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Nutritional Profile and Antioxidant Properties of Hemp (*Cannabis sativa* L.) Seed from Romania

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Abstract: This study evaluates the nutritional and phytochemical properties of six industrial hemp varieties from Romania. The proximate composition (proteins, lipids, ash, moisture, fiber, and carbohydrates), total polyphenol content (TPC), total flavonoid content (TFC), antioxidant activity (DPPH), and macro/microelements were analyzed. Two extraction methods were used: conventional and ultrasound-assisted extraction. The results showed a protein content of 20.92–25.39 g/100 g, lipid content 24.92–28.43 g/100 g, fiber 25.92–31.21 g/100 g, ash 4.71–6.38 g/100 g, moisture content 4.84–5.96 g/100 g, carbohydrates between 35.05 and 43.58 g/100 g, and energy value between 483.25 and 502.40 kcal/100 g. The TPC content varied between 732.36 and 1457.60 mgGAE/kg for conventional extraction methods and from 1003.48 to 1519.87 mg GAE/kg for ultrasound-assisted methods. The TFC content was 343.91–1013.40 mg QE/kg for conventional extraction methods and 511.92 to 1222.14 mg QE/kg for ultrasound-assisted methods. The results showed that the extraction method influenced the phytochemical compounds. Macroelements were dominated by potassium (5533.23 µg/g), magnesium (2616.34 µg/g), and calcium (1853.51 µg/g). Microelements showed the highest levels of iron (189.49 µg/g), followed by manganese (138.26 µg/g), zinc (75.25 µg/g), and copper (13.08 µg/g). Nickel and cadmium were found in trace amounts. Multivariate analysis (PCA) was used to correlate the data.

Keywords: hemp seed; cannabis sativa; proximate composition; mineral elements; total polyphenols content; antioxidant activity; extraction methods

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1. Introduction

Industrial hemp (*Cannabis sativa* L.), an annual herbaceous plant from the *Cannabaceae* family [1], has historically been a significant crop originating in Asia and extensively cultivated in Europe for textile and food production. Over the centuries, it has been regarded as one of the most essential crops, providing a range of materials and products, including rope, fabric, food, lamp oil, and medicinal products [2,3].

While hemp seeds are primarily used as animal feed, there is growing interest in their potential for human nutrition, leading to increased demand for hemp-derived products such as oil, meal, flour, and protein powder. These products offer a natural, nutrient-rich alternative for human consumption, providing all essential amino and fatty acids required for optimal health [4]. Nutrient-wise, hemp seeds comprise approximately 25–35% oil, 20–25% protein, 20–30% carbohydrates, 10–15% insoluble fibre, and a rich profile of vitamins and minerals [5].

Hemp seeds are a valuable source of essential macro- and microelements that contribute to human health. Mineral deficiencies, including iron (Fe), iodine (I), and zinc (Zn), are becoming a more significant dietary issue in human populations. Furthermore, the intake of key elements such as calcium (Ca), potassium (K), magnesium (Mg), and selenium (Se) may be insufficient in certain dietary patterns. However, hemp seeds might become crucial future candidates for addressing these deficiencies because of their balanced chemical composition [6].

Hemp seed oil is notably rich in polyunsaturated fatty acids, presenting a beneficial balance of α-linolenic acid (omega-6) and linoleic acid (omega-3) with an ideal ratio of 2.5–3.1, which is particularly favourable for human dietary needs [7]. These attributes contribute to the robust market value of hemp seeds and their predominant use in human food and nutritional supplements [8].

Hemp seeds comprise a significant abundance of natural antioxidants and a variety of bioactive compounds, which include phenolic substances such as coumarins, hydroxybenzoic acids, and multiple xanthone peptides. These polyphenols have significant anti-aging properties, protecting against various age-related conditions, including cardiovascular disease, cancer, and neurodegenerative disorders [9]. Phenolic compounds offer various physiological benefits, including cardioprotective and anti-inflammatory effects [10]. Recent research [11–13] supports the role of hemp-derived phenolic compounds in scavenging free radicals, contributing to anti-aging benefits and the prevention of oxidative stress-related diseases. Additionally, new studies [14] have strengthened the evidence supporting the cardioprotective and anti-inflammatory properties of flavonoids, including their modulation of key enzyme pathways.

Ensuring the availability of these bioactive compounds and achieving high recovery rates from any plant matrix is paramount. The extraction process plays a critical role in ensuring efficient acquisition of compounds while preserving their integrity and maximizing yield. It is a pivotal step in the overall process.

Standard extraction techniques for bioactive compounds from plant materials include Soxhlet extraction, maceration, and hydro-distillation [15]. These methods often involve lengthy extraction times, require expensive high-purity solvents, and can lead to the degradation of heat-sensitive compounds. These limitations have prompted researchers to explore and develop alternative extraction methods that are more efficient, cost-effective, and better at preserving the integrity of delicate compounds [16,17].

Ultrasound-assisted extraction (UAE) is a highly effective method that offers significant advantages over traditional techniques. These benefits include shorter processing times, simpler operational procedures, reduced solvent usage, lower temperatures, energy savings, and higher extraction yields [18]. UAE enhances extraction efficiency by utilizing cavitation effects and improving mass transfer [19].

UAE has been successfully used to extract valuable compounds like anthocyanins and phenolics from food-processing by-products. Studies such as those by Kruszewski et al. on blackcurrant pomace and Nour V. on bilberry pomace in apple juice [20,21] demonstrate the method's ability to enhance yields, improve antioxidant activity, and add nutritional value while promoting the sustainable use of by-products. With its efficiency, safety, and eco-friendly nature, UAE holds significant promise in food technology for extracting bioactive compounds and enriching functional foods.

While global research on hemp seeds has been extensive, data on the nutritional and phytochemical characteristics of industrial hemp varieties cultivated in Romania is limited.

Romania has a rich tradition of hemp cultivation and development of new hemp varieties, but studies on these varieties are scarce. This investigation aims to fill this gap by providing comprehensive data on six varieties of Romanian germplasm, including two varieties undergoing certification. This information could be valuable to the scientific community and future breeding programs.

Previous studies have established the efficacy of both conventional and ultrasound-assisted extraction methods. However, comparison of their application in hemp seeds, particularly in relation to polyphenols, flavonoids, and antioxidant compounds, has not been fully explored. Our study compares these methods in the context of Romanian hemp varieties, offering new insights into optimizing the recovery of bioactive compounds. This comparison will be of interest for practical applications in the food and nutraceutical sectors, as it points to more efficient extraction techniques.

This study aims to evaluate the nutritional, phytochemical, and antioxidant activity of six varieties of dioecious hemp seeds cultivated in Romania. For this purpose, the proximate composition and content of macro- and microelements in hemp seeds was determined. In addition, the impact of conventional extraction (CES) and ultrasound-assisted extraction (UAE) on the phytochemical profile and antioxidant activity was investigated.

2. Materials and Methods

2.1. Chemicals

The reagents ethanol, hydrochloric acid, Folin–Ciocalteu, gallic acid, quercetin standard, 1,1-diphenyl-2-picrylhydrazyl (DPPH), and ascorbic acid were purchased from Sigma–Aldrich Chemie GmbH (München, Germany), while sodium carbonate, sodium nitrite, and aluminium chloride were purchased from Geyer GmbH (Renningen, Germany). All the reagents utilized for the chemical analysis were of analytical quality.

2.2. Plant Material

The varieties and lines were developed and cultivated at the Lovrin Agricultural Research and Development Station, located at 45°57′03″ N 20°46′32″ E. The hemp seed varieties analyzed in this study were Lovrin 110 (HSLO), Silvana (HSSI), Armanca (HSAR), and Teodora (HSTE), while the LV 585 (HSLV585), and LV 300 (HSLV300) lines are in the process of being certified. The hemp seed was ground with a Grindomix GM200 (Retsch GmbH, Haan, Germany) mill before all analyses and used immediately.

