

Ministry of Education and Science of Ukraine
Black Sea Universities Network

ODESA NATIONAL UNIVERSITY OF TECHNOLOGY

International Competition of
Student Scientific Works

BLACK SEA SCIENCE 2022 PROCEEDINGS



ODESA, ONUT 2022

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BLACK SEA SCIENCE 2022

Proceedings

Odesa, ONUT 2022

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INTRODUCTION

International Competition of Student Scientific Works “Black Sea Science” has been held annually since 2018 at the initiative of Odesa National University of Technology (formerly Odesa National Academy of Food Technologies) with the support of the Ministry of Education and Science of Ukraine. It has been supported by Black Sea Universities Network (the Association of 110 higher education institutions from 12 countries of the Black Sea Region) since 2019, and by Iseki-FOOD Association (European Integrating Food Science and Engineering Knowledge into the Food Chain Association) since 2020.

The goal of the competition is to expand international relations and attract students to research activities. It is held in the following fields:

- Food science and technologies
- Economics and administration
- Information technologies, automation and robotics
- Power engineering and energy efficiency
- Ecology and environmental protection

The jury includes both Ukrainian and foreign scientists. In the 4 years that the competition has been held, the jury included scientists from universities of 24 countries: Angola, Azerbaijan, Benin, Bulgaria, China, Czech Republic, France, Georgia, Germany, Greece, Israel, Italy, Kazakhstan, Latvia, Lithuania, Moldova, Pakistan, Poland, Romania, Serbia, Slovakia, Switzerland, Turkey, USA.

At the same time, every year the geography has expanded and the number of foreign jury members has increased: from 46 jury members representing 25 universities from 12 countries in 2018, to 73 jury members of the 46 universities from 19 countries in 2022.

More than a thousand student research papers have been submitted to the competition from both Ukrainian and foreign institutions from 25 countries: China, Poland, Mexico, USA, France, Greece, Germany, Canada, Costa Rica, Brazil, India, Pakistan, Israel, Macedonia, Lithuania, Latvia, Slovakia, Romania, Kyrgyzstan, Kazakhstan, Bulgaria, Moldova, Georgia, Turkey, Serbia.

The interest of foreign students in the competition grew every year. In 2018, the students representing 15 institutions from 7 countries have submitted 33 works. In 2021 the number of submitted works increased to 73, authored by the students of 40 institutions from 18 countries.

The competition is held in two stages. In the first stage, student research papers are reviewed by members of the jury who are experts in the relevant fields. In the second stage of the competition, the winners of the first stage have the opportunity to present their work to a wide audience in person or online.

All participants of the competition and their scientific supervisors are awarded appropriate certificates, and the scientific works of the winners are included in the electronic proceedings of the competition. Every year the competition receives a large number of positive responses from Ukrainian and foreign colleagues with the desire to participate in the coming years.

1. FOOD SCIENCE AND TECHNOLOGIES

ISOLATION OF BIOACTIVE COMPOUNDS FROM HEMP (*Cannabis sativa* L.) BY CONVENTIONAL AND NOVEL EXTRACTION TECHNIQUES

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Abstract: *Industrial hemp (*Cannabis sativa* L.) is a source of valuable bioactive compounds which is used as a highly valuable product in food, pharmaceutical and cosmetic industries. Bioactive composition of hemp contributes to the prevention and treatment of some diseases, so it can be widely used in medicine. The main goal of this paper was to investigate the most convenient extraction approach for isolation of bioactive compounds (cannabinoids and phenols) from dry inflorescence of three hemp varieties. Modern extraction techniques were applied and compared with conventional techniques in order to obtain maximum yield. Essential oils were obtained by hydrodistillation (HD), microwave-assisted hydrodistillation (MWHD) and supercritical fluid extraction (SFE). Extracts from the primary raw material of hemp and SFE raffinate were processed by microwave-assisted extraction in order to isolate polyphenolic fraction. The obtained results showed that SFE provides the highest yield of hemp oil compared to HD and MWHD. The highest yield of lipid extract was obtained from variety Fedora 13 (14.01%), while the lowest yield was obtained from variety Marina (5.33%). The high content of phenols and flavonoids obtained from SFE raffinates indicates that the by-products of supercritical fluid extraction could be used as potential natural antioxidants.*

Keywords: *Cannabis sativa* L.; supercritical fluid extraction; microwave assisted extraction; essential oil; polyphenols

I. INTRODUCTION

Industrial hemp (*Cannabis sativa* L.) is one of the oldest medicinal plants that is a source of valuable bioactive compounds (fiber, protein, oil, cannabinoids, polyphenols, etc.). Due to the uniqueness of its composition, hemp is used as a highly valuable product in the food, pharmaceutical and cosmetic industries. The nutritious and bioactive composition of hemp contributes to the prevention and treatment of some diseases, so it can be used in medicine as: analgesic, antiepileptic, anticonvulsant, anti-neurodegenerative, antibacterial and anticancer agent [1]. Hemp is used to treat conditions, such as, rheumatoid arthritis, cardiovascular diseases, hormonal disbalance, psychological and psychiatric disorders, Alzheimer's, Parkinson's disease and schizophrenia [2]. In the past two decades, many countries have legalized the production of industrial hemp, which encouraged further studies focused on its health benefits. Without doubt, the huge interest in hemp cultivation is a consequence of the ability of this plant to biosynthesize various organic compounds called cannabinoids. In addition to cannabinoids, this plant also contains terpenes, polyphenols and fatty acids, which have also shown significant biological activity. Due to the presence of delta-9-tetrahydrocannabinol (Δ -9-THC), which is considered to be a psychoactive and

toxic substance for humans, cannabis is still a central topic of many legislative debates [3]. The main difference between industrial hemp and marijuana is primarily in the levels of Δ -9-THC production. Industrial hemp contains less than 0.3% Δ -9-THC, while marijuana contains more than 0.3% which causes psychoactive effects [4].

