DOI: https://doi.org/10.21323/2618-9771-2024-7-1-15-21

Received 18.08.2023
Accepted in revised 06.01.2024
Accepted for publication 26.01.2024

Available online at https://www.fsjour.com/jour
Original scientific article
Open access

GAS CHROMATOGRAPHY-MASS SPECTROMETRY CHARACTERIZATION OF THE AROMA OF OSSETIAN CHEESES

Andrey V. Gutnov[1]*, Gennady S. Kachmazov[1], Petrakis N. Chalikidi[1], Albina Yu. Tuaeva[2]

[1] North-Ossetian State University, Vladikavkaz, Russia
[2] National Research Center "Kurchatov Institute", Moscow, Russia

KEY WORDS:
Ossetian cheese, aroma, gas chromatography-mass spectrometry, metabolism, biochemistry

ABSTRACT

The aim of the study was the qualitative and quantitative determination of volatile aroma compounds and their formation pathways in brine Ossetian cheeses. Volatile components of cheeses were isolated by steam distillation and extraction with dichloromethane, with their subsequent determination and quantification by gas chromatography-mass spectrometry. The results of the analysis are presented according to the structural classes of the main chemical components and the corresponding microbial metabolic processes. Four processes were found to be the main contributors to flavor formation: lipolysis, proteolysis, glycolysis, and a number of oxidative enzymatic transformations. Lipolysis of the fatty fraction of cheeses is a source of formation of volatile carboxylic acids and their esters. Proteolysis of the casein fraction yields branched alcohols, aldehydes, and a number of aromatic and heteroaromatic compounds. Glycolysis of the carbohydrate fraction is a source of ethanol formation, which is the main cause of the dominance of ethyl esters in the ester fraction. Redox enzymatic transformations mainly determine the biosynthesis of unbranched aldehydes, ketones and lactones. A clear distinction between retail and homemade cheeses was observed, due to the different technological approaches to the cheese preparation. The structural-chemical and quantitative evolution of the volatile composition of the studied cheese samples during ripening is tentatively shown. From the authors' point of view, the aromatic composition of the Tib cheese sort is the most consistent with the Ossetian cheese standard. This study represents the first gas chromatographic study of Ossetian cheeses and aims to create objective criteria for controlling technological processes and product quality during production and storage in the food industry.

Поступила 18.08.2023
Поступила после рецензирования 06.01.2024
Принята в печать 26.01.2024

https://www.fsjour.com/jour
Научная статья
Open access

ГАЗОВАЯ ХРОМАТО-МАСС-СПЕКТРОМЕТРИЧЕСКАЯ ХАРАКТЕРИСТИКА АРОМАТА ОСЕТИНСКИХ СЫРОВ

Гутнов А. В.[1]*, Качмазов Г. С.[1], Чаликиди П. Н.[1], Туаева А. Ю.[2]

[1] Северо-Осетинский государственный университет, Владикавказ, Россия
[2] Научно-исследовательский центр «Курчатовский институт», Москва, Россия

КЛЮЧЕВЫЕ СЛОВА:
осетинский сыр, аромат, газовая хромато-масс-спектрометрия, метаболизм, биохимия

АННОТАЦИЯ

Целью исследования являлось качественное и количественное определение летучих ароматических соединений и путей их образования в рассольных осетинских сырах. Летучие компоненты сыров выделяли путем паровой дистилляции и экстракции дихлорметаном, с их последующим определением и квантификацией методом GC–MS. Результаты анализа представлены по структурным классам основных химических компонентов и соответствующим микробиологическим метаболическим процессам. Было установлено, что главный вклад в ароматообразование вносят четыре процесса: липолиз, протеолиз, гликолиз, а также ряд оксидативных ферментных превращений. Липолиз жирной фракции сыров является источником образования летучих карбоновых кислот и их эфиров. Протеолиз казеиновой фракции дает разветвленные спирты, альдегиды, а также ряд ароматических и гетероароматических соединений. Гликолиз углеводной фракции является источником образования этанола, главной причины доминирования этиловых эфиров в эфирной фракции. Окислительно-восстановительные ферментные превращения определяют главным образом биосинтез неразветвленных альдегидов и кетонов, а также лактонов. Отмечено четкое различие между розничными и домашними сырами, что связано с различными технологическими подходами к приготовлению сыров. Предварительно показана структурнохимическая и количественная эволюция летучего состава исследуемых образцов сыра в процессе созревания. С точки зрения авторов ароматический состав сорта сыра Тиб представляет собой наиболее соответствующий эталону осетинского сыра. Данное исследование представляет собой первое газхроматографическое исследование осетинских сыров и имеет целью создание объективных критериев для контроля технологических процессов и качества продукта при получении и хранении в пищевой промышленности.

1. Introduction

The most authentic Ossetian cheeses are produced in the highlands and foothills of the Republic of North Ossetia-Alania. This can be explained by the still preserved tradition of production, the floral characteristics of diverse alpine pastures, the characteristics of mountain cattle breeds and the specific microbiological diversity of mountain areas. Among many factors, bacteria and yeasts that occupy specific ecological niches are the most important factors determining the quality of raw milk, the microbiological composition of fermentation and, altogether, the brine cheeses produced. A comprehensive study of metabolites of bacterial and yeast communities in Ossetian cheeses could explain the processes developing throughout brine ripening and the formation of the best consumer characteristics of the final product. It should also be noted that the tradition of making Ossetian cheese is closely connected with the tradition of making Ossetian pies popular in Russia, where the former serves as one of the main components and cannot be replaced by other types of cheese without losing their original properties. Unfortunately, the tradition of making Ossetian cheese is currently under threat for a

FOR CITATION: **Gutnov, A. V., Kachmazov, G. S., Chalikidi, P. N., Tuaeva, A. Yu.** (2024). Gas chromatography-mass spectrometry characterization of the aroma of Ossetian cheeses. *Food systems,* 7(1), 15-21. https://doi.org/10.21323/2618-9771-2024-7-1-15-21

ДЛЯ ЦИТИРОВАНИЯ: **Гутнов, А. В., Качмазов, Г. С., Чаликиди, П. Н., Туаева, А. Ю.** (2024). Газовая хромато-масс-спектрометрическая характеристика аромата осетинских сыров. *Пищевые системы*, 7(1), 15-21. https://doi.org/10.21323/2618-9771-2024-7-1-15-21

number of reasons. Among them are the use of compositional bacterial starters, often not intended for this purpose, instead of traditional rennet, the regulatory requirement to use pasteurized milk instead of traditional raw milk, difficulties in keeping milk producing breeds and relatively low productivity of the mountain cattle, giving a competitive advantage to the industrial milk production. In this regard, the main task is not only to maintain the raw material base and preserve traditional methods of cheese production, but also to develop multiple analytical methods and, on their basis, objective criteria for assessing the conformity and quality of the product. The latter is the main goal of our current work and one of the first steps in this direction.

