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The Estimation of the Possibility of Bioethanol Production from Hemp Cellulose Using the HWE Method

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Abstract: This study investigates the effect of hot water extraction (HWE). The research investigates how different biomass fractions (0–4 mm, 4–8 mm, and 8–16 mm) respond to hydrothermal treatment, with cellulose content analyzed using the Kürschner–Hoffer method. Results indicate that cellulose loss varies across fractions, with the highest degradation observed in the 8–16 mm fraction and the lowest in stalks thinner than 4 mm. The HWE process removes both hemicellulose and lignin selectively, which helps improve enzyme accessibility and maximize bioethanol yields. The absence of fermentation inhibitors suggests that HWE is an effective alternative to acid-based pretreatment. Based on these findings, optimizing process parameters for sustainable bioethanol production from hemp biomass may be possible. Optimal HWE conditions and alternate pretreatment methods should be evaluated in future research to maximize efficiency and industrial feasibility.

Keywords: hydrothermal pretreatment; biorefinery approach; lignocellulosic fractionation; hemp bioethanol production; biomass valorization

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1. Introduction

The urgency to protect the environment drives modern industries to adopt environmentally friendly solutions [1]. The industrial paradigms are being fundamentally shifted not only by trend but by fundamental change as well. It is continuing to be the case that technologies are being modernized and adapted to use renewable sources. The modernization and adaptation of technologies using renewable resources continue, with biomass usage emphasized as a sustainable fuel source. Several studies have investigated the efficiency of *Miscanthus giganteus* in bioethanol production [2], while conducted studies focused on enzymatic hydrolysis and various pretreatment methods, including hydrothermal processing. Despite this, hemp biomass offers distinct advantages, including faster growth, reduced fertilizer requirements, and better adaptability to various environmental conditions. The study aims to extend existing research by investigating how hot water extraction (HWE) can be used to fractionate hemp biomass by examining its effect on particle size. The cellulose integrity of hemp is preserved during HWE, and enzyme digestion is improved compared to Miscanthus, which shows higher ash content and structural degradation. The research demonstrates hemp's potential for use as an alternative feedstock for bioethanol production by directly comparing it with previously studied biomass types.

Due to its favorable characteristics for bioethanol production, hemp (*Cannabis sativa* L.) has gained increasing attention among lignocellulosic sources. The advantages of hemp differ from those of traditional biomass sources, like wood and straw. Hemp grows rapidly, reaching maturity within 3–4 months, while wood-based biomass takes decades to replenish. The second advantage of hemp is its high cellulose content (50–70%), which is comparable to or higher than what is found in other agricultural residues, such as wheat straw (35–45%) or Miscanthus (40–60%) [1,3,4]. A high cellulose content translates into a greater potential for fermentable sugar release during hydrothermal pretreatment and enzymatic hydrolysis. The third benefit of hemp cultivation is that it requires relatively little fertilizer and pesticide inputs compared to conventional crops. As an additional benefit, hemp shives provide a valuable lignocellulosic feedstock without competing with food crops or requiring extra land [5,6].

The factors mentioned above justify using hemp over other lignocellulosic sources in this study for bioethanol production [7]. Having a high cellulose content, rapid growth, and little agricultural impact, hemp offers a promising alternative to conventional biomass feedstocks, bringing sustainability and circular economies together. The circular economy model emphasizes the use of resources efficiently, the reduction of waste, and the reusing of materials, which perfectly match the properties of biodegradable materials. More and more importance has also been attracted to natural materials, which can easily be managed and used. Increasing interest is due to their inherent renewability, reduced environmental impact, and compatibility with biological systems. Poland, for instance, has access to many biodegradable materials [8], of which biomass is the most prevalent [9]. Biomass is primarily obtained from the agricultural and forestry sectors, contributing significantly to the renewable energy mix in Poland. Industrial hemp cultivation has increased in Europe since its potential as a biomass feedstock has been recognized. The increased production of hemp in Poland is aligned with European trends favoring lignocellulosic biomass for bioethanol and bioenergy.

The rational management of plant materials creates a wide range of alternative uses. For instance, hemp shives can be hydrolyzed into simple sugars. They can be converted into ethanol (bioethanol) by hydrolyzing the polysaccharides left in the material [8]. Comparative studies have demonstrated that bioethanol yields depend on the feedstock type. The theoretical ethanol yield from lignocellulosic biomass varies widely. For instance, sugarcane bagasse can produce 82% of the theoretical yield, wheat straw between 60% and 75%, and Miscanthus around 70% [10]. According to studies, hemp can produce ethanol yields comparable or better than agricultural residues when hydrolyzed and fermented under optimal conditions [11]. According to these studies, hemp has a high potential for bioethanol production compared to conventional agricultural residues and woody biomass.

The growth of hemp biomass in Europe's bioeconomy has been highlighted by recent studies, and industrial hemp is being recognized as a viable alternative to traditional agricultural residues, such as wheat straw and Miscanthus. The expansion of its use in Poland and other European countries could enhance local bioethanol production. Regional economic development could be further stimulated by adopting widespread bioethanol production methods using locally sourced raw materials [12]. The next step in utilizing lignocellulosic [13,14] raw materials on the road to sustainability is modifying these materials through HWE based on innovative research. The utilization of lignocellulosic raw materials, including hemp (*Cannabis sativa* L.), can be further enhanced through HWE, a modification method traditionally used for lignocellulosic materials [15]. Hemp offers significant potential beyond bioethanol production due to its low ash content, resistance to soaking post-extraction, and high level of readily available cellulose after processing. Adapting HWE technology to local conditions, combined with eco-friendly materials, could lead to sustainable methods for manufacturing and other innovative materials, thus fostering a circular economy. The hemp processing technology for further applications is essential in developing and implementing local methodologies.

2. Materials and Methods

2.1. Characteristics of the Research Material

The material processing for this study was divided into several stages to ensure a comprehensive approach to the ongoing research. A carefully developed methodology was applied to select the physical and chemical parameters based on the initial assumptions. Given that the innovative research concept being developed is novel and not yet widely adopted, it requires in-depth analysis, and a research plan focused on fundamental material properties. The raw material underwent crushing and separation into fractions, which were essential for further analysis. The study used hemp grown in the Mazowieckie Voivodeship in Poland (52.2298° N, 21.0122° E) as the raw material, provided by Cannabotanique. Alternative lignocellulosic materials were also tested to compare the efficiency of bioethanol production, including wheat straw and Miscanthus. Materials selected for bioethanol production were selected based on their availability, composition, and documented potential. The processing conditions were kept consistent to compare the performance of these alternative raw materials with hemp shives. The plants were cultivated under temperate climate conditions, where the average daily temperature ranged from 15 °C to 24 °C throughout most of the growing season, ensuring optimal growth conditions.

Maintaining the correct moisture content of the material was a critical step to ensure consistency and accuracy in the analysis. Samples were dried in a laboratory dryer at 105 °C until they were dry. Moisture content was measured using a laboratory cuvette designed for this purpose [16], and the process was repeated every week to stabilize the moisture level at 12% [17]. A controlled laboratory environment was maintained during drying and conditioning to ensure consistent results, as environmental factors, such as temperature and humidity, can significantly impact the sample's moisture content. Calibration of the cuvette was conducted using a medium to ensure accuracy. Before conducting physical or chemical tests, each material sample's weight was verified using a Radwag MAC 50 (Radom, Poland) scale. The moisture content of lignocellulosic samples was determined following the PN-ISO 589:2006 standard [18]. These measures allowed for accurate determination of moisture content and ensured that the material was prepared consistently for subsequent testing and analysis.

2.2. The Hemp Fraction Preparation

This study aimed to determine whether hemp flakes could be sorted according to their fractions. The hemp analyzed contained fibers, straws, and leaf fragments from its original composition. Material and harvesting techniques are responsible for these characteristics. To conduct laboratory tests, the extracted materials had to be ground up before being analyzed. Segments of hemp were shredded using the Retsch SM200 (Haan, Germany) shredder with a power output of 9.6 kW (13 HP). This part of the section focuses on the fractionation of the biomass study, as one of the factors to be considered in facilitating the statistical analysis is the fractionality of the biomass.

To sort the material into fractions, it was shredded and then separated using an orbital shaker created by C.B.K.O Hydrolab (Warsaw, Poland) and a sorting machine. Using the diagonal mesh of the sieve, the material was divided into three fractions: minor, f_1 (<0 ÷ 4 mm), medium, f_2 (<4 ÷ 8 mm), and thickest, f_3 (<8 ÷ 16 mm). Several factors guided the selection of these specific fraction sizes. As for the tiniest fraction, the shredder's specifications, especially its ability to grind the material finely, played a role in determining its lower limit. After the selected fraction samples, the material was dried in a laboratory chamber at 105 °C. Each sample was weighed and placed into a cuvette [6,19] to ensure compliance with standardization requirements. The sets for separating fractions with the

C.B.K.O Hydrolab (Warsaw, Poland) separator and the screens used during separation are presented in Figure 1.

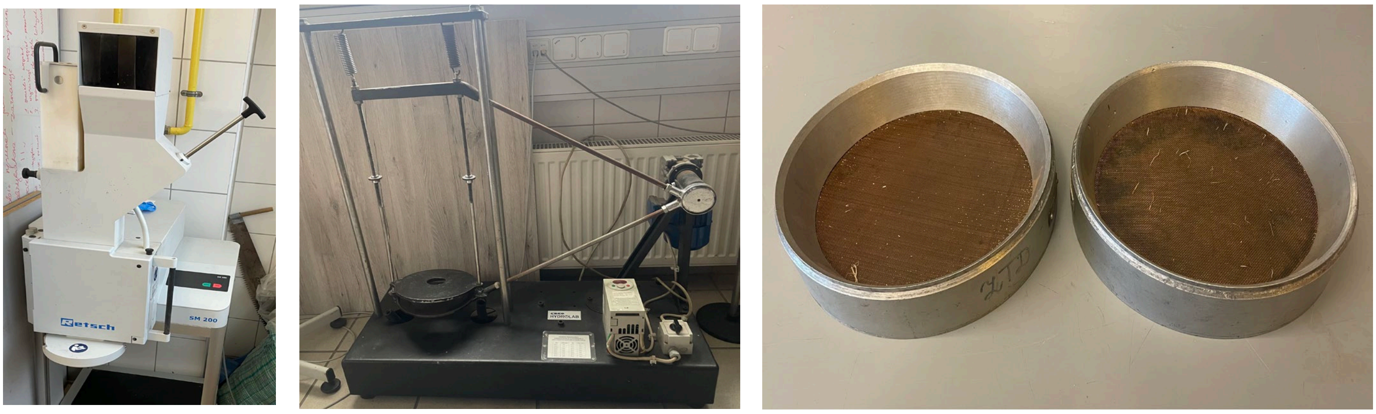

(**a**) (**b**) (**c**)

Figure 1. Preparation of the sets for separating fractions: (**a**) Retsch SM200 shredder, (**b**) C.B.K.O Hydrolab separator, and (**c**) sorting screens used for particle size separation.

