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Extracts of *Rhodiola rosea* L. and *Scutellaria galericulata* L. in functional dairy products

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Abstract:
Introduction. Modern scientific research into the biochemical composition and medicinal value of plants makes it possible to use them as functional ingredients in food technology. The research objective was to test rose root (*Rhodiola rosea* L.) and scullcap (*Scutellaria galericulata* L.) for biologically active substances and their potential use in functional dairy products.
Study objects and methods. The research featured biologically active substances (BAS) obtained from rose root and scullcap that grow in mountain areas or on rock outcrops along Siberian rivers. The BAS content was determined using high performance liquid chromatography (HPLC). The biologically active substances were screened and identified using HPLC, thin-layer chromatography (TLC), and infra-red identification (IR). The new functional products were based on whey and cottage cheese made from processed whole milk.
Results and discussion. The analysis of *Rhodiola rosea* rhizomes and roots showed the following BAS content (mg/g): rosavin – 16.9, salidroside – 14.3, rosin – 5.04, rosarin – 2.01, and methyl gallate – 6.8. The roots of *Scutellaria galericulata* had the following BAS content (mg/g): scutellarein – 22.27, baicalin – 34.37, baicalein – 16.30, apigenin – 18.80, chrysin – 6.50, luteolin – 5.40, and vogonin – 3.60. Whey served as a basis for a new functional whey drink fortified with BAS isolated from *Rhodiola rosea* 100 mL of the drink included 50 mL of whey, 20 mL of apple juice, 0.1 mL of rose root concentrate, 3 g of sugar, 0.5 g of apple pectin, 04 g of citric acid, and 30 mL of ionized water. The content of phytochemical elements ranged from 0.11 ± 0.001 to 0.49 ± 0.08 mg/100 g. Cottage cheese served as a basis for another dairy product fortified with BAS obtained from *Scutellaria galericulata*. The formulation included 81 g of cottage cheese, 10 mL of cherry jam, 9 g of sugar, and 0.025 mL of scullcap concentrate. The content of biologically active substances in the finished product varied from 0.09 ± 0.02 for luteolin to 0.48 ± 0.11 for baicalin. The whey drink fortified with the BAS extracted from *Rhodiola rosea* and the cottage cheese product fortified with the BAS isolated from *Scutellaria galericulata* satisfied 40–45% and 55–60% of the reference daily intake for phenolic compounds, respectively. The obtained data made it possible to recommend the new functional foods for commercial production.
Conclusion. A set of experiments was performed to isolate biologically active substances from *Rhodiola rosea* and *Scutellaria galericulata*. The research developed and tested formulations of two new functional products based on whey and cottage cheese.

Keywords: Medicinal plants, Functional food, biologically active substances, whey, cottage cheese

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INTRODUCTION

Public nutrition attracts attention of medical science and affects the development of biotechnology in food industry [1]. As a result, most solutions lie in the sphere of functional products designed for particular groups of population [2]. Miners, geologists, polar explorers, astronauts, submariners, athletes, and programmers are prone to various diseases as a result of adverse working conditions. Unsocial working hours make them vulnerable to diseases of digestive system, liver, thyroid gland, cardiovascular system, and bones. Low-income families still experience the consequences of unhealthy diet that lacks natural meat, dairy products, and fresh vegetables. As a result, a lot of people suffer from deficiency of proteins, vitamins, and other biologically active substances. Functional products can compensate for the missing elements as they are fortified with biologically active substances of plant origin, e.g. minerals, macro- and microelements, bioactive peptides, enzymes, etc. [3, 4, 20].

As a rule, food habits are as old as the nations or states they belong to. However, they were shaped not only by the local flora, fauna, climate, soil fertility, water availability, national traditions, and culture, but also by the genetic ability of the people to digest certain types of food [14–16]. Some researchers recommend introducing ancient Eastern traditions to the achievements of Western medicine. In fact, European diet includes less than 2–3% of edible plants while in the East people enjoy a variety of 1000 different edible species [17]. The Japanese, whose life expectancy is one of the longest in the world, consume equal amounts of meat and vegetables [24].