As the interests for the use of this plant grow, it is necessary to intensify research on the chemical composition of hemp, as well as the methods of extraction of bioactive compounds. High energy and solvent consumption and low yield of the desired compound are the biggest problems that can occur. Today, solutions that will enable reduction in the usage of organic solvents are being examined, and at the same time, these findings will be able to ensure the intensification of the process and cost-effective production of high-quality extracts. Novel techniques correspond to green chemistry concept that reduce the time of usage and energy consumption, lowers down the temperature, avoid usage of organic solvents. They should provide a high yield of targeted compounds by using alternative solvents (mainly water-based), preserves the high quality of extracts and reduce the number of operations in the production process [5].

Industrial hemp originates from central and northeastern Asia. *C. sativa* belongs to the family *Cannabaceae* and includes three species: *Cannabis sativa* L., *Cannabis indica* and *Cannabis ruderalis*. The plant is considered dioecious (dicotyledonous) and female inflorescences are densely covered with glandular trichomes containing resin, one of the most valuable cannabis products with various psychoactive and healing properties [6]. Historically, many products come from the seeds, fibers and trees of the hemp plant [7].

All ingredients of hemp can be divided into two groups: cannabinoids and non-cannabinoids [3]. Hemp is a plant known for its content of bioactive cannabinoids from the group of secondary metabolites unique to the genus Hemp. These compounds are produced in specialized glands in the leaf epidermis. Non-cannabinoid ingredients are: terpenes, carbohydrates, flavonoids, simple alcohols, aldehydes, ketones, acids, and esters. All these pharmacologically active ingredients of hemp are responsible for a wide range of its effects. Hemp contains four main groups of compounds with pronounced pharmacological activity: polyunsaturated fatty acids, cannabinoids, terpenes, and polyphenols. Oils from hemp seeds contain 70-80% of polyunsaturated fatty acids. Two essential fatty acids, linoleic and linolenic acid, are the most common. The ratio between fatty acids is 3:1, which is the optimal balance for people's diet and is unique in common herbal oils [8]. Cannabinoids are species of terpenophenols mostly synthesized in glandular trichome of female inflorescences. Cannabinoids interact with human endocannabinoid system, but those are different for their effects. For example, CBD is not psychoactive, whereas Δ -9-THC is psychoactive compound. As a result, these compounds are of great interest when it comes to their therapeutic potential in neurological disorders [9]. Typical and unique scent and aroma of this plant come from terpenes and polyphenols. Hemp essential oils are rich with monoterpenes (myrcene and pinene) and sesquiterpenes (β -caryophyllene and its derivatives) [10]. The greatest importance of phenolic compounds is their role as antioxidants. Antioxidants participate in hydrogen atom release reactions and free radical scavenging reactions. The most common flavonoids identified in cannabis are apigenin and quercetin [9].

II. LITERATURE ANALYSIS

Given that the demand for cannabis-derived compounds is constantly growing, despite strict regulations in some countries, the global market needs appropriate technologies to isolate the target compounds. Therefore, the subject of many studies was the isolation of bioactive compounds from hemp, using conventional (maceration, Soxhlet extraction (SOX), hydrodistillation (HD)), but also novel extraction techniques (microwave-assisted hydrodistillation (MWHD), ultrasonic-assisted extraction (UAE), microwave-assisted extraction (MAE) and supercritical fluid extraction (SFE)). Detailed research of innovative extraction techniques is indeed crucial for the extraction of phenolic compounds and terpenes from cannabis.

According to Naz et al. [11], the isolation of essential oils was examined from industrial and Indian hemp herb by different extraction methods (hydrodistillation and steam distillation) and supercritical fluid extraction. The content of essential oil in industrial hemp was higher than in Indian hemp, and the highest essential oil yield was obtained by HD. Drinić [12] researched the essential oil content of variety Helena which was determined by the HD. The essential oil content was 0.08% (v/m), which classifies this plant species in the group of plants poor in essential oil. The use of microwave technology in the extraction of bioactive compounds offers a number of advantages: fast heating, shorter processing time, reduced solvent consumption, higher reproducibility and improved yield [7]. Microwave-assisted hydrodistillation (MWHD) can be an effective alternative for isolation of terpenes from cannabis. Recently published studies have shown that microwaves can even improve oil extraction, reducing process time and increasing productivity, compared to conventional extraction methods [13]. In the work of Gunjević et al. [14], *C. sativa* flowers were used and conventional HD was performed to compare the yield of essential oil with MWHD. The optimized MWHD procedure gave a significantly higher yield of essential oil, compared to conventional (HD). Furthermore, Micalizzi et al. [6] applied MWHD to isolate cannabis essential oil from dried flowers of four different varieties of hemp. This study also showed that MWHD is an effective technique for isolating cannabis essential oil.

