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Synergistic Influence of Alkaline Pretreatment, Ultrasound, and Pulsed Electric Field on Cellulose Accessibility and Structural Characteristics of Lignocellulosic Biomass

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Abstract

The Management of agro-waste is a critical issue in India, as improper disposal contributes significantly to environmental pollution and health risks. Rice and wheat straw are among the primary agricultural residues generated, rich in lignocellulosic materials like cellulose and lignin. This study investigated the potential for isolating and enhancing cellulose from paddy and wheat straw using various pretreatment methods. Initially, the agro-residues were subjected to alkali pretreatment of different proportions: 2%, 5%, and 10% for 24 hrs, followed by a detailed analysis of their physical, chemical, and functional properties. Based on key chemical indicators such as cellulose, hemicellulose, and lignin content and functional properties. A 10% alkali concentration was optimized for both substrates. The optimized samples were subsequently exposed to non-thermal treatment of ultrasound at different power levels at 350, 450, and 550 W for 30 minutes and pulsed electric field (PEF) treatment at electric field strengths of 20, 30, and 40 kV/cm for 15 minutes. The findings revealed that applying ultrasound power (550 W) and PEF field intensity (40 kV/cm) markedly enhanced cellulose accessibility and extraction efficiency. Ultrasound treatment produced 82.3% cellulose from paddy straw and 84.3% from wheat straw, while PEF treatment resulted in efficiencies of 78% and 82.3%, respectively. Structural and morphological changes induced by the combined treatments were further confirmed through FTIR spectroscopy and SEM analysis, revealing enhanced fiber disruption, partial delignification, and improved cellulose exposure. Overall, the integrated alkaline, ultrasound, and PEF pretreatment strategies effectively enhanced cellulose recovery from lignocellulosic biomass, demonstrating their potential for value-added bioprocessing applications.

Keywords: *Cellulose; Paddy straw; Pulsed Electric Field; Ultrasound; Wheat straw*

1. Introduction

India generates 500–550 million tons of crop residues annually. It was an enormous quantity of agricultural waste. Among this, 66% of the overall production has been utilized for industrial fuel, livestock feed, and household firewood. The remaining 34% waste was 23 Mt of vegetables and fruits, 43 Mt of rice, 89 Mt of wheat, 47 Mt of cotton, and 56 Mt of sugarcane (Nagar and Kumar 2024). The annual production of agro-waste has increased significantly, and improper disposal leads to environmental pollution and health risks for both humans and animals. An important aspect contributing to air pollution is the burning of agro-waste. There has been a noticeable decline in air quality in recent years, which is extremely concerning. India's farmers have been burning their crops, though it is a friendly, low-priced, and less taxing method. An enormous issue in India and other Asian countries is managing crop waste (Chattopadhyay 2023). Many agricultural residues are abundant in bioactive compounds and are inadequately treated and underutilized. These agricultural waste derivatives have the potential to be transformed into valuable materials with antioxidant and antimicrobial properties. Due to its huge accessibility and potential value, utilizing agro-waste is now seen as a critical component of sustainability (Varghese 2023). So agro-residues like paddy straw, husk, sugarcane bagasse, corn straw, and wheat straw are rich in fiber and hold great potential as materials. The waste-to-wealth strategy has been shown to be advantageous for farmers and the environment.

Globally, around 731 million tons of rice straw (*Oryza sativa L.*) are produced. About 126.6 million tons of rice straw are annually produced in India, and 60% of this is burnt in the open fields by farmers (Bhattacharyya 2021). The main derivatives of rice straw are cellulose (33-40%), hemicellulose (12-19%), lignin (20%), and ash (20%). Utilizing rice straw cellulose as the primary source of raw materials for producing micro and nanocellulose

is a promising concept (Bangar 2023). The world produced nearly 760 million tons of wheat in 2020, with China, India, Russia, and the USA accounting for over 50 % of total wheat production. Annually, 50-54% is left on the field from 529 Mt. of wheat straw. Moreover, it burns in the open air, releasing greenhouse gases that harm the ecosystem. Wheat stalks are a valuable source for animal feed as they contain cellulose (38.8%), hemicellulose (39.5%), and lignin (17.1%)(Ahmed, Janaswamy, and Yadav 2024). Due to the high lignocellulosic content and low moisture content, its degradation occurs later during wheat cultivation, and is obtained as a by-product (Berthet 2015).

