Summer School and International Symposium
„Smart Sample Preparation: The Heart of Green Analytical, Food and Environmental Chemistry" within the CEEPUS network RS-1816-03-2526

BOOK OF ABSTRACTS

South-West University „Neofit Rilski"

Bachinovo education center, Blagoevgrad, Bulgaria

15-18.07.2026

Patronized by
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Published by:

University Publishing House "Neofit Rilski" -

Blagoevgrad, Bulgaria, September 2026

ISBN 978-954-00-0463-1

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Summer School and International Symposium „Smart Sample Preparation: The Heart of Green Analytical, Food and Environmental Chemistry" within the CEEPUS network RS-1816-03-2526

Conference Program

15.07.2026

12:00-14:00
Arrival and accommodation of participants

13:00 – 16:00
Registration

16:30-18:30
Workshop 1 Environmental sampling and analysis

18:45 – 19:00
Opening Ceremony
Central Auditorium of Conference Center
Chairman: Assoc. Prof Radoslav Chayrov, PhD

19:00-21:00
Welcome cocktail

16.07.2026

08:00-09:00
Breakfast

09:30-11:00
Workshop 2 Environmental sampling and analysis

11:30-13:00
Workshop 3 Environmental sampling and analysis

13:00-14:00
Lunch

Summer School and International Symposium „Smart Sample Preparation: The Heart of Green Analytical, Food and Environmental Chemistry" within the CEEPUS network RS-1816-03-2526

14:00-14:30

Plenary Lecture 1

Green Analytical Chemistry Principles1, V.Stankov Jovanović

14:30-15:00

Plenary Lecture 2

Green Analytical Chemistry Principles 2, Elma Omeragić

15:00-15:30

Plenary Lecture 3

Smart Sample-Preparation Strategies 1, Violeta Ivanova Petropulos

15:30-16:00

Plenary Lecture 4

Smart Sample-Preparation Strategies 2, Katarzyna Kalinowska-Wichrowska

16:00-16:30

Coffee break

16:30-17:00

Plenary Lecture 5

Sample preparation can make or break sensory panel Malgorzata Korzeniowska

17:00-18:00

Discussion

18:00-19:00

Coordination meeting

20:00-22:00

Official dinner

Summer School and International Symposium „Smart Sample Preparation: The Heart of Green Analytical, Food and Environmental Chemistry" within the CEEPUS network RS-1816-03-2526

17.07.2026

08:00-09:00
Breakfast

09:00-09:30
Plenary Lecture 6
Food Safety and Quality Assessment 2, Simona Vicas

09:30-11:00
Students' presentations

11:30-12:00
Coffee break

12:00-13:00
Discussion

13:00-14:00
Lunch

14:00-14:30
Plenary Lecture 7
Environmental Monitoring and Pollution Control 1, Jelena Nikolić

14:30-15:00
Plenary Lecture 8
Environmental Monitoring and Pollution Control 2, Mihaela Badea

15:00-15:30
Plenary Lecture 9
Instrumental Analysis & Data Interpretation 1, Violeta Ivanova

15:30-16:00
Plenary Lecture 10
Instrumental Analysis & Data Interpretation 2, Nicoleta Cioatera

16:00-16:30
Discussion

16:30-17:00
Coffee break

17:00-19:00
Students' presentations

19:00-20:00
Dinner

18.07.2026

08:00-09:00
Breakfast

09:00-10:00
Discussion

10:00-10:30
Coffee break

10:30-12:30
Round table

12:30-13:00
Lunch

13:30-14:30
Closing ceremony

Plenary Lectures

Green Analytical Chemistry Principles

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ABSTRACT

Green Analytical Chemistry (GAC) is integral to modern analytical science, as it seeks to mitigate the environmental impact of chemical analysis while maintaining reliable, accurate results. The central aim of GAC is to establish analytical methods that are safer, more rapid, and more sustainable by minimizing hazardous chemical usage, reducing waste, and decreasing energy consumption. These principles have become increasingly prominent in response to heightened environmental awareness and the growing demand for sustainable scientific practices.

Green Analytical Chemistry is based on several core principles, including reducing the amounts of chemicals and solvents used, replacing toxic substances with safer alternatives, minimizing sample sizes, and improving energy efficiency. Another key objective is to streamline analytical procedures by removing unnecessary steps when possible. The use of portable analytical instruments also enables on-site analysis, which reduces the need for sample transport and handling.

Recent developments in analytical chemistry have greatly supported the implementation of green principles. Miniaturized analytical systems, automation, microfluidic devices, and advanced sensor technologies achieve high-quality results while reducing resource consumption. Additionally, the use of chemometric tools and artificial intelligence for method optimization and data processing further improves both efficiency and sustainability.

Green Analytical Chemistry is widely applied in environmental monitoring, food quality control, pharmaceutical analysis, and clinical diagnostics. Its adoption improves laboratory safety, reduces operational costs, and supports sustainable development. Future progress

will rely on the ongoing development of environmentally friendly analytical methods and the education of researchers and students to apply green principles in both research and routine laboratory contexts.

Keywords: Green Analytical Chemistry, Sustainability, Green Chemistry, Analytical Methods, Miniaturization, Chemometrics

Smart Sample-Preparation Strategies 1
Innovative sample preparation techniques

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ABSTRACT

Analysis of different chemical compounds present in various types of samples, such as food, beverages, biological tissues and fluids, environmental matrices, natural waters, wastewater, medicinal plants, etc. is highly challenging. Usually, target compounds in these samples including proteins, lipids, vitamins, fatty acids, carbohydrates, polyphenols, amino acids, aroma compounds, biogenic amines, organic acids, various metabolites, elements, pesticides, etc. are present in low concentrations (μg/L) or in trace amounts (ng/L), and therefore highly selective methods for sample preparation are required for their identification as well as quantification. The mostly applied sample preparation techniques include liquid-liquid extraction (LLE), solid-phase extraction (SPE), solid-phase micro extraction (SPME), QuEChERS (quick, easy, cheap, effective, rugged, and safe), ultrasound assisted extraction (UAE), microwave-assisted extraction (MAE), supercritical fluid extraction (SFE), etc. Recent advances in analytical chemistry highlight a clear trend toward miniaturized and simplified sample preparation**,** aimed at reducing sample volumes and solvent waste. The smart and innovative sample preparation strategies are expected to be fast, sensitive and cheap, and to present an excellent accuracy and precision. Moreover, replacing the traditional and hazardous organic solvents with other friendly and green

solvents, these techniques protect the environment as well as the laboratory personnel involved in the experiments.