2.3. Determination of Proximate Composition and Energetical Values

The proximate chemical analysis of the hemp seed samples aligned with standardised methodologies established by the Association of Official Analytical Chemists (AOAC) [22]. Specifically, moisture content was determined using method 930.15, while ash content was analyzed using method 942.05. The crude protein content was measured by applying the Kjeldahl technique, as outlined in method number 2001.11, utilizing a Kjeltec system (Velp, Padua, Italy) for accuracy, with a conversion factor of 6.25. Method number 978.10 was used to assess crude fibre, with measurements taken via the Fibertec™ 2010 system (Foss, Padua, Italy). Lipid content was evaluated using method 920.39 through a Soxhlet Extraction System (Raypa, Barcelona, Spain, Raypa SX-6 MP). The carbohydrate content was not directly measured but instead was calculated by subtracting the total percentages of moisture, lipids, proteins, and ash from 100%. Additionally, the caloric or energy value of the samples was computed following the procedure detailed by Das P.C. et al. [23]. This method utilised Equation (1), applying the caloric conversion factors where each gram of carbohydrates contributed four calories, each gram of protein provided four calories, and each gram of fat supplied nine calories:

$$\text{Energy value (kcal/100 g)} = \text{carbohydrates (\%)} \times 4 + \text{lipids (\%)} \times 9 + \text{proteins (\%)} \times 4 \quad (1)$$

2.4. Preparation of Plant Extracts

The selection of UAE (ultrasound-assisted extraction) and CES (conventional extraction method) parameters was informed by previous studies [16] showcasing these methods and our own [17] extractions on various matrices.

The ground seeds of each variety were mixed with a 70% alcohol solution to prepare the sample for extraction. Subsequently, the mixture was subjected to two distinct extraction techniques: the traditional conventional extraction method (CES) and an alternative approach utilizing ultrasound-assisted extraction (UAE) (Figure 1).

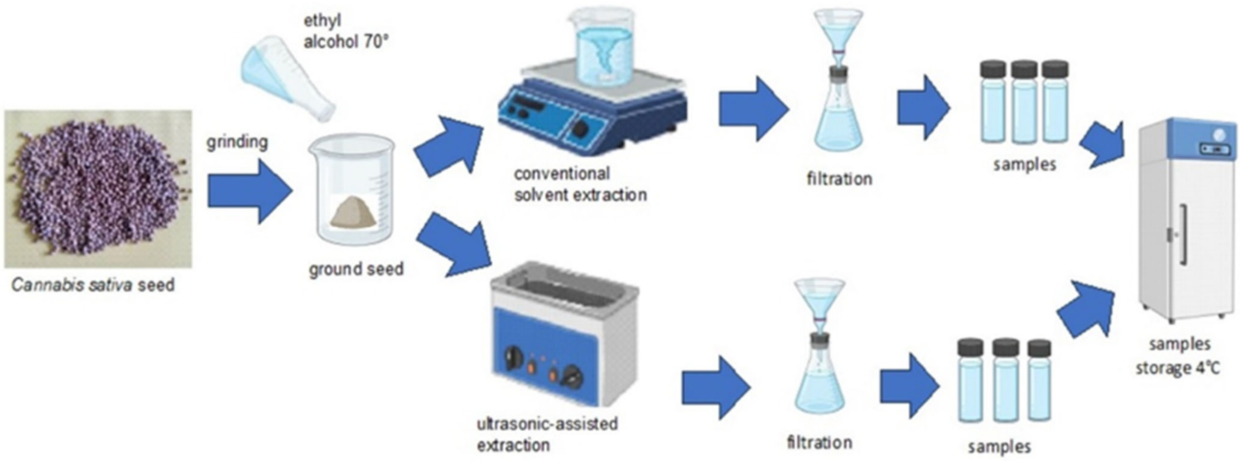


Figure 1. Preparation of hemp seed extract.

2.4.1. Conventional Extraction with Solvent (CES)

10 mL of 70 percent ethyl alcohol produced by Sigma-Aldrich, Merck KGaA, Darmstadt, Germany was used to extract a 1.0 g sample, which was then left at room temperature for an hour on a Holt plate stirrer (IDL, Freising, Germany). After the extraction, the mixture was filtered through filter paper and stored at 4 °C until analysis. This process was repeated three times.

2.4.2. Extraction of Antioxidant Compounds by UAE (Ultrasound-Assisted Extraction)

To ensure the comparability of results between the two extraction techniques, an identical 1.0 g sample was mixed with 10 mL of 70 percent ethyl alcohol and extracted in an ultrasonic water bath (FALC Instruments, Treviglio, Italy). The process was conducted at room temperature for 30 min, with the ultrasonic bath set at 216 W and 40 kHz. After extraction, the mixture was filtered through filter paper and stored at 4 °C for subsequent analysis. This procedure was repeated three times.

2.5. Determination of Macro- and Microelements

The elemental composition of *Cannabis sativa* seeds was analyzed after undergoing a calcination process at 800 °C in a muffle furnace (SLN 53 STD, POL-EKO-Aparatura SP, Wodzislaw, Poland). Following this, extraction was carried out using 20 percent hydrochloric acid (HCl) (Sigma-Aldrich Chemie GmbH, Munich, Germany). Atomic absorption spectrometry (AAS) was used to quantify these elements using a Varian Spectra 240 FS (USA) spectrophotometer. The working conditions are presented in Table 1.

Table 1. Working conditions for Varian Spectra 240 FS spectrophotometer.

Metal	λ (nm)	Lamp Current (mA)	Slit Width (mm)	Quantification (LOQ) Limit
copper (Cu)	324.8	4	0.5	0.0012 μg/L
cadmium (Cd)	228.8	4	0.5	0.0015 μg/L
nickel (Ni)	232	4	0.2	0.0158 μg/L
zinc (Zn)	213	5	1	0.008 μg/L
iron (Fe)	248.3	5	0.2	0.06 mg/L
manganese (Mn)	279.5	5	0.2	0.025 mg/L
calcium (Ca)	422.7	10	0.5	0.10 mg/mL
magnesium (Mg)	282.5	4	0.5	0.011 mg/mL
potassium (K)	414	4	0.5	0.252 mg/mL

Air–acetylene ratio 13.50:2. Nebulizer uptake rate: 5 L/min.

For calibration, standard solutions with concentrations ranging from 0.3 to 3 μg/L were prepared from ICP multielement standard solution 1000 mg/L. The results are presented in μg/g; each experiment was conducted in triplicate [24].

2.6. Determination of Antioxidant Profile

2.6.1. Determination of Total Polyphenols Content (TPC)

The total polyphenol content (TPC) of twelve hemp seed extracts was measured using the Folin–Ciocalteu method [25]. For this purpose, 0.5 mL filtered extract was mixed with 1.25 mL of 1:10 Folin–Ciocalteu aqueous solution (Sigma-Aldrich Chemie GmbH, Munich, Germany). The mixture was allowed to stand for 5 min at room temperature; then, 1 mL of 6% sodium carbonate (Geyer GmbH, Renningen, Germany) was added. A Memmert INB500 thermostat (Memmert GmbH & Co. KG—Schwabach, Germany) was used to maintain a temperature of 50 °C for 30 min. Absorbance was recorded at a wavelength of 750 nm using a Specord 205 UV-VIS spectrophotometer (Analytik Jena AG, Jena, Germany). Each sample was analyzed in triplicate, with the results expressed as the mean ± standard deviation (SD) in mg GAE·Kg^{-1}. Gallic acid served as the standard for establishing the calibration curve, which ranged from 0–200 μg·mL^{-1}, with the calibration equation given by y = 0.0174x + 0.0354 (R^2 = 0.9986).