Currently, SFE as a modern extraction method attracts a lot of attention due to its unique supercritical properties, which offer the advantages of liquid and gas phase, at the same time. Various authors have adopted this process for the isolation of cannabinoids from different parts of industrial hemp [15] as well as for the isolation of aromatic compounds from industrial hemp [8,11]. In the work of Moreno et al. [3], extracts were obtained from the buds of New Zealand varieties of industrial hemp with the help of SFE. The composition of the extracts and the influence of different extraction parameters and the addition of 5% co-solvent (ethanol) which improves the extraction of acidic forms were determined. According to Rovetto and Aieta [15], SFE has been shown to allow efficient extraction of cannabinoids. The total yield of the process under different extraction conditions and the effect of co-solvent (ethanol) were examined for four different varieties of *C. sativa*. It was concluded that the extraction results largely depend on the characteristics of the plant material. The addition of co-solvent improves the extraction process and the extraction speed. This

work demonstrates the efficacy of SFE cannabinoids with high yields of extracts. The use of extraction techniques such as UAE, MAE or SFE are good solutions for increased extraction efficiency with the shortest extraction time [9]. Marzorati et al. [16] investigated developed an extraction process that can provide cannabidiol-enriched products (CBD) from *C. sativa* inflorescences. They concluded that SFE enables selective extraction of cannabis oil without the use of toxic organic solvents. Although many works applied different extraction processes: maceration, SOX, UAE, etc., none satisfies all aspects in terms of efficiency and cost-effectiveness. Therefore, Devi and Khanam [17] investigated different processes of hemp oil extraction from techno-economic perspective at industrial level. Economic analyses for various processes revealed that SFE was the most useful extraction technique for recovery of hemp oil.

Several studies have reported that MAE was far better than the conventional method due to low solvent and energy consumption, shortened process time and higher yield of bioactive compounds [13, 19]. Maceration and SOX are considered to be the predominant methods with relatively low cost and high yield of essential oil. Despite this, they need more time to extract the oil and they also need chemical solvents. Some new methods have emerged such as MAE and UAE together with SFE for extraction of hemp oil from seeds [8,18,13] as a solution for these limitations. These methods offer large amounts of oil yield (especially in terms of microwave and ultrasound) and also significantly shorter extraction times compared to conventional ones [13]. MAE is a method proposed for the extraction of essential oils and hemp seeds due to its advantages in the production of high quality oil. Matešić et al. [19] extracted phenolic compounds from hemp with the help of microwaves and high yields of these compounds were observed.

III. OBJECT, SUBJECT, AND METHODS OF RESEARCH

The main objective of this paper was to investigate the most convenient extraction techniques for isolation of bioactive compounds (cannabinoids, phenols and fatty acids) from dry inflorescence of three *Cannabis sativa* L. varieties. In this research, modern extraction techniques (MWHF, MAE and SFE) were applied and compared with conventional technique (HD) in order to obtain of maximum yield. Since the chemical composition of the extracts depends on the extraction techniques and the applied conditions, this will enable a comparison of the examined techniques and the definition of the best extraction technique for the target group of compounds.

As a subject of research in this paper, dry inflorescence of three different varieties of *Cannabis sativa* L. were used: Helena, Marina and Fedora 13. Commercial crops were cultivated in the field in Bački Petrovac that belongs to the Institute of Field and Vegetable Crops in Novi Sad, Serbia.

Hydrodistillation was used as a conventional extraction technique in order to isolate the essential oil from dried inflorescences of the industrial hemp. The Clevenger apparatus (Figure 1a), was used in experimental work. Sample (50 g) was weighed into a 1 L flask and 400 mL of distilled water was added. The distillation lasted 2 h, at a power of 205 W. A continuous supply of water and heat was provided. The distillation

yield is expressed as the percentage of essential oil isolated from the plant material we used as (%; v/m). The obtained essential oil was stored in glass bottles in the refrigerator. The procedure was repeated three times for each variety of *C. sativa*.

Microwave assisted hydridistillation was applied as modern extraction technique, which uses microwaves for distillation of essential oil. The heating of the vessel in which the plant material and water are located, takes place due to the action of microwaves. For microwave distillation, a modified microwave oven (MM817ASM, Bosch, Germany) was used, in which a glass flask with an Unger distillation apparatus was placed, (Figure 1b). Sample (50 g) was weighed into a 1 L flask and mixed with 400 mL of distilled water. All experiments were performed at a constant frequency and power of 600 W microwave radiation for 2 h. Experiments were performed in triplicates and the oil yield was expressed as (%; v/m). The distillate was stored in glass bottles in the refrigerator at 4 °C.

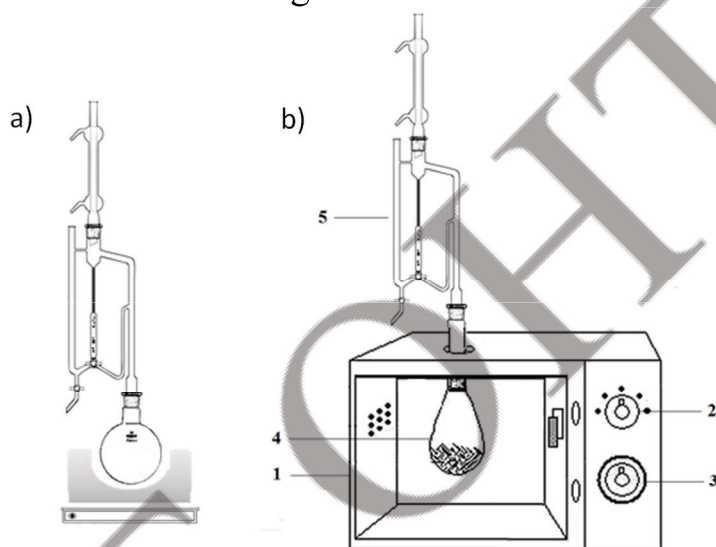


Figure 1. Schematic diagram of a) HD and b) MWHD apparatus: 1. microwave oven (MW); 2. irradiation power controller; 3. time controller; 4. glass flask; 5. Unger apparatus