To date there has been only one such study on Ossetian cheese, where polar water-soluble organic acids (lactic, acetic, etc.) in Ossetian cheese samples were determined and quantified by HPLC [1]. On the other hand, no studies of Ossetian cheese have yet been conducted using gas chromatography-mass spectrometry, despite the fact that this method allows characterization of a much wider range of volatile metabolites and therefore is routinely used in the literature, for example, for reliable qualitative and quantitative characterization of volatile composition of semi-hard Spanish goat cheeses, Gouda and Cheddar cheeses [2–5].

Volatile aroma substances play an important role in the flavor of cheese. The typical flavor of cheese is a result of a combination of volatile substances produced mainly by four processes: lipolysis of the fatty fraction of cheese, proteolysis of casein protein, microbiological metabolism of lactose, lactates and citrates, and some oxidative enzymatic transformations of the lipid fraction. All these transformations are carried out by the enzyme systems of microorganisms inhabiting both the interior and the surface of cheese heads. The microbiota of cheese is very complex and comprises different types of bacteria, yeasts and molds which have been the subject of numerous articles, reviews and books [6–7].

Lipolysis of the fat fraction produces fatty acids, which are flavor-forming compounds that give the product the main characteristic cheese flavors, especially short-chain and middle-chain fatty acids [8–9]. Lipolysis can occur when the milk fat is exposed to lipases or esterases [10–11]. The source of these enzymes is milk itself (lipoprotein lipase), psychrophilic bacteria present in the raw milk, lipase contamination during the cheese-making process, and numerous lipase-producing microorganisms that develop during cheese ripening, introduced either intentionally (starter bacteria, specific molds or yeasts) or unintentionally (non-starter lactic acid bacteria, molds, yeasts). In turn, fatty acids may be precursors to other compounds, such as esters resulting from esterification of fatty acids with alcohols by the action of mainly esterases of lactic acid bacteria, as well as lactones, ketones and aldehydes formed in oxidative processes from long-chain saturated and unsaturated fatty acids by lipoxygenases in *Penicillium roqueforti*, *Penicillium camemberti*, *Geotrichum candidum* and lipoxygenases produced by the rumen microflora migrating down the gastrointestinal tract.

Proteolysis of proteins during the cheese ripening plays an important role in the texture formation [12–13]. However, it also affects the flavor of cheese through the formation of peptides and free amino acids. Large peptides do not contribute directly to the cheese flavor, but shorter peptides have varying degrees of characteristic bitterness, many of them being bioactive [14]. The peptidases and proteinases that catalyze proteolysis come from several major sources, namely the coagulant (pepsin, proteinases found in *Rhizomucor*, *Cryphonectria parasitcia* or *Cynara cardunculus*), the milk itself (e. g. plasmin, the intrinsic proteinase in milk), lactic acid starters and other secondary inoculants (e. g. *Propionibacterium freudenreichii*, *Penicillium roqueforti*, *Penicillium camemberti*), as well as from the complex Gram-positive bacterial microflora formed on the surface of cheeses, and, in some cases, exogenous peptidases or proteinases added to milk or curd to accelerate ripening. The free amino acids produced by the hydrolytic processes are substrates for a number of further catabolic reactions that produce many important flavor compounds.

The catabolysis of amino acids in cheese (its most important direction) is initiated by the action of aminotransferases/deaminases, leading respectively to their deamination to α-keto acids and further decarboxylation of the latter to aldehydes [15–16]. Deaminases and aminotransferases capable of de- and transamination of branched-chain amino acids were found in the dairy *Lactobacillus plantarum*, *Lacticaseibacillus paracasei* and *Propionibacterium freudenreichii*. The breakdown of aromatic and branched amino acids leads to aromatic and branched aldehydes (benzaldehyde, phenylacetaldehyde, 3-methylbutanal, etc.), and further to branched volatile acids and/or alcohols (3-methylbutanoic acid, isoamyl alcohols, etc). Under the action of dehydrogenases from *Lactobacillus casei*, proteolytic oxidative breakdown of tryptophan yields the heterocyclic compounds such as faintly odorous indole and very potent 2-methylindole, while phenol and its derivatives are formed from tyrosine and phenylalanine [17,18]. Methionine and cysteine (to a lesser extent) are precursors for sulfur components like methional, dimethyl disulfide, dimethyl trisulfide [19] mainly as a result of metabolism by lactic acid bacteria.

In addition to lipids and proteins, lactose is another major component of milk for cheese flavor formation [20]. Lactose, lactates and citrates contribute to the formation of diacetyl, acetoin, ethanol, propanols, acetic, propionic and butyric acids. The role of ethanol itself in cheese flavor formation is insignificant, but it is a source of ethyl esters, the importance of which in cheese ripening cannot be overestimated. In some cases, ethanol is even added to cheese to increase the production of ethyl esters to improve flavor [21].

In conclusion, the planned studies using gas chromatography-mass spectrometry will make it possible to develop an auxiliary analytical procedure for the first time for Ossetian cheese. In the future, this will help, ultimately, to create an optimal bacterial-yeast starter complex capable of forming the most preferred metabolic products in cheese that will positively affect the physicochemical and consumer qualities of the final product.

2. Objects and methods

Nine samples of Ossetian cheese of different origin, maturity, age, and aroma were selected (Figure 1) for the study in order to cover the maximum possible area of organoleptic variations of this cheese type. In particular, commercial pasteurized cheeses of different degrees of maturity (Delikat, Nash, Fiagdon1) and domestic varieties were used: young (1–2 months) cheeses (Kobi2), mature (4–6 months) cheeses (Tib, Kobi1, Fiagdon2), and overripened (12–18 months) cheeses (Dargavs1, Dargavs2). The age was declared by the manufacturers. Fiagdon, Tib, Kobi and Dargavs refer to the geographical localization of the place of manufacture.