Tests and calculations were performed to determine the moisture content of the prepared mixture. Moisture content was measured by drying crushed samples at 105 °C until completely dry. The purpose of drying was to remove all water content from the samples. Following drying, the samples were weighed to standardize the moisture content measurement. This weighing step was crucial to ensure repeatable and comparable results. A professional laboratory scale Radwag WS30 (Radom, Poland) was used to enhance accuracy during testing during key measurement stages. Utilizing this device, we minimized potential measurement errors and improved the reliability of the moisture content data for the tested mixture.

2.3. Optical Microscope Image Analysis

A thorough understanding of lignocellulosic materials necessitates a detailed examination of their structural properties through microscopy. Microscopic observation reveals the inherent structural characteristics that define these materials. The initial stage of optical analysis involved a meticulous examination of the samples' internal structure using an optical microscope equipped with an integrated amplifier and an external light source. Digital cameras captured these microscopic observations, ensuring accurate documentation of the findings. The analysis assessed the samples' morphology, dimensions, and other key structural characteristics. A Nikon SMZ 1500 microscope (Nikon Corporation, Tokyo, Japan) and specialized software image analysis facilitated this detailed evaluation. The microscope's optical system, designed to correct axial and planar chromatic aberrations, produced highly accurate and detailed images. A 150-watt halogen illuminator provided optimal lighting conditions, enabling clear visualization of even the most intricate material features.

The microscopic images captured during this analysis revealed the materials intricate structural makeup, including detailed information about morphology and dimensions. The stereoscopic microscope, with its adjustable zoom range from 0.75× to 112.5× and a zoom ratio of 15:1, allowed for observation at macroscopic and microscopic scales. A comparative analysis across different specimens highlighted specific structural features that can serve as valuable standards for identifying and characterizing similar materials. By integrating the results of this comprehensive optical analysis, the study provided a thorough understanding of the materials' structural properties and their potential applications, such as in bioethanol production.

2.4. The Method of HWE Treatment

The raw material was modified by hydrothermal treatment (HWE) to achieve desired structural and chemical changes. As a result of selective hemicellulose solubilization and partial lignin modification, the HWE process facilitates cellulose accessibility. The elevated temperature and pressure cause glycosidic bonds in hemicelluloses to cleave hydrolytically, which leads to these bonds' dissolution. As a result of the disruption of hydrogen bonding networks within lignocellulosic fibers, cellulose surface exposure increases, potentially increasing its enzymatic digestibility in the subsequent production of bioethanol. Process parameters, such as temperature, retention time, and biomass fraction size, which were varied in this study, were used to observe their effect on the biomass structure and composition and determine the extent of these transformations. The process involved exposing the lignocellulosic complex (LCC) to steam generated from distilled water under controlled pressure and temperature.

The HWE process was conducted within a custom-designed apparatus consisting of a material container, a reactor, and a control system. The raw material was processed in measured quantities within the container. A specific water-to-biomass ratio was maintained within the reactor, which was filled with distilled water before each treatment. The apparatus facilitated controlled water flow through the material at a target temperature, regulated by a thermostat and monitored by a thermocouple connected to a computer. The gradual temperature increase prevented non-uniform heating. The HWE process involved repetitive treatment, lasting approximately 30 min each cycle. During performance testing, heated distilled water was applied to the test material in a single-pressure flow process. After each cycle, the material was replaced, and the reactor was refilled with fresh distilled water to start a new reaction. The methodology followed a structured workflow, as presented in Figure 2.

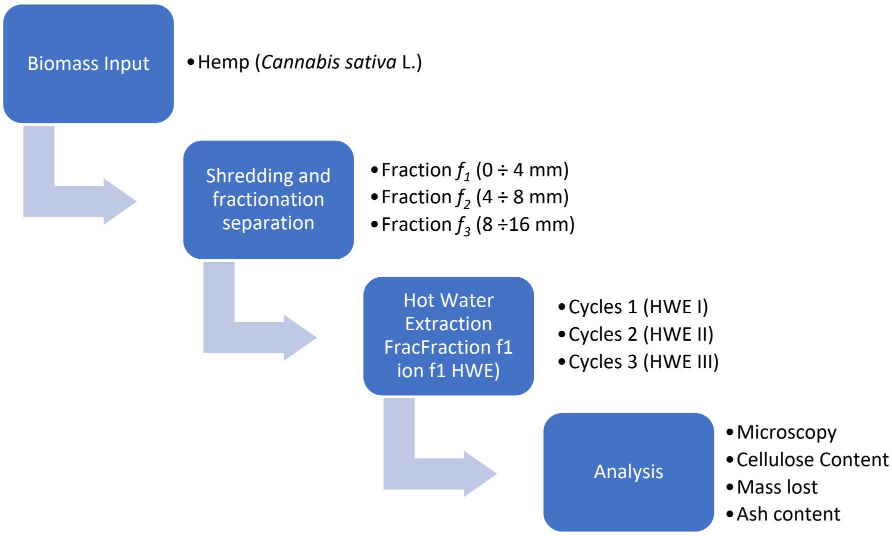


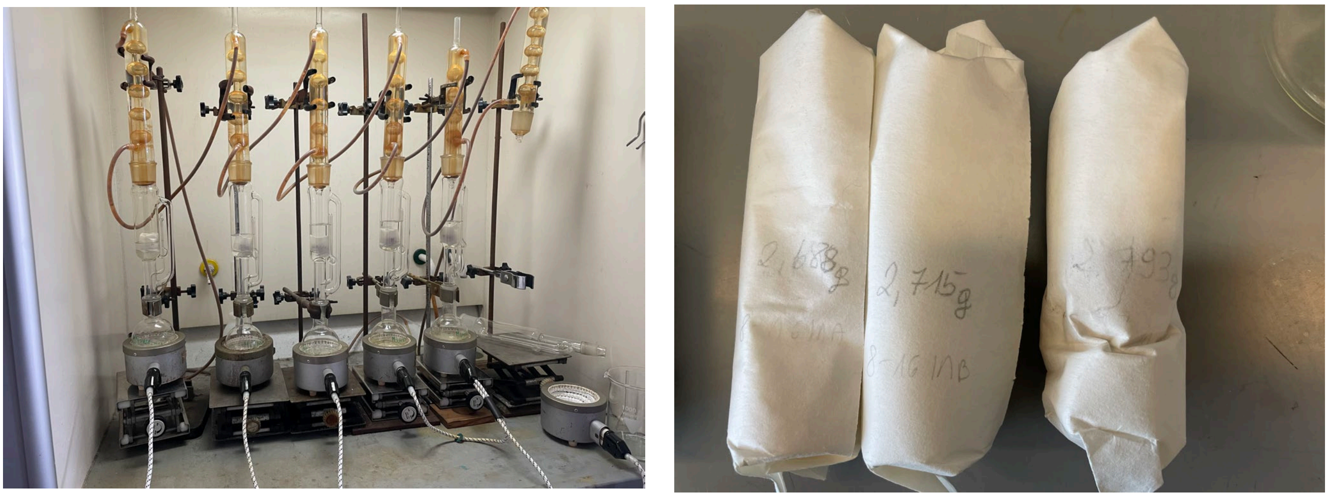
Figure 2. Diagram of the experimental workflow.

The raw material fractions were separated into three size categories (0–4 mm, 4–8 mm, and 8–16 mm) during the initial preparation. Each successive process was designated as HWE(I) for the first modification, HWE(II) for the second repetition, and HWE(III) for the third modification. After the HWE treatment, the modified material was stored in a labeled container and dried. The distilled water used during extraction was transferred to a separately labeled flask, weighed, and subjected to evaporation to determine the weight of the residue. Following HWE treatment, the modified material underwent further chemical analysis to statistically evaluate correlations between selected parameters.

2.5. Chemical Extraction and Determination of Cellulose

The chloroform–ethanol system is widely used for solubilizing lignin and hemicellulose but concerns over its environmental impact and scalability have led researchers to look at greener alternatives. It has been suggested that ethyl acetate and aqueous NaOH solutions can serve as potential replacements, as they are less toxic and biodegradable, while still maintaining lignin removal effectiveness [20,21]. Several studies should be conducted to determine whether these methods are feasible for large-scale applications with hemp-derived biomass. For nitro-lignin to pass into the solution, it needed to be dissolved in alcohol, which allowed it to enter the solution along with hemicelluloses. Each sample was divided into three batches, weighing approximately 2.7 g. To perform the test, extracting thimbles were prepared and filled with the test material before being placed in soufflés. The thimbles were then flooded with the chloroform–ethanol mixture during the extraction process.

Under standardized heating conditions, Soxhlet extractors (Alchem, Wrocław, Poland) were used for extraction. For efficient extraction, round-bottomed flasks (Alchem, Poland, 500 mL) were placed in bowls with an adjusted power level (Alchem, Poland). This extraction cycle took 10 h to complete. Afterward, the cooled flasks were removed from the extractor and evaporated separately to regenerate the chloroform–ethanol mixture and extract the precipitate from the flasks. The cellulose content of the material was determined using the Kürschner–Hoffer method [22]. The extraction set is presented in Figure 3.



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

Figure 3. The setup used for the extraction process: (**a**) complete extraction system, including Soxhlet apparatus, solvent reservoir, and heating system, and (**b**) thimbles containing the lignocellulosic material prepared for extraction.

Cellulose was quantified using the Kürschner–Hoffer method, which involves converting lignin into nitro-lignin and determining the dry weight with the addition of nitric acid and ethanol. The extracted samples were dried at 60 °C under 0.4 kPa pressure in air-dry weight. Approximately 1 g of the sample was placed into a 300 cm^3 conical flask, along with 20 cm^3 of ethanol and 5 cm^3 of 65% nitric acid. The ethanol and nitric acid solution was stirred gradually while being prepared. The flask containing the solution underwent three cycles of 65 min each in a water bath at 85 °C. The cycle time was measured from the moment the water started boiling. After each cycle, the flask contents were drained using a sterile Schott G3 filter (Alchem, Poland), dried, and weighed. The procedure was repeated twice by adding a fresh ethanol and nitric acid mixture for each test. The set for measuring cellulose by the Kürschner–Hoffer method is presented in Figure 4.