Keywords: sample preparation methods, extraction techniques, green solvents.

Sample preparation can make or break sensory panel

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ABSTRACT

Sensory evaluation is a powerful tool widely used in food science as well as in the food production sector. Nowadays, align with the growing consumer awareness and expectations sensory analysis is reaching new dimension becoming a necessary requirement for the industry before lunching products on the market. However, the validity of the sensory analysis data relies heavily on what happens before the product ever reaches the panelist. Preparation of the sensory samples is often treated as a daily routine, however it is a highly responsible step for the proper results feedback and it should be critically controlled. Even the most highly trained sensory panels cannot overcome the biases introduced by inconsistent serving temperatures, poor and uneven portion control, cross-contamination, or visual cues. That is why the sample preparation is such a crucial moment in this type of analyses. Together with the conditions of place and environment combined with the hidden operational factors connected with panelists they can directly impact panel performance and data integrity. The work presents common pitfalls, such as sensory fatigue and psychological bias, and contrast them with best practices for standardization and masking. By treating sample preparation not just as a logistical step, but as a rigorous scientific protocol, researchers can eliminate confounding variables, ensure reproducibility, and unlock the true sensory profile of food products.

Keywords: sensory analysis, sample preparation, sensory standards

Functional Foods and Bioactive Compounds: From Smart Sample Preparation to Quality and Safety Assessment

Functional Foods: From Bioactive Compounds to Quality and Safety Assessment

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ABSTRACT

Functional foods represent a rapidly expanding field at the interface of nutrition, food science, analytical chemistry and health-oriented product development. Beyond their nutritional value, these foods may provide physiological benefits and support health maintenance or disease-risk reduction. However, translating health-related claims into scientific evidence requires reliable analytical approaches and comprehensive quality assessment. The functional food concept is discussed, from its historical development with the Japanese FOSHU system to current international perspectives, together with the main categories of functional foods: naturally functional, intentionally modified and functionalized products. Particular attention is given to bioactive compounds, including polyphenols, carotenoids, glucosinolates, organosulfur compounds, omega-3 fatty acids, fibres, β-glucans and probiotics, which are frequently associated with the functional properties of foods. The importance of sample preparation as a key step for obtaining reliable analytical results from complex food matrices is highlighted. Because food matrices are complex and bioactive compounds may occur at low concentrations and show limited stability, extraction procedures must be selective, reproducible, efficient and environmentally sustainable. The influence of solvent polarity and the advantages of modern extraction techniques, including ultrasound-assisted extraction, microwave-assisted extraction, pressurized liquid extraction, supercritical CO_2 extraction and NADES-

based approaches, are discussed. Quality assessment should integrate extraction yield, chemical composition, stability, reproducibility and relevant functional activity. Antioxidant assays such as DPPH, ABTS, FRAP and CUPRAC provide valuable screening information but should be interpreted as complementary rather than standalone indicators of functionality. Overall, functional ingredients must demonstrate bioactivity, stability, reproducibility and safety, highlighting the importance of evidence-based evaluation of functional food products.

Keywords: functional foods; bioactive compounds; smart sample preparation; solvent polarity; green extraction; quality assessment

Environmental Monitoring and Pollution Control

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ABSTRACT

Environmental monitoring is crucial for assessing pollution level, protecting ecosystems and ensuring sustainable management of natural resources. Complexity of environmental matrices and occurrence of various contaminants at trace levels require analytical methods that are not only sensitive and reliable but also environmentally sustainable. As sample preparation is one of the most critical steps in analytical workflow, its optimization has a direct impact on analytical results quality and reliability.

Smart sample preparation strategies based on green analytical chemistry principles offer effective solutions for improving overall analytical method performance, minimizing consumption of solvent, waste generation, and energy requirements in the same time. Careful selection and optimization of extraction and preconcentration techniques enhance analyte recovery, reduce matrix effects and increase method sensitivity and reproducibility.

These approaches are particularly valuable for determination of inorganic and organic contaminants in environmental samples, especially water.

The implementation of sustainable sample preparation methodologies contributes to more efficient environmental monitoring and provides high-quality analytical data necessary for pollution assessment and environmental decision-making. The integration of innovative sample preparation techniques with green analytical principles supports the development of cost-effective, reliable, and environmentally friendly analytical methods, representing an important step toward more sustainable environmental monitoring and pollution control.

Keywords: environmental monitoring, green analytical chemistry, pollutants

Instrumental Analysis & Data Interpretation 2

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ABSTRACT

Modern research in materials science increasingly depends on the integrated use of complementary analytical techniques to establish robust structure–property relationships, particularly in complex systems. This lecture provides an overview of three essential characterisation methods—Raman spectroscopy, X-ray diffraction (XRD), and energy-dispersive X-ray spectroscopy (EDXS)—highlighting their fundamental principles, instrumentation, applications, and analytical capabilities. Raman spectroscopy is presented as a powerful tool for investigating vibrational properties, local structural arrangements, and disorder-related phenomena. XRD is examined as the primary technique for phase identification, crystal structure determination, and lattice parameter analysis, while EDXS is introduced as a valuable method for elemental analysis and spatial compositional mapping when coupled with electron microscopy. Particular attention is given to the complementarity of these techniques and their combined role in generating comprehensive

information on the vibrational, structural, and chemical characteristics of materials. Illustrative examples demonstrate the benefits of a multi-technique characterisation approach. The lecture further addresses data acquisition and interpretation strategies, representative applications, and the strengths and limitations of each method. By integrating theoretical concepts with practical considerations, the course aims to equip participants with the knowledge required to select suitable analytical techniques, critically evaluate experimental results, and effectively employ combined characterisation methodologies in advanced materials research.