European scientists believe that saturated fats and cholesterol in meat can be reduced by introducing safe fibers into processed foods [5, 6]. Nitrites and polycyclic aromatic hydrocarbons (PAH) are often found in processed meat products and can have a disastrous effect on human health [7]. Functional ingredients extracted from medicinal plants can significantly improve meat, fish, and dairy products [8, 9, 16]. Russian food science has achieved great success in developing new functional dairy products based on whey, cottage cheese, and buttermilk [14–16].

The relevance of the present research lies in the fact that a few plant species are actually used in functional products, including the rose root (*Rhodiola rosea* L.). According to scientific sources, it is usually used in herbal tea mixes, water tinctures, or wine products. As for the scullcup (*Scutellaria galericulata* L.), this plant is protected by law, and this is the first time it has become focus of the attention of food science. Its properties and prospects for functional food industry remain understudied. Thus, the research objective was to identify the biologically active substances that can be extracted from these plants and study their potential for the production of new functional foods based on whey and cottage cheese.

STUDY OBJECTS AND METHODS

The present research featured biologically active substances (BAS) extracted from two plants: the rose root (*Rhodiola rosea*) and the skullcap (*Scutellaria galericulata*).

The rose root can be found all over Russia, from its European part to the Far East. It is especially abundant on the fragmental soil of the Altai-Sayan mountain systems. The plant proliferates on the variety of local minerals and macro- and microelements. They add unique medicinal properties to the phytochemical composition of the plant organs [21, 22, 24]. In fact, the rose root has nearly become extinct due to uncontrolled herborization. As a result, it is now listed in the regional endangered-species lists and in the Red Book of Russia.

The scullcap is endemic to Eastern Siberia: it grows in the Tomsk and Kemerovo regions, in the Republic of Tuva, in Khakassia, and in the Mongolian areas of the Altai Mountains [13]. The plant prefers moist forest woodlands, steep river banks, and sandy terraces. The scullcap is a popular medicinal plant with unique adaptogenic, antioxidant, apoptotic, and antiviral properties. It is also known for its ability to inhibit the development of free radicals in cells [23–26]. The research featured aerial parts, rhizomes, and roots.

The content of BAS was determined using Shimadzu LC-20 Prominense chromatography unit. The device was equipped with a Shimadzu SPD20MA diode array detector and a RID refractometric detector with a Kromasasil C-18 250 × 4.6 mm column.

The TLC chromatography was performed using Sorbfil PTCX-AF-A plates with subsequent densitometry on a TLC Sorbfil plate. The experiment involved a densitometer with a Sony photofixation system (Handycam HDR-CX-405) purchased from IMID LLC, Russia. Sulfuric acid and 25% ethanolic solution of phosphoric-tungsten acid were used for targeted derivatization. After that, photofixation was performed at wavelengths of 254 and 365 nm in the visible range. Elution was conducted in mobile phase systems: chloroform – methanol – water (62:32:6) and ethyl acetate – formic acid – glacial acetic acid – water (100:11:11:26).

During the preparative stage, the chromatographic zones were excised and subjected to further analysis. The targeted BAS were screened and identified using HPLC, TLC, and IR. The obtained statistical data were processed using the Microsoft® Excel program. The tables show the arithmetic mean values. All experiments were performed in triplicates. The quantitative content of the BAS was determined using calibration curves constructed in the concentration range of 0.05–200 μg/mL.

The new functional products were based on whole milk whey and cottage cheese.