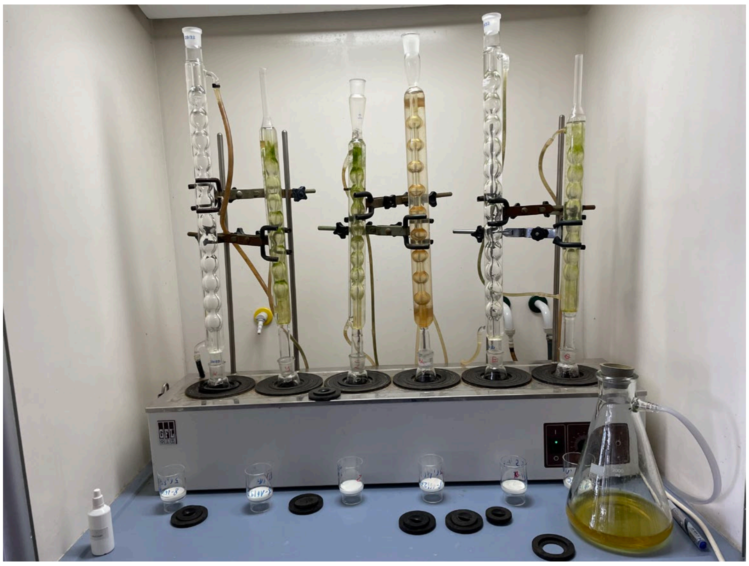


Figure 4. Experimental setup for cellulose content determination using the Kürschner–Hoffer method. The process involves lignin solubilization followed by cellulose isolation and quantification.

The sample was subsequently rinsed with 25 cm^3 of warm distilled water and boiled for 30 min to restore the test material to a usable state. The residue was thoroughly washed after draining the flask twice using the Schott G3 filter. In the final step, the flask's contents were transferred entirely to the Schott filter, and any remaining sediment was removed. The filter was washed with hot distilled water several times to achieve a neutral pH. The cellulose residue was then dried at 103 ± 2 °C. Afterward, the filter was rinsed with cold water and dried at room temperature. The desired cellulose product was carefully separated from the filter, and its yield was determined. The percentage of cellulose dry matter was calculated by comparing the amount obtained with the extracted sawdust's dry weight.

2.6. Soluble Content

The soluble content of the raw material is critical to understanding the characteristics of the solution during hemp processing. Soluble organic compounds were identified in the extracted liquid based on standard extraction methods. The extraction liquid is expected to contain hemicellulose-derived sugars, organic acids, and phenolic compounds owing to the nature of hot water extraction (HWE). These components have been extensively described in similar studies using lignocellulosic biomass [2]. Based on mass loss from the solid fraction and qualitative observations of color and viscosity changes, solubilized compounds were evaluated. A release of hemicellulose-derived sugars and lignin-related compounds was observed during the extraction process. The analysis provided valuable insights into the amount and nature of substances that were extracted from hemp during the modification process, which can affect the properties of the final product. The TAPPI Test Method T 207 cm-08 [23] was used to determine the content of cold and hot water extractives in hemp fractions. The pH of the water extracts was measured using an Oakton pH/temperature meter (Vernon Hills, IL, USA).

The soluble content was measured in three different ways: cold water extraction, hot water extraction, and ethanol extraction. For the cold water extraction, 0.25 g of dry matter of hemp fractions was extracted three times with the use of 2000 mL of water at room temperature, and the mixture was left for 48 h. The solution was then filtered through a dry circles filter paper with qualitative 4 of Whatman category No. 1004 125, with a known weight. The filter paper with the residue was dried and weighed. The pH of the collected filtrate was measured with the prepared aperture.

For the hot water extractions, the three-repetition cycle was performed for cannabis samples. Each dry sample containing a mass of 0.25 g was extracted in 2000 mL of hot water under a reflux condenser to prevent evaporation, and the mixture was left for 3 h. The solution was then filtered through a dry filter paper with a known weight, and the pH of the collected filtrate was measured after cooling. The filter paper of known weight with remaining residues on it and the collected solid sample after extraction were dried and weighed all together. To obtain the mass of the sample, the known mass of the blotting paper filter and container used had to be subtracted from the total mass.

2.7. Ash Content

Determining ash content involves a series of steps ensuring reliability and accuracy. Ash content measurement is a critical part of analyzing the chemical composition of solids, particularly for materials like hemp, where mineral saturation—measured through components such as iron, silicon, aluminum, magnesium, calcium, sodium, and potassium—impacts its performance and industrial usability. The systematic methodology included carefully burning samples in a controlled environment to eliminate contamination, then precision weighing the resulting ash using an analytical balance. Calibration and parameter control of the combustion and measuring apparatus are integral to this process. Regular calibration ensured that the combustion furnace and analytical balances provided consistent and accurate measurements. The repeatability and reliability of measurements were further enhanced by standardizing the operating environment and maintaining constant combustion temperatures and durations.

In this study, the ash content of hemp samples was determined using slow incineration in a muffle furnace, ensuring optimal conditions for accuracy. The procedure began with the preparation of specimens, which involved drying the samples before incineration to remove residual moisture. Each cycle involved approximately 2 g of sample material placed into pre-weighed crucibles. The samples were then incinerated in the muffle furnace, preheated to 805 °C, for 2 h to achieve complete combustion. After incineration, the crucibles containing the ash were placed in a desiccator to allow the samples to cool while avoiding exposure to external moisture. Once cooled, the weight of the crucible and ash was measured to determine the ash content.

2.8. Statistical Analysis

Statistical software (STATISTICA 13.3, TIBCO Software Inc., 2020) (Warsaw, Poland) evaluated the experimental design and validated relationships between key variables. The primary objective was to ensure the methodology's reliability and ability to detect significant effects on the tested parameters. To identify meaningful differences between groups, such as those influenced by fraction size and the number of extraction cycles, analysis of variance (ANOVA) was employed as the primary tool. This ensured that any observed differences were statistically significant and not the result of random variation when comparing mean values across multiple groups. After the ANOVA, Duncan's post hoc test was applied to isolate homogeneous groups and discover more nuanced differences between experimental conditions. The combination of these approaches enabled testing the suitability of variables and ensuring a rigorous and reproducible methodology for the experimental framework. This statistical analysis provided solid groundwork for further research and process optimization, validating the setup's reliability, identifying areas for refinement, and adapting the methodology to various settings.

3. Results

3.1. Material Analysis

3.1.1. Fraction Separation

Separating the raw material into three size ranges was the starting point for characterizing the size distribution of its fractions: 0–4 mm, 4–8 mm, and 8–16 mm. This became the primary framework of the study. During an exploratory phase, the mesh sizes of the sieves were temporarily increased to investigate the potential benefits of finer fractionation. A laboratory shaker with a 2 mm harmonic vertical vibration and a 25 Hz frequency was used to determine the percent distribution of fraction sizes. The hemp material was fractionated consistently and accurately by shaking for five minutes with a vibration amplitude of 3 mm. Maintaining uniformity across trials required careful material preparation, including ensuring a standardized bulk volume. The data provided insight into the composition of the material within the specified ranges, as detailed in Table 1.

Table 1. The size of the fractions in individual samples of woody biomass.

Fractions	Sample, %			Mean (SD), %
	I	II	III	
>16 mm	23.57	27.30	24.19	25.02 (2.00)
>8 mm	38.52	30.42	39.49	36.14 (4.98)
>4 mm	23.01	25.18	24.06	24.08 (1.09)
>2 mm	8.28	9.97	7.05	8.43 (1.47)
>1 mm	3.94	4.16	2.81	3.64 (0.72)
>500 µm	2.22	2.46	1.73	2.14 (0.37)
<500 µm	0.45	0.52	0.67	0.55 (0.11)

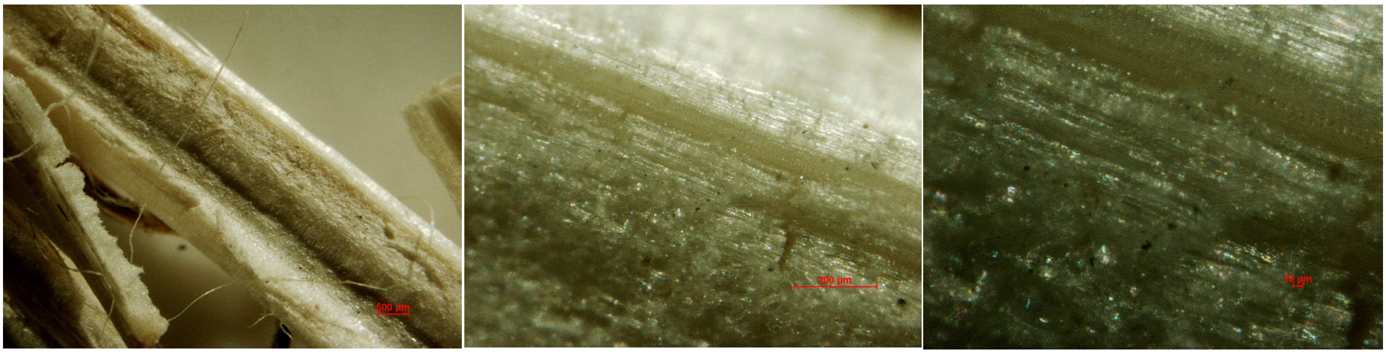
SD—standard deviation.

The table presents the percentage distribution of material fractions obtained during the sieving process for three samples (I, II, and III), along with their averages. Fractions were classified by particle size, ranging from greater than 16 mm to less than 500 µm. The most significant fraction consisted of particles greater than 16 mm, accounting for an average of 25.02%, with values ranging from 23.57% to 27.30%. The fraction greater than 8 mm was the most abundant, contributing an average of 36.14%, followed by the fraction more significant than 4 mm, which comprised 24.08%. Additionally, fractions greater than 2 mm and 1 mm constituted 8.43% and 3.64%, respectively. The tiniest fractions, greater than 500 µm and less than 500 µm, accounted for the lowest proportions, averaging 2.14% and 0.55%, respectively. This distribution indicates that most of the material was concentrated in more extensive ranges (>8 mm and >4 mm), while smaller fractions were less prevalent. The results provided valuable insights into the particle size distribution of the material, which was crucial for understanding its characteristics and optimizing processing methods.

3.1.2. Raw Material Optical Analysis

Optical analysis of the raw material at different magnifications enabled us to observe the fibers' overall arrangement and structural details, including constrictions and thickening of cell walls. A visual representation of the constrictions and thickening of cell walls was provided. According to these images, analyzing hemp fibers at different scales is necessary for understanding their structures, which differed depending on the fraction size. The fraction 8–16 mm was not included in the analysis since it was structurally the same as the fraction 4–8 mm. It was evident that the 0–4 mm and 4–8 mm fractions differed in structure, which was examined with a microscope.