Keywords: Raman spectroscopy, X-ray diffraction (XRD), Energy-dispersive X-ray spectroscopy (EDXS), materials characterization, phase analysis, multi-technique analysis

Oral Presentations

POSTER SESSION

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Mathematical modeling methods in energy – opportunities and challenges

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ABSTRACT

Energy efficiency is a key factor for the sustainable development of modern society. Along with the integration of renewable energy sources, they pose new challenges to the design, management and optimization of energy systems. These processes are accompanied by an increase in the complexity of the systems, the presence of many interconnected parameters and the need to analyze large volumes of data. In this regard, mathematical modeling is established as a basic tool for describing, analyzing and predicting the behavior of energy processes. Despite significant progress in the development of models, the choice of an appropriate method remains a challenge, as different approaches differ in accuracy and applicability. Therefore, a critical review of the main mathematical modeling methods used in the analysis and optimization of energy processes is of utmost importance.
Innovations in energy efficiency have important applications not only in residential or industrial lighting, but also in infrastructure projects such as road tunnels. The possibilities for integrating optimization algorithms, data processing methods and artificial intelligence techniques are considered, which expand the applicability of classical mathematical models. The presented analysis aims to outline the criteria for selecting an appropriate model depending on the specific engineering task and to identify the main scientific and practical challenges facing the development of mathematical modeling in the field of energy efficiency. In the current case, selecting an appropriate mathematical model in tunnel lighting in Bulgaria - especially through energy-efficient LED technologies and intelligent control, which help reduce energy costs, increase safety and create a more sustainable transport infrastructure.

Keywords: mathematical modeling, energy systems, energy efficiency, analytical models, numerical methods, optimization.

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Technologies for Simultaneous Wastewater Treatment and Metal Recovery

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ABSTRACT

Industrial wastewater streams frequently contain high concentrations of heavy metals, which pose serious environmental risks due to their non-biodegradability and tendency for bioaccumulation. A large portion of these metals are a valuable source of raw materials, the recovery of which aligns with circular economy goals. The following article evaluates contemporary approaches that bridge the gap between effective decontamination and metal reclamation. Emphasis is placed on adsorption, membrane-based processes, electrochemical recovery, ion exchange, and integrated hybrid configurations. The discussion centers on the core mechanisms driving metal separation, while also examining how variables like solution pH, initial metal loading, and operational parameters influence overall system performance. Combining distinct water treatment methods has proven effective in lowering toxic contaminant concentrations and facilitating the recovery of valuable metals. Consequently, both waste output and resource consumption are notably reduced. Yet, translating these approaches to large-scale operations highlights several unresolved challenges, primarily regarding membrane fouling, intense energy demands, and elevated operating expenses. Overcoming these barriers will require optimizing overall process efficiency, which can be achieved by pairing different treatment methods and incorporating eco-friendly materials. Aligning water purification goals with material extraction remains a highly sensible approach to support sustainable water management and secure critical raw materials.

Keywords: industrial wastewater, heavy metals, metal recovery, adsorption, circular economy; sustainable wastewater treatment.

This study was supported by the project RP-A2/26.

Summer School and International Symposium „Smart Sample Preparation: The Heart of Green Analytical, Food and Environmental Chemistry" within the CEEPUS network RS-1816-03-2526

Using virtual samples to evaluate the characteristics of a natural system

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ABSTRACT

The determination of the chemical composition of a natural system (water, minerals, etc.) is achieved by chemical and instrumental methods. The main categories of methods used are: optical methods; atomic absorption/emission spectrometry, chromatographic methods, etc. Many of these separation and detection methods are expensive. For the separation of species and the analysis of traces, laboratories use sophisticated techniques that involve high acquisition costs, maintenance, consumables and especially a complex preparation of the analyzed samples. The most important obstacle is the instability of the chemical species representative of the studied system. They can change their oxidation state or chemical structure very quickly during sampling and storage.

Alternatives to chemical and instrumental methods are the theoretical methods of analyzing the characteristics of various systems. The thermodynamic evaluation of speciation is an effective technique that currently works as a predictive and complementary tool, but with a potential to replace laboratory analyses. Even though they are less suitable for a strictly quantitative analysis, quantum methods are extremely effective in understanding how heavy metals behave in polluted waters.

The main objective of this study was to analyze how theoretical methods are suitable for determining the characteristics of a natural system (water, minerals, etc.). It was found that theoretical methods require the preparation of virtual samples in exchange for the sampling of real ones from the environment, virtual samples that do not require their preservation, their matrix can be adjusted according to the objectives of the analysis and there is no risk of their contamination.

Keywords: water, minerals, instrumental analysis, theoretical analysis, virtual samples

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Bioactivity of nanoemulsion encapsulated with Cannabis sativa through microfluidization

Nanoemulsion with Cannabis bioactive compounds

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ABSTRACT

Bioactive compounds from Cannabis sativa have significant potential as functional components in drug and food delivery systems. However, the low water solubility of these compounds creates a barrier to gaining maximum health benefits with higher bio accessibility. Therefore, this study aims to formulate and characterize a protective nanoemulsion delivery system with bioactive substances immobilized in coconut testa oil. The bioactive compounds of Cannabis (CBD) were immobilised (0mg/ml, 0.5mg/ml, and 1mg/ml) in oil-in-water emulsion with microfluidization (Coarse emulsion and after 3rd microfluidization passes) with testa oil (1%), Tween 80, Span 20 (3:2 ratio) and 1% sodium alginate. The particle size (108.63±5.00 nm), zeta potential (-15.70±1.38 mV), Lightness (75.80±4.97), and creaming index (0.00±0.00%) of the emulsion were significantly changed with the microfluidic cycles and CBD concentrations. The DPPH scavenging (2,2-diphenyl-1-picrylhydrazyl) activity (224.90±39.79 Trolox Eq mg/L), ABTS activity (2157.36±124.58 Trolox Eq mg/L), FRAP (66.99±5.48 Trolox Eq mg/L) and reducing power (12.95±0.99 Ascorbic acid Eq mg/L) of nano emulsion significantly increased with the 3rd microfluidic cycle, with 1mg/ml CBD concentration showing higher stability for future food applications.

