty acids Omega-3 and Omega-6, as well as vitamins A, C and E, compared to the control sample. The increased content of essential amino acids in the experimental sample, such as leucine, lysine and tryptophan, confirms the high biological value of the product. These changes can be attributed to the addition of collagen-containing concentrate, sea buckthorn, and rosehip extract, making the product more functional and healthy for consumers.

Several studies have explored using plant extracts to enhance the functional properties of cheese products. Caleja et al. (2016) investigated the incorporation of microencapsulated fennel and chamomile extracts into cottage cheese. Their findings suggest that microencapsulation offers a promising strategy to maintain higher

antioxidant activity after storage (7 days) compared to free extracts while preserving the nutritional profile of the cheese **[50]**.

Similarly, Ribeiro et al. (2016) examined the effects of rosemary extracts (both free and microencapsulated) on the antioxidant activity of cottage cheese. While both forms enhanced antioxidant activity, the free rosemary extract displayed a more pronounced effect at the initial time point (0 days) and after 7 days of storage **[51]**. These findings highlight the potential of rosemary for functional cheese development but also suggest that delivery methods may influence the extent and duration of its benefits.

Beyond rosemary and chamomile, Carocho et al. (2016) explored the use of basil leaves in "Serra da Estrela" cheese. Their research demonstrated that basil leaves, particularly in the form of decoctions, significantly increased the cheese's antioxidant activity, reduced moisture content, and preserved unsaturated fatty acids and proteins **[52]**. This study suggests that basil offers a multi-functional approach, improving the cheese's shelf life and nutritional value.

Investigation of shelf life of nonfat soft curd product by the indicators characterising the stability of its structure

A key factor affecting curd products' quality and consumer properties is its structure. The structural stability of the curd product plays an important role in forming texture and consistency and retaining these characteristics throughout shelf life. The structure's instability can lead to the separation of whey, change in consistency, and deterioration of the organoleptic parameters of the finished product **[53]**.

To determine the optimal conditions for storage of soft curd product, ensuring the maintenance of its texture and consistency throughout the shelf life, studies of changes in the coefficient of viscosity loss and mechanical stability of the product at different temperatures (0 °C, 2 ° C, 4 ° C, 6 ° C and 8 ° C) and storage time (24 hours, 48 hours, 72 hours and 96 hours are carried out.) The selected temperature regimes (0 °C, 2 °C, 4 °C, 6 °C and 8 °C) and time intervals (24 hours, 48 hours, 72 hours and 96 hours) allow a comprehensive evaluation of how the addition of collagen concentrate and extract of sea buckthorn and rosehip composition affects the preservation of the product structure during the shelf life. This will make it possible to develop recommendations on optimal storage conditions to ensure maximum conservation of the textural characteristics of the product and its consumer properties. The result of the study of change in the viscosity coefficient of soft curd product during storage is shown in Figure 10 at the initial value of viscosity loss coefficient after the addition of fillers 13.5%.

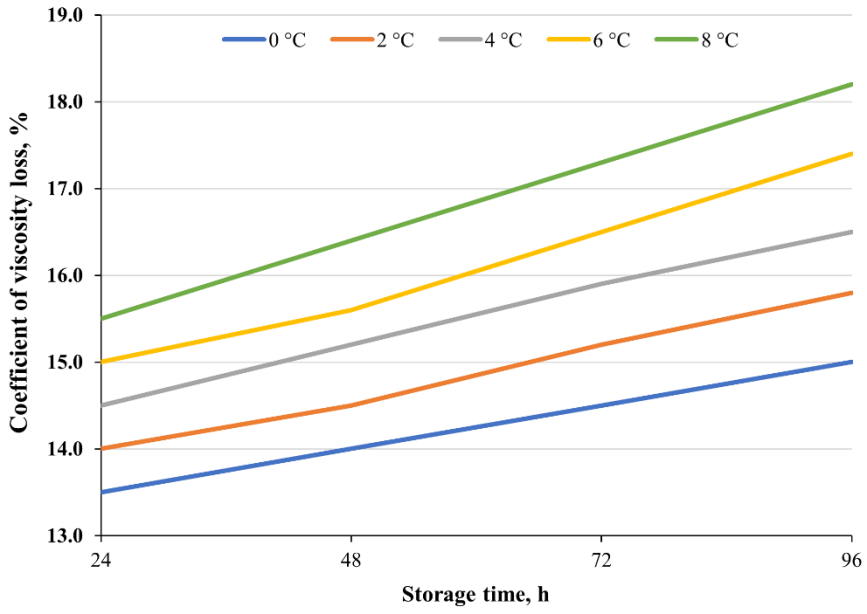

Figure 10 Change in coefficient of soft curd product viscosity loss during storage.