Since cellulose constitutes 32–65% of agricultural waste, as a biopolymer vital for sustainable agriculture. Cellulose is a naturally occurring organic substance that is primarily produced by plants in the biosphere. It is the richest organic substance in the world, with 5×10^{11} metric tons produced annually. The worldwide availability of cellulose derived from natural fibers is the driving force behind the search for sustainable material technologies. As a raw material for numerous industrial goods, cellulose has gained attention on a global scale. It can be extracted using several methods to be used in various sectors, including agriculture, food packaging, food binder, and medicine (Li and Chen 2020). Cellulose has generated significant attention these days.

One of the most effective ways to enhance the physicochemical characteristics of agricultural waste is by pre-treatment. Agro-waste pretreatment improves its grindability, hydrophobicity, density, and porosity. There are several pretreatment methods, such as chemical, physical, and biological. Since the delignification process that follows the breakage of ester linkages between cellulose, hemicellulose, and lignin, the alkaline pretreatment is particularly effective at increasing the availability of biomass for cellulose regions (Dixit and Yadav 2020). In addition to that, to enhance extraction efficiency and cost-effectiveness, physical pretreatment methods such as ultrasound (US) and pulsed electric fields (PEF) have emerged as promising alternatives.

Ultrasound, which uses high-frequency sound waves above 20 kHz, triggers cavitation within the plant material. During this process, tiny bubbles rapidly form and collapse, producing intense microturbulence that imparts mechanical, thermal, and chemical impact on the biomass (El khayat driaa 2025). The main aim of ultrasound is to improve the efficiency of delignification during thermal and chemical processes when used with suitable solvents. This technique improves biomass availability and reactivity, facilitating the breakdown of rigid cell walls by enzymes or chemicals and releasing soluble components. As a result, it leads to increased cellulose yields, accelerated reaction rates, and reduced enzyme and chemical consumption in subsequent processes, thereby making the entire process more sustainable and environmentally friendly (Abolore, Jaiswal, and Jaiswal 2024).

Pulsed electric fields (PEF) use short, strong electrical pulses to electro-permeabilize cell membranes, increasing their permeability while stimulating the release of intracellular bioactive substances (Vorobiev and Lebovka 2022). The rapid mechanical breakdown of plant tissue caused by the strong electric field enhances the yield or extraction efficiency of cellulose. The researchers explored PEF's significant effect on cellulose extraction (Sekar 2024). Further, PEF pretreatment may reduce processing time and energy consumption while increasing the extraction yield (Angky Wahyu Putranto 2025). Similarly, a high cellulose content of up to 97.5% was obtained from mendong fiber by pretreating it with NaOH-PEF while bleaching it with hydrogen peroxide (Suryanto, 2018)

Numerous organic substances, such as sugarcane bagasse, coffee grounds, cactus, and giant reed, can be used to extract cellulose and lignin (Moubarik 2013). However, significant quantities of paddy and wheat straw

are discarded annually, leading to environmental concerns, and the underutilization of a valuable resource has been noted in recent years. So, the valorization, extraction, and conversion of waste into value-added products help to solve these problems. The present study focused on the isolation of cellulose from paddy and wheat straw using the combined effect of chemical and non-thermal techniques. The impact of ultrasound and pulsed electric field on the efficiency of extracting cellulose was estimated. The characteristics of cellulose, such as physical, morphology (SEM), and functional groups (FTIR), were analyzed. This novel extraction technique has high potential for use in agro-processing, paper, and packaging industries.

2. Materials and methods

2.1. Materials

Paddy straw was procured from a local farm in the Trichy district of Tamil Nadu, while wheat straw (Pusa Tejas variety) was obtained from fields in Madhya Pradesh. Both straws were cut into 3–5 cm pieces and subjected to sodium hydroxide (NaOH) pre-treatment before assessing their degradation.