Keywords: Antioxidant properties, Cannabis sativa L., nano emulsion, microfluidic cycles

Development of a UHPLC-MS/MS Smart Analytical Workflow for Polyphenols Characterization in Experimental Red Wine

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ABSTRACT

Polyphenols are important bioactive compounds that contribute to the antioxidant capacity, sensory properties, and the overall quality of red wines. An accurate determination of these compounds is essential for food quality assessment and authenticity studies. The present study aimed to characterize the polyphenolic profile of an experimental red wine using a targeted UHPLC-MS/MS method and to evaluate the analytical performance of the method through a spike-recovery approach.

The analyses were performed on a Shimadzu UHPLC system coupled to an LCMS-8045 triple quadrupole mass spectrometer with an electrospray ionization source. Chromatographic separation was achieved on a reversed-phase C18 column using a water–methanol gradient containing 0.05% formic acid. Quantification was based on external

calibration curves prepared over the 50–5000 ng/mL range. The calibration data showed a good linear response, with R^2 values above 0.998.

Several polyphenolic compounds were detected in the experimental wine, with caffeic acid (4167.78 ng/mL), gallic acid (3686.10 ng/mL), quercetin (2639.52 ng/mL), and 3,5-dihydroxybenzoic acid (2052.34 ng/mL) occurring at the highest concentrations. p-Coumaric acid, ferulic acid, rutin, resveratrol, and rosmarinic acid were also identified. Following sample fortification, the concentrations of the target compounds increased as expected, and the recovery results indicated that the method was suitable for their quantitative determination.

The proposed UHPLC-MS/MS workflow provides a rapid, sensitive, and reliable approach for profiling wine polyphenols and represents a valuable tool for food quality assessment while supporting the principles of green analytical chemistry through reduced analysis time and solvent consumption.

Keywords: UHPLC-MS/MS; wine; polyphenols; food quality assessment; spike recovery

Declaration on the use of AI tools

During the preparation of this abstract, the authors used ChatGPT to assist with language editing, grammar correction, and improvement of clarity. The authors reviewed and revised the text and take full responsibility for the final content of the abstract.

Exploring New Approach Methodologies (NAMs) Across Sectors: the Role of In Vitro Toxicology in Food, Environmental, and Chemical Analysis

Diana Haj Ali[1,2,3], Dorina-Elena Coricovac[1,2*] , George-Andrei Drăghici[1,2], Iasmina-Alexandra Predescu[1,2,3], Cristina Adriana Dehelean[1,2]

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ABSTRACT

Toxicological assessment of food and environmental contaminants ensures chemical safety, hazard identification and human health protection. The increasing number of chemicals used for food industry, pharmaceutical sector, and agriculture, displaying complex mechanisms of action, has highlighted the limitations of conventional toxicity testing approaches, particularly those relying on animal models [1,2]. Consequently, in many countries, ethical concerns associated with animal experimentation have encouraged the replacement of conventional testing methods with New Approach Methodologies (NAMs), in accordance to the 3Rs' concept. Furthermore, conventional toxicity testing presents several limitations, including limited biological relevance, long experimental periods, and high financial and resource demands [2].

NAMs comprise advanced methods and technologies developed to improve toxicological testing by addressing the limitations of conventional animal-based models and providing mechanistic information and biological insights. In this context, some of the most advanced NAMs include in vitro models as 2D and 3D cell cultures, spheroids, organoids, microphysiological systems (MPS), organ-on-chip technologies, and multi-omics approaches. These methodologies offer several advantages over conventional testing, including the ability to perform reproducible analyses, improved human biological relevance, and, in the case of advanced 3D models, a closer representation of the structural and functional complexity of human tissues. Furthermore, they provide valuable mechanistic insights into chemical toxicity by enabling the investigation of changes in

gene expression, protein regulation, metabolic pathways [3,4]. Nevertheless, NAMs still present certain limitations.

Overall, even though these limitations have been highlighted, research is continuing towards finding ways to overcome them and improve the application of these NAMs in toxicological risks studies.

Keywords: NAMs, cell cultures, spheroids, organoids, microphysiological systems (MPS), organ-on-chip

Refrences:

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Declaration on the use of AI tools

During the preparation of this abstract, the authors used an AI-based tool (ChatGPT) to edit the language, correct grammar, improve clarity of the text. All content generated using AI was carefully checked and revised by the authors.

Summer School and International Symposium „Smart Sample Preparation: The Heart of Green Analytical, Food and Environmental Chemistry" within the CEEPUS network RS-1816-03-2526

Analysis of Whey Protein Concentrate Hydrolysates Obtained Using Different Proteolytic Enzymes

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ABSTRACT

Whey protein concentrate (WPC) is an excellent source of high-quality proteins that can be converted into bioactive peptides through enzymatic hydrolysis. The biological properties of the resulting hydrolysates are strongly influenced by the specificity of the proteolytic enzyme used. The aim of this study was to compare WPC hydrolysates produced using four proteolytic enzymes: pepsin, trypsin, chymotrypsin, and a serine protease isolated from *Cucurbita ficifolia*. A 2% WPC solution was subjected to enzymatic hydrolysis, and the obtained hydrolysates were characterized by determining the degree of hydrolysis, free amino group content, protein profile (SDS-PAGE and densitometric analysis), antioxidant activity (DPPH, ABTS and FRAP), and inhibitory activity against angiotensin-converting enzyme (ACE), dipeptidyl peptidase IV (DPP-IV), and α-glucosidase. All enzymes promoted progressive protein hydrolysis, although differences in hydrolysis efficiency were observed among proteases. The greatest DPPH radical scavenging activity was recorded for pepsin hydrolysates after 0.5 h of hydrolysis (202.6 ± 34.3 µmol TE/mL). In contrast, hydrolysates obtained with plant-derived enzyme maintained relatively high antioxidant activity throughout the process. Results demonstrate that serine protease isolated from *Cucurbita ficifolia* is a promising alternative to conventional commercial proteases for the production of bioactive WPC hydrolysates and highlights its potential application in the development of innovative functional food ingredients.