Supercritical fluid extraction (SFE) is an environmentally friendly extraction technique that uses a fluid in its supercritical state for extraction of lipid compounds. Extraction with supercritical CO₂ was performed at the laboratory device for high-pressure extraction (HPEP, NOVA - Swiss), whose scheme is shown in Figure 2. The extractor was first filled with the measured mass of plant material (50 g). The plant material in the extractor was first released of the present air by blowing CO₂ from the bottle (GC) in the gaseous state, after which the valve (V5) and the control valve (RV2) were closed. The heated temperatures in the ultrathermostats (UT1) and (UT2) maintained the operating temperatures in the extractor and separator (S), while the valve on the CO₂ bottle was closed (V1). Then, after opening the valve from V1 to V5 (V4 is closed), the compressor on which the roughly set operating pressure is started and the operating pressure is set to the set value by the control valve (RV1). After passing through the heat exchanger (IT1), carbon dioxide was introduced into the extractor by opening the valve (V5), while the control valve (RV2) was closed. By opening and adjusting the control valves (RV2) and (RV3), the desired pressure in the

separator was achieved, as well as maintaining the working flow of the extractant. Supercritical fluid extraction was performed under extraction conditions: temperature of 65 °C, flow of 0.3 kg CO₂/h and constant pressure of 400 bar [20]. The extraction process lasted for 3 h, and the extraction yield was determined after 15, 30, 45, 60, 90, 120 and 180 minutes, and was expressed in % (m/m). Two replicates were performed for each *C. sativa* variety. The obtained extracts were transferred to plastic bottles which were closed and stored in the refrigerator at 4 °C. With SFE, first the essential oils were extracted from the primary raw material, then the SFE raffinates were stored and used to obtain total polyphenols by MAE.

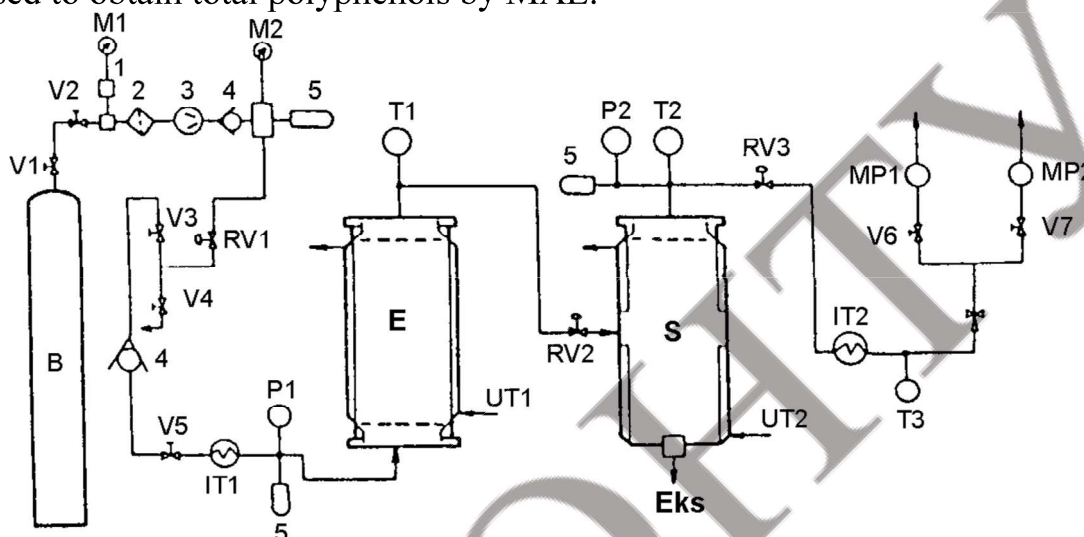


Figure 2. Schematic representation of the supercritical fluid extraction device:

1. Measuring connector; 2. Filter; 3. Diaphragm compressor; 4. Control valve; 5. Safety valve; B - Bottle with carbon dioxide; V - Valve; M - Manometer; RV - Control valve; IT - Heat exchanger; P - Pressure gauge; E - Extractor; T - Thermometer; UT - Ultrathermostat; S - Separator; Ex - Extract; MP - Flow meter.

Extracts from the primary raw material of hemp and SFE raffinate were processed by microwave-assisted extraction in order to isolate total phenol and flavonoids. MAE was performed on a modified microwave oven (MM817ASM, Bosch, Germany) in which a glass balloon with a reflux condenser was placed (Figure 3). Sample (2 g) was weighed and mixed with 60 mL of 25% ethanol, 30 mL/g was solvent-sample ratio [19]. The extraction was performed at a microwave power of 600 W for 25 minutes. When the extraction was completed, the obtained extracts were filtered through filter paper. MAE was performed with the primary raw material and SFE raffinate, twice for each variety of *C. sativa*. The obtained extracts were subjected to a dry evaporation process on a rotary vacuum evaporator to remove the solvent from the extract, after which the extraction yield was determined (%; m/m). The obtained extracts were stored in glass vials in a refrigerator at 4 °C.