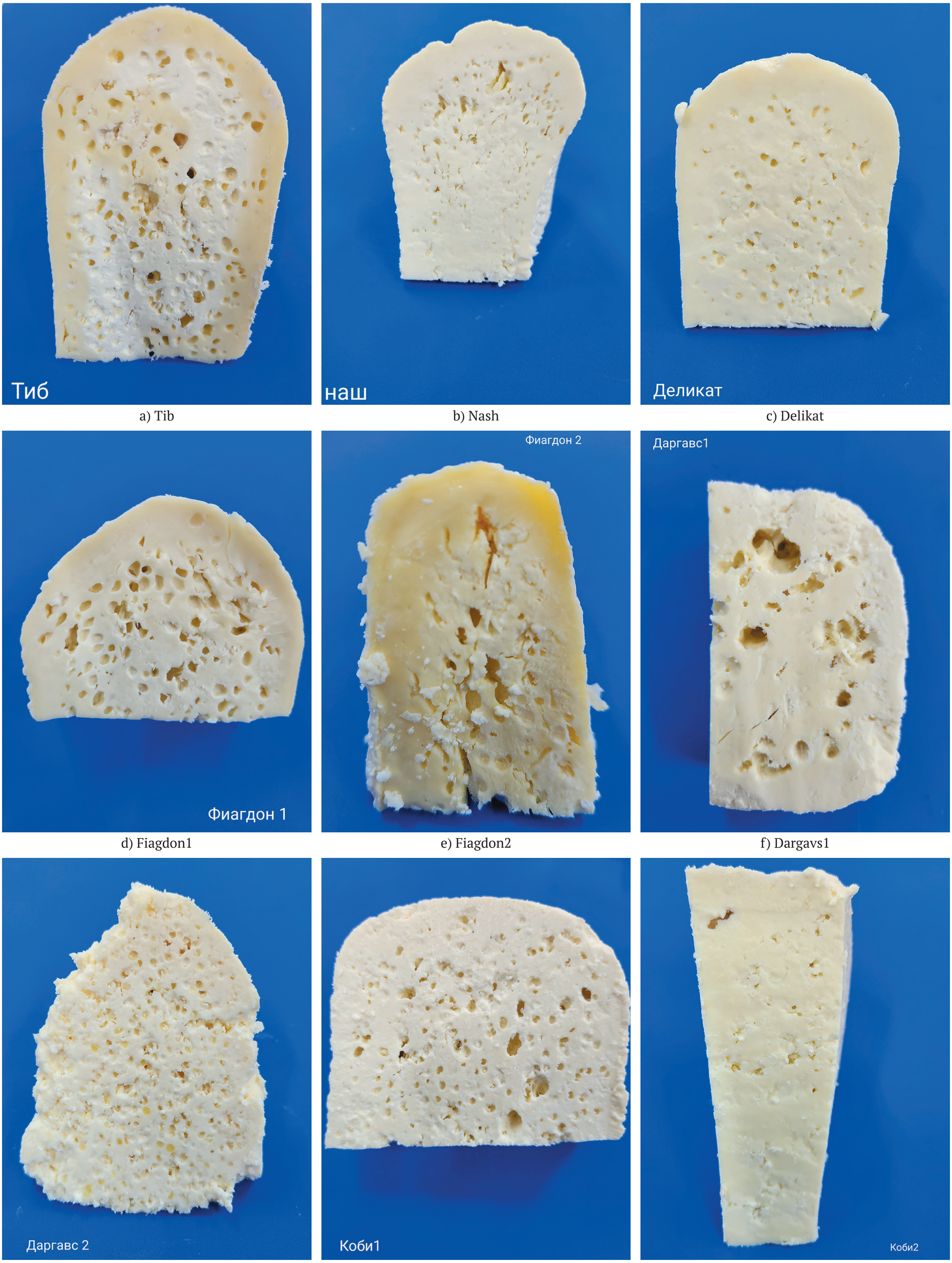
Cheese samples were taken in duplicate by cutting 100 g of a sample from the center to the outer part. The cheese samples were stored in a refrigerator at 5 °C until they were needed for analysis, but no longer than 3 days.

For the extraction of cheese volatiles, a sample of 100 g of shredded cheese was suspended with a blender (Stegler LB-2, Stegler Laboratory Instrumentation, USA) in 600 mL of water and steam distilled under reduced pressure (80–100 mbar) in an oil bath heated to 80–85 °C in order to keep the temperature of distillation at 40–50 °C as detailed earlier [22]. The resulting 200 ml of distillate was extracted with 2x20 ml of dichloromethane (Komponent-Reaktiv, pure for spectroscopy grade). To quantify the volatiles, dodecane was used as an internal standard and added to the solvent. Dodecane was not detected in the samples and was well separated from other cheese volatiles. After cooling to room temperature, the combined dichloromethane fractions were concentrated to a final volume of 0.5 mL on a rotary evaporator (Heidolph Hei-VAP Precision, Heidolph Instruments, Germany).

At the inlet of a gas chromatograph (Thermo Trace 1300, Thermo Fischer Scientific, USA) coupled to a mass spectrometer (ISQ MSD, Thermo Fisher Scientific, USA), 5 μL of a cheese extract was injected. The GC system was equipped with a TR-5MS capillary column (95% dimethylpolysiloxane, 5% phenylpolysilphenylphenylsiloxane, L 30 m x I.D. film thickness 0.25 mm x 0.25 mm; Thermo Fisher Scientific). Helium was used as a carrier gas at a flow rate of 1 mL/min. The column temperature was maintained at 30 °C for 15 min, then programmed at a rate of 10 °C/min to increase to 150 °C, which was maintained for 20 min. A split ratio of 1/10 was used and the injector and the ISQ MSD detector were maintained at 250 °C and 280 °C, respectively. The mass spectra were obtained by electron impact of 70 eV and the total ion current (TIC) chromatograms were recorded by monitoring the TIC in a scan range of 50–400 amu (atomic mass units). A solvent delay of 6.0 min was used to avoid excessive solvent disturbing the MS source. Identification of the cheese volatiles was made by comparing the mass spectra of the different volatiles with the mass spectra of a commercial Mass Spectral Library, Wiley275 (Wiley, Somerset). Semi-quantitative data of the isolated aroma compounds were calculated by relating the peak area of the cheese volatile to the peak area of dodecane. It was assumed that the volatile compounds have the same response factor to that of the internal standard. Quantitative results were obtained by calculating the average of three cheese sample analyses. Coefficients of variation (CoVs) were calculated by repeating the steam-distillation-extraction procedures with subsequent analysis of Kobi1 cheese in order to determine the repeatability of the analysis. The CoVs of most peaks were lower than 10% and never exceeded 15%, which was considered to be a satisfactory result for this type of analysis.