To compare the TPC content obtained through the two extraction methods, UAE and CES, the following indicator was used:

$$\text{Increasing } TPC_{UAE/CES}\ (\%) = \left[\frac{\text{TPC UAE} - \text{TPC CES}}{\text{TPC CES}}\right] \times 100 \qquad (2)$$

where TPC_{UAE} represents the TPC of the UAE sample (mg GAE/Kg), and TPC_{CES} refers to the TPC of the CES sample (mg GAE/Kg)

2.6.2. Determination of Total Flavonoid Content (TFC)

The total flavonoid content was determined by the methodology proposed by Cocan et al. [26] Precisely 1 mL of the extract was placed into a test tube, followed by the addition of 0.3 mL of a 5% $NaNO_2$ (Geyer GmbH, Renningen, Germany) solution and 0.3 mL of a 10% $AlCl_3$ (Geyer GmbH, Renningen, Germany) solution. The mixture was left undisturbed at room temperature, and after 6 min, 2 mL of 4% NaOH solution (Chemie GmbH, Munich, Germany) was added. The final volume of 10 mL was achieved using a 70 per cent ethyl alcohol solution. After 15 min, a UV-VIS spectrophotometer (Specord 205;

Analytik Jena AG, Jena, Germany) was employed to measure the absorbance at 415 nm, with the 70 per cent ethyl alcohol solution selected as the blank control. The analysis was performed in triplicate, with the results reported as mean ± standard deviation (SD) in mg QUE·100 g^{-1}. For calibration, quercetin (Sigma-Aldrich Chemie GmbH, Munich, Germany) was prepared within a concentration range of 0–500 μg·mL^{-1}, resulting in the calibration equation y = 0.0013x + 0.0081 (R^2 = 0.9955).

To compare the TFC content obtained through the UAE and CES extraction methods, the following indicator was used:

$$\text{Increasing TFC}_{\text{UAE/CES}}\ (\%) = \left[\frac{\text{TFC UAE} - \text{TFC CES}}{\text{TFC CES}}\right] \times 100 \quad (3)$$

where TFC_{UAE} is the TFC of the UAE sample (mg GAE/Kg), and TFC_{CES} is the TFC of the CES sample (mg GAE/Kg)

2.6.3. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Assay for Quantification of Antioxidant Capacity

To evaluate the radical scavenging activity using the DPPH method, five concentrations of each extract were prepared: 100.00 mg/mL, 40 mg/mL, 20 mg/mL, 13.33 mg/mL, and 5 mg/mL, for each of the twelve tested samples. A 0.1 mM DPPH solution (Sigma-Aldrich, Taufkirchen, Germany) in ethyl alcohol was prepared to evaluate antioxidant activity. A 1 mL extract was mixed with 2.5 mL DPPH solution, shaken, and incubated in the dark at room temperature for 30 min. Concurrently, the antioxidant activity of five ascorbic acid solutions at various concentrations (0.02–0.1 mg/mL) was measured as a positive control. Absorbance was measured at 518 nm using a UV-VIS spectrophotometer (Specord 205, Analytik Jena AG, Jena, Germany). Each sample was tested in triplicate, and the mean RSA (%) was calculated using the designated formula:

$$\text{RSA}\ (\%) = \left(\frac{\text{A control} - \text{Asample}}{\text{Acontrol}}\right) \times 100 \quad (4)$$

where A control and A sample are the absorbance values of the control and sample extract.

The IC_{50} value was calculated and compared against ascorbic acid to determine the antioxidant capacity.

To compare the RSA content obtained through the two extraction methods (UAE and CES), the following indicator was used:

$$\text{Increasing RSA}_{\text{UAE/CES}}\ (\%) = \left[\frac{\text{RSA UAE} - \text{RSA CES}}{\text{RSA CES}}\right] \times 100 \quad (5)$$

where RSA_{UAE} refers to the RSA of the UAE sample (mg GAE/Kg) and RSA_{CES} represents the RSA of the CES sample (mg GAE/Kg).

2.7. Statistical Analysis

JASP 0.19 was selected for statistical analysis. Mean values and standard deviation were calculated for data summarization. Group comparisons were evaluated through ANOVA, followed by the Tukey test. The threshold for statistical significance was set at $p < 0.05$. Principal component analysis (PCA) with varimax rotation for dimensionality reduction was conducted using OriginPro 2025 to provide a concise and optimal description of the data.

3. Results

3.1. Determination of the Proximate Composition of Hemp Seed Varieties

Table 2 comprehensively summarises the variability in moisture content, protein, mineral, lipid, fiber, and carbohydrate composition across the various hemp seed samples.

Table 2. Proximate composition of the samples.

Sample	Moisture	Dry Matter	Ash	Lipids	Proteins	Fiber	Carbohydrates	Energy Values
	(g/100 g)	(g/100 g)	(g/100 g)	(g/100 g)	(g/100 g)	(g/100 g)	(g/100 g)	(kcal/100 g)
HSLO	4.84 ± 0.06 ^{a}	95.16 ± 0.85 ^{c}	5.05 ± 0.04 ^{b}	27.86 ± 0.53 ^{bc}	20.93 ± 0.03 ^{a}	29.77 ± 0.04 ^{e}	40.79 ± 0.07 ^{d}	502.40 ± 0.12 ^{f}
HSSI	5.96 ± 0.04 ^{c}	94.04 ± 0.70 ^{a}	5.03 ± 0.03 ^{b}	28.43 ± 0.05 ^{c}	21.78 ± 0.04 ^{c}	29.37 ± 0.03 ^{d}	38.73 ± 0.18 ^{c}	498.36 ± 0.07 ^{e}
HSAR	5.52 ± 0.11 ^{b}	94.48 ± 0.54 ^{b}	5.07 ± 0.06 ^{b}	27.13 ± 0.31 ^{b}	20.92 ± 0.03 ^{a}	28.02 ± 0.07 ^{c}	41.05 ± 0.10 ^{d}	494.85 ± 0.10 ^{c}
HSTE	5.63 ± 0.05 ^{b}	94.37 ± 0.36 ^{b}	4.71 ± 0.04 ^{a}	24.92 ± 0.07 ^{a}	21.15 ± 0.05 ^{b}	31.21 ± 0.04 ^{f}	43.58 ± 0.07 ^{e}	483.25 ± 0.05 ^{a}
HSLV585	5.52 ± 0.06 ^{b}	94.48 ± 0.41 ^{b}	6.38 ± 0.03 ^{c}	27.67 ± 0.03 ^{bc}	25.39 ± 0.03 ^{e}	26.80 ± 0.02 ^{b}	35.05 ± 0.06 ^{a}	490.75 ± 0.06 ^{b}
HSLV300	5.54 ± 0.04 ^{b}	94.46 ± 0.47 ^{b}	5.03 ± 0.06 ^{b}	27.03 ± 0.54 ^{b}	23.72 ± 0.03 ^{d}	25.92 ± 0.08 ^{a}	38.13 ± 0.13 ^{b}	495.59 ± 0.09 ^{d}
Average	5.50	94.50	5.21	27.17	22.31	28.52	39.55	494.20

hemp seed varieties: HSLO—Lovrin 110; HSSI—Silvana; HSAR—Armanca; HSTE—Teodora; and hemp seed lines HSLV585—LV 585; HSLV300—LV 300. Results are expressed as the mean value of three determinations ± standard deviation (SD). The different lower-case letters (a–f) represent the significant differences ($p < 0.05$) according to the ANOVA between samples in the same row.

The moisture content across the samples ranged from 4.84 ± 0.06% in HSLO to 5.96 ± 0.04% in HSSI, with an average of approximately 5.50%. Ash, representing the total mineral content, averaged 5.21% across the samples, with HSTE having the lowest (4.71 ± 0.04%) and HSLV585 the highest (6.38 ± 0.03%). The highest protein content was found in HSLV585 at 25.39 ± 0.03%, while HSAR had the lowest at 20.93 ± 0.03%. The average protein content was 22.31%.

Each sample demonstrated notably high lipid content. HSSI had the highest lipid concentration at 28.43 ± 0.05 g/100 g, while HSTE had the lowest at 24.92 ± 0.07 g/100 g. The mean lipid concentration was 27.17 g/100 g.