RESULTS AND DISCUSSION

In the industrially developed regions of Siberia,

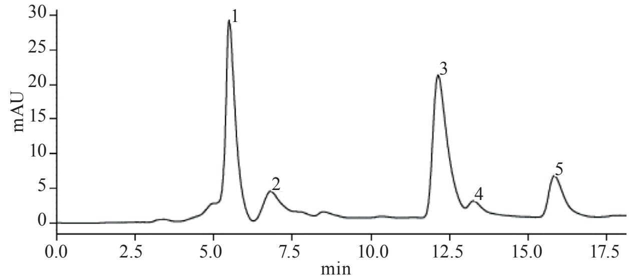

Figure 1 Chromatogram of ethanol extract from rhizomes and roots of *Rhodiola rosea* L.

public health is especially vulnerable. Its maintenance requires an active use of biological resources in food biotechnology [22, 23]. The present research featured the content of BAS in the rhizomes and roots of the rose root (*Rhodiola rosea*) harvested in the subalpine zone of the Kuznetsk Alatau mountains (Figs. 1 and 2). The BAS were isolated using chromatographic methods (Figs. 1 and 2). Rosavin (peak 1) and salidroside (peak 3) appeared to be the most abundant substances. Methyl gallate (peak 5), rosin (peak 2), and rosarin (peak 4) also proved significant. Rosavin, rosarian, and rosin belong to phenylpropanoids.

These compounds possess a lot of beneficial properties. First of all, they have scientifically proven adaptogenic and antioxidant properties [17]. Phenylpropanoids (rosavin, rosin, rosarin) are known to have tonic, antiviral, and immunomodulatory properties. Salidroside is regarded as one of the most promising substances for solving gerontology problems. This fact confirms the hypothesis that BAS extracted from rose root can be used in functional food industry. The actual value of rosavin was 16.9 mg/g, which exceeded other BAS by 15.4–88.2%.

Baicalin has good antioxidant properties. It also neutralizes oxidation processes and prevents the formation of free radicals. Scutellarein and vogonin exhibit mutual synergism and have anticonvulsant and antitoxic properties. Luteolin and vogonin have apoptotic, anti-inflammatory, and other useful properties. The BAS complex obtained from the *Scutellaria* genus is actively used for the treatment and recovery of cancer patients.

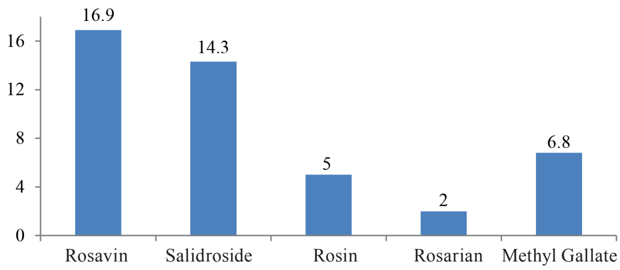

Figure 2 Content of biologically active substances in *Rhodiola rosea* L., mg/g

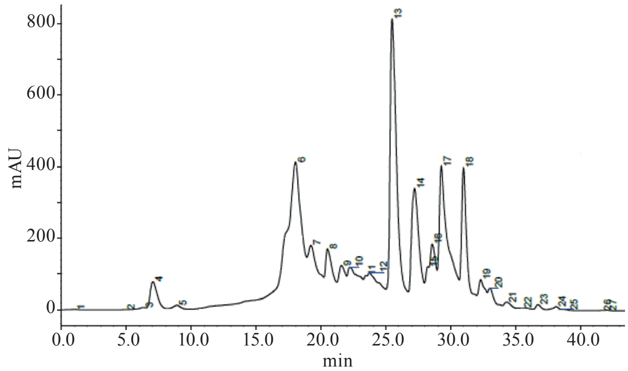

Figure 3 Chromatogram of ethanol extract from roots of *Scutellaria galericulata* L.

The analysis of scullcap roots showed high concentrations of the following BAS: baicalin (peak 13), scutellarein (peak 6), baicalein (peak 17), apeginin (peak 18), chrysin (peak 14), luteolin (peak 16), and vogonin (peak 7) (Figs. 3 and 4).