The concentrations of the volatiles were expressed as ng/g^{-1} of cheese.

A representative GC/MS spectrum of Tib cheese is shown in Figure 2.



a) Tib b) Nash c) Delikat
d) Fiagdon1 e) Fiagdon2 f) Dargavs1
g) Dargavs2 h) Kobi1 i) Kobi2

Figure 1. **Appearance of the selected Ossetian cheeses**
Рисунок 1. **Внешний вид выбранных осетинских сыров**

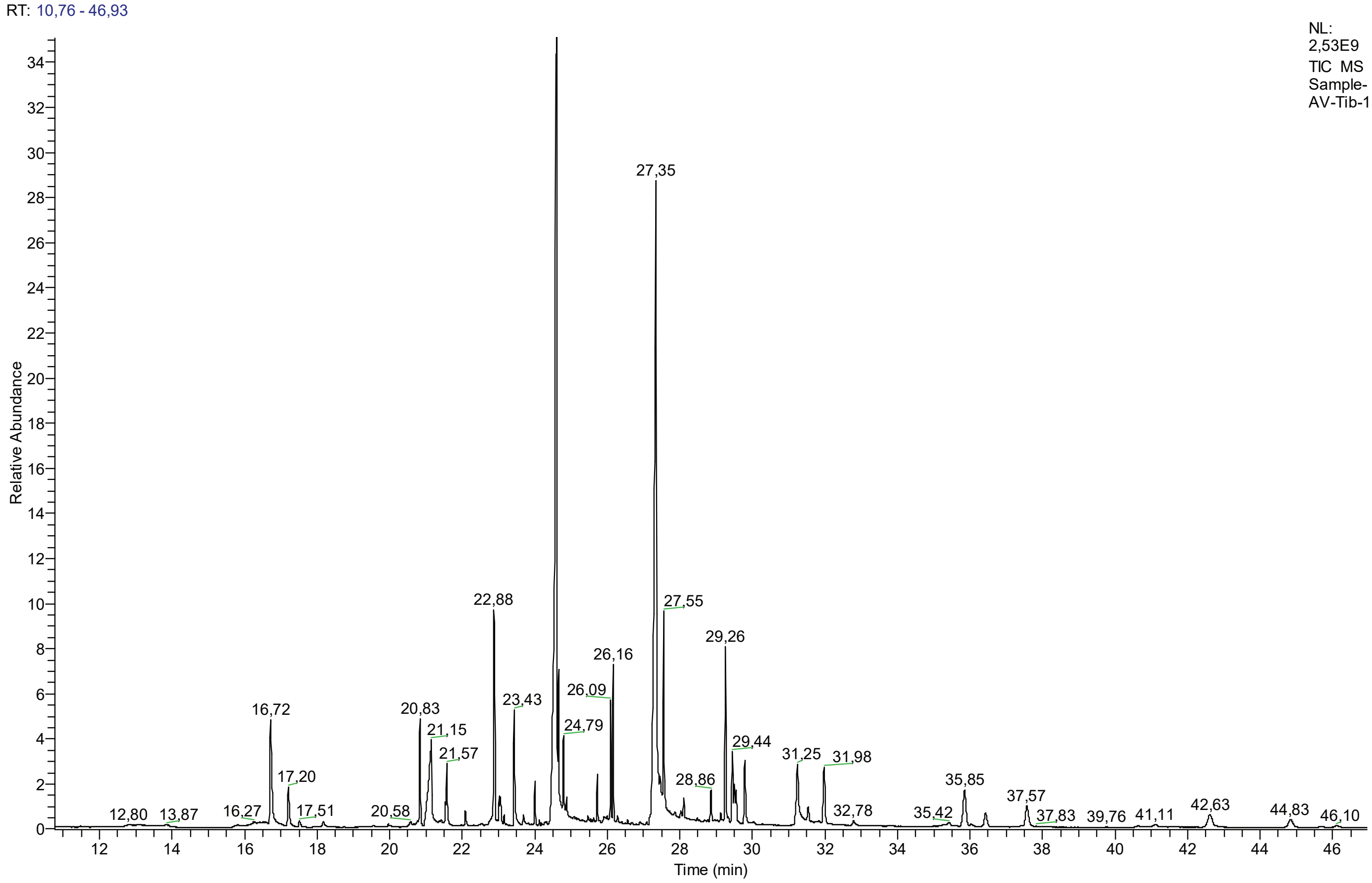


Figure 2. **GC/MS spectrum of Tib cheese. Major components: 2-heptanone (16.72), propyl butanoate (17.20), ethyl hexanoate (20.83), hexanoic acid (21.15), 2-ethylhexane-1-ol (21.57), phenylacetaldehyde (22.08), 2-nonanone (22.88), phenethyl alcohol (23.43), dodecane (24.6), propyl octanoate (26.1), 2-undecanone (26.17), decanoic acid (27.35), ethyl decanoate (27.55), propyl decanoate (29.26), 2-tridecanone (29.44), dodecanoic acid (31.25), ethyl dodecanoate (31.98), propyl dodecanoate (35.85), 6-heptyl-tetrahydropyran-2-one (37.57), ethyl tetradecanoate (42.63)**

Рисунок 2. **Спектры ГХ/МС сыра Тиб. Основные компоненты: 2-гептанон (16,72), пропилбутаноат (17,20), этилгексаноат (20,83), гексановая кислота (21,15), 2-этилгексан-1-ол (21,57), фенилацетальдегид (22,08), 2-нонанон (22,88), фенетиловый спирт (23,43), додекан (24,6), пропилоктаноат (26,1), 2-ундеканон (26,17), декановая кислота (27.35), этилдеканоат (27,55), пропилдеканоат (29,26), 2-тридеканон (29,44), додекановая кислота (31,25), этилдодеканоат (31,98), пропилдодеканоат (35,85), 6-гептил-тетрагидропиран-2-он (37,57), этилтетрадеканоат (42,63)**

3. Results and discussion

Eight ketones were detected in the cheeses (Table 1). Ketones are common components of most milk products and can be biochemically reduced to secondary alcohols. Methyl ketones are formed from fatty acids by β-oxidation or from β-keto acids and are known primarily for contributing to the flavor of moldy cheeses. They have typical odors (fruity, floral, mushroom or musty notes) and a low perception threshold. The concentration of ketones is moderately dependent on the method of manufacture and age of cheese. Their lowest concentrations were found in young (Kobi2) and commercial pasteurized cheeses (Delikat, Nash, Fiagdon1). The highest concentrations of ketones were recorded in overripened cheese Dargavs2.

