**UDC 543.544.5.068.7; 615.074**

***Oleksandr SYROTCHUK***
*Candidate of Chemical Sciences, Assistant at the Department of Medicinal Chemistry and Toxicology, Bogomolets National Medical University, Shevchenko Boulevard, 13, Kyiv, Ukraine, 01601 (Syrotchuk@gmail.com)*
***ORCID:*** *0000-0003-3262-4224*
***SCOPUS:*** *57156668400*

***Olga GLUSHACHENKO***
*Candidate of Chemical Sciences, Associate Professor at the Department of Medicinal Chemistry and Toxicology, Bogomolets National Medical University, Shevchenko Boulevard, 13, Kyiv, Ukraine, 01601 (g_o_a@ukr.net)*
***ORCID:*** *0000-0002-7017-3914*
***SCOPUS:*** *58484886100*

***Nataliia KHROMOVA***
*Analyst Chemist, State Enterprise "Central Laboratory for Quality Control of Medicines and Medical Products", Kudriavska Street, 10-H, Kyiv, Ukraine, 04053 (nnatasha_art@ukr.net)*
***ORCID:*** *0009-0002-4879-0729*

***Iryna DIDUKH***
*Candidate of Chemical Sciences, Deputy Director State Enterprise "Central Laboratory for Quality Control of Medicines and Medical Products", Kudriavska Street, 10-H, Kyiv, Ukraine, 04053 (irdidukh@gmail.com)*
***ORCID:*** *0009-0001-0194-8704*
***SCOPUS:*** *6508107162*

***Iryna NIZHENKOVSKA***
*Doctor of Medicine, Professor, Head of the Medicinal Chemistry and Toxicology Department, Bogomolets National Medical University, Shevchenko Boulevard, 13, Kyiv, Ukraine, 01601 (iryna.nizhenkovska@gmail.com)*
***ORCID:*** *0000-0001-5065-3147*
***SCOPUS:*** *6506649044*

**To cite this article:** Syrotchuk O., Glushachenko O., Khromova N., Didukh I., Nizhenkovska I. (2025). Vstanovlennia markeriv yakosti nadkrytychnykh $CO_2$ -ekstraktiv prosa [Determination of quality markers in supercritical $CO_2$. extracts of millet]. *Fitoterapiia. Chasopys – Phytotherapy. Journal*, 2, 218–227, doi: https://doi.org/10.32782/2522-9680-2025-2-218

## DETERMINATION OF QUALITY MARKERS IN SUPERCRITICAL $CO_2$ EXTRACTS OF MILLET

***Actuality.*** *The article presents the results of a study on $CO_2$ extracts of millet to determine their quality using various analytical methods. Establishing quality markers for millet extracts is crucial for the food, pharmaceutical, and cosmetic industries to assess raw material quality.*

*The purpose of this study is to identify and establish reliable quality markers for assessing the composition and value of millet supercritical $CO_2$ extracts.*

***Material and methods.*** *A Shimadzu GCMS-QP2010 Ultra gas chromatograph with a DB-WAX column (30 m × 0,32 mm × 0,25 µm) was used to identify extract components. The fatty acid composition of the extracts was determined using a GC-2010AF Plus gas chromatograph with a flame ionization detector and an HP-INNOWAX column (30 m × 0,53 mm × 1 µm). Quantification of squalene was performed using a Shimadzu Nexera LC-30 liquid chromatograph with a photometric detector and a Symmetry C18 column (150 × 4,6 mm, 3,5 µm).*

***Research results.*** *The following compounds were identified in the millet extract: palmitic, stearic, oleic, linoleic, and linolenic fatty acids, as well as squalene and miliacin. The fatty acid composition of the oil in the millet extract was considered a potential quality marker. It was found that the fatty acid composition in the selected samples was nearly identical, except for one commercial extract, which showed a significant difference in fatty acid ratios. An eco-friendly method for quantifying squalene using liquid chromatography with ethanol and isopropanol as solvents was developed, reducing environmental impact. Significant differences in squalene content were observed among the extracts: 1,05, 1,01, and 0,45% in flour, meal, and groats extracts, respectively, while only trace amounts of squalene were found in one commercial extract. Miliacin was detected in all extracts except for the same low-quality commercial sample.*

***Conclusion.*** *Based on the results obtained, the fatty acid composition can be considered an indicator of millet extract quality. Squalene and miliacin were identified as key quality markers of $CO_2$ millet extracts.*

***Key words:*** *$CO_2$ extraction, millet, squalene, miliacin, fatty acid composition.*

***Олександр СИРОТЧУК***
*кандидат хімічних наук, асистент кафедри хімії ліків та лікарської токсикології, Національний медичний університет імені О.О. Богомольця, бульв. Тараса Шевченка, 13, м. Київ, Україна, 01601 (Syrotchuk@gmail.com)*
***ORCID:*** *0000-0003-3262-4224*
***SCOPUS:*** *57156668400*

***Ольга ГЛУШАЧЕНКО***
*кандидат хімічних наук, доцент кафедри хімії ліків та лікарської токсикології, Національний медичний університет імені О.О. Богомольця, бульв. Тараса Шевченка, 13, м. Київ, Україна, 01601 (g_o_a@ukr.net)*
***ORCID:*** *0000-0002-7017-3914*
***SCOPUS:*** *58484886100*

***Наталія ХРОМОВА***
*хімік-аналітик, ДП «Центральна лабораторія з аналізу якості лікарських засобів і медичної продукції», вул. Кудрявська, 10-Г, м. Київ, Україна, 04053 (nnatasha_art@ukr.net)*
***ORCID:*** *0009-0002-4879-0729*

***Ірина ДІДУХ***
*кандидат хімічних наук, заступник директора, ДП «Центральна лабораторія з аналізу якості лікарських засобів і медичної продукції», вул. Кудрявська, 10-Г, м. Київ, Україна, 04053 (irdidukh@gmail.com)*
***ORCID:*** *0009-0001-0194-8704*
***SCOPUS:*** *6508107162*

***Ірина НІЖЕНКОВСЬКА***
*доктор медичних наук, професор, завідувач кафедри хімії ліків та лікарської токсикології, Національний медичний університет імені О.О. Богомольця, бульв. Тараса Шевченка, 13, м. Київ, Україна, 01601 (iryna.nizhenkovska@gmail.com)*
***ORCID:*** *0000-0001-5065-3147*
***SCOPUS:*** *6506649044*

**Бібліографічний опис статті:** Сиротчук О., Глушаченко О., Хромова Н., Дідух І., Ніженковська І. (2025). Встановлення маркерів якості надкритичних $CO_2$-екстрактів проса. *Фітотерапія. Часопис*, 2, 218–227, doi: https://doi.org/10.32782/2522-9680-2025-2-218