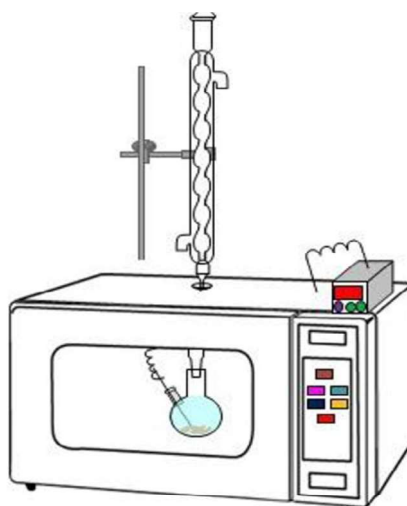


Figure 3. Schematic diagram showing vacuum assisted MAE model

The content of total phenols (TP) in liquid hemp extracts was determined by the Folin-Ciocalteu spectrophotometric method [21]. This method is based on measuring the reduction capacity of polyphenolic compounds. Appropriate dilution of the liquid extract or dry hemp extract solution (0.1 mL) was mixed with 7.9 mL of distilled water, 0.5 mL of Folin-Ciocalteu reagent and 1.5 mL of 20% Na_2CO_3 . In the preparation of the trial, 0.1 mL of distilled water or an appropriate ethanol-water mixture used as an extractant in MAE extraction was added instead of 0.1 mL of sample. The reaction mixture was incubated for 1 h at room temperature and the absorbance of the sample was measured on a VIS spectrophotometer at 750 nm. All measurements were performed in triplicate. TP yield in extraction procedures is expressed in g of gallic acid equivalents per 100 g of sample (g GAE/100 g of sample).

The content of total flavonoids (TF) in hemp extracts was determined by the Markham colorimetric method [22]. The determination of the content of total flavonoids is based on their ability to build appropriate metal-organic complexes with metals. The reaction mixture was prepared by mixing 1 mL of sample (appropriate dilution of liquid extract or dry hemp extract dissolved in distilled water) with 4 mL of distilled water and 0.3 mL of 5% NaNO_2 . The mixture was then incubated at room temperature for 6 minutes and 0.3 mL of 10% $\text{AlCl}_3 \times 10 \text{H}_2\text{O}$ was added thereto and 5 mL of 1 M NaOH 5 minutes later. The resulting mixture was made up with distilled water (3.4 mL) to a total volume of 10 mL. The absorbance of the sample was measured on a VIS spectrophotometer at a wavelength of 510 nm and all measurements were performed in triplicate. TF yield is expressed in g of catechin equivalents per 100 g of sample (g CE/100 g of sample).

Data was analyzed by using analysis of variance (ANOVA) with Tukey's multiple comparison test at $p < 0.05$. Statistica 10.0 (StatSoft, Inc., Tulsa, OK).

IV. RESULTS

4.1. Hydrodistillation (HD) and microwave-assisted hydrodistillation (MWHD)

After hemp hydrodistillation of all three varieties we obtained golden to pale yellow color essential oil. The yield of essential oil obtained in the process of HD and MWHD, is shown in Figure 4.

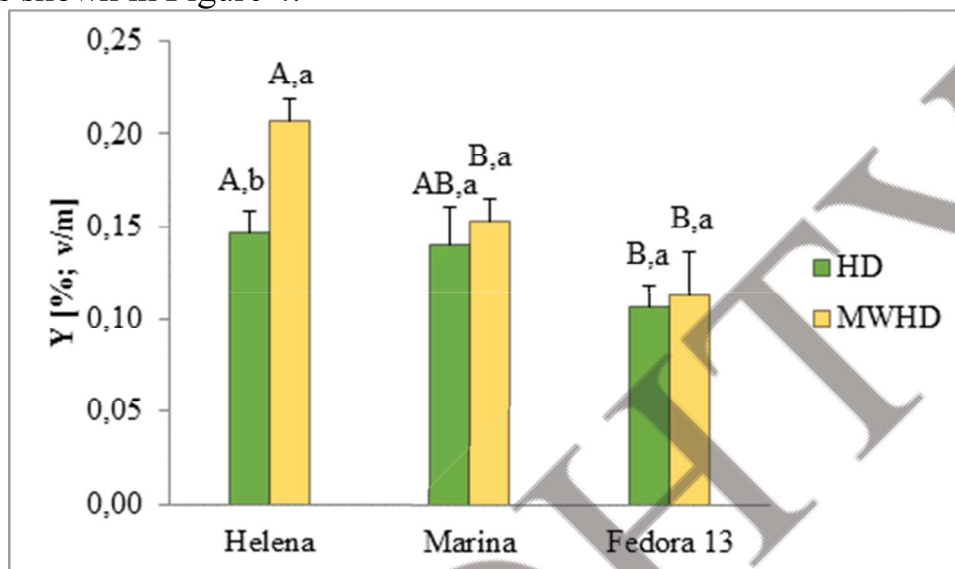


Figure 4. Yield of essential oil of three different varieties of *C. sativa* in HD and MWHD. Results were expressed as mean \pm standard deviation (SD) and different uppercase letters represent statistically significant differences ($p < 0.05$) among hemp varieties, while different lowercase letters represent differences among applied techniques according to Tukey's test.