As for quantification, the content of BAS within this group varied from 5.4 to 34.4 mg/g. Baikalin had the biggest share compared with other BAS: 34.37 mg/g. Its advantage over other components was 35.2–84.3%. The

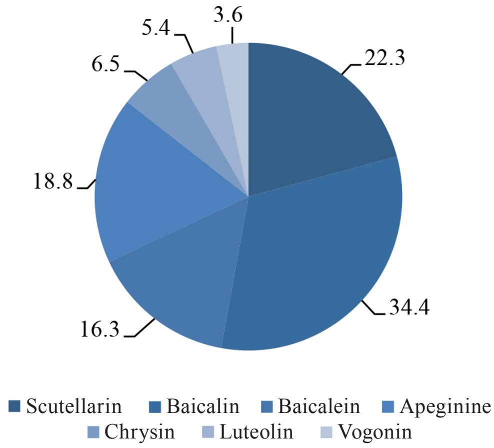

Figure 4 Content of biologically active substances in *Scutellaria galericulata* L., mg/g

Table 1 Formulation of the whey drink fortified with *Rhodiola rosea* L. concentrate

Component	Amount					
	1	2	3	4	5	6
Whey, mL	70.0	60.0	50.0	70.0	60.0	50.0
Apple juice, mL	30.0	40.0	50.0	20.0	20.0	20.0
Sugar, g	3.0	3.0	3.0	3.0	3.0	3.0
Apple pecin, g	0.5	0.5	0.5	0.5	0.5	0.5
Concentrate of *Rhodiola rosea*, mL	0.1	0.1	0.1	0.1	0.1	0.1
Lemon acid, g	0.04	0.04	0.04	0.04	0.04	0.04
Drinking water, mL	–	–	–	10.0	20.0	30.0

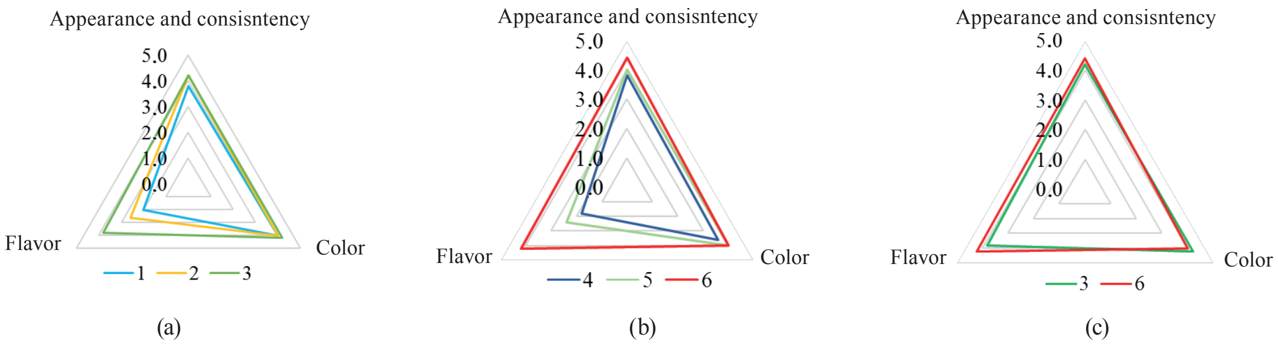

Figure 5 Sensory evaluation of the whey drink: (a) formulations 1–3; (b) formulations 4–6; (c) formulations 3 and 6, which proved optimal

obtained data prove that both plants have good prospects for functional food industry.

Whey and cottage cheese are high-protein dairy products and are beneficial for human health. They served as bases for formulations of two new functional products: a whey drink fortified with BAS extracted from rose root concentrate and cottage cheese fortified with BAS extracted from scullcap roots.

The formulation of the whey drink included cottage cheese whey, apple juice, sugar, rose root concentrate, and drinking water. Citric acid served as a regulator of acidity, while apple pectin was used as a stabilizer (Table 1). We tested six formulations of the new product. The first three samples had a different amount of apple juice. The remaining three samples differed in the amount of water, while the volume of apple juice remained the same. Water affects sensory properties and regulates the acidity of the finished product.

In order to determine the optimal formulation, the drink underwent a sensory evaluation for appearance, consistency, flavor, and color on a five-point scale (Fig. 5).