Six alcohols were identified in the cheeses (Table 2). Primary alcohols are formed by the reduction of aldehydes by alcoholdehydrogenases. They give fruity and nutty notes to the cheese, but their high content may also cause odor defects. Secondary alcohols are also formed by the enzymatic reduction of the corresponding methyl ketones. They have similar but heavier aromatic notes than the methyl ketones. Phenylethyl and benzyl alcohols are considered to be products of the enzymatic breakdown of phenylalanine, and isoamyl alcohol is formed proteolytically from leucine.

Also, eight fatty acids were identified in the cheeses (Table 3). Fatty acids are important odor components of all types of cheese. Long-chain fatty acids (> 12 carbon atoms) play a minor role in flavor formation because of their relatively high perception threshold. Short- and medium-chain fatty acids with an even number of carbon atoms (C4-C12) have a much lower perception threshold and characteristic notes (vinegar, sourness). In addition, free fatty acids serve as precursors to methyl ketones, alcohols, lactones and esters. On the other hand, high concentrations of free fatty acids can cause an unpleasant rancid odor as the cheese matures, e. g., Dargavs1 and Dargavs2.

The occurrence of the short-chain butyric acid in mature and partially overripened Kobi1 and Dargavs2 cheeses requires a special mention. It is not related to lipolysis but to the anaerobic metabolism of lactose by, for example, *Clostridium acetobutyricum* or other butyric acid bacteria to butyric acid, gaseous CO_2 and H_2, and leads to a defect known as late gas blowing [23], which causes cracks in cheese during ripening and the development of an unpleasant rancid odor characteristic for butyric acid. The late gas blowing is a problem especially occurring in brine-salted cheeses because of the time required for NaCl to diffuse into cheese and to reach inhibitory concentrations.

Twenty-seven esters were detected in the cheeses (Table 4). The esters are common volatiles in cheese. Esterification reactions occur between short- and medium-chain fatty acids formed by lipolysis and alcohols. Most esters in cheeses are described as having sweet, fruity and floral notes. Some of them have a very low perceptual threshold and their contribution to odor is enhanced by synergistic effects. They can also contribute to the aroma of cheese, minimizing, for example, the harshness and bitterness imparted by fatty acids. The amount of esters, numerically and by mass, is most high in overripened Dargavs1 and Dargavs2 cheeses, and is mostly absent in young and pasteurized commercial cheeses (Delikat, Nash, Fiagdon1).

Eight aldehydes were identified in the cheeses (Table 5). Linear aldehydes can be formed by β-oxidation of unsaturated fatty acids or from amino acids by Strecker degradation. Benzaldehyde and phenylacetic aldehyde are formed from the degradation of phenylalanine by both enzymatic and non-enzymatic pathways, such as Strecker degradation. It should be noted that methional aldehyde in Dargavs2 and Kobi2 cheeses is a product of deep proteolytic degradation of methionine, and the presence of furfural in Fiagdon1 and Fiagdon2 cheeses may be an indication of the pentosan-rich local corn diet of the animals. Aldehydes are transient compounds in cheese because they are rapidly reduced to primary

Table 1. **Ketones** / Таблица 1. **Кетоны**

Compound	Tib	Delikat	Nash	Kobi1	Dargavs1	Fiagdon1	Dargavs2	Fiagdon2	Kobi2
2-hexanone	—	—	—	—	—	—	29.2	—	—
2-heptanone	339.2	619.1	570.2	530.3	405.7	618.7	4444.4	639.3	175.4
2-octanone	2.4	7.7	—	—	—	—	87.7	6.4	—
2-nonanone	458.8	791.6	356.8	633.8	1736.8	834.9	8245.6	724.1	166.5
8-nonen-2-one	—	—	—	—	245	—	2149.1	33.7	45
2-undecanone	179.4	653.6	228.1	672.6	548.2	731.4	277.8	387.3	63.6
2-tridecanone	89.7	807	164.5	918.4	493.4	331	251.1	226.9	21.6
2-pentadecanone	74.2	762.9	80.6	569.2	208.3	206.8	142.3	316.2	16.5

Table 2. **Alcohols** / Таблица 2. **Спирты**

Compound	Tib	Delikat	Nash	Kobi1	Dargavs1	Fiagdon1	Dargavs2	Fiagdon2	Kobi2
2-heptanol	15.5	—	—	—	131.6	—	1155	245.2	28
2-nonanol	—	—	—	—	—	—	1052.6	247.5	
phenethyl alcohol	176.3	168.7	311.2	698.5	892.1	676.9	160.8	5960.4	202.1
2-ethylhexane-1-ol	127.2	74.8	192.2	—	—	171.1	—	121.5	30.5
benzyl alcohol	—	—	—	—	—	—	131.5	—	—
iso-amyl alcohol	—	—	—	—	—	—	—	1251.2	32.2

Table 3. **Fatty acids** / Таблица 3. **Жирные кислоты**

Compound	Tib	Delikat	Nash	Kobi1	Dargavs1	Fiagdon1	Dargavs2	Fiagdon2	Kobi2
butanoic acid	—	—	—	1940.3	—	—	482.5	—	—
3-methylbutanoic acid	—	—	—	—	—	—	—	29.8	—
hexanoic acid	440.2	47.9	—	24939	25043.9	1086.9	11769	61.2	—
heptanoic acid	—	—	—	207	—	—	—	59.6	—
octanoic acid	2	—	—	49711	29561.4	129.7	31564.3	149	—
decanoic acid	2139	3737.8	—	26841	9638.2	2055.2	59502.9	646.2	—
dodecanoic acid	228.9	40.3	—	3078.6	767.5	24.4	4590.6	55	—
tetradecanoic acid	10.3	—	—	129	—	—	149.3	—	—