2.2. Alkali treatment

The 100 g of paddy and wheat straw samples were chopped and washed. The sample was immersed in a 500 mL NaOH solution of different proportions: 2%, 5%, and 10% for 24 hrs. Following a 24 hrs soaking period in the NaOH solution, the sample was removed, rinsed, and cleaned with deionized water to remove any remaining NaOH residue until it achieved a neutral pH and turned colorless. The sample was placed in a hot air oven to remove the excess moisture. The method was carried out according to (K. Kaur & Phutela 2016). Then the sample was ground and stored in polyethylene bags and analyzed for physical properties, chemical properties, and functional characteristics. The process for the determination of the above-mentioned properties was also carried out for the control sample.

2.3. Determination of physical properties

2.3.1. Moisture content

A moisture content was determined using the AOAC 931.04; 2019 method. The sample was first weighed to measure its initial moisture content. It was then dried in a hot air oven at 105 °C until a constant weight was achieved, cooled to room temperature, and subsequently weighed again to obtain the final weight.

2.3.2. Color

The Color of paddy straw and wheat straw was measured using a Hunter Lab colorimeter (ColorFlex EZ model: 4510, Hunter Associates Laboratory, Reston, VA). Before the experiment, the color values were calibrated using a white tile and a black tile provided by the manufacturer. The color characteristics of the samples were evaluated using the L*, a*, and b* parameters, where L* measures the sample's lightness, a* measures its position on the red-green spectrum, and b* measures its position on the yellow-blue spectrum. The total color differences (ΔE) between the various alkali-treated and control samples were then calculated according to (Rao 2021)

$$\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad \text{---(1)}$$

2.3.3. Density properties

The bulk density, tapped density, and true density of both the alkali-treated and control samples were measured following the method outlined by (Ejikeme 2008). The bulk density (g/mL), a known volume of 50 mL

was taken, and the sample was filled and weighed. For tapped densities (g/mL) a dry 250 mL measuring cylinder was carefully filled with 20 g of sample, and the volume occupied by the sample was noted. The new volume, V₂₀₀, was also measured following 200 taps at the cylinder's base. True density is known as the ratio of bulk density to tapped density. Where ρ represents the sample's density (g /mL), W_2 is the total weight of the sample and container (g), W_1 is the container's weight (g), and V is the container's volume (mL). The density value is calculated as per equations 2 and 3.

$$\rho = \frac{(W_2 - W_1)}{V} \quad \text{---(2)}$$

$$\text{True density} = \frac{\text{Bulk density}}{\text{Tapped density}} \quad \text{---(3)}$$

2.3.4. Porosity

The porosity of paddy straw and wheat straw was determined by measuring the void space between particles relative to the total volume of the sample. Where P represents the porosity of the sample (%), B.D denotes the bulk density of the sample and T.D denotes the true density of the sample.

$$P (\%) = (1 - \frac{B.D}{T.D}) \times 100 \quad \text{---(4)}$$

2.4. Determination of Chemical Composition

2.4.1. Separation of holocellulose content

The holocellulose content was determined according to (Rattanawongkun 2020). Three grams of paddy and wheat straw were taken in a 250 mL beaker, and 0.5 mL of glacial acetic acid was added, containing 160 mL of distilled water, with the addition of 2 g of sodium chloride. The beaker was placed in a water bath for 1 hr at 80°C with periodic stirring. A consistent amount of reagent was added to the beaker at hourly intervals for 3 hrs. The mixture was then cooled to 10 °C in an ice bath, settled, and washed with a series of solvents such as ethanol, distilled water, and acetone. Finally, the resulting residue was dried at 105°C in a hot air oven until it reached a constant weight. The residue was weighed to quantify the holocellulose, and each estimation was calculated as per equation (5) given below.

$$\text{Holocellulose (\%)} = \frac{\text{Remaining dried weight (g)}}{\text{Initial weight (g)}} \times 100 \quad \text{---(5)}$$

2.4.2. Separation of alpha-cellulose and Hemicellulose content

Two grams of holocellulose were added to a 100 mL beaker containing 10 mL of 17.5% NaOH solution (Rattanawongkun 2020) The mixture was stirred continuously. The reagent was added at 5 min intervals for a total of 30 min. The beaker was kept in a water bath at 25 °C. After adding 20 mL of distilled water allow it to stand for an hr. The sample was then subjected to a series of rinses and filtrations using distilled water, sodium hydroxide (8.3%), and acetic acid (15%), until a neutral pH was achieved. Finally, the leftover sample was dried in a hot air oven at 105 °C until its weight stabilized. After this process, the sample was weighed to quantify the alpha-cellulose. Each estimation was determined as per the equations (6) and (7) given below.