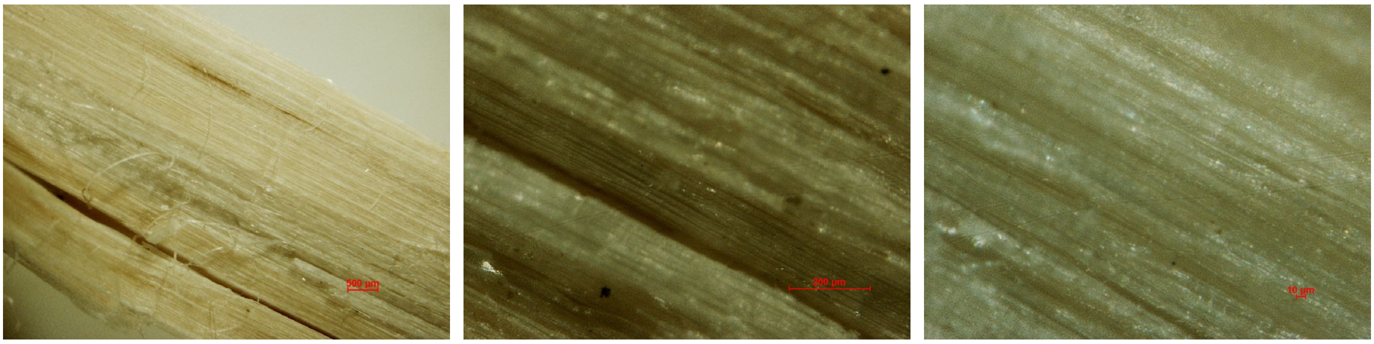
An image of the fraction 0–4 mm showed characteristic elongated cells with thickened walls arranged evenly in bundles. With a magnification ratio of 0.75×, the photo revealed the general arrangement of the fibers, while with magnification ratios of 5× and 11.25×, details of the cell structure could be seen. Fiber diameters remained constant despite appearing to increase with magnification, which was about 0.75 m. Understanding the hemp fiber structures requires an understanding of their structures at different scales. The microscope images of the 0–4 mm fraction hemp at magnifications are presented in Figure 5.



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

Figure 5. The microscope images of the 0–4 mm fraction hemp: (**a**) 0.75× magnification, (**b**) 5× magnification, and (**c**) 11.25× magnification.

Microscopic images exposed distinct structural differences between 4 and 8 mm of hemp. A picture of hemp fibers between 4 and 8 mm was visible with a magnifying power of approximately 0.75×. Fibers were arranged in longitudinal bundles parallel to one another, having a noticeable thickness, which is characteristic of this fraction [24]. Several spaces between the bundles were also visible, suggesting the presence of other plant tissues. Numerous dense bundles of longitudinally arranged fibers were visible in the image at approximately 5× magnification. Despite being relatively thick, the fibers were pretty thin. The fiber structure can be seen as elongated cells with a distinct structure. In the 11.25× zoom image, fiber bundles were densely arranged along longitudinal axes. Hemp fibers are relatively thick, so they are typical for this fraction. The microscope images of the 4–8 mm fraction hemp at magnifications are presented in Figure 6.



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

Figure 6. The microscope images of the 4–8 mm fraction hemp: (**a**) 0.75× magnification, (**b**) 5× magnification, and (**c**) 11.25× magnification.

The hemp fibers of the 0–4 mm fraction had a uniform diameter (about 0.75 m) and parallel arrangements of thickened walls, whereas the fibers of the 4–8 mm fraction were thicker, formed dense bundles, and had distinct cellular structures. An analysis of microscopy images of a cannabis stalk revealed its complex structure, including long fibers, vessels for nutrient and water transport, crumb cells, and thick cell walls. Analyzing photographs taken at magnifications of 0.75×, 5×, and 11.25× revealed more and more details about the fiber structure in the stem, such as the cell shape, thickening of the walls, and cracks and irregularities on the surface. The outer pores, however, remained invisible at low magnifications, suggesting they require a higher microscope magnification to observe. The pictures were captured using a digital camera and analyzed with NIS-Elements BR (Nikon Corporation, Tokyo, Japan). Tracing the contours of individual fibers in captured images gave us an estimate of their length. The results were averaged after measuring 30 fibers per sample. Within each sample group, standard deviation values were calculated to represent variability in fiber length. The mathematical characteristics of the microscopic image for each magnification are listed in Table 2.

Table 2. Mathematical characteristics of the microscopic image for individual magnification.

Fraction	Zoom	Average Length of Fibers (SD), μm	Average Thickness of Fibers (SD), μm
f_1 (0–4 mm)	0.75×	2670 (3295.12)	26.67 (18.86)
	5×	400 (494.97)	8 (8.49)
	11.25×	7.11 (8.80)	0.356 (0.01)
f_2 (4–8 mm)	0.75×	5330 (6595.89)	53.34 (37.71)
	5×	800 (989.95)	8 (8.49)
	11.25×	47.56 (54.09)	0.71 (0.75)

SD—standard deviation.

This table contains a detailed mathematical microscopic analysis of cone-pi fibers at different magnifications (0.75×, 5×, and 11.25×) and fractions (0–4 mm and 4–8 mm). The study measured the fibers' average length, maximum length, and minimum thickness. Hemp fibers displayed an uneven surface shape with a long, thin, densely packed arrangement. There was a significant difference between the fibers in the 4–8 mm fraction and the 0–4 mm fraction as far as length and thickness were concerned. The image detail increased with magnification, and the numerical values decreased, but the proportions between the parameters remained stable, confirming the consistency of the analysis. Analysis of the 4–8 mm fraction at 5× and 11.25× magnification revealed similar morphological features of the fibers.

3.2. Evaporation of Distilled Water Used in the HWE Process

The laboratory tests were conducted using the research methodology that proceeded during the study. In preparing the native and modified HWE process materials, the reagents were chemically ready to enable the extraction and determination of cellulose within the stalks of cannabis. A moisture content test was conducted on the stalks used, which resulted in 12% for all samples. The amount of sludge left over after evaporating the distilled water used during the HWE process was further analyzed. Measurements were performed following the HWE procedure. As a result, distilled water washed out part of the organic compounds from the raw material, resulting in a solution in a receiving container with water. After the HWE process, distilled water was evaporated and weighed to determine the mass of the leached sludge further. There was the smallest amount of sludge in stalks of fractions 0–4, which then increased in fractions 4–8 and reached its highest level in fractions 8–16. Study results showed that sludge increased with each repeat of the HWE process, a relationship characterized by consistency. The result of the three HWE processes was a significant amount of sediment in samples after evaporating the mixture with stalks. In the third cycle of the HWE process, there appeared to be only two exceptions: fractions 4 and

8. The mass of the stalks subjected to the HWE process was approximately 10 g. Table 3 presents the mass of sludge after evaporation of the water used for HWE.

Table 3. The mass of sludge in the HWE process after the evaporation of water.

Fractions	Number of Repetitions of the HWE Process	Weight of Sludge Remaining After Evaporation of Distilled Water Used in the HWE Process (SD), g
f_1 (0 ÷ 4 mm)	HWE(I)	0.143 (0.058)
	HWE(II)	0.216 (0.146)
	HWE(III)	0.262 (0.124)
f_2 (4 ÷ 8 mm)	HWE(I)	0.154 (0.096)
	HWE(II)	0.217 (0.038)
	HWE(III)	0.052 (0.020)
f_3 (8 ÷ 16 mm)	HWE(I)	0.163 (0.079)
	HWE(II)	0.235 (0.113)
	HWE(III)	0.375 (0.130)

SD—standard deviation.

The compressive mass of sludge was statistically analyzed to determine the intensity of washing based on the fractions of the analyzed raw material tested. The amount of sediment generally increased with each extraction stage, and the amount of sediment in thicker fractions was higher. Statistical analysis revealed no significant difference in the sizes of the raw material fractions and sludge as residue after the HWE process, as indicated by the p-value = 0.206 and for an empirical statistic of $F_{(2,\,15)}$ = 1.7581. Based on approximate Duncan probabilities, no homogeneous group could be isolated since the degree of significance (p) of the measurement exceeded 0.05, which is a high value. In the range of the tiniest fractions of 0 ÷ 4 and 4–8, it was 0.198, and at 8 ÷ 16, it was 0.654. There was a difference of 0.107 when the combination of the fractions 4 ÷ 8 and 8 ÷ 16 was used. As a result of the combination between the groups, there were no significant differences between the measured parameters. The effects of the study parameters are displayed according to the impact analysis in Figure 7.

In a statistical analysis of the effect of the size fraction of raw material tested on sludge residue after the HWE process, the significance level was higher than the alpha level assumed. The analysis showed no considerable differences in the mean effects. The value of results was grouped into only one homogeneous group in that case. The analysis results also provided information on the amount of sludge left after evaporation of the distilled water used in HWE. According to what fraction of the material fraction was used, sludge was left behind in various amounts. After the third repetition of the hot water extraction (HWE(III)) process, the weight of the sludge was 2.62 g, about 2.62% of the initial material weight. Sludge after HWE(III) was only 0.52 g, about 0.52% of the initial weight, higher than the previous fraction. This group had lower values, and the amount of sludge after HWE(III) was much smaller. In contrast, in the f_1 (8 ÷ 16 mm) fraction, the amount of sediment was even higher, reaching 3.75 g after HWE(III), which was about 3.75% of the initial weight. It followed that the thicker f_1 (8 ÷ 16 mm) fractions tended to leave a higher amount of sludge than the thinner f_2 (4 ÷ 8 mm) and f_1 (0 ÷ 4 mm) fractions.