Table 4. **Esters** / Таблица 4. **Сложные эфиры**

Compound	Tib	Delikat	Nash	Kobi1	Dargavs1	Fiagdon1	Dargavs2	Fiagdon2	Kobi2
isoamyl acetate	—	—	—	—	—	—	63.9	61.9	—
ethyl butanoate	—	—	—	64.7	219.3	—	26.1	—	5.3
propyl butanoate	105.2	—	—	4.6	—	—	—	—	—
sec-butyl butanoate	—	—	—	—	—	—	87.7	—	—
pentyl butanoate	—	—	—	—	—	—	248.5	25.2	—
benzyl butanoate	—	—	—	—	—	—	117	—	—
ethyl hexanoate	146.4	—	—	349.3	4287.3	21	570.1	11.5	6.7
propyl hexanoate	29.4	—	—	—	—	—	—	—	—
isopropyl hexanoate	—	—	—	—	—	—	424	—	—
butyl hexanoate	—	—	—	—	106.6	—	102.3	—	—
isobutyl hexanoate	—	—	—	—	20	—	994.1	—	—
isoamyl hexanoate	—	—	—	—	953.4	—	687.1	—	5.3
ethyl octanoate	117.5	2.4	23.4	294.7	6688.6	—	1549.7	—	7.6
propyl octanoate	137.1	—	—	—	514.9	—	745.6	—	—
isopropyl octanoate	—	—	—	—	—	—	760.2	—	—
isobutyl octanoate	—	—	—	—	70.2	—	891.8	—	—
ethyl decanoate	281.4	—	11.7	1474.6	8662.3	—	—	—	28
propyl decanoate	335	—	—	62	164.5	—	1666.7	—	—
sec-amyl decanoate	—	—	—	—	132.9	—	1549.7	—	—
iso-amyl decanoate	—	—	—	—	563.4	—	409.4	—	—
ethyl dodecanoate	168	—	6.7	1241.8	7785.1	21	1271.9	26.5	14.7
propyl dodecanoate	175.3	—	—	—	458.3	—	293	—	—
i-Pr dodecanoate	—	—	—	—	—	—	599.4	—	—
ethyl tetradecanoate	104.1	—	—	983.1	7236.8	—	628.7	—	21.6
i-Pr tetradecanoate	—	—	—	—	—	—	248.5	—	—
phenethyl acetate	69.1	—	—	49.6	603.1	—	—	3380	10.1
phenethyl propionate	—	—	—	—	—	—	—	50.4	4.7

alcohols or oxidized to the corresponding acids. They are characterized by a grassy or hay-like aroma and can be very unpleasant if their concentration exceeds certain values. As discussed above, the concentration of aldehydes decreases as cheese matures due to their enzymatic reduction to alcohols or oxidation to carboxylic acids.

Four lactones were detected in the cheeses (Table 6). Lactones are associated with oxidative breakdown of lipids. The aromatic characteristics of lactones vary: oily, fruity, coconut. Lactones are initially present in the lipid fraction of milk and undergo a slow but complete breakdown as the cheese ages, as in the case of the overripened Dargavs2 cheese.

Also, three compounds that do not fit into any of the above groups, namely phenol, indole and skatole, were found in some samples (Table 7). All three are products of deep proteolytic breakdown of phenylalanine and tryptophan. Skatole (3-methylindole) is particularly known for its distinct fecal-rotten and musty odor. The highest concentrations of indoles are found in Cobi1 and especially Cobi2 cheeses and are decisive in their flavor.

In general, based on GC–MS data, it can be concluded that the ripening process of Ossetian cheeses is determined by metabolic accumulation of lipolysis products (especially Tib, Delikat, Dargavs1, Dargavs2) and proteolysis products (especially Kobi2). In some cases, both processes are present simultaneously (Cobi1, Fiagdon1, Fiagdon2). In one case (young commercial cheese Nash), only very small amounts of all metabolites were detected.

Volatiles found in Tib and Fiagdon2 cheeses exhibited reasonable similarities to white brined cheeses analyzed and described in the literature [24–26] both qualitatively and quantitatively, especially in regard to the most significant flavor forming substances from the class of carboxylic acids, esters, ketones and aldehydes. Unlike them, the cheeses Delikat, Nash, Kobi1, Kobi2, Dargavs1, Dargavs2, Fiagdon1 showed significant deviations in many ways when compared to the known brine-ripened cheeses.

4. Conclusion

For the first time, volatile aromatic substances of nine homemade and commercial Ossetian cheeses have been analyzed. Aliphatic ketones, alcohols, fatty acids, esters, aldehydes, lactones and a number of heterocyclic compounds of indole and furan series were detected in the samples in different concentrations. This provides an evidence of the predominance of lipolytic and proteolytic processes during ripening of the studied cheeses. From the authors' point of view, the Tib cheese is the most compliant with the classical variant of Ossetian cheese. Overall, we were able to use the GS-MS method to document the qualitative and quantitative volatile profile of Ossetian cheese both for its classic version and for its local deviations from the standard, which can further serve as an objective analytical criterion for its production. In the future, the authors consider it necessary to study the ripening processes in dynamics.

Table 5. **Aldehydes** / Таблица 5. **Альдегиды**

Compound	Tib	Delikat	Nash	Kobi1	Dargavs1	Fiagdon1	Dargavs2	Fiagdon2	Kobi2
phenylacetaldehyde	24.7	14	38.3	138	—	246.3	—	34.4	30.5
nonanal	10.8	17.3	36.7	—	—	35.7	28.8	6.9	6.4
dodecanal	36.1	53.7	63.6	—	—	39.1	—	—	—
tetradecanal	18.6	82.4	—	65.9	—	35.6	—	52.7	—
octadecanal	61.9	180.2	—	159.4	595.2	138.9	—	229.2	22.1
benzaldehyde	27.5	—	—	55	—	112.8	—	9.2	—
methional	—	—	—	—	—	—	43.9	—	54.7
furfural	—	—	—	—	—	39.2	—	20.6	—