Keywords: ***Whey protein concentrate; enzymatic hydrolysis; protein hydrolysates; serine protease;***

Summer School and International Symposium „Smart Sample Preparation: The Heart of Green Analytical, Food and Environmental Chemistry" within the CEEPUS network RS-1816-03-2526

Long-term changes in the species composition and structure of natural stands featuring pedunculate oak (*Quercus robur* L.) in protected areas of north-eastern Poland

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ABSTRACT

Currently, the insufficient density of natural regeneration, particularly of the main forest-forming tree species, poses a threat to the long-term sustainability of forest ecosystems. The aim of this study was to analyse long-term changes occurring in forest stands of selected nature reserves in north-eastern Poland with a substantial proportion of pedunculate oak, located on fertile fresh forest sites, and to assess the prospects for maintaining the conservation objectives of these protected areas.

The study was conducted between 2017 and 2020 in the Stara Dębina, Grąd Radziwiłłowski, Koryciny, Kalinowo, and Rycerski Kierz Nature Reserves. Permanent research plots established in the 1970s were relocated and re-established in the field. Trees with a diameter greater than 5 cm at 1.3 m above ground level were measured. Regeneration was assessed on smaller circular subplots by recording seedling and sapling height, species identity, and evaluating their vitality and quality.

Stand density and stand basal area changed over the study period. The proportion of oak declined. A marked reduction in the density of young trees with DBH from 0.1 to 5.0 cm was observed. No natural oak regeneration was recorded during the final measurement. Regeneration densities on most plots did not exceed 3,500 individuals ha^{-1} and consisted predominantly of hornbeam or maple seedlings.

The observed changes in the investigated stands are characteristic of mature forest ecosystems. Although the current density of the younger tree generation does not guarantee successful stand regeneration, there are indications that it may still be too early to expect the establishment of abundant natural regeneration.

Keywords: natural regeneration, tree stands, environmental changes, long-term changes

Summer School and International Symposium „Smart Sample Preparation: The Heart of Green Analytical, Food and Environmental Chemistry" within the CEEPUS network RS-1816-03-2526

The Use of Electrochemical Impedance Spectroscopy for Electrode Characterization: From Nyquist Plots to Interfacial Insight

EIS for Electrode Characterization

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ABSTRACT

Electrochemical impedance spectroscopy (EIS) is an important technique for investigating electrode interfaces, yet its full potential is often underused in electroanalytical chemistry. In many sensor-related studies, EIS is primarily used to present Nyquist plots and estimate charge-transfer resistance in the presence of a redox probe, while other valuable representations, such as Bode plots, phase-angle analysis, complex capacitance, and measurements in the supporting electrolyte alone, are frequently overlooked. However, these approaches can provide important information about double-layer capacitance, surface heterogeneity, ion accessibility, interfacial stability, and the influence of electrode modification or activation on charge-transfer processes.

In this work, EIS is presented as a comprehensive tool for characterizing selected carbon-based electrode systems: a 5% CeO_2-modified carbon paste electrode, an SWCNT-

modified carbon paste electrode, and an MWCNT-modified glassy carbon electrode. The electrodes were examined under both non-Faradaic conditions and Faradaic conditions. The comparison of impedance responses enabled evaluation of changes in solution resistance, effective capacitance, charge-transfer resistance, constant-phase-element behavior, and diffusion-related features.

The results show that electrode modification and electrochemical activation can change interfacial properties, which cannot always be fully captured by voltammetric measurements alone. When it's interpreted through complementary plots and equivalent-circuit analysis, EIS provides important insight into the physicochemical and electrochemical behavior of sensor interfaces.

Keywords: impedance, characterization, modification, nanotubes, ceria, activation

ZrO_2-Graphite Based Sorbents and Photocatalysts for Dye Removal: The Role of Sample Preparation and Instrumental Characterization

ZrO_2-Graphite Sorbents and Photocatalysts

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ABSTRACT

The growing presence of synthetic dyes in wastewater is a serious environmental concern because of their persistence, complex chemical structures, and potential toxicity. Among other treatment approaches, sorption and photocatalytic degradation of dyes have attracted considerable attention as efficient, relatively simple, and environmentally relevant methods. In this context, zirconium dioxide (ZrO_2) is an interesting material due to its chemical stability, surface reactivity, and potential for remediation. Combining ZrO_2 with graphite could improve material performance by influencing surface properties, dispersion, and interactions with dye molecules. This work focuses on the preparation and evaluation of ZrO_2-graphite based materials for removing dyes from aqueous solutions via sorption and photocatalytic processes.Special attention was given to sample preparation, since the conditions of synthesis, component ratio, dispersion of particles, and characteristics of the surface can strongly affect the interaction between the material and dye molecules. The prepared materials were characterized using appropriate instrumental techniques in order to investigate their morphology, structural properties, surface features, and potential changes upon interaction with dyes. Such characterization was important for understanding the mechanisms responsible for dye removal, including surface adsorption, electrostatic interactions, and photocatalytic degradation under irradiation. This work aims to provide more comprehensive insight into the performance of ZrO_2-graphite based sorbents and photocatalysts.

Keywords: zirconia, graphite, sorption, degradation, photocatalysis, preparation

Evaluation of Various Sorbents for Antibiotic Analysis

Evaluation of Sorbents for Antibiotic Analysis

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ABSTRACT

The increasing occurrence of antibiotic residues in aquatic environments has highlighted the need for efficient, cost-effective, and environmentally friendly sample preparation techniques. This study evaluated the performance of two natural sorbents, diatomaceous earth and clinoptilolite, in a dispersive solid-phase extraction (dSPE) procedure for the determination of cephalexin in environmental water samples by HPLC-DAD.