## ВСТАНОВЛЕННЯ МАРКЕРІВ ЯКОСТІ НАДКРИТИЧНИХ $CO_2$-ЕКСТРАКТІВ ПРОСА

***Актуальність.*** *У статті представлено результати дослідження $CO_2$-екстрактів проса з метою визначення їхньої якості за допомогою різних аналітичних методів. Встановлення маркерів якості екстрактів проса важливе в харчовій, фармацевтичній і косметичній промисловості для оцінювання якості сировини.*

***Мета дослідження*** *– ідентифікація та встановлення надійних маркерів для оцінювання якості надкритичних $CO_2$-екстрактів проса.*

***Матеріал і методи.*** *Для ідентифікації компонентів екстрактів було використано газовий хроматограф із мас-детектором "Shimadzu GCMS-QP2010 Ultra" і хроматографічною колонкою "DB-WAX" 30 м × 0,32 мм × 0,25 мкм. Для встановлення жирно-кислотного складу екстрактів проса використано газовий хроматограф "GC-2010AF Plus" із полумяно-іонізаційним детектором і хроматографічною колонкою "HP-INNOWAX" 30 м × 0,53 мм × 1 мкм. Для кількісного визначення сквалену було використано рідинний хроматограф "Shimadzu Nexera LC-30" зі спектрофотометричним детектором і хроматографічною колонкою "Symmetry C18 column" (150 × 4,6 мм, 3,5 мкм).*

***Результати дослідження.*** *Було ідентифіковано такі сполуки в екстракті проса, як: пальмітинова, стеаринова, олеїнова, лінолева та ліноленова жирні кислоти, а також сквален і міліацин. Як потенційний маркер якості розглянуто жирно-кислотний склад олії, що міститься в екстракті проса. Було встановлено, що жирно-кислотний склад у вибраних зразках є практично однаковим, за винятком одного з комерційних екстрактів, який показав істотну відмінність у співвідношенні жирних кислот. Для оцінювання вмісту сквалену було розроблено екобезпечну методику для кількісного визначення методом рідинної хроматографії з використанням екобезпечних розчинників, а саме етанолу й ізопропанолу, що дозволяє зменшити негативний вплив на навколишнє середовище. Виявлено значні відмінності у вмісті сквалену серед екстрактів: в екстрактах борошна, мучки та крупи проса вміст сквалену становив 1,05, 1,01 та 0,45% відповідно, тоді як в одному з комерційних екстрактів сквален виявлено лише у слідових кількостях. Так само міліацин був виявлений в усіх екстрактах, окрім одного, що підтверджує низьку якість цього екстракту.*

***Висновок.*** *На основі отриманих результатів можна зробити висновок, що жирно-кислотний склад є одним із показників, за якими можна визначати якість екстракту проса. Більш специфічними маркерами є наявність сквалену та міліацину, які були визначені як ключові маркери якості $CO_2$-екстрактів проса.*

***Ключові слова:*** *$CO_2$-екстракція, просо, сквален, міліацин, склад жирних кислот.*

**Introduction. Actuality.** Millet (Panicum miliaceum L.) is an important cereal crop and a valuable component of the human diet. It is known that millet possesses effective medicinal and nutritional properties, including antioxidant, anticancer, and anti-inflammatory effects (Liang et al., 2010). Millet oil could be a good source of linoleic acid and tocopherols, which may have benefits for health (Sarita & Singh, 2016). Consumption of foxtail millet and proso millet decreased serum triglyceride and C-reactive protein levels in hyperlipidemic rats, but did not affect oxidative status (Lee et al., 2010). Millet seed oil promotes hair growth by activating β-catenin signaling and increasing hair follicle size and number (Lee et al., 2023). Millet consumption has shown promising effects on lipid profiles and overall health. Studies indicate that millet intake can significantly reduce total cholesterol, triglycerides, while increasing HDL-C (good cholesterol) (Anitha et al., 2021). Moreover, millet contains oil, which is a source of oleic, linoleic, and linolenic fatty acids, essential components of the diet. The authors (Nizhenkovska et al., 2024) demonstrated that the content and ratio of higher fatty acids in tissues serve as an indicator of the severity of the pathological process and a marker for its correction.

Currently, $CO_2$ extraction is not widely used in the production of millet extracts for the food industry. However, $CO_2$ extraction offers several advantages over other methods: a) solvent safety: $CO_2$ is non-toxic and does not pollute the environment. This makes the method more environmentally friendly compared to traditional organic solvents extraction such as chloroform or hexane, which can be toxic and difficult to utilize (King, 2002), b) no residual solvents and lower metal content: The extract contains no residual solvents, and the levels of metals are significantly lower (Wen-lan, 2009), c) the process operates at low temperatures, allowing for the preservation of heat-sensitive (thermolabile) compounds.

$CO_2$ extraction provides the opportunity to obtain the highest quality and quantitative characteristics of valuable components from the by-products of millet processing (Glew et al., 2008; Bossard et al., 2013). The extraction of fatty oils, triglycerides, and phospholipids from millet flour is one of the most effective methods. Accordingly, the extracts themselves are valuable raw materials and subjects of research. Researchers have identified the nutritional characteristics of various millet products, including the fatty acid composition (Zhang et al., 2015; Ji et al., 2019; Shen et al., 2018; Bora et al., 2019; Slama et al., 2020), amino acid content (Glew et al., 2008; Shen et al., 2018; Sharma et al., 2024), minerals (Glew et al., 2008; Sharma et al., 2024), squalene (Ji et al., 2019), miliacin (Bossard et al., 2013).

Currently, the fatty acid composition of millet seed oil and its processed products is being intensively studied, with a focus on variations depending on the variety and growing conditions.

In the study (Zhang et al., 2015) 35 samples of foxtail millet were analyzed, including 7 varieties grown in 5 different regions of China. The fatty acid composition of these varieties, including the 5 main fatty acids, varied within the following ranges: for palmitic acid, 7,05–7,86%; for stearic acid, 5,92–7,34%; for oleic acid, 15,01–17,09%; for linoleic acid, 65,51–68,30%; and for linolenic acid, 2,25–2,77%. A similar fatty acid composition was also reported in the study (Liang et al., 2010).

In the paper (Slama et al., 2020), the fatty acid composition of pearl millet was determined. Five main fatty acids were identified, with the relative content of palmitic acid at 16,79%, stearic acid at 5,02%, oleic acid at 27,07%, linoleic acid at 47,50%, and linolenic acid at 2,15%. A similar fatty acid composition was found for palmitic acid at 15,7%, stearic acid at 5,02%, oleic acid at 34,1%, and linoleic acid at 42,0% in little millet (Panicum sumatrense Roth.)(Sharma et al., 2024).