$$\text{Alpha cellulose (\%)} = \frac{\text{Leftover dried weight (g)}}{2 \text{ g of holocellulose}} \times 100 \quad \text{---(6)}$$

The difference between holocellulose and alpha-cellulose was used to assess the hemicellulose amount.

$$\text{Hemicellulose (\%)} = (\text{holocellulose content (g)} - \text{alpha cellulose (g)}) \times 100 \quad \text{---(7)}$$

2.4.3. Separation of Lignin

The lignin content was determined based on the acid hydrolysis method with a slight modification of (Rattanawongkun 2020). A round-bottomed flask containing two grams of paddy and wheat straw was filled with 15 mL of 72% sulphuric acid and stirred. At room temperature, the sample was stirred for 2 hrs in a water bath. Add 200 mL of distilled water, followed by heating the mixture for 2 hrs. After filtering, the residue was removed with hot distilled water to measure the amount of lignin, then the sample was dried at 105°C in a hot air oven until its weight stabilized.

2.4.4. Extraction of cellulose

Cellulose from paddy and wheat straw was extracted by the method of (Thongsomboon, Baimark, and Srihanam 2023) with slight modifications. The wheat and paddy straw were immersed in hexane for 3 hrs to extract and remove waxes and other impurities. Then the residue was allowed to dry at room temperature. The 2 g of dewaxed substrate were soaked in a 7.5% NaOH solution and kept in a water bath at 70 °C for 2 hrs to remove lignin, hemicellulose, and other organic complexes from the samples. The sample was then filtered and treated with distilled water to neutralize its pH. The paddy and wheat straw were bleached with 5% sodium hypochlorite at 70 °C for 1 hr. The bleaching step was repeated twice until the white and fine fibers occurred. Then the sample residue was filtered and rinsed with distilled water. Finally, the extracted cellulose was dried at 80°C until a constant weight was obtained.

2.4.5. Ash content

The ash contents were determined by the standard AOAC 923.03 method and were measured by burning the pre-weighed test sample of 1 g dry weight of paddy straw and wheat straw in a muffle furnace for 5 h at 550 °C. Followed by 5 hr, the crucible was kept in a desiccator till it cooled to remove the temperature. The final weight of the crucible was taken. The ash content was estimated using equation (9) given below. Where, W_1 = initial amount of sample with crucible in (g); W_2 = final amount of sample in the crucible in (g); W = weight of the sample in (g)

$$\text{Ash content} = \frac{(W_1 - W_2)}{(W_1 - W)} \times 100 \quad \text{---(8)}$$

2.5. Determination of Functional Properties

2.5.1. Water holding capacity and Oil holding capacity

The water holding capacity (WHC) and oil holding capacity (OHC) of paddy and wheat straw were measured according to (He et al 2023). 1 g of the dried material was weighed and transferred to a centrifuge tube containing 10 mL of deionized water or vegetable oil. The sample was thoroughly mixed using a vortex meter for 2 min. Following mixing, the sample was subjected to centrifugation at 3000 rpm for 30 min, after which the clear liquid layer (supernatant) was decanted and removed, and the weight of the precipitate along with the centrifuge tube was recorded. The increase in weight of the sample was used to determine the water or oil holding capacity (WHC/OHC), with the result expressed as the ratio of grams of water or oil absorbed to grams of sample.

2.5.2. Water Swelling Capacity and Solubility Index

One gram of the sample was weighed, taken into a centrifuge tube with 20 mL of distilled water, and the mixture was continuously agitated at 90 °C in a water bath for 30 min (Rao et al. 2021). After the sample had cooled to room temperature, it was centrifuged for 20 min at 3000 rpm. After being decanted onto a weighed evaporating petri dish, the supernatant was evaporated at 100 °C for 15 min, and then the mass of the resulting dried residue, along with the sediment, was measured and recorded. The water swelling capacity (WSC mL/g) and solubility (S%) were estimated as per equations (9) and (10) given below. Where v_0 is the volume of the dried sample and m_0 is the mass of the dried sample.