Four river water samples collected in Serbia were spiked with cephalexin at concentrations of 0.1 and 0.5 mg L^{-1}. The influence of sample pH on extraction performance was evaluated by comparing untreated samples with samples adjusted to pH 3 using a phosphate buffer. Method performance was assessed based on recovery values obtained for different sorbents and sample preparation parameters. The obtained LOD and LOQ values were 0.01 and 0.03 mg L^{-1}, respectively.

Recovery values ranged from **74.13 to 115.26%** for samples extracted without pH adjustment, whereas pH-adjusted samples exhibited improved and more consistent recoveries, ranging from **81.03 to 114.58%**. The results demonstrated that extraction efficiency depended on the sorbent type, sample matrix, and pH conditions, while pH adjustment improved the reliability of cephalexin determination. The evaluated natural sorbents showed promising potential for dSPE-based sample preparation, providing a simple and sustainable approach for the analysis of antibiotic residues in environmental water samples.

Keywords: antibiotics; dSPE ; sorbents

Green Sample Preparation: A Key Step Toward Sustainable Analytical Chemistry

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ABSTRACT

Sample preparation is a critical stage in the analytical process, significantly influencing the accuracy, sensitivity, and reliability of analytical results. However, it is often the most time-intensive step and the primary source of chemical waste in laboratory settings. Green sample preparation seeks to minimize solvent consumption, energy usage, and waste generation while preserving high analytical performance.

Conventional sample preparation methods typically require substantial sample quantities, extended extraction times, and considerable volumes of organic solvents. Green Analytical Chemistry advocates replacing these procedures with safer, faster, and more efficient alternatives. Contemporary green sample preparation techniques emphasize miniaturization, automation, and the utilization of environmentally benign solvents and materials.

Numerous innovative techniques have been developed- solid-phase microextraction (SPME), stir bar sorptive extraction (SBSE), dispersive liquid–liquid microextraction (DLLME), single-drop microextraction (SDME), ultrasound-assisted extraction, and microwave-assisted extraction. These approaches decrease solvent consumption, reduce extraction time, and enhance extraction efficiency. Additionally, natural deep eutectic solvents, supercritical fluids, and bio-based extraction media are increasingly regarded as promising alternatives to traditional organic solvents.

Green sample preparation has been effectively implemented in environmental, food, pharmaceutical, forensic, and clinical analyses. Future advancements are expected to

prioritize novel sorbent materials, portable extraction devices, automation, and the integration of artificial intelligence for method optimization. Through the combination of sustainable technologies and high analytical standards, green sample preparation occupies a central position in modern analytical chemistry and supports environmentally responsible laboratory practices.

Keywords: Green Sample Preparation, Microextraction, Sustainable Extraction, Sample Preparation, Green Solvents, Analytical Chemistry

Valorization of Molasses as an Alternative Carbon Source in Kombucha Fermentation: A Physicochemical Study

Molasses-Based Kombucha Fermentation

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ABSTRACT

Kombucha fermentation usually relies on sweetened tea as the main substrate for the activity of a symbiotic culture of bacteria and yeasts. In this study, beet molasses was tested as a partial or total replacement for refined sugar, considering its content of fermentable carbohydrates, minerals, pigments, and other compounds that may affect the properties of the final beverage. Three kombucha variants were prepared using black tea as the fermentation base: a control with refined sugar, a formulation with beet molasses, and a formulation containing both sugar and molasses. After inoculation with raw kombucha starter, the samples were fermented for seven days at room temperature. The fermentation process was followed by monitoring pH, total soluble solids, titratable acidity, optical density, and antioxidant capacity using the DPPH assay. During fermentation, all samples showed a decrease in pH and an increase in titratable acidity, indicating the progression of acidification. Total soluble solids decreased, suggesting substrate consumption by the microbial culture. The molasses-containing variants showed more evident changes in

optical density, probably due to the natural colour of molasses, microbial development, and compounds formed during fermentation. Antioxidant capacity also increased during the fermentation period, suggesting an improvement in the functional profile of the beverages. These preliminary results indicate that beet molasses can be used as an alternative carbohydrate source in kombucha fermentation. Its use may contribute to the valorization of an agro-industrial by-product and to the development of fermented beverages with improved functional characteristics.

Keywords: kombucha; beet molasses; fermentation; antioxidant capacity; physicochemical properties

Green Extraction and Stability Evaluation of Natural Pigments from Red Onion
Extraction and Stability of Red Onion Anthocyanins

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ABSTRACT

Red onion (*Allium cepa* L.) contains anthocyanins responsible for its red–purple colour, with potential applications as natural food pigments. This study evaluated the influence of drying temperature on anthocyanin recovery and colour stability in red onion powders. Fresh red onion bulbs were sliced and dried at different temperatures ranging from 40 to 80°C, then milled into powders. Anthocyanins were extracted using food-grade ethanol. The samples were evaluated immediately after processing and after 30 days of storage at room temperature. Total monomeric anthocyanin content was determined, and colour stability was assessed using CIE L*, a*, and b* parameters. Preliminary results showed that drying temperature influenced both pigment recovery and colour stability. Moderate drying temperatures, particularly in the 50–60°C range, were associated with better anthocyanin recovery and improved preservation of red–purple colour during storage.

Compared with samples dried at higher temperatures, these conditions showed a more favourable balance between pigment retention and colour stability. In contrast, powders dried at 70–80°C showed more pronounced changes in the b* coordinate, suggesting a tendency toward yellow–brown colour development during storage. Overall, the results indicate that drying temperature is an important factor affecting the recovery and stability of anthocyanins from red onion powders. The use of ethanol as a food-grade extraction solvent may represent a suitable green approach for obtaining natural pigment-rich extracts from dried red onion.

Keywords: Allium cepa L*, anthocyanins; red onion powder; drying temperature; colour stability; ethanol extraction.*

Comparative Evaluation of 70% and 96% Ethanol for the Green Extraction of Bioactive Compounds from Wild Garlic (*Allium ursinum* L.)