In the research (Ji et al., 2019) the fatty acid composition of different fractions of foxtail millet bran extract was determined. The fatty acid composition of the fractions, including the 5 main fatty acids, varied within the following ranges: palmitic acid, 7,43–8,51%; stearic acid, 4,59–5,04%; oleic acid, 12,86–17,08%; linoleic acid, 65,21–68,8%; and linolenic acid, 3,00–3,65%. In the work (Glew et al., 2008) the fatty acid composition of finger millet was analyzed, revealing the following: palmitic acid 22,7%, stearic acid 2,1%, oleic acid 44,0%, linoleic acid 20,2%, and linolenic acid 3,7%. In conclusion, the fatty acid composition of millet varies depending on the species and growing conditions, but it is generally found that the main fatty acids present in the extracts are oleic, linoleic, palmitic, and linolenic acids. Specifically, oleic and linoleic acids make up the majority of the fatty acids, making millet a promising source of these beneficial compounds.

Squalene content was determined in the study (Ji et al., 2019), where it was found to be in the range of 10–13 mg per 100 g of millet oil extracted from various bran fractions. By the authors (Sharma et al., 2024) identified squalene using liquid chromatography, however, no quantitative determination was performed. No information has been found regarding the squalene content in millet extracts obtained through the CO2 extraction method, highlighting the need for further investigation.

The presence of miliacin in ancient civilization vessels is considered by some researchers as a marker for the use of millet in their diet (Jacob et al., 2008), while other

researchers approach this idea with caution (Bossard et al., 2013). However, all agree that millet contains significant amounts of miliacin, and this crop can be distinguished by the identification of this compound

The use of millet extracts is also highly popular in the production of dietary supplements and cosmetic products. Therefore, this study focuses on developing quality assessment criteria for supercritical $CO_2$ extracts of millet, obtained from various raw materials and commercially available from different manufacturers. To investigate the qualitative and quantitative characteristics that could indicate the quality of millet extract, $CO_2$ extraction was performed on different millet products, namely: millet flour, ground millet groats, and millet bran – by-products from millet milling. Commercial extracts were also purchased from online stores for comparison.

**The purpose ot this work** is to identify quality markers for $CO_2$-extracts of millet by studying their fatty acid composition, squalene content, and miliacin, which will allow for the assessment of the authenticity and quality of the extracts, determine the peculiarities of the raw material's chemical composition, and also develop criteria for the identification and standardization of millet-based products.

**Materials and methods.** Research objects: $CO_2$-supercritical extracts of millet flour, millet groats, and millet bran (obtained at the M.P. Semenenko Institute of Geochemistry, Mineralogy and Ore Formation of the National Academy of Sciences of Ukraine) under the following conditions: $CO_2$ pressure – 20 MPa, temperature 33 °C, extraction time – 1 hour. Commercially available extracts: CO2-supercritical millet extract 1 (Germany), CO2-supercritical millet extract 2 (Ukraine).

Standard samples: standard sample of flaxseed oil, PHR2980 Supelco; Squalene, S3626, Sigma-Aldrich.

Reagents: heptane (cat. number 34873) Honeywell; potassium hydroxide (cat. number 30603), Sigma-Aldrich; methanol (gradient grade, cat. number 20864.320) VWR BDH Chemical; 2-propanol (cat. number 34863), Sigma-Aldrich, Ethyl alcohol (96 vol. %, Lux) State Enterprise "Ukrspyrt", chromatography water, obtained on Simplicity UV system, Millipore, USA; carrier gas – helium (99.999%).

Component identification in millet extract. Chromatographic conditions: Shimadzu GCMS-QP2010 Ultra. Column: DB-WAX 30 m × 0,32 mm × 0,25 μm. Temperature program: initial 80 °C, then +10 °C/min to 240 °C, 20 minutes at 240 °C. Carrier gas: helium. Injector temperature: 220 °C. Split ratio: 20 : 1, flow rate: 5,00 ml/min. Injection volume: 0,5 μl. Mass detector parameters: ion source temperature: 230 °C. Temperature: 250 °C. Sample preparation for component identification in millet extract: weight of about 0.1g (accurate weight) of each $CO_2$-supercritical extract and standard flaxseed oil placed into a 20 ml flask. Add 5 ml of heptane and 10 ml of potassium hydroxide solution in methanol (38 g in 100 ml of methanol). Shake intensively for 3 minutes. The upper layer was use for chromatography.

Determination of the fatty acid composition of millet extracts was carried out according to the methodology of the European Pharmacopoeia monograph 2.4.22 "Composition of fatty acids by gas chromatography". Apparatus: GC-2010AF Plus with a flame ionization detector. Column: HP-INNOWAX 30 m × 0,53 mm × 1 μm. Temperature program: initial 170 °C, then +3 °C/min to 230 °C. Carrier gas: helium. Injector temperature: 250 °C. Split ratio: 50 : 1, carrier gas flow rate: 5,44 ml/min. Injection volume: 0,5 μl. Flame ionization detector temperature: 250 °C. Samples prepared for components identification were used.

The determination of squalene was carried out by HPLC using a Shimadzu LC-30 liquid chromatograph with a spectrophotometric detector, and a Symmetry C18 column (150 × 4,6 mm, 3,5 μm). The mobile phase used was 96 vol. % ethanol. The column temperature was maintained at 40 °C. The injection volume was 2 μl. The flow rate of the mobile phase was 1.0 ml/min. Sample preparation for quantitative determination of squalene: 0,2 g samples of five $CO_2$-supercritical extracts (three samples of each) were dissolved in a small amount of solvent (2-propanol) and brought to the mark in a 10 ml volumetric flask with the same solvent. Standard squalene substance samples (0,1 g, accurate weight) were prepared in duplicate, dissolved in a small amount of solvent (2-propanol), and brought to the mark in a 50 ml volumetric flask, resulting in a concentration of 2,0 mg/ml squalene standard. For calibration and verification of the chromatographic system's suitability, six dilutions of the above standard solution were prepared with concentrations 0,03; 0,24; 0,20; 0,16; 0,12; 0,08 mg/ml. Resolution solution: squalene and tocoferol acetate solution with concentration of 0,2 mg/ml in 2-propanol.

**Calculations:**

Resolution: $Rs = 1{,}18\ (t_2 - t_1)/(w_{h1} + w_{h2})$, where $t_1$, $t_2$ – retention times of the peaks; $w_{h1}$, $w_{h1}$ – peak widths at half height.

Column efficiency: $N = 5{,}54 \times (t_R/w_h)^2$, where $t_R$, $t_2$ – retention time; $w_{h1}$ – peak width at half height.

Symmetry factor: $As = w_{0.05}/2d$, where $w_{0.05}$ – width of the peak at one-twentieth of the peak heigh; d – distance between the perpendicular dropped from the peak maximum and the leading edge of the peak at one-twentieth of the peak height.