Fiber content varied considerably, from 25.92 ± 0.08 g/100 g in HSLV300 to 31.21 ± 0.04 g/100 g in HSTE, with an average of 28.52 g/100 g.

Total carbohydrates were calculated by subtracting moisture, protein, lipid, and mineral contents from 100%. Carbohydrate levels ranged from 35.05 ± 0.06% in HSLV585 to 43.58 ± 0.07% in HSTE.

Finally, the energy content derived from carbohydrates, lipids, and proteins showed that HSLO had the highest energy value at 502.40 ± 0.12 kcal per 100 g, while HSTE had the lowest at 483.25 ± 0.05 kcal per 100 g.

3.2. Macro- and Microelement Composition of Hemp Seed Varieties

The experimental results for the mineral composition, as shown in Table 3, showed that potassium was the macronutrient with the highest concentration in the analyzed seed samples, followed by magnesium and calcium. Potassium, calcium, and magnesium contribute to the development of vital cellular functions, particularly in relation to the heart's excitability. They are key elements in the movement of the heart muscle and the activation of enzyme systems [27].

The potassium content across the samples ranged from 5533.20 µg/g in sample HSLV300 to 3743.08 µg/g in sample HSSI, with an average of 4391.95 µg/g reported in all analyzed samples. Among the certified and traditionally grown varieties in Romania, HSLO stood out with a potassium content of 5000.95 µg/g, while the other three varieties had similar potassium levels. Both varieties undergoing certification also exhibited high potassium content.

Table 3. Macro- and microelement composition of hemp seed varieties (μg/g).

Sample	HSLO	HSSI	HSAR	HSTE	HSLV 585	HSLV 300	Average
Cu	9.56 ± 0.12 [b]	9.48 ± 0.19 [a]	9.81 ± 0.09 [b]	13.08 ± 0.08 [d]	12.56 ± 0.06 [c]	9.29 ± 0.07 [a]	10.69
Zn	46.70 ± 4.53 [b]	39.88 ± 4.77 [a]	53.93 ± 6.09 [e]	52.70 ± 8.41 [d]	75.25 ± 7.53 [f]	49.14 ± 6.07 [c]	52.93
Fe	135.10 ± 5.18 [d]	107.48 ± 13.97 [b]	159.59 ± 11.60 [e]	96.94 ± 11.99 [a]	189.49 ± 3.30 [f]	129.72 ± 10.51 [c]	136.39
Ca	1853.51 ± 89.76 [e]	1167.19 ± 32.37 [a]	1305.97 ± 98.18 [c]	1255.45 ± 56.78 [b]	1161.22 ± 62.77 [a]	1168.27 ± 32.96 [d]	1368.6
Mg	2571.18 ± 30.97 [e]	1969.95 ± 97.59 [a]	2313.52 ± 197.4 [c]	2258.62 ± 69.17 [b]	2616.34 ± 51.45 [f]	2491.38 ± 146 [d]	2370.17
Cd	0.041 ± 0.009 [a]	0.079 ± 0.015 [ab]	0.085 ± 0.025 [ab]	0.088 ± 0.013 [b]	0.079 ± 0.021 [ab]	0.092 ± 0.008 [b]	0.077
Ni	0.80 ± 0.05 [b]	0.17 ± 0.08 [a]	1.36 ± 0.11 [c]	0.87 ± 0.12 [b]	2.82 ± 0.09 [e]	1.99 ± 0.07 [d]	1.34
Mn	101.96 ± 17.49 [b]	105.82 ± 6.86 [d]	102.45 ± 7.78 [c]	87.70 ± 4.53 [a]	138.26 ± 9.58 [f]	109.33 ± 9.12 [e]	107.59
K	5000.95 ± 183.4 [e]	3743.08 ± 258.1 [a]	3912.92 ± 113.8 [b]	3974.79 ± 172.0 [c]	4186.75 ± 188.3 [d]	5533.23 ± 90.4 [f]	4391.95

hemp seed varieties: HSLO—Lovrin 110; HSSI—Silvana; HSAR—Armanca; HSTE—Teodora; and hemp seed lines: HSLV585—LV 585; HSLV300—LV 300. Cu—copper, Zn—zinc, Fe—iron, Ca—calcium, Mg—magnesium, Cd—cadmium, Ni—nickel, Mn—manganese, K—potassium. Results are expressed as the mean value of three determinations ± standard deviation (SD). Different lower-case letters (a–f) represent significant differences ($p < 0.05$) according to the ANOVA between samples in the same row.

Magnesium was the next most prevalent macroelement in the analyzed hemp seeds. The magnesium content ranged from 1969.9 μg/g in sample HSSI to 2616.3 μg/g in sample HSLV585. Calcium content in the analyzed samples varied between 1161.2 μg/g in sample HSLV585 and 1853.51 μg/g in sample HSLO, with no significant differences ($p > 0.05$) between samples HSLV585 and HSSI.

Among the varieties, HSLO had the highest quantities of potassium, calcium, and magnesium. This was followed by the varieties undergoing certification, which had high potassium and magnesium levels but lower calcium content compared with the already cultivated varieties.

Among the microelements listed in Table 3, iron was found in the highest concentrations in all hemp resources, followed by manganese, zinc, and copper, while nickel and cadmium were present in lower amounts.

The iron content across the samples ranged from 96.94 μg/g in sample HSTE to 189.49 μg/g in sample HSLV585, with an average of 136.39 μg/g reported in all analyzed samples. The current research revealed significant variation in zinc concentrations across various hemp varieties, with values fluctuating between 39.88 μg/g and 75.25 μg/g. Similarly, the copper concentrations ranged from 9.29 μg/g in sample HSLV300 to 13.08 μg/g in sample HSTE. No statistically significant differences ($p > 0.05$) were found between the zinc content of samples HSLV300 and HSSI and between samples HSLO and HSAR.

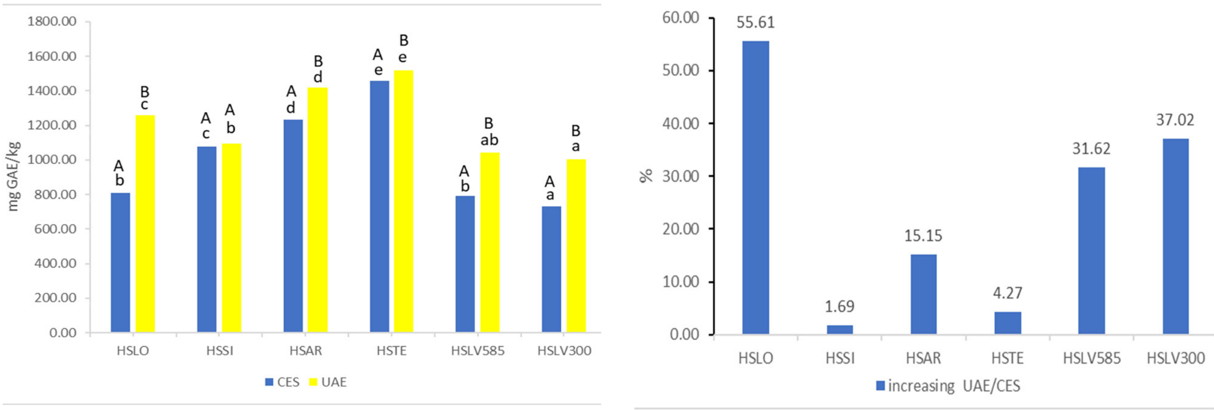
The manganese content in the analyzed samples ranged from 87.70 μg/g in sample HSTE to 138.26 μg/g in sample HSLV585, with an average of 107.59 μg/g reported in all analyzed samples. Nickel, which typically occurs in soil naturally at low concentrations, was found in the samples at levels ranging from 0.17 μg/g to 2.82 μg/g, at an average of 1.34 μg/g. No significant differences ($p > 0.05$) were observed between the samples HSLO and HSTE.