Ethanol Concentration Effect on Wild Garlic Bioactive Extraction

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ABSTRACT

The recovery of phenolic compounds and antioxidant constituents from *Allium ursinum* L. leaves depends strongly on the polarity of the extraction solvent. In this study, two ethanol-based food-grade solvents were compared in order to select a suitable green extraction system for dried wild garlic leaves. Dried and ground *A. ursinum* leaves were extracted using 70% and 96% ethanol under the same experimental conditions. The obtained extracts were evaluated for total phenolic content using the Folin–Ciocalteu method, antioxidant

activity by DPPH, ABTS and FRAP assays, and chlorophyll content by spectrophotometric analysis.The preliminary results showed that the hydroethanolic solvent was more efficient than 96% ethanol. The extract obtained with 70% ethanol contained 8.86 mg GAE/g DW, compared with 5.25 mg GAE/g DW for the 96% ethanol extract, representing an increase of 68.7%. A similar trend was observed for antioxidant capacity. DPPH radical scavenging activity was approximately 70.3% higher in the 70% ethanol extract, while FRAP values increased by 12.2% compared with the extract obtained using 96% ethanol. Overall, the results indicate that the presence of water in the extraction solvent improves the recovery of phenolic compounds and enhances the antioxidant potential of *A. ursinum* extracts. Therefore, 70% ethanol may represent a more appropriate solvent for obtaining wild garlic extracts intended for further food-related applications.

Keywords: Allium ursinum L.; *ethanol extraction; phenolic compounds; antioxidant activity; chlorophylls; green extraction.*

Evaluation of Three-Step and Five-Step Sequential Extraction Procedures in Zeolite Analysis of Heavy Metals

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ABSTRACT

The presence and mobility of heavy metals in natural zeolites are very important factors for environmental protection and for agricultural use. For environmental protection it is very important to know concentration of heavy metals and their mobility. Sequential extraction procedures are widely used for these types of analysis because this procedures give us information about mobility, bioavailability and concentration of metals. In this

work sequential procedures were used in order to determine concentration of toxic metals and their mobility and their bioavailability in natural clinoptilolite. Two types of sequential extraction procedures were performed on natural clinoptilolite: three step and five step, and the results gotten from both procedures were compared. The concentration of heavy metals in individual fractions were measured by ICP-OES. The two procedures differed in their mobility to separate metals from zeolite structure. The results revealed that three step procedure is suitable for fast analysis and determine bioavailability of heavy metals, while five step procedure gave us more detailed information of distribution of heavy metals in sample. The concentration of metals in each fraction in both procedures were low which indicated low mobility and low environmental risk, only concentration of Cd and Hg exceeded limit concentration in residual fraction. The evaluation of both procedures revealed that both procedures are suitable for environmental protection investigations, where three step is suitable for quick analysis, while five step gives more information of distribution of metals through zeolite structure.

Keywords:sequential extraction, heavy metals, ICP-OES

Differential Regulation of Angiogenesis-Related Genes by Melissa officinalis Extracts in the Chick Chorioallantoic Membrane Model

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ABSTRACT

Melissa officinalis (MO) is a medicinal plant widely recognized for its antioxidant, anti-inflammatory, and potential anti-angiogenic properties, largely attributed to its rich content of phenolic compounds and bioactive metabolites. The present study evaluated the effects of ethanolic extracts obtained from commercially available and cultivated *M. officinalis* on the expression of genes involved in angiogenesis and cell survival using the chick chorioallantoic membrane (CAM) model. Relative gene expression analysis performed by quantitative real-time PCR demonstrated a differential modulation of key angiogenic markers following treatment with MO extracts. VEGF expression was reduced in the MO4-treated sample compared with the untreated CAM control, suggesting suppression of pro-angiogenic signaling. In contrast, PIK3CA expression was upregulated, indicating activation of intracellular pathways associated with cell survival and adaptive cellular responses. Furthermore, FGF expression was downregulated in both treated samples, with a more pronounced decrease observed in MO2 than in MO4, supporting an inhibitory effect on fibroblast growth factor-mediated angiogenesis. These findings indicate that *M. officinalis* extracts differentially regulate molecular pathways involved in vascular development, suggesting a favorable anti-angiogenic profile while maintaining cellular adaptive mechanisms. Collectively, the results highlight the therapeutic potential of *M. officinalis* as a promising natural source of bioactive compounds for the modulation of angiogenesis and encourage further mechanistic and translational investigations.

Keywords: Melissa officinalis; angiogenesis; gene expression; RT-qPCR; chick chorioallantoic membrane (CAM); PIK3CA

Summer School and International Symposium „Smart Sample Preparation: The Heart of Green Analytical, Food and Environmental Chemistry" within the CEEPUS network RS-1816-03-2526

Sequential Analysis of Clinoptilolite: A Comparison Between Three-Step and Five-Step Procedures for Essential Metals

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ABSTRACT

Natural cliniptilolite has the ability to retain and slowly release adsorbed substances and is recognized for its ion exchange and soil supplementation capacities. A good assessment of the distribution of nutrients in a mineral requires the application of analytical methods that can distinguish a certain number of geochemical fractions. Total elemental analysis cannot provide information on the mobility and bioavailability of elements, while sequential extraction can, providing a more detailed assessment. Determination of the distribution of essential metals calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), lead (Fe), manganese (Mn), zinc (Zn), copper (Cu) and others in natural clinoptilolite was done by sequential extraction and further analysis using induced-coupled plasma optical emission spectrometry (ICP-OES). Three- and five-step extraction are two variants of this analysis, and by comparing them we get information about the differences in the efficiency of extraction from fractions using different reagents. The three-step procedure provided a faster assessment of the more mobile elements compared to the more detailed results obtained from the five-step procedure. The final results showed that the essential elements are found mostly in the stable mineral fraction, which is excellent for application in agriculture and the environment. The extraction results of Ca, Mg, Si, and Zn were several times higher using the three-step procedure, while the five-step procedure was better for K and Na. These differences provide a lot of help in choosing the appropriate procedure, in accordance with the quality of the information, the speed and time of work and the use of reagents.