From the Figure 4, it can be seen that the highest yield of essential oil was achieved in the process of HD, in the variety Helena (0.15%), while Marina (0.14%) and Fedora 13 (0.11%) gave a lower yield ($p < 0.05$). In MWHD, the highest yield of essential oil was given by the variety Helena (0.21), and the lowest yield was given by the variety Fedora 13 (0.11%) ($p < 0.05$), while the variety Marina gave a yield of 0.15%. Optimized MWHD provided higher essential oil yields than conventional HD. Unlike Helena and Marina, the yields of essential oil obtained with the Fedora 13 variety are the same using HD and MWHD.

According to Drinic [12], the content of essential oil of industrial hemp herb was determined by the HD, where it was determined that the content of essential oil was 0.08% (v/m). Comparing the results obtained in this work, it can be seen that the yield of essential oil in the HD process was slightly higher than the results obtained in the aforementioned study [12]. MWHD has been optimized for the isolation of cannabis essential oil from dried flowers of four different varieties of hemp [6]. The variety Kompolti gave the highest yield of 0.27%. This study showed that MWHD is an effective technique for isolating cannabis essential oil from dried hemp flowers. Generally, the use of MWHD could facilitate extraction and increase the yield of essential oil. In the work of Gunjević et al. [14], conventional HD was performed to compare the yield of essential oil with MWHD. The optimized MWHD procedure gave $0.35 \pm 0.02\%$ (v/v) of essential oil, while the HD yielded $0.12 \pm 0.01\%$ (v/v). The yield

of essential oil obtained in HD is therefore about three times lower than that obtained in optimized MWHD. The yield of essential oil in this work in the variety Helena obtained by MWHD (0.21%) is significantly higher than the yield obtained by HD (0.15%). It can be concluded that MWHD provides certain advantage in terms of yield.

4.2. Supercritical fluid extraction (SFE)

SFE was used to isolate the lipophilic fraction from industrial hemp varieties. The extraction yield depended on the initial composition of the plant material and the yield increased linearly with the extraction time, when non-polar and easily volatile compounds were isolated. Extraction yields (Y) for three different varieties of *C. sativa* are shown in Figure 5.

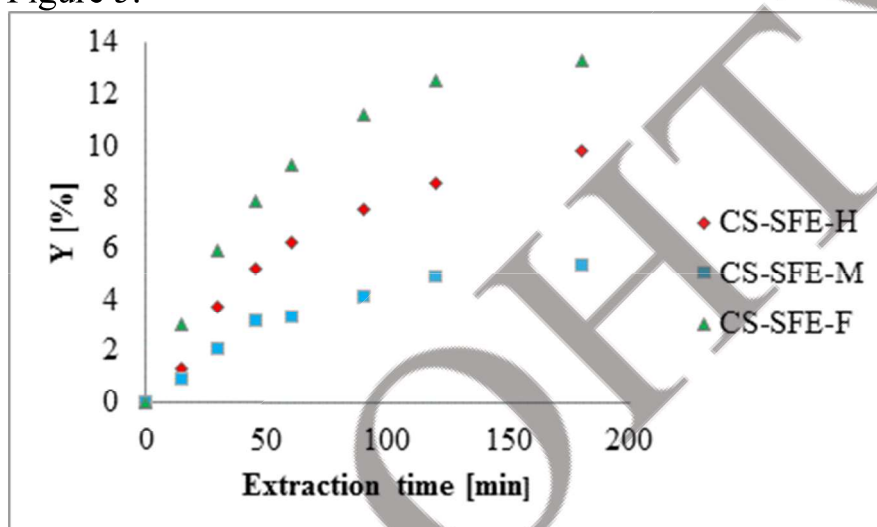


Figure 5. Kinetics of SFE lipophilic compounds from three different varieties of *C. sativa*

The highest yield of essential oil was given by the variety Fedora 13 (14.01%), while Helena achieved a slightly lower yield (9.77%), and the variety Marina had the lowest yield of essential oil (5.33%). When we compared SFE with traditional HD and MWHD to isolate essential oils from *C. sativa*, it was noticed that SFE provided better oil yield. Low temperature, increased pressure, and the use of supercritical CO₂ increased the yield of essential oils from *C. sativa*. So SFE at previously defined optimal conditions [19] has given preference to the use of modern extraction techniques over conventional approach.

Naz et al. [11] compared SFE with traditional steam distillation and HD for isolation of volatile aromatics from *C. indica* and *C. sativa* leaves. SFE was able to provide better oil recovery comparing to applied distillation techniques, while SFE at low pressure (85 bar) and temperature of 45 °C provided the highest oil yield (0.031 and 0.039%). By comparing the results obtained in this paper, it can be concluded that the parameters used were better in the process of isolating essential oil from the literature parameters. SFE has been established method for production of CBD-rich oil from *C. sativa* inflorescence. Marzorati et al. [16] applied heat treatment (100 °C, 6h) of Finola variety inflorescence for decarboxylation of CBDA to CBD which was followed by SFE at 380 bar and 60 °C to obtain 14% of total extraction yield with ≈50% CBD content. The yield of essential oil obtained in this work in the variety Fedora 13 is in accordance with the literature data.