**Results and discussion.** For the investigation of qualitative and quantitative characteristics that could

indicate the quality of millet extract, $CO_2$-extraction was performed on different millet products, specifically: millet flour, ground millet grains, and millet bran – waste from the milling process. Millet flour: this product may contain a high amount of carbohydrates and proteins, as well as phytochemical compounds that could be useful in dietary supplements. Ground millet: This contains a higher amount of fiber and may be a source of less soluble components. Millet bran (waste after milling): This material may have a different composition, as it often retains residual seed husk particles, which could affect the content of vitamins, minerals, or other active compounds. Additionally, commercial extracts were purchased from online stores.

**Identification of the components of $CO_2$ extracts of millet.** The use of gas chromatography (GC) combined with mass spectrometry (MS) for the identification of components in $CO_2$-extracts of millet is a highly effective approach for analyzing the composition of extracts. This combination allows the identification of specific compounds that may serve as potential quality markers. Mass spectrometry libraries are an important tool for compound identification, as they contain information about the mass and fragmentation of molecules from various compounds, enabling automatic comparison of analysis results with databases. The identification results using the NIST (National Institute of Standards and Technology, USA) library spectra are presented in the table 1.

Table 1

**Component identification of supercritical extracts**

| Compound | Millet flour | Ground millet | Millet Gran | Extract 1 | Extract 2 |
|---|---|---|---|---|---|
| Palmitic acid | + | + | + | + | + |
| Stearic acid | + | + | + | + | + |
| Oleic acid | + | + | + | + | + |
| Cis-13-oleic acid | – | – | + | – | + |
| Linoleic acid | + | + | + | + | + |
| Linolenic acid | – | + | + | + | + |
| Eicosanic acid | – | + | + | + | + |
| Cis-13-eicosanic acid | – | + | – | + | + |

The table 1 shows the identification of fatty acids that are components of triglycerides in millet extracts. The fatty acid composition and triglyceride content in $CO_2$ extracts of millet can provide important information about the origin and quality of the extract, although fatty acids are not direct markers of millet quality. In particular, the fatty acid composition and triglyceride concentration may indicate specific features of the extraction technology or the presence of specific components that define the nutritional or pharmacological value of the extract. Additionally, squalene and miliacin were identified, as shown in the table 2, which may serve as marker substances, as they are found only in certain crops. Squalene is a natural tetraterpenoid found in many plants and animal fats, but it can be found in significant amounts in oils from crops such as amaranth and olives (olive oil).

Miliacin is a specific compound that occurs only in millet, making it a unique marker for this crop. As a natural compound, miliacin is an important element that can indicate the authenticity and quality of the extract. Its presence is an indicator that the extract truly originates from millet, as miliacin is not found in other plants. This allows millet extracts to be distinguished from other plant oils or supplements that may have similar properties but do not contain miliacin.

Table 2

**Identification of squalene and miliacin in extract samples**

| Compound | Millet flour | Ground millet | Millet Gran | Extract 1 | Extract 2 |
|---|---|---|---|---|---|
| Squalene | + | + | + | + | Traces |
| Miliacin | + | + | + | + | – |

The absence of positive identification for miliacin and squalene in the Extract 2 is an important signal for checking the quality and authenticity of this product. Since miliacin is a specific marker for millet and squalene is usually present in significant amounts in millet oil, its absence may indicate several potential issues: a) the raw material did not contain millet, which may suggest that the extract was not obtained from millet but from another plant source that does not contain these specific markers (for example, from the oil of other crops that contain squalene but lack miliacin); b) dilution with oil of different origin: if the extract contains other oils with similar physical properties (such as amaranth or olive oil, which also contain squalene but lack miliacin), it may reduce the concentration of millet markers; c) insufficient concentration of active components from millet due to improper or ineffective extraction methods, which may lead to a decrease in the levels of squalene and miliacin.

**Fatty acids composition.** The application of the European Pharmacopoeia Monograph 2.4.22 for determining the fatty acid composition using gas chromatography (GC) is a reliable approach for the accurate identifica-

tion and quantitative determination of fatty acids in CO2 extracts. Comparing with a known standard, such as flaxseed oil, ensures precise determination. In this case, the European Pharmacopoeia 2.4.22 methodology involves using fatty acid methyl esters, which is a standard procedure for determining the fatty acid composition of oils. Flaxseed oil was chosen as the standard for comparison due to its well-known fatty acid composition, allowing identification of key fatty acids: palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1, omega-9), linoleic acid (C18:2, omega-6), and linolenic acid (C18:3, omega-3), fig. 1. The identification results for flaxseed oil were used to identify the corresponding fatty acids in millet extracts.

The fatty acid composition of the millet extracts was determined, and the results were obtained by normalization, calculating the proportion of the signal of a specific fatty acid relative to the total signal of all fatty acids, results are shown in table 3.

The results of the fatty acid composition analysis of the extracts show significant differences between Extract 2 and other millet extracts, which requires further investigation. According to the data in the table 3, the following conclusions can be made: the content of oleic acid in all extracts, except for commercial extract 2, ranges from 22,5 to 24,7%. The content of linoleic acid in millet extracts varies from 66,5 to 68,9%, which corresponds to the fatty acid content in the Millet Foxtail variety. The main fatty acid composition of millet extracts includes a significant amount of linoleic acid (omega-6), which is typically found in plant oils. The content of oleic acid (monounsaturated omega-9 acid) is also quite high. The ratio of oleic to linoleic acid. In the first four extracts, the ratio of oleic to linoleic acid ranges from 2,69 to 2,96. This indicates that linoleic acid predominates in these extracts, but oleic acid still occupies a significant portion. In extract 2, the amount of oleic acid is twice as much as linoleic acid. The ratio of oleic to linolenic acid in this extract is 0,54, which is significantly lower compared to the ratios in other extracts. This ratio is close to fatty acids ratio in Finger Millet variety (Slama et al., 2020), with the distinction that the content of oleic and linoleic acids is lower, while the content of palmitic acid in this variety is four times higher, approximately 23%.