Keywords:sequential extraction, essential elements, ICP-OES

POLYPHENOL PROFILE IN NATURAL PODUCTS

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ABSTRACT

Natural bee products, such as honey and propolis, play a vital role in supporting the human immune system and serve as rich sources of dietary antioxidants and bioactive nutrients.
This study aimed to investigate the effects of enriching honey with alcoholic propolis extracts of varying geographical and botanical origins on its antioxidant capacity and polyphenolic profile, ensuring that core honey quality parameters remained uncompromised.
A total of 21 honey samples and 10 alcoholic propolis extract samples were collected, primarily from the Federation of Bosnia and Herzegovina. Total antioxidant activity was evaluated using the Ferric Reducing Antioxidant Power (FRAP) assay. Total polyphenol content was measured via the Folin-Ciocalteu (FC) technique, while the individual polyphenolic profile was characterized using High-Performance Liquid Chromatography (HPLC).
While honey alone proved to be an excellent dietary source of antioxidants, the addition of alcoholic propolis extract significantly enhanced its antioxidant potential, yielding an average increase of 82.65%. HPLC and FC analyses confirmed that this enrichment significantly boosted the concentrations of plant secondary metabolites, including phenolic glycosides, free phenols, flavonoids, catechins, and anthocyanins. Specifically, key phenolic acids such as p-hydroxybenzoic, p-coumaric, cinnamic, gallic, ferulic, and caffeic acids were successfully identified and enriched within the samples.
The incorporation of propolis extracts into honey provides a powerful synergetic effect, dramatically increasing its functional antioxidant capacity and enriching its polyphenol profile without degrading its original quality.

Keywords: antioxidants, honey, propolis, polyphenols, immunity, bioactive supstances

Spectroelectrochemical and Kinetic Analysis of Thiamine Degradation in Halide Media: Influence of Silver Nanoparticles

Ag Nanoparticles in Thiamine Degradation

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ABSTRACT

The electrochemical stability and reactivity of biologically active molecules are strongly governed by the composition of the supporting electrolyte and by interfacial processes occurring at electrode surfaces. In this work, the spectroelectrochemical behavior and degradation kinetics of thiamine (Th, vitamin B1) were systematically investigated on platinum electrodes in halide-containing media (NaF, NaCl, NaBr), both in the absence and presence of silver nanoparticles (AgNPs). A multi-technique approach integrating UV-Vis spectrophotometry, cyclic voltammetry, chronopotentiometry, and kinetic modeling was employed to elucidate the influence of halide ions and nanoparticle–molecule interactions under both steady-state and dynamic electrochemical conditions. Tauc plot analysis revealed pronounced halide-dependent variations in optical band gaps, indicating strong electronic coupling between thiamine and AgNPs and reflecting significant changes in interfacial charge-transfer dynamics. Electrochemical and kinetic analyses demonstrate that thiamine degradation proceeds via competing pathways, including direct surface-controlled oxidation and indirect halogen-mediated processes, with their relative contributions governed by the nature of the halide. Silver nanoparticles exhibit a dual role, enhancing oxidation under potentiodynamic conditions while exerting an overall inhibitory effect during prolonged galvanostatic electrolysis, likely due to interfacial sequestration

phenomena. These findings provide mechanistic insight into the complex interplay between halide ions, nanoparticles, and thiamine at electrochemical interfaces, highlighting key factors that control redox behavior, degradation kinetics, and molecular stability. Such understanding is essential for the rational design of controlled electrochemical and bioelectrochemical systems.

Keywords: Thiamine degradation, Spectroelectrochemistry, Silver nanoparticles, Halide media, Electrochemical kinetics

Comparative Study of Polyphenolic Content and Antioxidant Activity of Nettle Root and Seed Extracts

Polyphenolic Content and Antioxidant Activity of Nettle Extracts

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ABSTRACT

Nettle (Urtica dioica L.) and preparations based on this plant have long been used in traditional medicine due to their beneficial biological properties. Different parts of the plant contain numerous bioactive compounds, particularly polyphenols and flavonoids, which are associated with antioxidant activity.

In this study, aqueous, methanolic, ethanolic, and acetone extracts of nettle roots and seeds collected in April 2026 in the Tuzla region were prepared using two extraction techniques: maceration and ultrasound-assisted extraction.

The extracts were analyzed for total polyphenolic content, total flavonoid content, and antioxidant activity using the FRAP and DPPH assays. The results demonstrated a clear relationship between the content of bioactive compounds and antioxidant activity. Ultrasound-assisted extraction generally provided higher extraction efficiency than maceration, while water and methanol proved to be the most effective extraction solvents.

Nettle seed extracts exhibited higher polyphenolic and flavonoid contents, as well as stronger antioxidant activity than nettle root extracts.

Keywords: Urtica dioica, polyphenols, flavonoids, maceration, ultrasonic- assisted extraction, natural antioxidants

Determination of aroma profile of grape brandies applying GC-FID technique

GC-FID analysis of aroma in brandy

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ABSTRACT

The GC-FID technique was applied to analyze the aroma profile in 52 grape brandies from various varieties, produced in domestic conditions in the Republic of N. Macedonia. The following compounds have been identified and quantified in the samples: acetaldehyde, 1-butanol, isobutanol, hexanol, 2-methyl-1-butanol, methanol, 1-propanol, 2-phenylethanol, ethyl acetate, ethyl lactate, isoamyl acetate and propyl acetate. Separation of the compounds was performed on an HP 6890 N gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) using the column ZB WAX (30 m x 320 µm x 1 µm). The applied temperature program was as follows: 40 °C 1 min., ramp 4 °C/min., final temperature 184 °C, total time 37 min. Ethyl acetate was the dominant compound within the group of esters. Among the alcohols, 1-propanol (which serves as an indicator of contamination) and 2-methyl-1-butanol (which contributes to a fruity aroma) were present in relatively high amounts. Moreover, 2-methyl-1-butanol was the most prevalent compound in the aromatic Temjanika grape brandy.

Keywords: grape brandy, aroma compounds, GC-FID.