The mean cadmium concentration in the analyzed samples was 0.077 μg/g, with a minimum of 0.041 μg/g in sample HSLO and a maximum of 0.092 μg/g in sample HSLV300. No statistically significant differences ($p > 0.05$) were observed between samples HSSI, HSAR, HSTE, and HSLV585.

3.3. Antioxidant Profile

3.3.1. Determination of Total Polyphenols Content (TPC)

Hemp seeds are abundant in phenolic compounds and recognized for their antioxidant, antimicrobial, and anti-inflammatory benefits. Figure 2 reports the total phenolic content (TPC) for samples processed using CES and UAE methods. Figure 2a shows TPC values derived from use of the CES and UAE methods, expressed in mg GAE/kg. Figure 2b illustrates the percentage change in TPC after different sample preparations, calculated using Equation (2).



(**a**) (**b**)

Figure 2. Comparative TPC content following CES and UAE: (**a**) TPC values using CES and UAE methods. (**b**) Increasing TPC using UAE/CES methods. Distinct lower-case letters (a–e) signify significant differences ($p < 0.05$) among samples derived from the same extraction procedure. Distinct capital letters (A, B) denote significant differences ($p < 0.05$) between samples derived from various extraction procedures.

According to the findings for CES (Figure 2a), the total phenolic content (TPC) values ranged from 732.36 to 1457.60 mg GAE/kg. In comparison, the UAE results showed TPC values ranging from 1003.48 to 1519.87 mg GAE/kg. Regarding the conventional extraction, no statistically significant differences ($p > 0.05$) were found between samples HSLV585 and HSLO. In the case of UAE, no statistically significant differences ($p > 0.05$) were observed between samples HSSI and HSLV585, nor between HSLV585 and HSLV300. Additionally, sample HSSI showed no statistically significant differences ($p > 0.05$) in polyphenol content when comparing the two methods, CES and UAE.

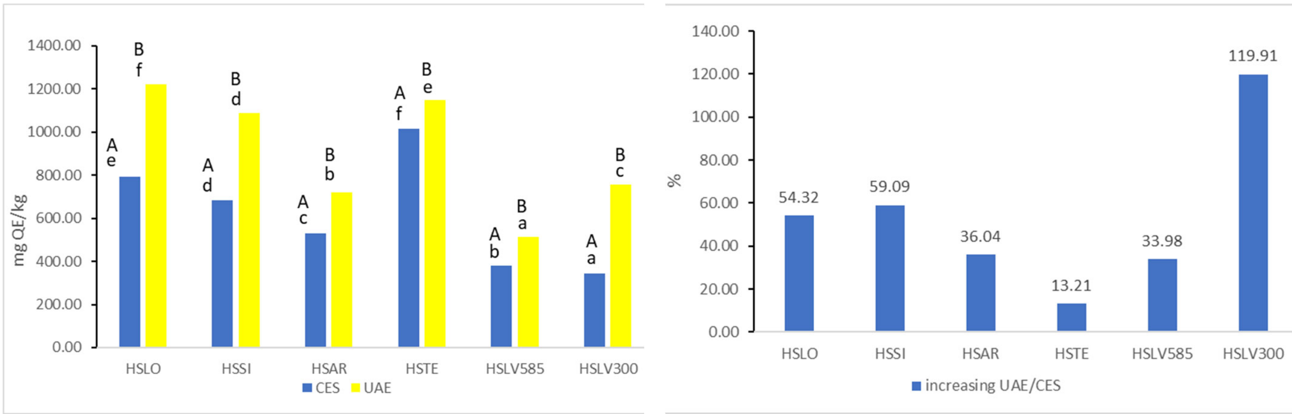
A notable increase in TPC was observed following UAE compared with CES (Figure 2b). The maximum increase was for the HSLO extract (55.61%), while the minimum was for the HSSI extract (1.69%).

3.3.2. Determination of Total Flavonoid Content (TFC)

The antioxidant capacity of flavonoids is influenced by their molecular structure, specifically the number and position of hydroxyl (–OH) groups, the effects of conjugation and resonance, the surrounding environment that affects the preferred antioxidant site, and the specific antioxidant mechanism of each compound [14].

Figure 3 presents the total flavonoid content (TFC) for samples processed using CES and UAE methods. Figure 3a shows TFC values obtained from the CES and UAE methods, expressed as mg QE/kg. Figure 3b illustrates the percentage variation in TFC following different sample preparations, calculated using Equation (3).

TFC levels ranged from 343.91 to 1013.40 mg QE/kg in samples extracted via the CES method, and from 511.92 to 1222.14 mg QE/kg in those obtained using the UAE method. The highest flavonoid content for the CES procedure was observed in HSLO and HSTE extracts, while for the UAE process, the highest values were noted in HSTE and HSLO extracts. Statistical analysis showed significant differences ($p < 0.05$) between samples extracted using CES and UAE methods. A significant increase in TFC was observed with the UAE method compared with CES (Figure 3b), with the most substantial increase in the HSLV300 extract (119.91%) and the smallest increase in the HSTE extract (13.21%).



(**a**) (**b**)

Figure 3. Comparative TFC content following CES and UAE: (**a**) TFC values using CES and UAE. (**b**) Increasing TFC using UAE/CES methods. Lower-case letters (a–f) signify significant differences ($p < 0.05$) among samples derived from the same extraction procedure. Distinct capital letters (A, B) denote significant differences ($p < 0.05$) between samples derived from various extraction procedures.

3.3.3. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Assay for Antioxidant Capacity Quantification

Ascorbic acid at the maximum dose (0.1 mg/mL) demonstrated 91.45% inhibition (Table 4). The IC_{50} representing the concentration required for each extract to achieve 50% inhibition of DPPH, expressed as mg·mL^{-1}, is presented in Table 5.

Table 4. Radical scavenging activity (% inhibition) following CES and UAE.

Sample		Concentration (mg/mL)				
		5	13.33	20	40	100
		(% inhibition)				
HSLO	CES	2.51 ± 0.21 ^{b}	6.86 ±0.05 ^{g}	10.53 ± 0.02 ^{j}	14.84 ± 0.03 ^{o}	45.21 ± 0.01 ^{w}
	UAE	4.44 ± 0.04 ^{b}	8.87 ± 0.03 ^{e}	12.60 ± 0.007 ^{j}	17.61 ± 0.01 ^{n}	54.20 ± 0.01 ^{v}
HSSI	CES	3.15 ± 0.08 ^{c}	6.92 ± 0.03 ^{g}	9.52 ± 0.05 ^{i}	12.24 ± 0.02 ^{l}	32.91 ± 0.02 ^{t}
	UAE	3.67 ± 0.04 ^{a}	9.59 ± 0.03 ^{fg}	11.75 ± 0.02 ^{i}	19.40 ± 0.01 ^{o}	42.80 ± 0.03 ^{s}
HSAR	CES	3.18 ± 0.09 ^{c}	8.64 ± 0.02 ^{h}	12.43 ± 0.02 ^{m}	18.87 ± 0.02 ^{s}	48.28 ± 0.01 ^{x}
	UAE	6.48 ± 0.02 ^{c}	7.57 ± 0.95 ^{d}	16.96 ± 0.02 ^{m}	21.31 ± 0.003 ^{p}	51.74 ± 0.004 ^{u}
HSTE	CES	4.35 ± 0.05 ^{e}	11.37 ± 0.004 ^{k}	17.07 ± 0.02 ^{p}	18.02 ± 0.02 ^{r}	56.63 ± 0.01 ^{y}
	UAE	6.25 ± 0.05 ^{c}	15.38 ± 0.05 ^{l}	21.39 ± 0.01 ^{p}	31.59 ± 0.01 ^{q}	60.48 ± 0.05 ^{w}
HSLV585	CES	2.30 ± 0.04 ^{a}	6.68 ± 0.02 ^{f}	9.49 ± 0.02 ^{i}	13.41 ± 0.04 ^{n}	33.54 ± 0.02 ^{u}
	UAE	4.28 ± 0.009 ^{b}	9.37 ± 0.03 ^{ef}	10.06 ± 0.04 ^{gh}	15.29 ± 0.06 ^{l}	39.41 ± 0.009 ^{r}
HSLV300	CES	3.90 ± 0.03 ^{d}	9.49 ± 0.02 ^{i}	12.42 ± 0.03 ^{m}	17.49 ± 0.10 ^{q}	38.01 ± 0.01 ^{v}
	UAE	4.39 ± 0.01 ^{b}	10.24 ± 0.01 ^{h}	13.27 ± 0.02 ^{k}	17.79 ± 0.03 ^{n}	47.62 ± 0.07 ^{t}
Ascorbic acid		28.02	45.94	57.09	70.59	91.45