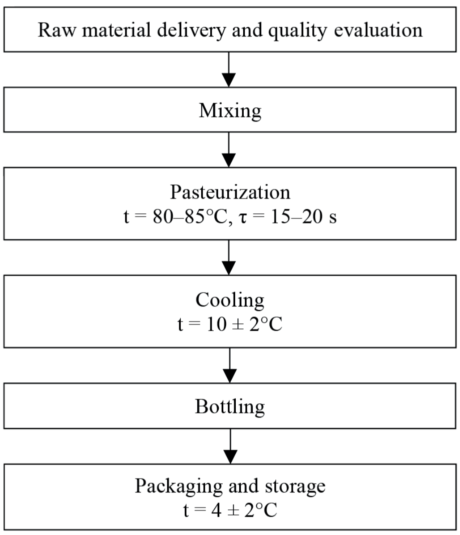

Figure 6 Flow chart for the whey drink fortified with biologically active substances extracted from *Rhodiola rosea* L.

Variants 3 and 6 received the highest score. When they were compared with each other, preference was given to variant 6. It had the highest sensory evaluation both in terms of flavor and color. Therefore, variant 6 was selected for the production of the functional product.

The technological process for the whey drink fortified with rose root concentrate included the following stages: raw material delivery and sensory evaluation, mixing the components, pasteurization, cooling, bottling, packaging, and storage (Fig. 1).

At the first stage, the raw material was evaluated according to the main quality indicators. Raw materials that met the requirements of regulatory and technical documentation passed on to the next stage. The initial mix was made up of the main ingredients, i.e. whey and drinking water, which entered the tank through a pipeline. Apple juice and rose root concentrate were introduced manually. The rose root concentrate was a dense, homogeneous dark brown mass. The dry ingredients, i.e. sugar, pectin, and citric acid, were gradually added to the resulting solution. A continuously working stirrer prevented lump formation. To suppress the development of vegetative microorganisms, the mix was pasteurized at 80–85°C for 15–20 s. The resulting drink was cooled to 10°C, bottled, and capped in uniform vessels.

Tables 2 and 3 show the content of BAS in the finished product and the results of sensory, physicochemical, and microbiological evaluation. All the BAS introduced into the formulation of the functional drink were represented in quantities that were found sufficient for practical use. State-issued Recommended

Table 2 Biologically active substances in the functional whey drink fortified with *Rhodiola rosea* concentrate L.

Component	Content in the concentrate, mg/g	Content in the finished drink, mg/100 g
Rosavin	16.89 ± 2.11	0.31 ± 0.077
Salidroside	14.35 ± 2.52	0.49 ± 0.08
Rosin	5.04 ± 0.93	0.11 ± 0.001
Rosarin	2.01 ± 0.37	0.17 ± 0.012
Methyl gallate	6.8 ± 1.05	0.12 ± 0.032

Table 3 Sensory, physico-chemical, and microbiological indicators of the functional whey drink fortified with *Rhodiola rosea* L. concentrate

Index	Property
Appearance and texture	Opaque liquid with slight phase layering
Color	Intrinsic, uniform
Taste and smell	Characteristic, no extraneous flavors and odors; tastes a little sour
Mass fraction of solids, %	9.7 ± 0.3
Mass fraction of fat, %	0.02 ± 0.03
Acidity, °T	47.5 ± 0.8
Release temperature, °C	4 ± 2
Coliform bacteria, per 0,01 cm^3	Not detected
Yeast and mold, CFU/cm^3	≤ 1,0×10–^{1}
Pathogens, including salmonella	Not detected

Practice MP 2.3.1.1915-04 highlights the level of BAS consumption. According to the data provided in the document, the new functional drink satisfied 40–45% of the reference daily intake for phenolic compounds and phenylpropanoids. The performed evaluation of sensory, physico-chemical, and microbiological properties of the drink showed that it corresponded to another state-issued standard – Technical Requirements 10.51.55-001-02068309-2019.