Table 6. **Lactones** / Таблица 6. **Лактоны**

Compound	Tib	Delikat	Nash	Kobi1	Dargavs1	Fiagdon1	Dargavs2	Fiagdon2	Kobi2
6-pentyl-tetrahydropyran-2-one	62.7	40.3	—	—	—	—	—	41.2	—
6-hexyl-tetrahydropyran-2-one	141.2	580.8	118.1	—	219.3	342.2	—	355.2	21.6
6-heptyl-tetrahydropyran-2-one	110.3	467.7	85.5	401	142.5	216.2	—	210.8	30.5
gamma-dodecalactone	—	34.5	—	—	—	584.8	—	—	—

Table 7. **Other compounds** / Таблица 7. **Другие компоненты**

Compound	Tib	Delikat	Nash	Kobi1	Dargavs1	Fiagdon1	Dargavs2	Fiagdon2	Kobi2
phenol	—	—	—	34.8	—	—	—	—	575.8
indole	—	—	—	711.4	—	327.2	—	55	4542.7
3-methylindole	—	—	—	—	—	—	—	—	8.9

REFERENCES

1. Kochetkova, T.V., Grabarnik, I.P., Klyukina, A.A., Zayulina, K.S., Gavirova, L.A., Shcherbakova, P.A. et al. (2023). The Bacterial microbiota of artisanal cheeses from the Northern Caucasus. *Fermentation*, 9(8), Article 719. https://doi.org/10.3390/fermentation9080719
2. Poveda, J.M., Sánchez-Palomo, E., Pérez-Coello, M.S., Cabezas, L. (2008). Volatile composition, olfactometry profile and sensory evaluation of semi-hard Spanish goat cheeses. *Dairy Science and Technology*, 88, 355–367. https://doi.org/10.1051/dst:2007021
3. Alewijn, M., Sliwinski, E.L., Wouters, J.T.M. (2005). Production of fat-derived (flavour) compounds during the ripening of Gouda cheese. *International Dairy Journal*, 15(6), 733–740. https://doi.org/10.1016/j.idairyj.2004.09.009
4. Van Leuven, I., Van Caelenberg, T., Dirinck, P. (2008). Aroma characterisation of Gouda-type cheeses. *International Dairy Journal*, 18(8), 790–800. https://doi.org/10.1016/j.idairyj.2008.01.001
5. Wang, J., Yang, Y.J., Xu, L.Y., Wang, B., Zhang, J.H., Li, B.Z. et al. (2021). Key aroma compounds identified in Cheddar cheese with different ripening times by aroma extract dilution analysis, odor activity value, aroma recombination, and omission. *Journal of Dairy Science*, 104(2), 1576–1590. https://doi.org/10.3168/jds.2020-18757
6. Zheng, X., Shi, X., Wang, B. (2021). A review on the general cheese processing technology, flavor biochemical pathways and the influence of yeasts in cheese. *Frontiers in Microbiology*, 12, Article 703284. https://doi.org/10.3389/fmicb.2021.703284
7. Centeno, J.A., Carballo, J. (2023). Current advances in cheese microbiology. *Foods*, 12(13), Article 2577. https://doi.org/10.3390/foods12132577
8. Thierry, A., Collins, Y.F., Mukdsi, M.C.A., McSweeney, P., Wilkinson, M.G., Spinnler, H.-E. (2017). Lipolysis and Metabolism of Fatty Acids in Cheese. Chapter in a book: Cheese. Academic Press, 2017. https://doi.org/10.1016/B978-0-12-417012-4.00017-X
9. Collins, Y.F., McSweeney, P., Wilkinson, M.G. (2003). Lipolysis and free fatty acid catabolism in cheese: A review of current knowledge. *International Dairy Journal*, 13(11), 841–866. https://doi.org/10.1016/S0958-6946(03)00109-2
10. Deeth, H.C. (2020). Lipase Action on Milk Fat. Chapter in a book: Dairy Fat Products and Functionality. Springer, 2020. https://doi.org/10.1007/978-3-030-41661-4_2
11. Macedo, A.C., Xavier, F. (2015). Milk Fat Hydrolysis. Chapter in a book: Encyclopedia of Membranes. Springer. 2015. https://doi.org/10.1007/978-3-642-40872-4_387-7
12. Ardö, Y., McSweeney, P., Magboul, A.A.A., Upadhyay, V.K., Fox, P.F. (2017). Biochemistry of Cheese Ripening: Proteolysis. Chapter in a book: Cheese. Academic Press. 2017. https://doi.org/10.1016/B978-0-12-417012-4.00018-1
13. Fox, P.F. (1989). Proteolysis during cheese manufacture and ripening. *Journal of Dairy Science*, 72, 1379–1400. https://doi.org/10.3168/jds.S0022-0302(89)79246-8
14. Rangel, A.H.d.N., Bezerra, D.A.F.V.d.A., Sales, D.C., Araújo, E.d.O.M., Lucena, L.M.d., Porto, A.L.F. et al. (2023). An overview of the occurrence of bioactive peptides in different types of cheeses. *Foods*, 12(23), Article 4261. https://doi.org/10.3390/foods12234261
15. Curtin, A.C., McSweeney, P.L.H. (2004). Catabolism of amino acids in cheese during ripening. *Cheese: Chemistry, Physics and Microbiology*, 1, 435–454. https://doi.org/10.1016/S1874-558X(04)80077-0
16. Yvon, M., Rijnen, L. (2001). Cheese flavour formation by amino acid catabolism. *International Dairy Journal*, 11(4–7), 185–201. https://doi.org/10.1016/S0958-6946(01)00049-8