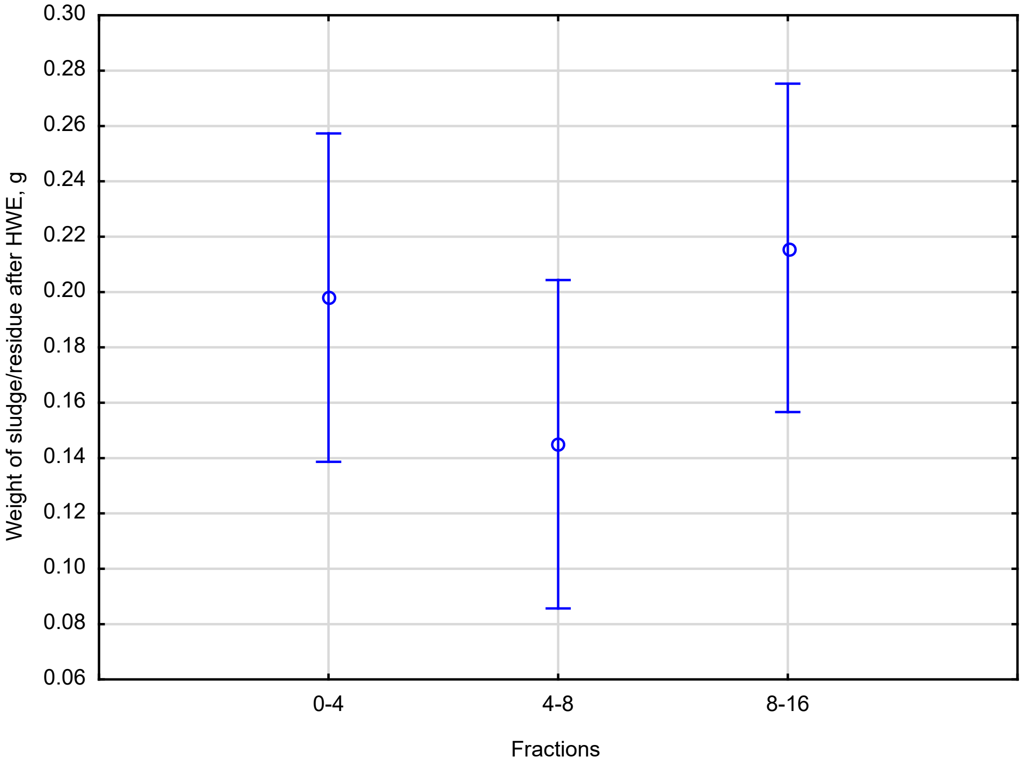


Figure 7. Effect of the size of the fractions of the raw material tested on the weight of sludge in the form of residue after the HWE process.

The results within a specific fraction showed that the amount of sludge left over increased with successive HWE cycles. For example, in fraction f_1 ($0 \div 4$ mm), the sediment weights were 0.143 g after HWE(I), 0.216 g after HWE(II), and 0.262 g after HWE(III), respectively. Also, in the f_2 fraction ($4 \div 8$ mm), the sediment after HWE(I) was 0.154 g, after HWE(II) was 0.217 g, and after HWE(III) was only 0.052 g. Similar results could be seen in the f1 fraction ($8 \div 16$ mm). The findings of this study suggested that with every successive round of HWE, a more significant amount of sludge was produced. The HWE process also led to substantial differences in fractions after consecutive repetitions. In fraction f_2 ($4 \div 8$ mm), for example, the amount of sludge after HWE(III) was significantly lower than in other fractions. The evidence may indicate differences in material structures that affected extracting substances. Observing the effect of the HWE process on different fractions was possible by determining the percentage of sludge. The test material could be effectively cleansed through the process, as the values were relatively low. Based on the evaluation of the process and its effect on the test material, the impact of material fractions and the number of repetitions were essential.

3.3. Chemical Extraction and Determination of Cellulose Tests

3.3.1. Sludge Content in the Extraction Process

The relationship between the influence of fraction size, extraction amount, and sludge content in the extraction process after the chloroform–ethanol mixture had evaporated was also essential to observe. The mass of sludge after evaporation of the chloroform–ethanol mixture was measured. The chloroform–ethanol mixture leached more substances with each successive co-repetition of the HWE process for each fraction. The most significant number of substances was leached from fractions 0–4, while the least was leached from fractions 8–16 in the form of sludge released after extraction. The reason may be that finer stalks had a greater surface area in contact with the solvent, contributing to efficient substance extraction [25]. As HWE was repeated, the remaining sludge weighed more each time, except the third repetition of HWE in fractions 4 ÷ 8. This relationship may be explained by thicker stalks releasing more substances than thinner ones [26]. There was approximately 2.7 g worth of stalks used for extraction. After extracting the stalks with fractions 0–4, the most significant amount of sediment was found, followed by fractions 8–16 and 4–8, which were in the middle of the range. However, the lowest amount was found when fractions 4–8 were filtered. The mass of the precipitate formed after evaporation of the chloroform–ethanol mixture is presented in Table 4.

Table 4. Mass of residue after evaporation of chloroform–ethanol mixture.

Fractions	Number of Repetitions of the HWE Process	Mass of Precipitate After Evaporation of Chloroform–Ethanol Mixture from Flask (SD), g	Percentage of Precipitate After Evaporation of Chloroform–Ethanol Mixture from Flask (SD), %
f_1 (0 ÷ 4 mm)	Native	0.034 (0.022)	1.246 (0.766)
	HWE(I)	0.047 (0.015)	1.740 (0.546)
	HWE(II)	0.060 (0.005)	2.130 (0.149)
	HWE(III)	0.066 (0.040)	2.445 (1.546)
f_2 (4 ÷ 8 mm)	Native	0.030 (0.002)	1.113 (0.061)
	HWE(I)	0.039 (0.014)	1.420 (0.504)
	HWE(II)	0.051 (0.013)	1.852 (0.483)
	HWE(III)	0.065 (0.024)	2.409 (0.878)
f_1 (8 ÷ 16 mm)	Native	0.027 (0.003	0.979 (0.096)
	HWE(I)	0.041 (0.011)	1.479 (0.415)
	HWE(II)	0.042 (0.004)	1.543 (0.132)
	HWE(III)	0.054 (0.007)	1.981 (0.264)

SD—standard deviation.

The high temperatures in the HWE extraction process can cause lignin and hemicelluloses, also found in hemp, to leach out. The decrease in cellulose content caused by HWE may be due to its decomposition under elevated temperatures and pressures. It should be noted that water is also used during HWE extraction, which can cause a further decrease in the cellulose content of the product [27]. The extraction process may cause the stalks' structures to be affected by the extracted compounds and other components, such as lignin, hemicelluloses, or pectin [28]. The study's results were statistically analyzed to determine the relationship between fraction size, amount of extraction, and sludge remaining after extraction for individual samples of the analyzed material. The degree of significance was 0.99704, with a significance level of p-value > 0.05, and an empirical value of $F(6, 24)$ of 0.08720 was obtained. Statistical differences between the measured parameters were observed in some studies. The Duncan test could not isolate homogeneous groups based on the approximate probabilities of the test. The results of the combination values between the groups did not indicate a difference between the measured parameters in the two groups. The effects of each parameter that followed the analysis of their influence are presented in Figure 8.

The amount of sludge remaining after the HWE process was an important aspect. Considering the fraction of material and the number of times the HWE process was repeated, the study determined the amount of sludge remaining. According to the methodology, a post hoc Duncan's test was used to make a breakdown into homogeneous groups. The significance level (p), as determined by statistical analysis of selected parameters, was lower than the alpha value of 0.05, the mean significance level. This means that there was a difference in the mean effects. The data showed that the remaining sludge amount varied with the fraction of the material. Fraction f_1 (0 ÷ 4 mm) tended to leave more sludge compared to f_2 (4 ÷ 8 mm) and f_1 (8 ÷ 16 mm). Based on this, thinner fractions will likely remove substances from materials more efficiently in the HWE process. The amount of sludge produced by the HWE process increased as the process was repeated, which may reduce the efficiency of the final process.

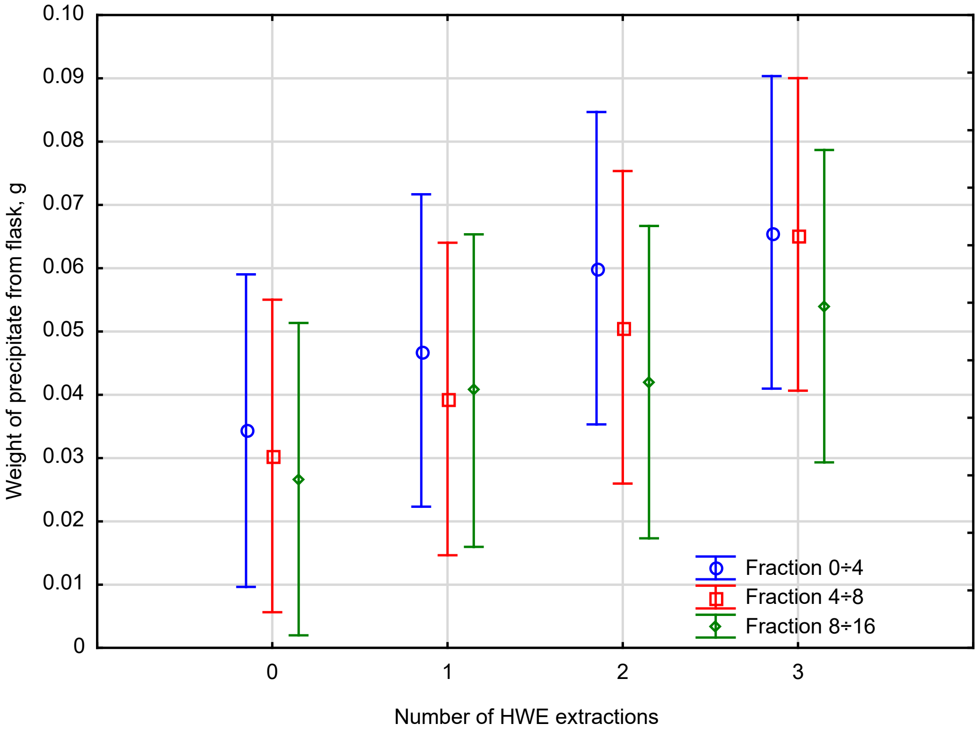


Figure 8. The influence of fraction size, extraction amount, and sludge content in the extraction process.

Another important aspect was the number of times the HWE process was repeated. Results showed that with each successive repetition, sludge amounts increased. The significance of this is that repetition of the process may result in sludge accumulation, which could influence the process's performance and efficiency. However, for the f_2 fraction (4 ÷ 8 mm), the amount of sludge dropped sharply after the third repetition of HWE, giving rise to an interesting phenomenon for further research. The percentage of sludge calculated allowed for a better comparison of results between fractions and repetitions of the HWE process. The HWE process removed substances from the test material based on the relatively low values. Based on the results, there was a difference in the residual sludge amounts depending on the fraction and the number of repetitions. The results of this study will be used for further research on optimizing the process of substance extraction from biomass to maximize its efficiency. The mechanism of sludge production was random, especially for unexpected changes, such as a reduction in sludge when the HWE process was repeated three times in the f_2 fraction (4 ÷ 8 mm). The results provide an essential basis for further research into the HWE process and its optimization. Biomass treatment processes need to take into account the difference between fractions, as well as how repetitions affect sludge generation.