The unique properties of skullcap, or *Scutellaria galericulata*, have never become an object of food technology. However, it is rich in flavonoids, and a functional product fortified with its BAS will have a beneficial effect on various systems of human body. Using the above techniques, we obtained another functional dairy product – cottage cheese fortified with skullcap concentrate. The experiment involved five variants: a control sample, two samples with cranberry jam, and two samples with cherry jam.

Table 4 demonstrates the formulation, while Fig. 7 shows the flow chart for the producing of cottage cheese enriched with skullcap concentrate.

Table 4 Formulation of the cottage cheese fortified with *Scutellaria galericulata* L. concentrate

Component	Amount				
	1	2	3	4	5
Cottage cheese, g	91.0	86.0	81.0	86.0	81.0
Cherry jam, mL	–	5.0	10.0	–	–
Cranberry jam, mL	–	–	–	5.0	10.0
Sugar, g	9.0	9.0	9.0	9.0	9.0
Concentrate of *Scutellaria galericulata*, mL	0.025	0.025	0.025	0.025	0.025

The sensory analysis of the cherry jam samples revealed good monogenicity, consistency, and appearance in both variants. Variant 3 was given the best scores for flavor (Fig. 7a). This sample contained 81 mL of cherry jam, 9 g of sugar, and 0.025 mL of scull-cap concentrate per 81 g of cottage cheese. The samples with cranberry jam showed no significant differences. After a comparative analysis of all the options, variant 3 was announced best according to taste properties.

The technology for the new cottage cheese product included the following stages: preparation of the raw material, mixing, heating, homogenization, cooling, packaging, and storage (Fig. 8). Raw materials were evaluated according to the main quality indicators and regulatory documentation. To prepare the mix, cottage cheese was put into the kneading machine. Jam, sugar, and scull-cap concentrate were added manually. The obtained mix underwent a thermal treatment at 62°C for 15–20 s to suppress the development of vegetative microorganisms. To obtain a homogeneous texture, the cottage cheese was homogenized at 62°C. After that, the finished cottage cheese was cooled to 20°C and packaged. The product was stored at 4 ± 2°C.

A biochemical analysis of the finished product revealed sufficient quantities of BAS (Table. 5). Table 6 shows sensory, physico-chemical, and microbiological indicators of the fortified cottage cheese.

Figure 7 shows the results of the sensory evaluation of appearance, consistency, flavor, and color on a five-point scale.

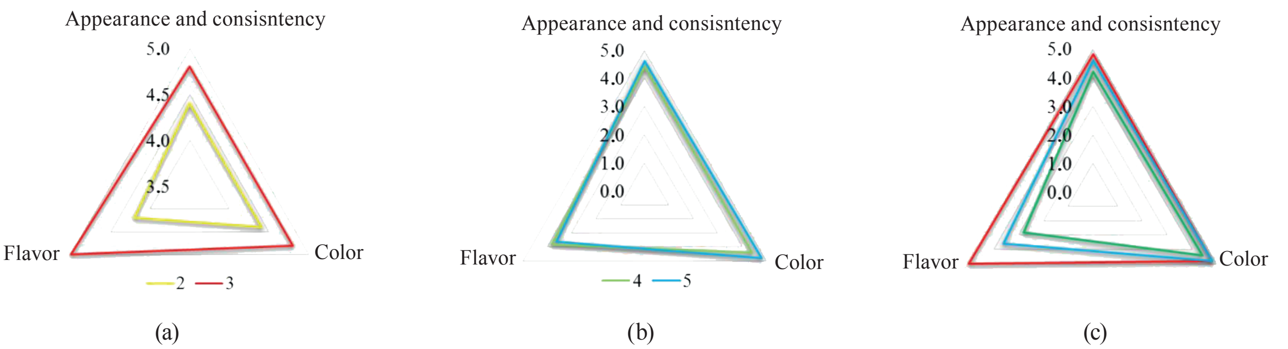

Figure 7 Sensory evaluation of the cottage cheese: (a) formulations 2 and 3; (b) formulations 4 and 5; (c) control formulation 1 and formulations 3 and 5, which proved optimal