As can be seen from Figure 10, when the curd product was stored at 0-2 °C for 96 hours, the coefficient of viscosity loss was in the range of 15.0-15.8%. Under these conditions, the product retained its typical soft and creamy consistency, which is common for soft curd. This effect may be due to the effect of collagen concentrate on the microstructure of the curd product, which helps to increase its stability and maintain textural characteristics under the specified storage conditions.

At a temperature of 4 °C for 72 hours, the viscosity loss coefficient was 15.9%; at a temperature of 6 °C for 48 hours - 15.6%; at a temperature of 8 °C for 24 hours - 15.5%. When the viscosity loss coefficient exceeded 16%, there was whey release and a decrease in the curd product's creaminess during storage, probably due to the destruction of the protein-collagen network.

At the next stage, the change in the mechanical stability coefficient of the soft curd product during storage at its initial value of 1.4 was investigated. The results of the study are presented in Figure 11.

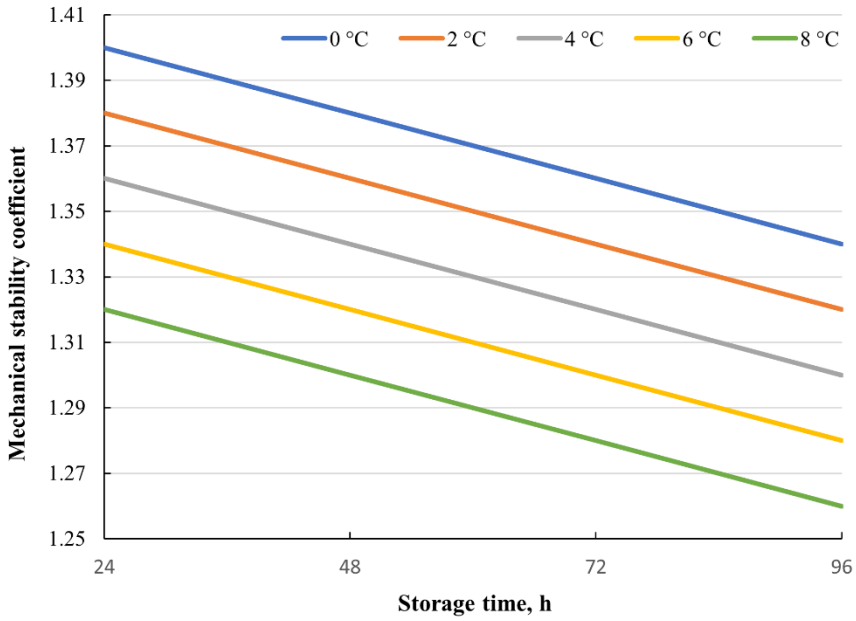

Figure 11 Change of mechanical stability coefficient of soft curd product during storage.

As shown from Figure 11, the mechanical stability coefficient gradually decreases with increasing temperature and storage time, indicating a gradual degradation of the product's structural integrity. At lower temperatures (0-2 °C), the decrease in stability is slower than at higher temperatures (6-8 °C), at which the product loses mechanical stability much faster. When the mechanical stability coefficient falls below 1.28 (after 96 hours of storage at 4-6 °C or 72 hours at 6-8 °C), the curd product has a loose consistency with a pronounced whey release. These data emphasise the importance of maintaining a low temperature to preserve the structural properties of the curd product during long storage times.

CONCLUSION

This study developed a functional soft cottage cheese product enriched with dry collagen concentrate and antioxidant-rich extracts from sea buckthorn and cinnamon rosehip. The formulation, which includes 8% collagen concentrate and 4% antioxidant extract, significantly enhances the nutritional value of the product, providing an optimal balance of polyunsaturated fatty acids, essential vitamins (A, C, and E), and minerals (such as calcium, phosphorus, magnesium, and potassium). Compared to the control cheese product, the enriched version notably increased omega-3 and omega-6 fatty acids. Additionally, the enriched product contained higher levels of vitamins A, C, and E. The study demonstrated that the enriched cottage cheese product maintained structural stability and textural integrity during storage at 0-2°C for 96 hours. The balance between collagen and plant extracts allowed the product to achieve desirable water-holding capacity, viscosity, and mechanical stability. The inclusion of collagen improved the product’s gelation properties, while the antioxidants from plant extracts provided additional health benefits without compromising the sensory attributes of the cottage cheese. Overall, the developed soft cottage cheese product delivers enhanced nutritional and biological value and retains its sensory qualities, making it a promising option for athletes and health-conscious consumers seeking functional dairy products to support their active lifestyle.

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