The identified difference in the ratio of oleic to linolenic acid in extract 2 is significant. Oleic acid significantly predominates in this extract, which may indicate

Table 3

**Fatty acid composition**

| Compound | Millet flour | Ground millet | Millet Gran | Extract 1 | Extract 2 |
|---|---|---|---|---|---|
| Palmitic acid | 5,5 | 7,5 | 7,0 | 5,7 | 5,9 |
| Stearic acid | 1,5 | 3,0 | 1,2 | 1,1 | 3,0 |
| Oleic acid | 24,3 | 22,5 | 24,7 | 23,6 | 57,4 |
| Linoleic acid | 68,7 | 66,5 | 66,5 | 68,9 | 30,8 |
| Linolenic acid | 0,0 | 0,6 | 0,6 | 0,7 | 2,9 |

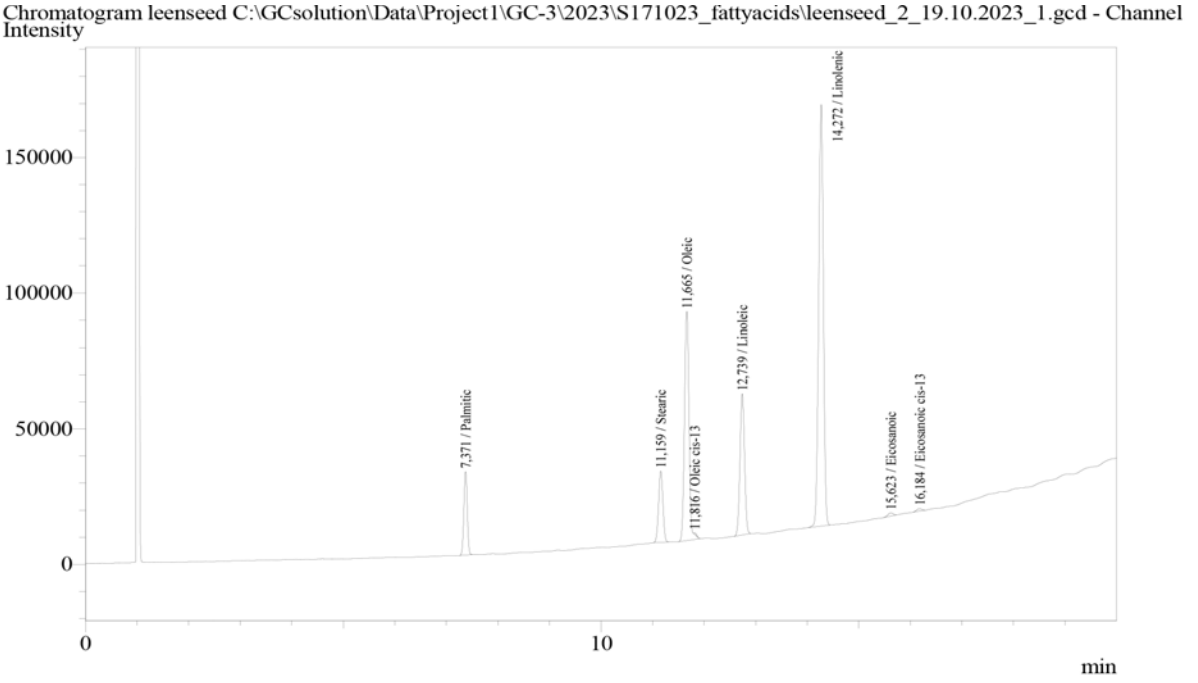


**Fig. 1. Flaxseed oil standart solution chromatogram**

mixing with other oils: It is likely that oils rich in oleic acid (such as olive oil or sunflower oil) were added to extract 2, altering its fatty acid composition. This may explain the high content of oleic acid and the significantly lower content of linolenic acid. The mixing with other oils could be an attempt to improve its economic efficiency.

**The content of triglycerides of fatty acids in the extracts.** In addition to the fatty acid composition, the mass content of triglycerides in the millet extracts can be assessed using a flaxseed oil standard sample. During the determination of the fatty acid composition of millet extracts, the triglyceride content was established. The results are presented in table 4. The results of the oil content analysis in different millet extracts show significant differences between the extracts, confirming the influence of the raw material on the final product composition.

Table 4

**Triglicerides content in millet extracts**

| Compound | Millet flour | Ground millet | Millet Gran | Extract 1 | Extract 2 |
|---|---|---|---|---|---|
| Oil content, mass. % | 7,6 | 99,0 | 43,6 | 75,8 | 73,4 |

The oil content in the flour extract was found to be around 7,6%, which indicates that the flour contains a relatively low amount of fatty acid triglycerides. This extract also appeared the darkest in color and remained solid at room temperature. The millet grain extract, on the other hand, contained 99,0%, which means that most of the substance in this extract is composed of fatty acid triglycerides. This extract also appeared light in color and remained in a liquid state at room temperature. The oil content in the bran extract was 43,6%, while in the commercial extract1, it was 75,8%, and in the commercial extract 2, the oil content was 73,4%. Since the oil fraction content in different extracts can vary significantly depending on the raw material used for extraction, this parameter cannot be used as a marker to verify the quality of the extract.

**Squalene determination**. For the quantitative determination of squalene, the principles of "green" chemistry were applied to minimize environmental impact. Eco-friendly solvents – ethanol and 2-propanol – were used as the mobile phase and sample solvent, respectively. These solvents are less toxic compared to traditional organic solvents often used in the analysis of lipophilic compounds, such as chloroform or hexane. They comply with the principles of "green" chemistry, as they have low toxicity and break down quickly in the environment, reducing the risk of environmental pollution. Prior to the determination, the metrological characteristics of the method were established, and the results are presented below.

**Chromatography system suitability.** Chromatogram of standard solution is shown on fig. 2. The chromatographic system is considered suitable if the following conditions are met in the chromatogram of standard solution and resolution solution:

1. RSD (relative standard deviation), % peak area of 6 injections of the standard sample (0,2 mg/mL) does not exceed 1,0%. Obtained 0,05%.

2. RSD, % retention times of 6 consecutive injections of the standard sample (0.2 mg/mL) does not exceed 0,5%. Obtained 0,09%.

3. The column efficiency (N) of squalene in the chromatogram of the suitability test solution is not less than 5 000 theoretical plates. Obtained 10 271 theoretical plates.