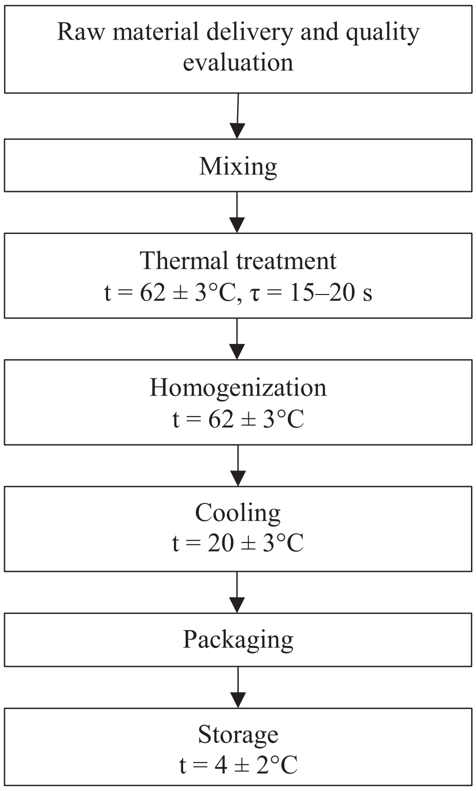

Figure 8 Flow chart for the cottage cheese fortified with biologically active substances extracted from *Scutellaria galericulata* L.

Table 5 Main flavonoids in the cottage cheese fortified with biologically active substances extracted from *Scutellaria galericulata* L.

Component	Content in the concentrate, mg/g	Content in the finished cottage cheese, mg/100 g
Scutellarein	22.27 ± 2.23	0.26 ± 0.019
Baicalin	34.37 ± 3.47	0.48 ± 0.11
Baicalein	16.3 ± 2.19	0.26 ± 0.019
Apigenin	18.80 ± 1.98	0.23 ± 0.019
Chrysin	6.50 ± 1.13	0.14 ± 0.012
Luteolin	5.40 ± 1.00	0.09 ± 0.02
Vogonin	3.60 ± 0.90	0.12 ± 0.014

According to the Recommended Practice MP 2.3.1.1915-04, the new functional cottage cheese product satisfied 55–60% of the reference daily intake for phenolic compounds. The performed sensory, physico-chemical, and microbiological evaluation of the cottage cheese showed that it corresponded to Technical Requirements 10.51.55-001-02068309-2019.

Table 6 Sensory, physico-chemical, and microbiological indicators of the cottage cheese fortified with biologically active substances extracted from *Scutellaria galericulata* L.

Index	Property
Appearance and consistency	Homogeneous, pasty, soft
Color	White, with the hue characteristic of the introduced components
Flavor	Pure, sour-milk, sweet, with a touch of added ingredients
Moisture content, %	59.3 ± 3.9
Mass fraction of protein, %	12.4 ± 0.7
Mass fraction of fat, %	3.8 ± 0.7
Acidity, °T	149.3 ± 10.9
Release temperature, °C	4 ± 2
Lactic acid microorganisms, CFU/cm^3	1×10^7
Coliform bacteria, per 0,01 cm^3	Not detected
Yeast and mold, CFU/cm^3	Not detected
Pathogens, including salmonella	Not detected

CONCLUSION

The present research established the content of biologically active substances obtained from two medicinal plants of the Kemerovo Region. It featured the rhizomes and roots of *Rhodiola rosea* harvested in the subalpine belt of the Kuznetsk Alatau mountains and the roots of the *Scutellaria galericulata* harvested on the rocky outcrops along the Tom' River.

The biomass was tested for biologically active substances and revealed good pharmacological prospects, i.e. high antioxidant, anti-inflammatory, antibacterial, antiviral, and apoptotic properties.

A set of experiments resulted in two formulations of new functional dairy products: a whey drink fortified with biologically active substances extracted from *Rhodiola rosea* concentrate and cottage cheese fortified with biologically active substances extracted from *Scutellaria galericulata*.

CONTRIBUTION

The authors were equally involved in writing the manuscript and are equally responsible for plagiarism.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest related to the publication of this article.

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