3.3.2. Measurement of the Percentage of Cellulose Content

The study found that the HWE process decreased the amount of cellulose over time. Hydrothermal degradation of amorphous cellulose regions and the progressive removal of hemicelluloses were responsible for this reduction. Dissolving hemicelluloses made the biomass porous and fibers separate, increasing porosity. The changes in morphology suggested that HWE increased cellulose accessibility in bioethanol by loosening the lignocellulosic matrix. Biomass characteristics and process conditions determined the extent of these modifications. Based on the measurements made after each repetition of the HWE process, there was a total decrease in the cellulose content of about 2 to 5%, with the decline varying slightly due to the fraction. The highest reduction in cellulose content was observed in the hemp stalks of fractions 0 and 4, which was observed after just three repetitions of thermal treatment. This resulted in a total decrease of 11.7% in the cellulose content. The most minor decrease could be observed in the fractions 4 and 8, where the level was 8.21%. The cellulose content of the material depleted as the number of repetitions of the HWE process increased. A lower loss could be observed after the third HWE process compared to the first two. This may be attributable to the reduced easily extractable compounds during the first two extraction processes. The percentages of cellulose content are presented in Table 5.

Table 5. The percentages of cellulose content.

Fractions	Number of Repetitions of the HWE Process	Mass of Extracted Dry Sawdust (m_0) (SD), g	The Amount of Obtained Dry Matter Cellulose (m_c) (SD), g	Percentage of Cellulose Content (SD), %
f_1 (0 ÷ 4 mm)	Native	0.741 (0.021)	0.446 (0.049)	60.071 (6.116)
	HWE(I)	0.726 (0.013)	0.407 (0.007)	56.145 (1.202)
	HWE(II)	0.716 (0.011)	0.367 (0.029)	51.241 (3.311)
	HWE(III)	0.746 (0.009)	0.361 (0.013)	48.359 (1.224)
f_2 (4 ÷ 8 mm)	Native	0.730 (0.005)	0.357 (0.007)	48.925 (0.807)
	HWE(I)	0.733 (0.020)	0.350 (0.010)	47.778 (0.745)
	HWE(II)	0.749 (0.012)	0.323 (0.013)	43.186 (2.398)
	HWE(III)	0.754 (0.020)	0.307 (0.004)	40.713 (1.560)
f_1 (8 ÷ 16 mm)	Native	0.754 (0.030)	0.405 (0.062)	53.456 (6.574)
	HWE(I)	0.750 (0.026)	0.374 (0.031)	50.021 (5.724)
	HWE(II)	0.728 (0.006)	0.333 (0.008)	45.741(1.021)
	HWE(III)	0.738 (0.016)	0.320 (0.024)	43.381 (4.067)

SD—standard deviation.

The use of alternative solvent systems, such as ethyl acetate or alkaline extraction to replace traditional chloroform–ethanol mixtures is also recommended, given the environmental impact of chemical extraction. Using these approaches may improve the sustainability of lignin removal while maintaining the effectiveness of the process. In particular, cellulose is a central component of lignocellulose and a potential source of biofuels, such as bioethanol and bio-butanol [29]. The raw material for bioethanol production is lignocellulose, composed primarily of cellulose and used to produce enzymes in bioethanol production [30]. Potential biofuel production depends on minimizing cellulose losses during HWE to achieve the desired quantity and quality of the end product. To determine the statistical relationship between the fraction size, extraction amount, and residual cellulose content, it was necessary to compare the size of the fraction, extraction amount, and residual cellulose content. Statistics were used to analyze the study results, comparing the influence of various parameters and determining their correlation.

In most cases, the measured parameters had no statistically significant difference. The p-value of 0.934 indicated that the measurement was substantial. According to the empirical value computed by the $F(6, 24)$ statistic, it was 0.293181. A high level of significance (p) of the measurement of the Duncan test did not isolate homogeneous groups, as the degree of significance exceeded 0.05 in the approximate probability test. There was no evidence that the measured parameters differed between groups in the combination. The effects of the analysis of the influence of the studied parameters are presented in Figure 9.

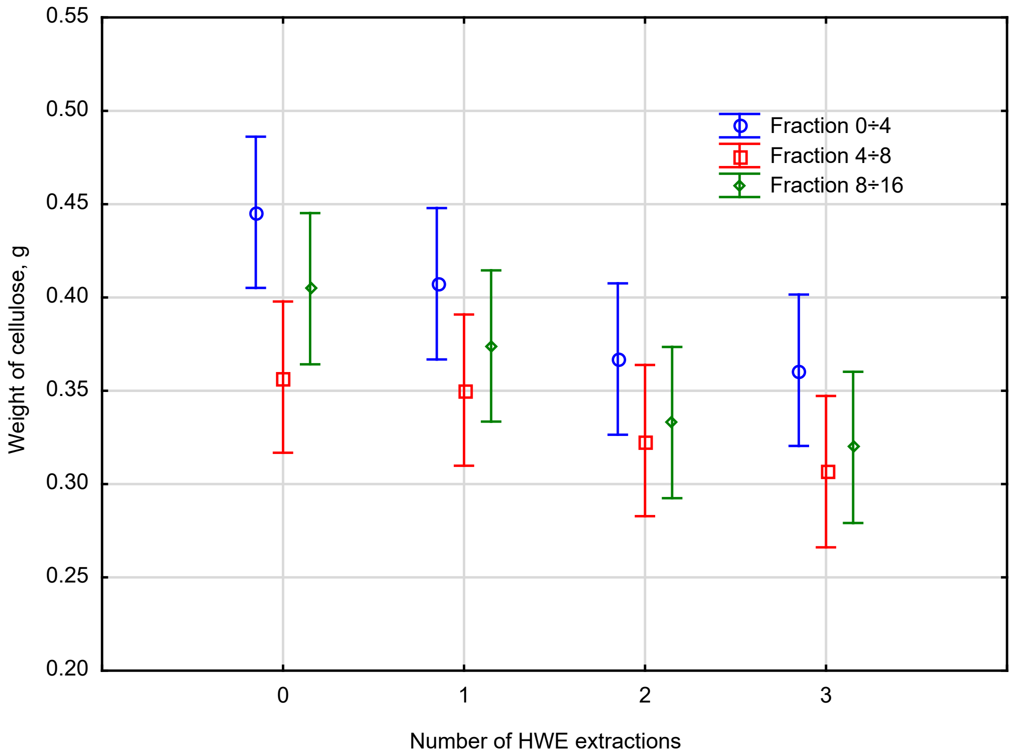


Figure 9. The influence of fraction size, extraction amount, and residual cellulose content.

The degradation of cellulose often occurs when hemp components are selectively extracted from the cellulose surface. The HWE method is an effective process for extracting lignocellulosic substances from hemp. Investigating possible substrate sources for bioproduction [31] and maintaining sustainable efficiency are also important. In the HWE process, critical substances, such as cellulose, hemicelluloses, and lignin, can be lost, resulting in significant reductions in production efficiency. Therefore, production costs are elevated, with the undesirable consequence of raising them. Cellulose is one of the critical components of bioproduction. The degradation of the feedstock during HWE might compromise its quality, decreasing the efficiency of total production. To minimize the loss of these essential substances, it is necessary to consider strategies to reduce loss. Extracting cellulose, hemicelluloses, and lignin must be cost-effective and sustainable. Providing alternatives to cellulose during the process can also mitigate the effects of cellulose degradation.

Analysis of the test results also showed that cellulose content was not the main factor affecting HWE modification properties. The results confirmed some correlation between the cellulose content and the size of the fraction of the material used. Native material fractions with a finer structure and a higher specific surface area exhibited a slightly higher cellulose content, likely due to a greater available surface for hydrolysis processes during the Kürschner–Hoffer treatment. In light of these observations, the conclusion is that the key factor determining the properties of the biocomposite structure was not so much the cellulose content itself but the size of the material fraction and its effect on interactions with ABS thermoplastic. The next step in this research was to measure the strength of composites using various material fractions and analyze how the particle size affected adhesion between the filler and ABS. This analysis will allow optimization of the composite composition to achieve the desired mechanical properties. It should also be noted that while the HWE process can lead to cellulose degradation, it also allows for cellulose extraction, a valuable by-product that should not be overlooked in the analysis of the profitability and sustainability of biocomposite production. Based on the results of the HWE, the cellulose content decreased with the increasing number of repetitions. The third HWE process had a less pronounced effect, suggesting that optimizing the number of repetitions to minimize cellulose loss and maximize hemp biomass's potential is possible.

3.4. Characteristics of Soluble Content

To understand the characteristics of the solution during processing, it is vital to evaluate the soluble compounds extracted from hemp. The soluble fraction extracted during HWE was dark, indicating lignin-derived phenolic compounds. The mass loss in the solid fraction after extraction indicated the removal of hemicellulose-derived sugars and weak organic acids, like acetic acid. As previous studies on lignocellulosic biomass extraction predicted, hot water pretreatment led to partial degradation of lignin and solubilization of hemicellulose [32,33]. Performing this analysis can provide valuable insights into how much and what kinds of substances are extracted from hemp. Two different methods were used to determine the soluble content. Each extraction method measured the mass of the extracted substances and the solution pH. For each extraction, the temperature of the solution was also recorded. The results of the soluble content analysis are presented in Table 6.

Table 6. The characteristics of soluble content.

Modification	**Extraction Type**	**Repetition Number**	**Sample Mass, g**	**Temperature, °C**	**pH Value**
Native	Cold	1	0.2532	18.6	6.84
		2	0.2346	18.1	6.13
		3	0.2581	18.5	6.35
	Hot	1	0.2502	18.6	6.76
		2	0.2556	17.5	6.41
		3	0.2444	17.4	6.10
HWE	Cold	1	0.2338	18.3	5.34
		2	0.2424	18.3	5.45
		3	0.2501	17.9	5.42
	Hot	1	0.2482	18.1	5.67
		2	0.2510	18.0	5.57
		3	0.2532	18.1	5.71

SD—standard deviation.

This study investigated the impact of various modifications and extraction methods on the pH of cannabis solutions. Cannabis samples were either unmodified (Native) or underwent a pretreatment, potentially denoted as HWE. Extractions were conducted using Tappi and cold or hot water. To ensure reproducibility and reliability, each extraction was repeated multiple times. The cannabis sample mass ranged from 0.2338 g to 0.2581 g, while extraction temperatures varied between 17.4 °C and 18.6 °C. The pH of the resulting solutions, a measure of their acidity or alkalinity, was subsequently measured. Temperature differences observed between hot and cold extractions were due to controlled laboratory conditions, where measurements were taken at ambient temperatures, which reduced variation. The material had a low thermal conductivity, meaning rapid temperature changes were not possible, and the extraction duration was short, so significant thermal accumulation was not allowed. This comprehensive approach allowed for a thorough assessment of how different modifications and extraction methods influenced the pH of the final cannabis solutions. The average values of the material modification type on the pH value are presented in Figure 10.