17. Kemmer, H., Dehnhard, M., Claus, R. (1997). Screening of indoles in cheese. *Zeitschrift für Lebensmitteluntersuchung und -Forschung A*, 205, 433–436. https://doi.org/10.1007/s002170050194
18. Broadbent, J.R., Gummalla, S., Hughes, J.E., Johnson, M.E., Rankin, S.A., Drake, M.A. (2004). Overexpression of Lactobacillus casei D-hydroxyisocaproic acid dehydrogenase in cheddar cheese. *Applied and Environmental Microbiology,* 70(8), 4814–4820. https://doi.org/10.1128%2FAEM.70.8.4814-4820.2004
19. Weimer, B., Seefeldt, K., Dias, B. (1999). Sulfur metabolism in bacteria associated with cheese. *Antonie Van Leeuwenhoek,* 76, 247–261. https://doi.org/10.1023/A:1002050625344
20. Tekin, A., Hayaloglu, A.A. (2023). Understanding the mechanism of ripening biochemistry and flavour development in brine ripened cheeses. *International Dairy Journal,* 137, Article 105508. https://doi.org/10.1016/j.idairyj.2022.105508
21. Thierry, A., Maillard, M.-B., Richoux, R., Lortal, S. (2006). Ethyl ester formation is enhanced by ethanol addition in mini swiss cheese with and without added propionibacteria. *Journal of Agricultural and Food Chemistry*, 54(18), 6819–6824. https://doi.org/10.1021/jf060673m
22. Lawrence, R.C. (1963). Formation of methyl ketones as artifacts during steam distillation of Cheddar cheese and butter-oil. *Journal of Dairy Research*, 30(2), 161–170. https://doi.org/10.1017/S0022029900011341
23. Kaya, H.I., Simsek, O., Akgunoglu, O. (2023). Diversity of *Clostridium* spp. causing late blowing in Kaşar cheese and their behaviour against various antimicrobials. *International Dairy Journal,* 139, Article 105560. https://doi.org/10.1016/j.idairyj.2022.105560
24. Massouras, T., Zoidou, E., Baradaki, Z., Karela, M. (2023). Physicochemical, microbiological and sensory characteristics of white brined cheese ripened and preserved in large-capacity stainless steel tanks. *Foods*, 12(12), Article 2332. https://doi.org/10.3390/foods12122332
25. Plessas, S., Ganatsios, V., Mantzourani, I., Bosnea, L. (2021). White brined cheese production by incorporation of a traditional milk-cereal prebiotic matrix with a candidate probiotic bacterial strain. *Applied Sciences*, 11(13), Article 6182. https://doi.org/10.3390/app11136182
26. Hayaloglu, A.A., Bansal, N. McSweeney, P.L.H. (2012). Influence of brine immersion and vacuum packaging on the chemistry, biochemistry, and microstructure of Mihalic cheese made using sheep's milk during ripening. *Dairy Science and Technology,* 92, 671–689. https://doi.org/10.1007/s13594-012-0083-4

AUTHOR INFORMATION	СВЕДЕНИЯ ОБ АВТОРАХ
Affiliation	**Принадлежность к организации**
Andrey V. Gutnov, Candidate of Chemical Sciences, Docent, Department of Chemistry and Biotechnology, North-Ossetian State University 44–46, Vatutina str., Vladikavkaz, North Ossetia — Alania, 362025, Russia Tel.: +7–903–483–73–49 E-mail: gutnov@mail.ru ORCID: https://orcid.org/0000-0002-4492-7639 * corresponding author	**Андрей В. Гутнов** — кандидат химических наук, доцент, кафедра химии и биотехнологии, Северо-Осетинский государственный университет 362025, Республика Северная Осетия — Алания, Владикавказ, Ватутина, 44–46 Тел.: +7–903–483–73–49 E-mail: gutnov@mail.ru ORCID: https://orcid.org/0000-0002-4492-7639 * автор для контактов
Gennady S. Kachmazov, Candidate of Veterinary Sciences, Docent, Department of Food Technology, North-Ossetian State University 44–46, Vatutina str., Vladikavkaz, North Ossetia — Alania, 362025, Russia Tel.: +7–918–829–90–99 E-mail: kgssogutfi@yandex.ru ORCID: https://orcid.org/0009-0007-2330-7485	**Геннадий С. Качмазов** — кандидат ветеринарных наук, доцент, кафедра технологии пищевых продуктов, Северо-Осетинский государственный университет 362025, Республика Северная Осетия — Алания, Владикавказ, Ватутина, 44–46 Тел.: +7–918–829–90–99 E-mail: kgssogutfi@yandex.ru ORCID: https://orcid.org/0009-0007-2330-7485
Petrakis N. Chalikidi, Candidate of Chemical Sciences, Senior Researcher, Department of Chemistry and Biotechnology, North-Ossetian State University 44–46, Vatutina str., Vladikavkaz, North Ossetia — Alania, 362025, Russia Tel.: +7–918–709–29–81 E-mail: chalikidi@gmail.com ORCID: https://orcid.org/0000-0001-5283-6596	**Петракис Н. Чаликиди** — кандидат химических наук, старший научный сотрудник, кафедра химии и биотехнологии, Северо-Осетинский государственный университет 362025, Республика Северная Осетия — Алания, Владикавказ, Ватутина, 44–46 E-mail: chalikidi@gmail.com ORCID: https://orcid.org/0000-0001-5283-6596
Albina Y. Tuaeva, Graduate Student, Laboratory of the Molecular Center of Yeast Genetics, Kurchatov Complex of NBICS-Nature-Like Technologies (KK NBICS-PT) Center for Genomic Research "Kurchatov Genomic Center", National Research Center "Kurchatov Institute», 1, Akademika Kurchatov Square, Moscow, 123098, Russia Tel.: +7–918–704–57–90 E-mail: tuaeva_97@bk.ru ORCID: https://orcid.org/0009-0007-2214-0935	**Альбина Ю. Туаева** — аспирант, Лаборатория молекулярной генетики дрожжей, Центр геномных исследований «Курчатовский геномный центр» Курчатовского комплекса НБИКС — природоподобных технологий, Научно — исследовательский центр «Курчатовский институт» 123098, Москва, пл. Академика Курчатова, 1 Тел.: +7–918–704–57–90 E-mail: tuaeva_97@bk.ru ORCID: https://orcid.org/0009-0007-2214-0935
Contribution	**Критерии авторства**
Andrey V. Gutnov performed laboratory work, analyzed data, wrote the text of the article. **Gennady S. Kachmazov** performed laboratory work, corrected the text of the article. **Petrakis N. Chalikidi** obtained GC–MS data. **Albina Y. Tuaeva** reviewed literature sources related to the study. The authors are equally related to the writing of the manuscript and are equally responsible for plagiary.	**Гутнов Андрей Владимирович** выполнял лабораторную работу, анализировал данные, написал текст статьи. **Качмазов Геннадий Созырович** выполнял лабораторную работу, корректировал текст статьи. **Чаликиди Петракис Николаевич** получал данные GC–MS. **Альбина Юрьевна Туаева** провела обзор литературных источников по исследуемой проблеме. Авторы в равных долях имеют отношение к написанию рукописи и одинаково несут ответственность за плагиат.
Conflict of interest	**Конфликт интересов**
The authors declare no conflict of interest.	Авторы заявляют об отсутствии конфликта интересов.