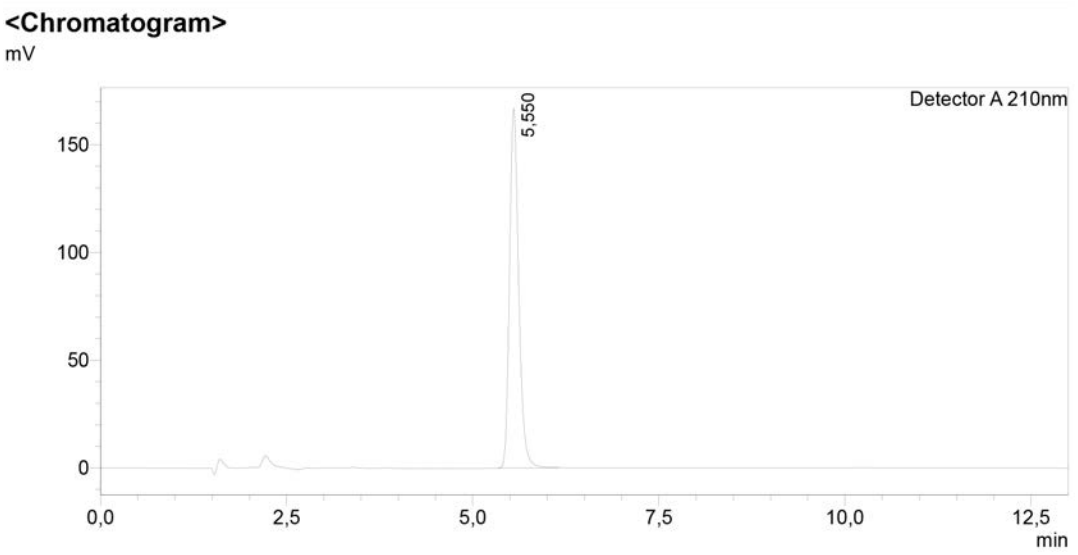


**Fig. 2. Chromatogram of standard solution**

4. Resolution (Rs) of squalene from alpha-tocopherol acetate is at least 2,0 in the chromatogram of the resolution solution. Obtained 7,8.

5. The symmetry (As) of the squalene peak in the chromatogram of the standard solution should be between 0,8–1,8. Obtained 1,2.

To establish the dependence of peak area on concentration, 6 solutions were prepared by diluting the stock solution to concentrations of 40, 60, 80, 100, 120, 150% of the nominal concentration of 0,2 mg/ml, in the range of 0,08–0,3 mg/ml. The equation obtained is y = 6 898 543,5 × x + 28 500,2. The standard quadratic deviation for the intercept of the equation was SD (a) = 3 636,0, and the standard deviation for the coefficient b (slope) was SD (b) = 17 876,35. The regression coefficient $r^2$ = 1,000, which meets the correlation coefficient requirements of at least 0,998. The intercept a is practically negligible, as its signal accounts for 2,0 % of the signal from the standard sample with a concentration of 100% (0,2 mg/ml).

From the calibration curve, the limit of quantification for squalene in the extract was established at 0,026 mass % (using the formula 10 × SD (a)/b), and the detection limit was established at 0,009 mass % (using the formula 3,3 × SD (a)/b).

**Squalene determination in samples.** Squalene was quantified in the extracts using the developed method, and the results are presented in table 5. Fig. 3 shows the

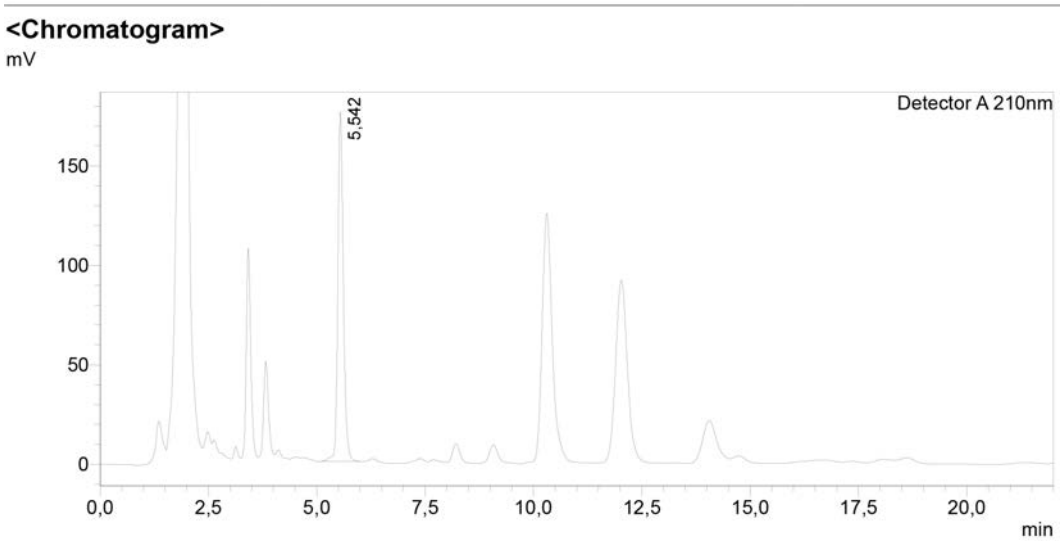


**Fig. 3. Chromatogram of millet gran extract solution**

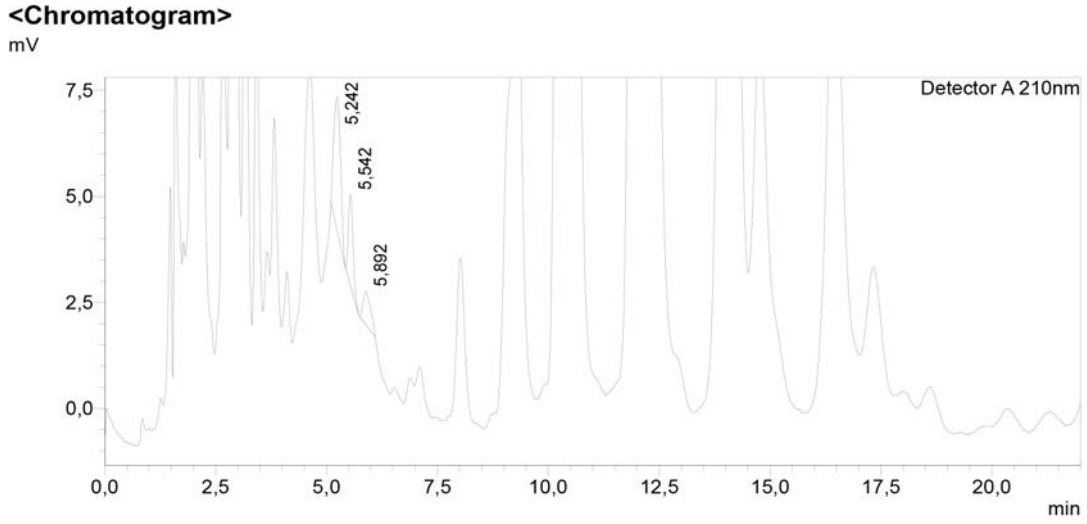


**Fig. 4. Presents the chromatogram of commercial extract 2, where squalene is identified as a trace peak at 5,54 min**

chromatogram of the millet gran extract, where squalene is identified at 5,54 min.

Assay results for squalene are presented in Table 5. The obtained data indicate that squalene is present in millet extracts derived from CO2 extraction of millet flour, ground millet, and millet gran. Additionally, squalene was detected in commercial extract 1 but was absent in commercial extract 2, supporting the suspicion of adulteration.