hemp seed varieties: HSLO—Lovrin 110; HSSI—Silvana; HSAR—Armanca; HSTE—Teodora; and hemp seed lines: HSLV585—LV 585; HSLV300—LV 300; CES—conventional extraction with solvent; UAE—ultrasound-assisted extraction. Results are expressed as the mean value of three determinations ± standard deviation (SD). Different lower-case letters (a–y) represent significant differences ($p < 0.05$) according to the ANOVA between samples at different concentrations within the same method.

Table 5. The IC_{50} values of sample extracts vs. ascorbic acid.

Sample	IC_{50} (mg/mL)	
	CES	**UAE**
HSLO	6.64 ± 0.07 ^{C}	5.81 ± 0.14 ^{cB}
HSSI	8.72 ± 0.09 ^{C}	6.70 ± 0.05 ^{eB}
HSAR	6.16 ± 0.06 ^{C}	5.80 ± 0.07 ^{cB}
HSTE	5.56 ± 0.07 ^{C}	4.85 ± 0.10 ^{bB}
HSLV585	8.33 ± 0.06 ^{C}	7.50 ± 0.09 ^{fB}
HSLV300	7.43 ± 0.07 ^{C}	6.33 ± 0.14 ^{dB}
Ascorbic acid	2.43 ± 0.07 ^{aA}	

hemp seed varieties: HSLO—Lovrin 110; HSSI—Silvana; HSAR—Armanca; HSTE—Teodora; and hemp seed lines: HSLV585—LV 585; HSLV300—LV 300; CES—conventional extraction with solvent; UAE—ultrasound-assisted extraction. The results are presented as mean values of three measurements ± standard deviation (SD). Uppercase letters A–C indicate significant differences ($p < 0.05$) between samples extracted using different methods. Lowercase letters (a–f) represent significant differences ($p < 0.05$) between UAE samples.

As shown in Table 4, the highest radical scavenging activity was observed at the maximum concentration (100 mg/mL) for all samples. For the conventional extraction method, the highest antioxidant activity was found in the HSTE extract (56.6%), followed by HSAR (48.28%) and HSLO (45.21%). The lowest radical scavenging activity was seen in HSLV300 (38.01%), followed by HSLV585 (33.54%) and HSSI (32.90%).

Following the ultrasound extraction method, the HSTE extract exhibited the highest antioxidant activity (60.48%), followed by HSLO (54.20%), HSAR (51.74%), and HSLV300 (47.62%). HSSI (42.80%) and HSLV585 (39.41%) had the lowest radical scavenging activity.

Statistically significant differences ($p < 0.05$) were found in most samples extracted using the conventional method, across all tested concentrations. However, exceptions included HSSI and HSAR, which did not show significant differences ($p > 0.05$) at a concentration of 5 mg/mL; HSLO and HSSI showed no significant differences at 13.33 mg/mL; and HSAR and HSLV300 exhibited no significant differences at 20 mg/mL.

No statistically significant differences ($p > 0.05$) were observed between HSAR and HSTE, nor between HSLV585 and HSLV300 at a 5 mg/mL concentration following ultrasonic extraction. Additionally, at a concentration of 13.33 mg/mL, no statistically significant differences ($p > 0.05$) were observed between HSLO and HSLV585.

Regarding the influence of the extraction technique, Figure 4 shows that ultrasound-assisted extraction (DPPH—UAE) led to increases in DPPH values ranging from 1.72% to 103.77% compared with the values obtained using the conventional extraction method (DPPH—CES).

Table 5 shows that the highest antioxidant activity for all samples was achieved with the UAE process, followed by the CES process. Significant differences were observed between the two methods across all analyzed samples. With the UAE method, the IC_{50} values of the samples varied significantly, highlighting differences in their antioxidant efficacy. Sample HSTE exhibited the highest antioxidant activity, with an IC_{50} value of 4.85 mg/mL, while sample HSLV585 had the lowest activity, with an IC_{50} value of 7.50 mg/mL. Most varieties of hemp seeds analyzed showed significant differences ($p < 0.05$), except for sample HSLO, which was paired with sample HSAR.

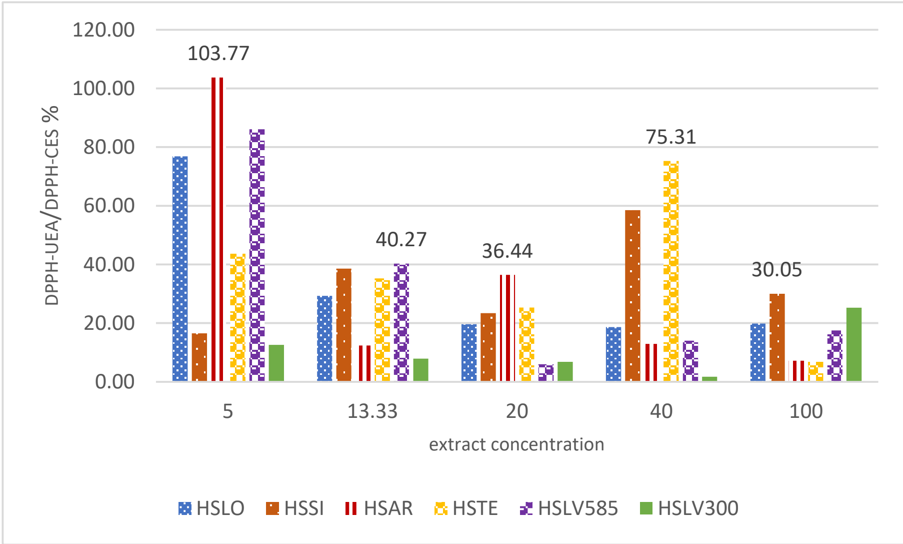


Figure 4. Increase in DPPH radical scavenging activity values using UAE method compared with CES method.

The biplot reveals two principal components: PC1, accounting for 46.25% of the variance, and PC2, accounting for 23.55% of the variance (Figure 5). Together, these components explain a significant portion of the total variance in the data. Zn, Fe, Mn, Ni, Ash, and Prot, located on the right side of the plotted graph, significantly influence PC1, indicating that this component predominantly reflects the mineral and protein composition of the hemp seeds. TC, Fib, TPC, TFC, and DPPH100, located on the left side, have lower values for PC1 and are inversely correlated with those on the right, suggesting that hemp varieties with higher mineral and protein content have lower levels of antioxidants and phenolic compounds. This inverse relationship provides valuable insight into the nutritional and functional properties of hemp seeds.