4.3. Microwave-assisted extraction (MAE)

MAE was performed under optimal conditions, which predict the maximum yield of the extract. The yields of extracts from the primary raw material and SFE raffinates were compared and shown in Figure 6.

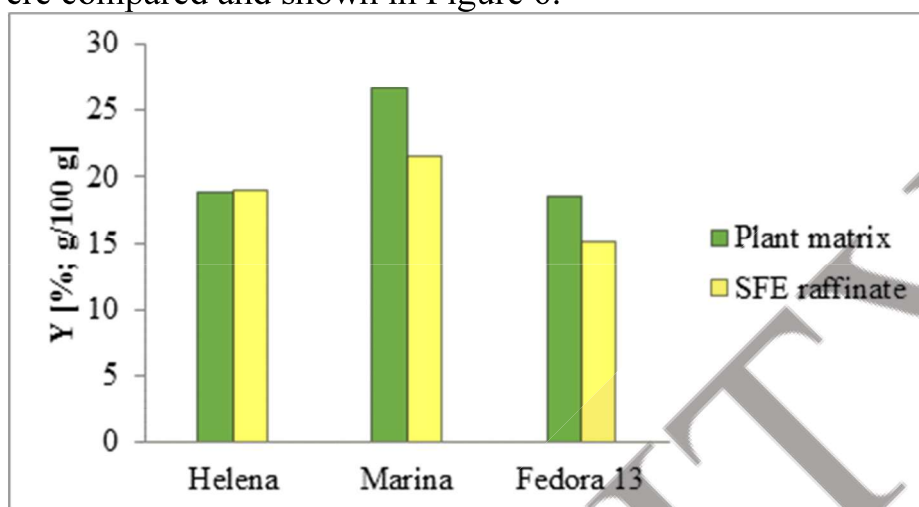


Figure 6. Yield of extraction from primary raw material and SFE raffinate for three different varieties of *C. sativa*

The yield of the extract is higher from the primary raw material than from the SFE raffinate, which is expected, but the amount obtained from SFE raffinate is not insignificant. The highest yield of primary raw material was given by the variety Marina (26.76%), and the lowest Fedora 13 (18.57%). The highest yield of SFE raffinates was given by the variety Marina (21.63%), and the lowest Fedora 13 (15.15%). From the obtained results it can be concluded that the highest yield of extract was in the primary raw material and in SFE raffinate at variety Marina. It is interesting that in variety Helena SFE raffinate has the same extraction yield as the primary raw material.

A recent study investigated efficiency of MAE in recovery of linoleic acid and linolenic acid oil from hempseed [13]. Oil yield obtained under MAE optimal conditions (33.91% w/w) was lower comparing to SOX (37.93% w/w). However, oils obtained with MAE exhibited higher tocopherol content and oxidative stability comparing to SOX due to shorter extraction procedure and lower temperatures used in MAE. In this paper, slightly lower yields of extracts were obtained in relation to the literature. Another study compared performance of MAE, UAE, heat reflux extraction (HRE), SOX and SFE in isolation of cannabinoids from hemp seeds [23]. The highest total CBDs yield was obtained with MAE (6.09 $\mu\text{g/g}$) comparing to all other extraction procedures (3.61-5.81 $\mu\text{g/g}$). This was supported with morphological observations on microstructure level, whereat hempseed cell wall breaking was significant after MAE which allowed easier diffusion of target compounds towards extraction medium. Furthermore, MAE was the fastest technique and required the smallest solvent to plant ratio indicating that MAE is rapid and cost-effective technology for CBDs isolation.

4.4. Content of total phenols (TP) and total flavonoids (TF)

The values of total phenols content (TP) in hemp extracts by MAE from primary raw material and SFE raffinate were the result of experiment. Figure 7 shows the yield of total phenols expressed in g GAE/100 g for three different varieties of *C. sativa*.

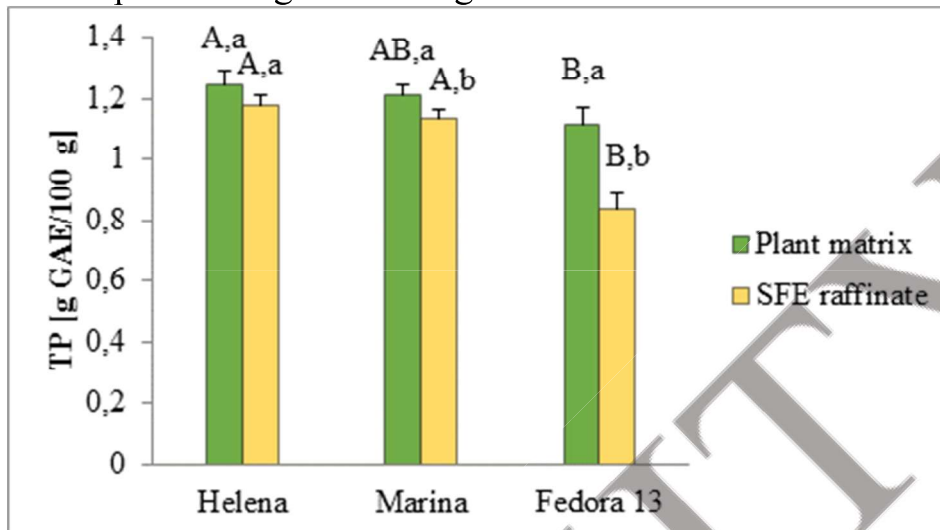


Figure 7. Yield of total phenols expressed in g GAE/100 g from raw material and SFE raffinate. Results were expressed as mean \pm standard deviation (SD) and different uppercase letters represent statistically significant differences ($p < 0.05$) among hemp varieties, while different lowercase letters represent differences among applied techniques according to Tukey's test.