Table 5

**Squalene content in $CO_2$-ecstracts**

| Compound | Millet flour | Ground millet | Millet Gran | Extract 1 | Extract 2 |
|---|---|---|---|---|---|
| Squalene, % | 1,05 | 1,01 | 0,45 | 0,25 | Traces |

**Conclusions. It has been established that palmitic, stearic, oleic, linoleic, and linolenic fatty acids, as well as squalene and miliacin, were identified in supercritical $CO_2$ extracts of millet obtained from flour, millet grain, and bran, as well as in samples of commercial extracts. In commercial extract 2, neither squalene nor miliacin was detected, indicating a potential case of adulteration.**

**It was found that supercritical $CO_2$ extracts contain varying amounts of oil: the flour extract – around 8%, the millet grain extract – around 99%, the bran extract – around 44%, commercial extract 1– around 76%, and commercial extract 2 – around 73%. However, the fatty acid composition is more consistent across most extracts. Specifically, the oleic acid content ranges from 22,5 to 24,7%, and the linolenic acid content ranges from 66,5 to 68,9% in all extracts except for commercial extract 2, where the linolenic acid content was 30,8% and the oleic acid content was 57,4%.**

**An eco-friendly, selective method for determining squalene in millet extracts by liquid chromatography was developed. The squalene content was determined as follows: CO2-supercritical flour extract – 1,05%; CO2-supercritical bran extract – 1,01%; CO2-supercritical millet grain extract – 0,45%; CO2-supercritical commercial extract 1 – 0,25%; CO2-supercritical commercial extract 2 – trace amounts of squalene were detected.**

**Thus, it has been established that the markers for determining the quality of millet extracts may include the fatty acid composition of the extract, presence and content of squalene, as well as the presence of the main millet marker – miliacin.**

**The proposed approaches for identifying key biomarkers such as squalene and miliacin, as well as the fatty acid profile, can serve as a basis for the standardization and quality control of supercritical $CO_2$ millet extracts. This is essential for preventing product adulteration and ensuring compliance with food or pharmacological quality requirements.**

**BIBLIOGRAPHY**


Anitha, S., Botha, R., Kane-Potaka, J., Givens, D. I., Rajendran, A., Tsusaka, T. W., Bhandari, R. K. Can Millet Consumption Help Manage Hyperlipidemia and Obesity?: A Systematic Review and Meta-Analysis. *Frontiers in Nutrition.* 2021. Vol. 8. P.1–12. https://doi.org/10.3389/fnut.2021.700778.

Bora, P., Ragaee, S., Marcone, M. Characterisation of several types of millets as functional food ingredients. *International Journal of Food Sciences and Nutrition*. 2019. Vol. 70 (6). P. 714–724. https://doi.org/10.1080/09637486.2019.1570086.

Bossard, N., Jacob, J., Le Milbeau, C., Sauze, J., Terwilliger, V., Poissonnier, B., Vergès, E. Distribution of miliacin (olean-18-en-3β-ol methyl ether) and related compounds in broomcorn millet (Panicum miliaceum) and other reputed sources: Implications for the use of sedimentary miliacin as a tracer of millet. *Organic Geochemistry.* 2013. Vol. 63. P. 48–55. https://doi.org/10.1016/j.orggeochem.2013.07.012.

Glew, R., Chuang, L., Roberts, J., Glew, R. Amino acid, fatty acid and mineral content of black finger millet (Eleusine coracana) cultivated on the Jos Plateau of Nigeria. *Food.* 2008. Vol. 2 (2). P. 115–118. URL: http://edoc.ypu.edu.tw:8080/ paper/ biotech/LT #44.pdf.

Jacob, J., Disnar, J. R., Arnaud, F., Chapron, E., Debret, M., Lallier-Vergès, E., Desmet, M., Revel-Rolland, M. Millet cultivation history in the French Alps as evidenced by a sedimentary molecule. *Journal of Archaeological Science*. 2008. Vol. 35 (3). P.814–820. https://doi.org/10.1016/j.jas.2007.06.006.

Ji, J., Liu, Y., Ge, Z., Zhang, Y., & Wang, X. Oleochemical properties for different fractions of foxtail millet bran. *Journal of Oleo Science*. 2019. Vol. 68 (8). P. 709–718. https://doi.org/10.5650/jos.ess19063.

King, J. Supercritical Fluid Technology for Lipid Extraction, Fractionation, and Reactions. *Lipid Biotechnology.* 2002. December. P. 663–689. https://doi.org/10.1201/9780203908198.ch34.

Lee, E., Seo, H. D., Kim, D., Park, S. H., Kim, S. R., Hyun, C., Hahm, J. H., Ha, T. Y., Ahn, J., Jung, C. H. Millet seed oil activates β–catenin signaling and promotes hair growth. *Frontiers in Pharmacology.* 2023. Vol. 14 (May). P. 1–10. https://doi.org/10.3389/fphar.2023.1172084.

Lee, S. H., Chung, I. M., Cha, Y. S., Park, Y. Millet consumption decreased serum concentration of triglyceride and C-reactive protein but not oxidative status in hyperlipidemic rats. *Nutrition Research.* 2010. Vol. 30 (4). P. 290–296. https://doi.org/10.1016/j.nutres.2010.04.007.

Liang, S., Yang, G., Ma, Y. Chemical characteristics and fatty acid profile of foxtail millet bran oil. *Journal of the American Oil Chemists' Society*. 2010. Vol. 87 (1). P. 63–67. https://doi.org/10.1007/s11746-009-1475-3.

Nizhenkovska, I., Kuznetsova, O., Narokha, V. Effect of vitamin B3 / niacin complex with germanium (VI ) on the content of fatty acids in the brain, liver and kidneys of alcohol-exposed rats. *Current topics in Pharmacology*. 2024. Vol. 28. P. 29–36.

Sarita, Singh E. Potential of Millets: Nutrients Composition and Health Benefits. *Journal of Scientific and Innovative Research.* 2016. Vol. 5 (2). P. 46–50. https://doi.org/10.31254/jsir.2016.5204.

Sharma, R., Kumar, D., Gupta, R. K. Phytochemical profiling of little millet (Panicum sumatrense Roth.). *Indian Journal of Natural Products and Resources*. 2024. Vol. 15 (4). P. 555–564. https://doi.org/10.56042/ijnpr.v15i4.9518.

Shen, R., Ma, Y., Jiang, L., Dong, J., Zhu, Y., Ren, G. Chemical composition, antioxidant, and antiproliferative activities of nine Chinese proso millet varieties. *Food and Agricultural Immunology.* 2018. Vol. 29 (1). P. 625–637. https://doi.org/10.1080/09540105.2018.1428283.

Slama, A., Cherif, A., Sakouhi, F., Boukhchina, S., Radhouane, L. Fatty acids, phytochemical composition and antioxidant potential of pearl millet oil. *Journal Fur Verbraucherschutz Und Lebensmittelsicherheit.* 2020. Vol. 15 (2), P. 145–151. https://doi.org/10.1007/s00003-019-01250-4.