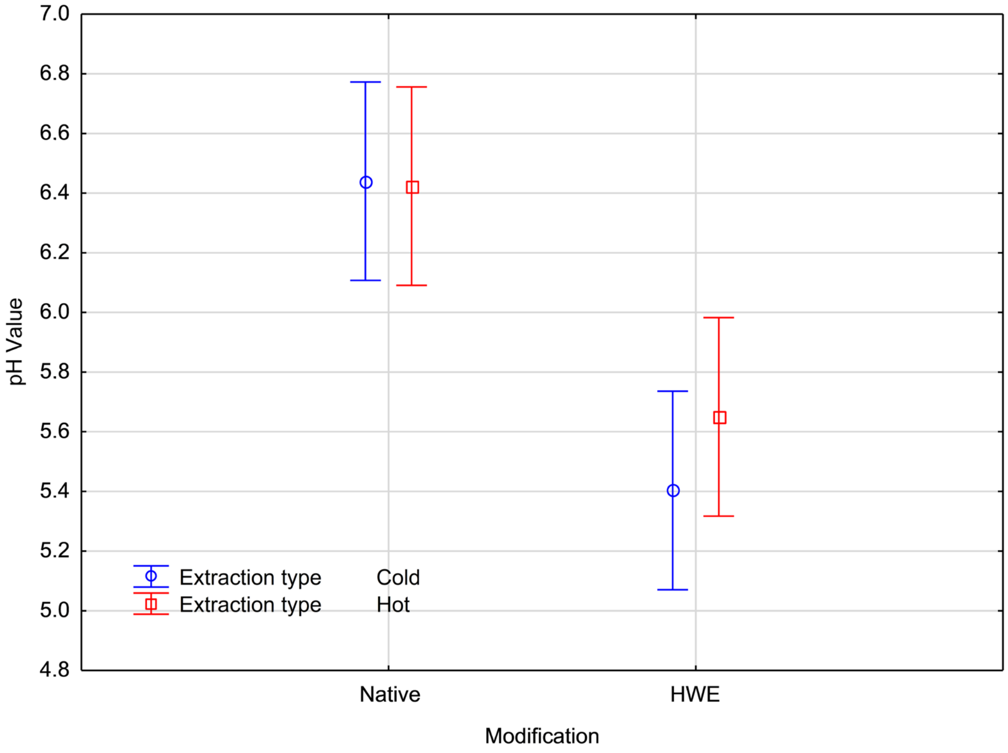


Figure 10. The average values of the material modification type on the pH value.

A two-way ANOVA was conducted to determine the impact of different modifications and extraction methods on the pH of cannabis solutions. According to this analysis, modification ($F = 2.457$, $p = 0.092$) had a significant effect, whereas extraction type ($F = 0.039$, $p = 0.543$) and their interaction ($F = 0.052$, $p = 0.500$) had no significance. The mean pH values of the different treatment groups were then compared via a post hoc test, likely Duncan's multiple range test. As a result of this analysis, it showed that the HWE Cold and HWE Hot groups were statistically similar, forming a distinct group with mean pH values of 5.40 and 5.65, respectively. Native Hot (mean pH = 6.42) and Native Cold (mean pH = 6.44) also had statistically similar pH values, forming their group. The statistical approach comprehensively evaluated how different modifications and extraction methods affected the cannabis solution pH.

3.5. Ash Content Tests

The final step in the study involved determining the ash content of the sample that had been collected. To determine the sample's mineral composition and thermal stability, the material was burned to determine its ash content. An investigation into hemp fiber properties and potential applications focused on its ash content, which was systematically analyzed for ash content. Using standardized procedures, the ash content was measured with accuracy and reproducibility. The weight of the crucible, including the ash, was then calculated based on the weight of the ashes. Table 7 shows the percentages of ash in the samples of hemp native and after-modification materials.

Table 7. The percentage shares of ash are broken down by type.

Modification	Sample Weight, g	Ash Weight, g	Ash Content, %	Average Ash Content, % (SD)
Native	0.937	0.029	3.095	2.527 (0.534) [a]
	1.081	0.022	2.035	
	1.020	0.025	2.451	
HWE(I)	0.912	0.027	2.949	2.118 (0.762) [a]
	1.482	0.022	1.452	
	1.461	0.029	1.952	
HWE(II)	1.331	0.040	3.021	2.995 (0.482) [a]
	1.231	0.043	3.463	
	1.122	0.028	2.501	
HWE(III)	1.090	0.025	2.293	1.840 (0.647) [a]
	1.081	0.023	2.128	
	0.972	0.011	1.099	

[a]—Homogenous group; SD—standard deviation.

The main objective of this study was to determine how much ash was present in the material in the extractions after the HWE method had been used to extract the samples. The statistical analysis allowed the study results to be characterized in terms of the relationship between the type of modification made to the raw material analyzed and the ash content percentage based on the study results. According to the statistical analysis, no differences were found between the measured parameters. For the results to be derived from the empirical value of $F(3, 8) = 2.004$, where p-value = 0.219, it needs to be considered in light of its significance for the empirical value of $F(3, 8) = 2.192$. The average values of the material modification type on the percent values of ash hemp content are presented in Figure 11.

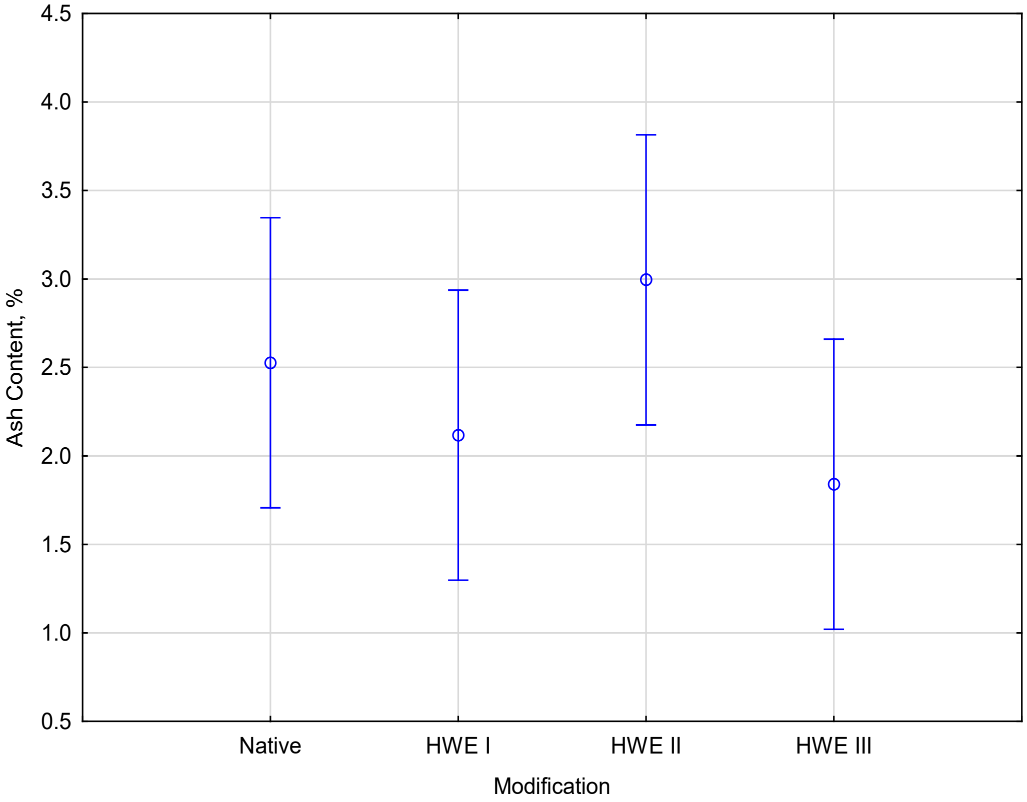


Figure 11. The average values of the material modification type on the percent values of ash hemp content.

According to the statistical analysis of the impact parameters, p was more significant than the alpha significance threshold of 0.05, which was accepted as a threshold for significance. The test classified all parameters into one homogeneous group based on the results. The methodology used Duncan's post hoc test to divide the groups into homogeneous ones. No parameters showed a homogenous grouping for each individual. Materials with a high ash content would also harm their mechanical and physical properties. Depending on the sample, the ash percent may differ, affecting the material's overall durability and mechanical properties [34]. An analysis of the ash content results of different modifications showed that the extraction process significantly impacted this property. Original samples and samples subjected to HWE(I) and (II) extractions had a higher ash content than those subjected to HWE(III). An extraction process can reduce the ash content of a material by removing soluble substances, thus altering its chemical composition. The ash content of HWE(III)-modified samples was the lowest of all extracted samples.

4. Discussion

The HWE process aims to extract valuable substances from plant materials during usage. It enhances enzymatic hydrolysis efficiency by selectively removing hemicellulose and lignin without degrading cellulose. Bioethanol conversion is more stable with HWE than with acid hydrolysis due to decreased inhibition of fermentation-by-fermentation inhibitors. It is also environmentally friendly and does not require chemical catalysts. HWE also has limitations at high temperatures, such as the need for multiple extraction cycles for optimal efficiency. The process generates soluble by-products, including organic acids and phenolic compounds, which must be treated before fermentation occurs. A thorough understanding of these factors is vital to optimizing HWE parameters and evaluating its industrial applicability. According to the observed changes in the color of the extraction liquid and the estimated mass of dissolved material, these compounds may be hemicellulose-derived sugars and lignin. In previous studies, oligomeric sugars, organic acids, and phenolic derivatives have been released during hydrothermal treatment, influencing subsequent enzymatic hydrolysis and fermentation [32,33]. To maximize sugar recovery while minimizing fermentation inhibitor formation, these compounds must be eliminated from the extraction process before downstream bioethanol production.

In the HWE process, modified sludge is washed out and collected in the upper reactor container after being washed with water [35]. In three repetitions of HWE, cellulose loss minimizes, suggesting an extraction efficiency saturation point. There are several potential explanations for this behavior. In subsequent cycles, soluble material becomes less available after hemicellulose and low-molecular-weight lignin fractions are depleted in the early extraction stages. The cellulose-rich cellulose matrix becomes more resistant to degradation, as few amorphous regions remain. Also, it should be considered that solubilized compounds may reprecipitate. The lignin and hemicellulose fragments released during earlier extractions may undergo condensation at elevated temperatures, leading to partial redeposition onto cellulose fibers and limiting additional loss. As a result of partial dehydration and rearrangement of fibrils, the biomass structure may become more compact, reducing water penetration and slowing the solubilization rate.