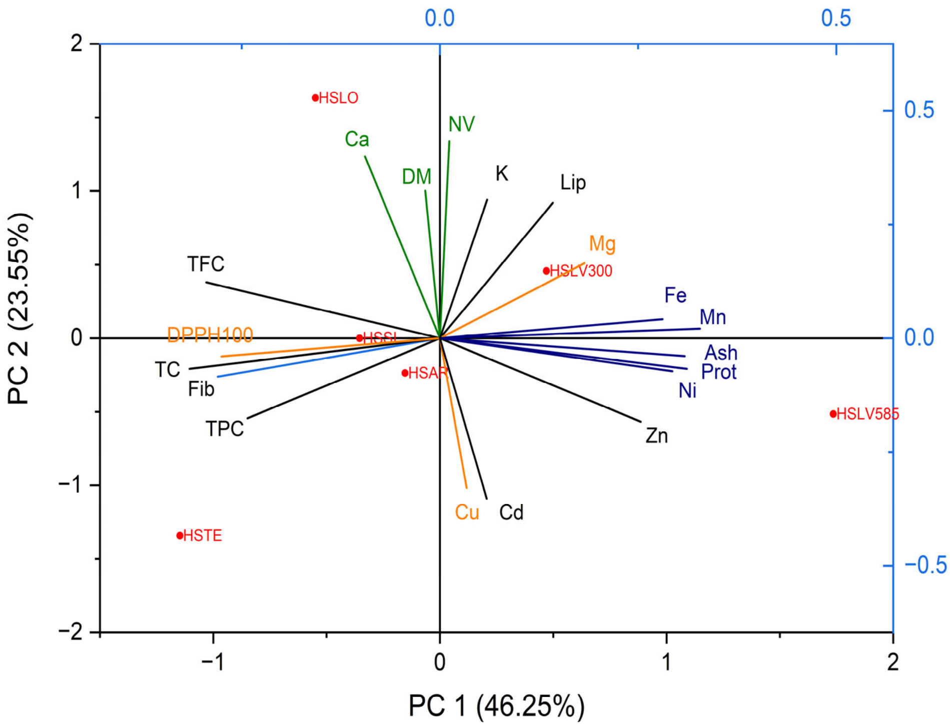


Figure 5. PCA Biplot.

Ca, DM, and NV, represented by green vectors pointing upwards, primarily influence PC2, reflecting the nutritional matrix. The strong contribution of Ca, DM, and NV to this component suggests that these variables influence the antioxidant performance of hemp seeds.

4. Discussion

4.1. Proximate Composition of Hemp Seeds

According to Leonard et al. and El-Sohaimy et al., hemp seeds have a proximate composition of 18–23% protein, 25–30% oil, 30–40% fiber, and 6–7% moisture [5,28]. The moisture content obtained aligned with findings from other studies. Allonso-Esteban et al. [29] reported moisture values ranging between 4.53 and 7.06 g/100 g for eight hemp varieties, while Siano et al. found a moisture content of 7.3% in Fedora cultivar seeds [6]. Rosso et al. noted moisture content from 6.54% to 7.9% in three different hemp varieties cultivated in Central Italy [30]. These variations could be attributed to environmental and storage conditions, particularly temperature.

The ash content is consistent with the reports by Vonapartis et al. [7], who observed ash values ranging from 5.1 to 5.8 g per 100 g in ten different hemp cultivars, and Siano et al., who reported 5.3 g per 100 g in Fedora seeds [6]. Similar results were obtained by Arango et al. (2024), with ash values between 4.40% and 7.49% in 29 hemp varieties [31].

Hemp seed proteins are mainly storage proteins, with albumin accounting for 25–37% and the legumin protein edestin for 67–75% [32]. These proteins make hemp seeds particularly beneficial for vegetarian diets. Our results regarding the protein content of hemp seeds are consistent with other findings. Oseyko et al. [33] reported protein content of 22.5% in hemp seeds, while Siano and colleagues found 24.8% [6]. House et al. observed protein content ranging from 21.3 to 27.5 mg/100 g across 30 hemp seed samples [8]. Hemp proteins are primarily located in the internal layer of the seed, with minimal quantities in the hull [9].

The lipid composition of the analyzed hemp seed varieties was in close alignment with the existing research. Siano et al. reported a lipid content of 24.5%, House et al. found 25.4–33.0 mg per 100 g, and Vonapartis et al. observed 26.9 to 30.6 mg per 100 g [6–8]. Rosso et al. found crude fiber content varying between 20.98 and 25.58 g/100 g [30].

Reduced carbohydrate content in the improved samples suggests potential hypoglycemic benefits and suitability for diabetic-friendly diets. Siano et al. found a carbohydrate prevalence of 38.1% [6]. Callaway et al. [4] reported 27.6 g per 100 g in Finola hemp seeds, while Mattila et al. found carbohydrate content comparable to flaxseed (34.4 ± 1.5 g/100 g vs. 29.2 ± 2.5 g/100 g) [34]. Mattila et al. reported an energy value of 2301 kJ/100 g dw for hemp seeds [34].

Observed differences in chemical composition can be attributed to environmental conditions, soil characteristics, agricultural practices, and natural botanical variation [6,7,30,31].

4.2. Macro- and Micronutrient Composition of Hemp Seed Varieties

Due to the differences in hemp varieties analyzed across various studies, comparing mineral content results can be challenging. Our findings on potassium levels in hemp seeds surpassed those reported by Siano [6] but were lower than those obtained by other researchers studying macroelements in hemp seeds [4,29,34–36]. Magnesium is an essential mineral crucial for activating numerous biochemical processes vital to human health [37], and its content in certain hemp seeds ranges from 240 mg/100 g [33] to 694 mg/100 g [35], exceeding the values obtained in our study. Calcium levels reported in prior research varied from 90 mg/100 g [33], 94 mg/100 g [6], 94–121 mg/100 g [36], 127 mg/100 g [34], 145 mg/100 g [4], to 175.6 mg/100 g [29], aligning with our findings.

Iron is essential in the human diet to enable hemoglobin production. However, inorganic iron such as is commonly found in plant-based foods is less readily absorbed by the body compared with organic (heme) iron present in animal products [38]. Consequently, vegetarians are advised to increase their iron intake by up to 80% above the recommended levels to compensate for the lower bioavailability of plant-based iron [39]. Reports in the literature describing iron concentrations in hemp seeds show significant variations [4,6]. High values of up to 240 mg/100 g have been associated with iron-rich soils used in experimental hemp cultivation [40].

Typical zinc levels in hemp seeds range from 4 to 7 mg per 100 g, as documented by Mihoc, Oseyko, and Mattila [33,34,40]. Copper concentrations in hemp seeds span from 0.5 mg to 2 mg per 100 g, according to Siano and Callaway [4,6]. Our study's results for both zinc and copper are consistent with these previous findings.

Manganese concentrations in earlier studies varied from 6 mg/100 g [33] to 12–15 mg/100 g [36], matching our results. In their research on heavy metal and micronutrient content in hemp seeds from northwest Turkey, Korkmaz and colleagues reported nickel content between 0.36 and 1.66 mg/kg, comparable to our findings [41]. Mihoc reported higher micronutrient values of 1.6 to 6.1 mg/kg in hemp seeds, exceeding our study's results [40].

Cadmium, a heavy metal present in soil, poses significant environmental and health risks due to its toxicity and ease of absorption by plants. The EU limits cadmium levels in seeds used for oil production and cereals to 0.1 mg/kg [42]. Korkmaz's study of 21 hemp seed samples from northwest Turkey revealed cadmium concentrations ranging from 0.5 to 2.3 µg/100 g, significantly higher than those in our studied hemp varieties [41].

Mineral profiles in hemp seeds can vary significantly due to environmental conditions, soil mineral composition, fertilizer application, and plant variety. Proper fertilization and nutrient management can enhance mineral accumulation. Additionally, antagonistic interactions occur when one mineral inhibits the uptake or utilization of another, leading to deficiencies or imbalances. For instance, high levels of calcium, magnesium, and potassium can compete with iron and zinc absorption. Anti-nutrient components like phytates and oxalates in hemp seeds can also bind minerals, reducing their bioavailability [43].

4.3. Total Polyphenols Content (TPC)

Phenolic compounds in hemp seeds can mainly be categorized into three primary classes: flavonoids, stilbenes, and lignans [5,9]. These compounds are crucial in combating diseases related to oxidative stress, due to their potent antioxidant properties. Among the specific phenolic compounds in hemp seeds are ferulic acid, p-coumaric acid, and N-trans-caffeoyl tyramine [32].