Wen-lan, H. U. O. Supercritical Fluid Extraction and GC-MS Analysis of Oil from Millet Bran. *Food Science*. 2009. Vol. 30 (20). P. 324–326.

Zhang, A., Liu, X., Wang, G., Wang, H., Liu, J., Zhao, W., Zhang, Y. Crude fat content and fatty acid profile and their correlations in foxtail millet. *Cereal Chemistry.* 2015. Vol. 92 (5). P. 455–459. https://doi.org/10.1094/CCHEM-12-14-0252-R.

**REFERENCES**

Anitha, S., Botha, R., Kane-Potaka, J., Givens, D.I., Rajendran, A., Tsusaka, T.W., & Bhandari, R.K. (2021). Can Millet Consumption Help Manage Hyperlipidemia and Obesity?: A Systematic Review and Meta-Analysis. *Frontiers in Nutrition*, Vol. 8, 1–12. https://doi.org/10.3389/fnut.2021.700778.

Bora, P., Ragaee, S., & Marcone, M. (2019). Characterisation of several types of millets as functional food ingredients. *International Journal of Food Sciences and Nutrition*, Vol. *70* (6), 714–724. https://doi.org/10.1080/09637486.2019.1570086.

Bossard, N., Jacob, J., Le Milbeau, C., Sauze, J., Terwilliger, V., Poissonnier, B., & Vergès, E. (2013). Distribution of miliacin (olean-18-en-3β-ol methyl ether) and related compounds in broomcorn millet (Panicum miliaceum) and other reputed sources: Implications for the use of sedimentary miliacin as a tracer of millet. *Organic Geochemistry*, Vol. 63, 48–55. https://doi.org/10.1016/j.orggeochem.2013.07.012.

Glew, R., Chuang, L., Roberts, J., & Glew, R. (2008). Amino acid, fatty acid and mineral content of black finger millet (Eleusine coracana) cultivated on the Jos Plateau of Nigeria. *Food*, Vol. *2* (2), 115–118. Retrieved from: http://edoc.ypu.edu.tw:8080/paper/biotech/LT #44.pdf.

Jacob, J., Disnar, J. R., Arnaud, F., Chapron, E., Debret, M., Lallier-Vergès, E., Desmet, M., & Revel-Rolland, M. (2008). Millet cultivation history in the French Alps as evidenced by a sedimentary molecule. *Journal of Archaeological Science*, Vol. *35* (3), 814–820. https://doi.org/10.1016/j.jas.2007.06.006.

Ji, J., Liu, Y., Ge, Z., Zhang, Y., & Wang, X. (2019). Oleochemical properties for different fractions of foxtail millet bran. *Journal of Oleo Science*. Vol. 68 (8), 709–718. https://doi.org/10.5650/jos.ess19063.

King, J. (2002). Supercritical Fluid Technology for Lipid Extraction, Fractionation, and Reactions. *Lipid Biotechnology,* December, 663–689. https://doi.org/10.1201/9780203908198.ch34.

Lee, E., Seo, H.D., Kim, D., Park, S.H., Kim, S.R., Hyun, C., Hahm, J.H., Ha, T.Y., Ahn, J., & Jung, C.H. (2023). Millet seed oil activates β–catenin signaling and promotes hair growth. *Frontiers in Pharmacology*, Vol. 14 (May), 1–10. https://doi.org/10.3389/fphar.2023.1172084.

Lee, S.H., Chung, I.M., Cha, Y.S., & Park, Y. (2010). Millet consumption decreased serum concentration of triglyceride and C-reactive protein but not oxidative status in hyperlipidemic rats. *Nutrition Research*, Vol. 30 (4), 290–296. https://doi.org/10.1016/j.nutres.2010.04.007.

Liang, S., Yang, G., & Ma, Y. (2010). Chemical characteristics and fatty acid profile of foxtail millet bran oil. *Journal of the American Oil Chemists' Society*, Vol. *87* (1), 63–67. https://doi.org/10.1007/s11746-009-1475-3.

Nizhenkovska, I., Kuznetsova, O., & Narokha, V. (2024). Effect of vitamin B3 / niacin complex with germanium (VI) on the content of fatty acids in the brain, liver and kidneys of alcohol-exposed rats. *Current topics in Pharmacology*, Vol. *28*, 29–36.

Sarita, Singh, E. (2016). Potential of Millets: Nutrients Composition and Health Benefits. *Journal of Scientific and Innovative Research*, Vol. *5* (2), 46–50. https://doi.org/10.31254/jsir.2016.5204.

Sharma, R., Kumar, D., & Gupta, R.K. (2024). Phytochemical profiling of little millet (Panicum sumatrense Roth.). *Indian Journal of Natural Products and Resources*, Vol. 15 (4), 555–564. https://doi.org/10.56042/ijnpr.v15i4.9518.

Shen, R., Ma, Y., Jiang, L., Dong, J., Zhu, Y., & Ren, G. (2018). Chemical composition, antioxidant, and antiproliferative activities of nine Chinese proso millet varieties. *Food and Agricultural Immunology*, Vol. *29* (1), 625–637. https://doi.org/10.1080/09540105.2018.1428283.

Slama, A., Cherif, A., Sakouhi, F., Boukhchina, S., & Radhouane, L. (2020). Fatty acids, phytochemical composition and antioxidant potential of pearl millet oil. *Journal Fur Verbraucherschutz Und Lebensmittelsicherheit*, Vol. *15* (2), 145–151. https://doi.org/10.1007/s00003-019-01250-4

Wen-lan, H.U.O. (2009). Supercritical Fluid Extraction and GC-MS Analysis of Oil from Millet Bran. *Food Science*, Vol. *30* (20), 324–326.

Zhang, A., Liu, X., Wang, G., Wang, H., Liu, J., Zhao, W., & Zhang, Y. (2015). Crude fat content and fatty acid profile and their correlations in foxtail millet. *Cereal Chemistry,* Vol. *92* (5), 455–459. https://doi.org/10.1094/CCHEM-12-14-0252-R.

*Стаття надійшла до редакції 30.12.2024*
*Стаття прийнята до друку 25.04.2025*

**Конфлікт інтересів:** відсутній.
**Внесок авторів:**
**Сиротчук О.А.** – ідея, дизайн дослідження, коректування статті;
**Глушаченко О.О.** – участь у написанні статті, збір та аналіз літератури;
**Хромова Н.А.** – виконання експериментальної роботи, обробка даних;
**Дідух І.Р.** – коректування статті, анотації;
**Ніженковська І.В**. – коректування статті, висновки, загальна візія.

**Електронна адреса для листування з авторами:**
***syrotchuk@gmail.com***