Several cycles of HWE are necessary to reach an optimal extraction state, beyond which additional treatments yield diminishing returns. The extraction rates of lignocellulosic biomass have shown similar trends after an initial rapid phase when pretreated with hydrothermal methods [36]. The saturation effect is crucial for optimizing bioethanol production efficiency and minimizing unnecessary energy inputs. Study results also showed that subsequent HWE cycles increased sludge mass, leaving a residue. The sludge mass did not follow the trend of increasing after the third repetition of HWE in fractions

4–8. Sample fractal thickness plays a vital role in this phenomenon. The performance of the HWE process may be affected by the physical properties of the biomass. More substances may be released from thicker stalks [37]. Distilled water is poured over the thickest fraction of stalks to find the most sediment. HWE can dissolve cellulose, hemicellulose, and lignin in hemp shavings. To use these substances as biofuel substrates, it is crucial to ensure that HWE extracts them efficiently [31]. As a result of the loss of these substances, HWE can reduce the efficiency and cost of biofuel production. More research is required to identify the substances removed during the HWE process by distilled water [38,39]. Dynamic processes affect the consequential results of each iteration, thus affecting the nature of the process.

The HWE process is designed to extract valuable substances from plant materials when they are being used. During the HWE process, the modified sludge is washed out and collected in the upper reactor container after being rinsed with water. The transformations during HWE include hemicellulose solubilization, lignin redistribution, structural reorganization of cellulose, and organic compound release. At elevated temperatures, hemicellulose glycosidic linkages dissolve in the extraction liquid, enabling enzymes to digest cellulose more easily. Increasing temperatures and pressure may cause lignin to soften and partially depolymerize, which alters the structural integrity and reduces the resiliency of lignocellulosic fibers. The removal or modification of hemicellulose or lignin increases the surface area of cellulose fibrils, which may contribute to a higher bioethanol yield through enzymatic hydrolysis. Hydrothermal conditions also facilitate the release of organic acids and phenolic compounds, which impact downstream processing and the chemical environment of the extraction liquid. The physical and chemical properties of lignocellulosic biomass are optimized through these transformations. The balance between hemicellulose removal and cellulose preservation is crucial to maximizing efficiency. Excessive thermal exposure can result in cellulose degradation, reducing ethanol yields overall.

Hemp shavings reduced cellulose content by a considerable amount following the HWE process. In addition, the trend toward a lower cellulose content after the HWE process is crucial. The least cellulose was lost in fractions 4–8 after the third post-HWE test, which showed the slightest deviation. The lower loss of cellulose after the third repetition of HWE suggested that there might be a reduction in the amount of easily extractable compounds. In light of this, it was necessary to optimize the number of repetitions to increase the overall efficiency of the process. Selective extraction has been introduced, showing that cellulose is not the only structural component that can dissolve during HWE—it also includes components near the surface. Considering that different elements can be affected simultaneously, this introduces complexity into the process, potentially altering the final composition.

As a result of HWE, the cellulose content of this feedstock remains comparable to other lignocellulosic feedstocks used for bioethanol production, thereby ensuring that it can be hydrolyzed and fermented using enzymes. Based on three repetitions of HWE, the cellulose yield for 0–4 mm fractions was 48.4%, 40.7% for 4–8 mm fractions, and 43.4% for 8–16 mm fractions. According to this study's results, HWE preserved sufficient cellulose for bioethanol conversion, which aligned with those from other bioethanol feedstocks, such as wheat straw (35–45%) and Miscanthus (40–60%). In bioethanol production, lignocellulose consists primarily of cellulose, an essential enzyme feedstock. Biofuel conversion efficiency was adversely affected by cellulose loss during the HWE process, mainly caused by high temperatures and pressures [30]. According to the results of this study, different lignocellulosic materials yielded different bioethanol yields. While providing high conversion efficiencies, the pretreatment requirements and inhibitor formation of sugarcane bagasse and wheat straw often pose challenges to fermentation.

Studies by Mussatto et al. (2010) and Song et al. (2013) have shown that HWE efficiently solubilizes hemicellulose while preserving cellulose integrity [39,40]. In addition to the previous research, it has been demonstrated that HWE has a similar effect on hemp biomass as observed on Miscanthus and wheat straw. Although Miscanthus is rich in lignocellulose, it exhibits variable ethanol yields depending on the hydrolysis method [2,41]. Compared to conventional feedstocks, hemp shives produce bioethanol yields that are on par or higher than these conventional feedstocks, proving its potential as a raw material for second-generation bioethanol production. Minimizing this loss is critical for maintaining both the quality and quantity of biofuel. Adjusting the parameters of the HWE process can reduce its aggressiveness and improve cellulose preservation. However, less aggressive parameters might also lower the efficiency of extracting other valuable substances, such as hemicelluloses and lignin, impacting the overall process performance [42,43]. An informed parameter modification supported by thorough experimental studies is essential. Beyond bioethanol, the versatility of HWE allows it to be considered for producing biocomposites or pellets. As the HWE process is modified, cellulose extraction is enhanced, while its structural integrity is preserved, making it more adaptable to producing sustainable materials.

Research in this area provides insight into the complexity of the HWE process and emphasizes the need for optimization to meet the requirements of various industrial applications. The results of this study are consistent with those reported by Roman et al. (2024) who investigated the impact of hot water extraction (HWE) on *Miscanthus giganteus*. According to their research, HWE increases biomass density and reduces ash content, increasing its suitability for bioethanol production [2]. Based on the results of the study, the cellulose content after HWE ranged from 48.4% for the 0–4 mm fraction, to 40.7% for the 4–8 mm fraction, to 43.4% for the 8–16 mm fraction, which falls within the range reported for other lignocellulosic feedstocks, like *Miscanthus giganteus*. The results demonstrated that HWE preserved cellulose integrity while selectively removing hemicellulose and lignin, making the material suitable for enzymatic hydrolysis. As reported in the studies, the absence of fermentation inhibitors in HWE-treated biomass suggests that the process is more ideal for bioethanol production than acid hydrolysis.

As a result of this study, the average ash of hemp samples analyzed averaged around 2.5%. The value was significantly higher than the typical ash content reported for hemp hurds, usually between 1% and 1.5% [34,35]. Several factors, including the type of hemp used, the growing conditions, and the harvesting and processing methods, may contribute to the high ash content found in this study [35]. The potential negative impacts of high ash content in hemp hurds warrant considering strategies for reducing it. Optimizing harvesting and processing methods could minimize using non-woody materials, such as leaves and bark, which tend to have higher ash content [35]. It is possible to remove ash-forming minerals from hemp hurds using chemical or physical treatments. More understanding of these strategies and developing effective methods for reducing ash in hemp hurds require further research.

The results of this study suggested that hemp biomass can contribute to regional economic growth from a socioeconomic perspective. The HWE process can be optimized to reduce the production costs for bioethanol, making renewable energy sources more affordable and accessible. This technology can also create jobs in biomass processing, bioethanol production, and sustainable material development. The scalability of the process would also enable rural and agricultural communities to benefit from the emerging bioeconomy by reducing their reliance on non-renewable energy sources and strengthening their economic standing [44–46].

5. Conclusions

The study aimed to plan and further develop the HWE method, focusing on utilizing hemp biomass as a lignocellulosic raw material. Hemp biomass extracted at different stages of the production process often has little commercial value due to insufficient utilization. To address this, the research focused on understanding hemp's potential for hydrothermal processing and its applications in bioethanol production. Laboratory results revealed a decrease in cellulose content after extraction, with the highest loss observed in the 0–4 mm fraction and an increase in evaporated water from the 8–16 mm fraction with successive HWE cycles. These findings provided insights into the structural and compositional changes occurring during HWE, contributing to a better understanding of its role in biomass valorization. The study demonstrated that after HWE treatment, the cellulose content in hemp biomass remained comparable to other commonly used lignocellulosic feedstocks for bioethanol production.

The process selectively removed hemicellulose and lignin, improving enzyme accessibility and enhancing bioethanol yield. Additionally, the absence of fermentation inhibitors suggested that HWE provided a more stable and efficient alternative to acid-based pretreatments. Hydrothermal pretreatment also facilitated the dissolution of organic components, including hemicellulose-derived sugars and phenolic compounds, into the extraction liquid. This finding aligned with other hydrothermal pretreatment studies and indicated that optimizing HWE can maximize sugar recovery while minimizing inhibitory compounds affecting fermentation efficiency. The study identified key mechanisms involved in HWE, including hemicellulose solubilization, lignin redistribution, and increased cellulose accessibility. The results showed that cellulose degradation stabilized after multiple HWE cycles, suggesting an extraction efficiency threshold. A new study provides insight into hydrothermal pretreatment by showing that hemp biomass can produce more efficient bioethanol than other biomass [2]. Hemp offers additional advantages, such as reduced ash content and reduced structural degradation, making it a more stable and sustainable feedstock. The research identified mechanisms that control fraction-dependent cellulose retention in hemp and confirmed that fermentation inhibitors were absent in HWE-treated hemp. The long-term scalability of hemp and other lignocellulosic materials should be examined in future studies.

The results of this study provided valuable insights into the potential applications of HWE-treated hemp biomass in sustainable energy production. By optimizing HWE conditions, cellulose became more accessible for bioethanol production, which can be adapted for industrial and small-scale, decentralized energy systems alike. The process also reduced the formation of inhibitory by-products, resulting in an efficient and environmentally friendly biomass processing method. Optimizing process parameters ensured the sustainability and scalability of bioethanol production by balancing effective biomass fractionation with minimal unnecessary cellulose loss.

Further refinement of HWE conditions could enhance sugar release and reduce inhibitory by-products, improving the overall bioethanol yield. Reducing the ash content, which averaged 2.5% across all modifications, was another critical aspect for improving process efficiency. By examining cellulose content changes in hemp shives, the study established a baseline understanding of the effects of HWE. Advanced analytical techniques, such as FTIR, should assess the potential degradation or modification of cellulose caused by HWE. Further studies comparing alternative pretreatment methods, including alkaline treatment, steam explosion, and enzyme-assisted extraction, may help determine the most effective method for modifying hemp biomass. Investing in fermentation optimization strategies and exploring the potential applications of extracted compounds could further improve the industrial feasibility of HWE for bioethanol production.

The findings of this study have practical applications beyond biofuel production. Biodegradable packaging, eco-friendly composites, and textile innovations could be developed from optimized HWE-treated hemp biomass. The increased efficiency of biomass processing could result in renewable household energy solutions, making bioethanol a more affordable and accessible fuel. Energy and material industries can benefit from these applications, which align with global sustainability efforts.

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