Studies have shown that the phenolic content can vary based on factors such as the geographical location where the hemp is grown and the specific hemp cultivar.

The determined values for TPC aligned with those documented in previous research. Siano et al. reported a TPC value of 767 mg GAE/kg for seeds in their comparative study of the chemical, biochemical, and characteristic properties of seeds, oil, and flour from the Fedora hemp cultivar [6]. Mattila and colleagues found a total phenolic content of 96 ± 35 mg GAE/100 g for whole hemp seeds [44]. Moccia et al. reported a total polyphenol content of 0.92 ± 0.04 mg GAE/g for polar extracts from Fedora hemp seeds, flour, and oil [45]. Frasinetti et al. documented a higher total polyphenol content of 2.33 ± 0.07 mg GAE/g dw for hemp seeds and sprouts [46]. Similarly, Vonapartis et al. analyzed ten industrial hemp cultivars approved for cultivation in Canada, revealing TPC values from 1.37 to 5.16 mg GAE/g [7]. Irakli and colleagues documented an average TPC of 588.8 mg GAE/100 g in their study of seven hemp seed varieties grown in Greece, which was higher than previously reported values [47].

Polyphenol levels exhibit quantitative variation due to biotic and abiotic factors affecting their biosynthesis pathways. Ultrasound-assisted extraction (UAE) yields plant extracts with higher concentrations of active compounds and improved biological activity. The advantages of this method over traditional techniques are well established.

4.4. Total Flavonoid Content (TFC)

The current findings regarding TFC align with previously documented research in the specialist literature. In their comparative study on hemp seeds and steam, Allo et al. identified active principles and biological properties, reporting a total flavonoid content of 107.502 mg/100 g CAE [48]. Barčauskaitė and colleagues, in their investigation of the potential for industrial hemp cultivation in the Nordic-Baltic region, reported TFC values ranging from 2.52 to 4.74 mg/g RUE [49]. Frassinetti et al., in their study on the nutraceutical potential of hemp seeds and sprouts, reported a total flavonoid content of 2.93 ± 0.23 mg QE/g DW, which was higher than the values found in our study [46].

4.5. Antioxidant Capacity by 1,1-Diphenyl-2-picrylhydrazyl (DPPH)

Antioxidant activity is a crucial biological property with significant implications for industries such as cosmetics, food, and beverages. Since antioxidant potential is vital for determining the therapeutic benefits of plants, this study employed antioxidant assays, specifically the radical scavenging (DPPH) method. This assay is favored for its simplicity, speed, reproducibility, and cost-effectiveness, making it an ideal tool for evaluating the antioxidant activity of plants.

Our study’s findings on DPPH radical scavenging activity are consistent with those reported in the literature. Siano et al. observed a DPPH free radical scavenging activity value of 51.5% for hemp seed extracts [6]. Frasinetti et al. reported the antioxidant activity of hemp seeds as 40 ± 2% DPPH inhibition [46].

Judžentienė et al., in their study on the chemical composition and antioxidant activity of various hemp parts cultivated in Lithuania, reported a DPPH radical scavenging activity Trolox value of 1.556 ± 0.004 mmol/L for seed extracts [50].

In another study by Babiker E. et al., the effects of different roasting durations (7, 14, and 21 min) at 160 °C on the proximate composition, color properties, bioactive compounds, and the fatty acid profile of hemp seeds were examined. The DPPH radical scavenging capacity was 18.37% for unroasted seeds, decreasing to 6.65% after 7 min of roasting and reaching 10.99% after 14 min. Interestingly, the antioxidant activity of the seeds significantly increased with longer roasting times, reaching 33.08% after 21 min [51].

The findings related to the extraction method are consistent with the literature. In a study by Szydłowska-Czerniak, A. et al., two extraction methods were used to obtain total antioxidants from winter and spring rapeseed cultivars: ultrasound-assisted extraction (UAE) and conventional solid–liquid extraction. The researchers found that rapeseed extracts obtained after 18 min of ultrasonication had the highest levels of total antioxidants, indicating that UAE was more effective than traditional extraction techniques in extracting antioxidant compounds [52].

Regarding IC_{50} values, Benkirane et al. reported IC_{50} values ranging from 1.64 to 4.37 mg/mL in the DPPH test results for two varieties of hemp seed collected from four different Moroccan regions [53]. Chen et al. extracted forty samples from defatted kernels and hulls of two varieties of hemp seed using ten polar solvent systems, reporting IC_{50} values ranging from 0.58–4.55 mg/mL for defatted kernels and 0.09–2.21 mg/mL for hulls [54].

Manosroi and colleagues evaluated the antioxidant activity of ethanolic extracts from Thai hemp seeds, finding that the extract had an IC_{50} value of 14.39 mg/mL in the DPPH free radical scavenging assay [55].

Alonso-Esteban et al. studied the chemical composition and biological activity of eight varieties of hemp seeds, both hulled and dehulled, reporting DPPH test (EC_{50}) antioxidant activity ranging from 2.5 to 9.2 mg/mL for hulled seeds [29].

Additionally, Rosso et al. examined three distinct varieties of hemp seeds grown in a Mediterranean area, reporting DPPH test (EC_{50}) antioxidant activity ranging from 0.06 to 0.13 mg/mL [30].

Analysis of the biplot revealed that the HSTE variety was characterized by high values of antioxidant activity (DPPH100) and TC, and HSLV by a high content of ash, protein, Zn, Fe, Mn, and Ni, while HSLO had a high content of Ca and DM.

The Mn vector is closely aligned with DPPH100, indicating a strong influence on the antioxidant capacity of hemp seeds. Similarly, the NV vector aligns well with DPPH100, suggesting a significant contribution, while the Lip vector points in a similar direction, implying that lipids also influence DPPH100. Overall, the biplot indicates that Mn, NV, and Lip are the key variables influencing DPPH100, as they have vectors pointing in a similar direction to DPPH100, indicating a strong relationship. Other variables exhibited moderate or different contributions to the principal components. The variables related to antioxidants and phenolic compounds (TPC, TFC, Fib, TC, DPPH100) were correlated with each other, and DPPH100 was closer to this group, suggesting a link between antioxidants and these compounds. The results for DPPH100 were almost opposite to those for the mineral variables (Zn, Fe, Mn, Ni, Ash, Prot), indicating that samples rich in antioxidants (DPPH100) had lower levels of minerals and vice versa. The negative correlation between Ca and DM (pointing upwards) and TPC and Fib (pointing downwards) suggests that products high in Ca and DM tend to have lower levels of TPC and Fib.

5. Conclusions

Our study reveals that Romanian hemp varieties contain approximately 20–25% easily digestible protein, 35–43% carbohydrates, 25–31% fiber, and several biologically important minerals. Examining the influence of the extraction technique, our findings indicate that ultrasound-assisted extraction resulted in increased TPC, TFC, and DPPH values compared with the conventional extraction method. PCA analysis demonstrated that the HSTE variety was characterized by high antioxidant activity (DPPH100) and TC. HSLV showed a high content of ash, protein, Zn, Fe, Mn, and Ni, while HSLO had a high content of Ca and DM. The compounds with higher antioxidant activity and consequently, the higher antioxidant capacity of hemp seeds obtained using the UAE method compared with the CES method are of significant interest to the food and pharmaceutical industries, as UAE reduces extraction times and solvent quantities. Hemp seeds are a valuable ingredient for the food industry, particularly in the rapidly expanding market of plant-based alternatives. They boast a high content of phenolic compounds and possess strong antioxidant capacity, making them ideal functional ingredients. Due to their rich protein and fiber content, hemp seeds are well suited for developing functional food products with nutritional claims such as "source of/high in protein" and "high in fiber". By incorporating hemp seeds with their favourable nutritional profile, these products can effectively meet both nutritional and product development needs.

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