It can be observed that the highest yield of TP was recorded in the sample of the variety Helena (1.24g GAE/100 g) from the primary raw material (Figure 7). The highest yield of total phenols from SFE raffinate was recorded in the variety Helena (1.17g GAE/100 g), while the lowest yield was achieved with the Fedora 13 variety (0.84 g GAE/100 g) ($p < 0.05$).

Figure 8 shows the yield of total flavonoids (TF) expressed in g CE/100 g from the primary raw material and SFE raffinate for three different varieties of *C. sativa*.

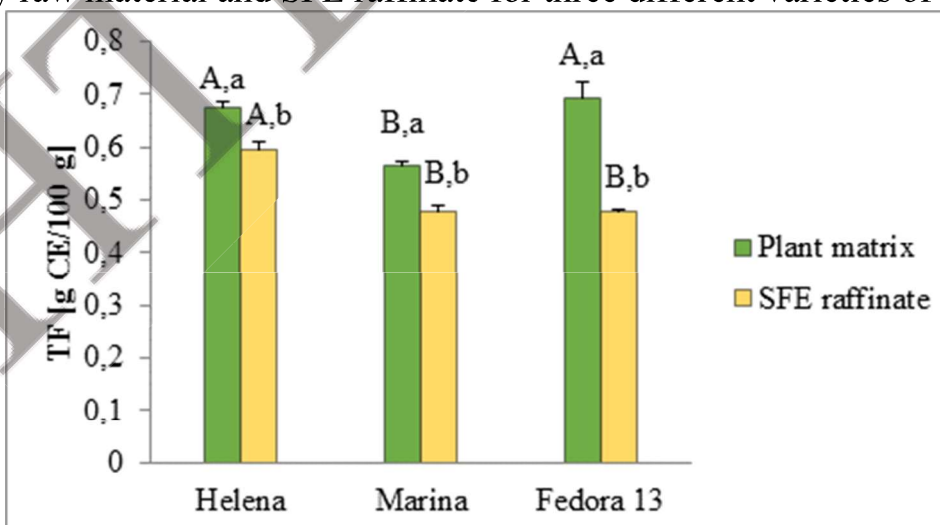


Figure 8. Yield of total flavonoids expressed in g CE/100 g from primary raw material and SFE raffinate. Results were expressed as mean \pm standard deviation (SD) and different uppercase letters represent statistically significant differences ($p < 0.05$) among hemp varieties, while different lowercase letters represent differences among applied techniques according to Tukey's test.

As for flavonoids, the yields of these compounds in hemp extracts were obtained using MAE. The highest yield of total flavonoids (TF) was obtained in the variety Fedora 13 (0.69 g CE/100 g) from the primary raw material, in contrast to the variety Marina in which the lowest yield of (0.56 g CE/100 g) was recorded ($p < 0.05$). In case of SFE raffinates, the obtained results showed that the highest TF yield was achieved in the Helena variety (0.60 g CE/100 g), while the yield in Fedora 13 was significantly lower (0.48 g CE/100 g) ($p < 0.05$).

Matešić et al. [19] extracted phenolic compounds from *C. sativa* using microwaves. The effect of each parameter separately on the yield of total phenols and flavonoids was determined. The highest amount of polyphenols (35.514 ± 1.289 mg GAE/ g) was measured for hemp extract. The maximum yield of total flavonoids was $3.04 \pm 0.62\%$. Comparing the results from the literature, it is concluded that a lower content of phenols and flavonoids was obtained in this paper. The results of the content of TP and TF were compared with the study of Drinić [12]. The content of total phenols (2.71 g GAE/100 g) and flavonoids (1.42 g CE/100 g) was observed to be significantly higher than the content of phenols (1.24 g GAE/100 g) and flavonoids (0.69 g CE/100 g) determined in this study.

V. CONCLUSIONS

Although the interest of the scientific community for investigation of the hemp is constantly growing, there is still room for further improvements. Therefore, it is very important to improve the extraction techniques to obtain a product enriched with bioactive compounds in an optimal ratio that ensures maximum biological activity of the product. This step may include further investigation of the extraction processes, application of different solvents, optimization of the extraction, and improvement of the downstream processes (isolation and purification of/from the crude extract). Based on the conducted experiments and the obtained results, it can be concluded that supercritical fluid extraction is the best technique for obtaining essential oil from *Cannabis sativa*, when compared to HD and MWHD. The high content of phenols and flavonoids obtained from SFE raffinates indicates that the by-products of supercritical fluid extraction are a good natural source of antioxidants that can be used in the pharmaceutical and cosmetic industries. It is also very important to elaborate the mass production of the products and making them available to the customers. This step includes scaling-up the production processes which have to be in accordance to the good manufacturing practices (GMP) and HACCP, as well as good laboratory practices (GLP). This includes intensive funding and further investigations and market analysis which will provide necessary data for accomplishing mentioned goals. Finally, hemp-based extracts exhibited significant health benefits against the different types of diseases and disorders. Therefore, the required large-scaled studies and investments will be justified by development of the different products which will help people to fight these diseases.

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