$$WSC = \frac{(V_1 - V_0)}{m_0} \quad \text{---(9)}$$

$$S (\%) = \frac{\text{dry supernatant weight}}{\text{initial weight of a sample}} \times 100 \quad \text{---(10)}$$

2.6. Effect of Non-Thermal Assistance on Extraction of Cellulose

2.6.1. Ultrasound-Assisted Alkaline Extraction

The sample was processed using an ultrasonic probe sonicator (LABMAN, PRO-650 model). It was immersed in distilled water (1:10) and subjected to ultrasound treatment for 30 minutes, with 5-minute intervals. Sonication was carried out at power levels of 350 W, 450 W, and 550 W using a high-intensity probe system. To prevent overheating, the sample was placed in an ice bath, ensuring it remained within the range of 25-30 °C. The ultrasonication was performed in cycles with a 10 s “on” time followed by a 3 s “off” time at each power level. The probe was immersed 1 cm beneath the liquid’s surface. Following extraction, the sample was filtered. The residues are washed with deionized water until a neutral pH is attained. This method was done according to (Singh, Lim, and Manickavasagan 2020). The dewaxing process involved heating a toluene-ethanol solution (2:1) in a Soxhlet apparatus for six hrs. The resulting dewaxed sample was then filtered, rinsed with ethanol, and dried in an oven at 60 °C. Subsequently, 2 g of the sample were treated with 7.5% NaOH in a water bath at 70 °C for two hrs, followed by filtration and washing with water until a neutral pH was achieved. The Sample was treated with sodium hypochlorite (5%, w/v), and boiled in a water bath for 1 hr at 70°C to get bleached. For cellulosic samples, the bleaching step was repeated until white and fine fibers occurred. The sample was then filtered and rinsed repeatedly with distilled water to remove all traces of the bleach solution. Finally, the samples were dried in a hot air oven at 80°C until their weight remained stable. The cellulose extracted from both paddy straw and wheat straw was done with a slight modification (Thongsomboon, Baimark, and Srihanam 2023).

2.6.2. Pulsed Electric Field (PEF) Assisted Alkaline Extraction

A pulsed electric field (PEF) generator equipment (PEF generator 40KV-A2103096) was purchased from Surview Instruments from Thane, Maharashtra. The experimental setup consists of a pulsed electric field generator, treatment chamber, control panel, and oscilloscope. The samples were submerged in distilled water at a 1:10 ratio. The PEF was performed with an electric field strength of 20 kV/cm, 30 kV/cm, and 40 kV/cm, with a treatment time of 15 min, a pulse width of 100 µs, and a pulse-off time of 50 ms. After PEF treatment, the sample was extracted to obtain cellulose content (Suryanto 2018).

2.6.3. Morphological and Characterization by SEM

Morphological characteristics of wheat and paddy straw cellulose samples were studied using the scanning electron microscope (Model: EVO-18, CAREL ZEISS). A double-sided carbon tape was attached to an aluminium sample holder, and a thin layer of the sample material was applied on top of the tape. High-resolution images were taken with magnifications of 300 x, 500 x, 1 kx, 1.5 kx, 2.5 kx, and 3.0 kx with a higher voltage of 10 kV. To improve its electrical conductivity the gold layer was coated on the sample before the analysis (Singh, Lim, and Manickavasagan 2020).

2.6.4. Fourier transform infrared spectroscopy (FTIR) analysis

The morphology of the sample's surface and its functional groups were examined using FTIR spectroscopy. The pretreated and untreated samples of FTIR spectra were analyzed. (Model: PERKIN ELMER Spectrum RX I). FTIR spectra were recorded with a spectral resolution of 4 cm^{-1} , scanning a wavelength range of $4000\text{--}400\text{ cm}^{-1}$.

2.7. Statistics analysis

IBM SPSS Statistics (version 23) was used to perform all statistical analyses needed for the experimental data. A one-way analysis of variance (ANOVA) was employed to compare the data from different treatments, with Duncan's multiple comparison test used to identify significant differences at a probability level of $P \leq 0.05$. Each experiment was conducted in triplicate, and the results are presented as the mean value $\pm$ standard deviation.

3. Results and discussions

3.1. Effect of alkali pretreatment on Physical Properties

3.1.1. Moisture content

The paddy and wheat straw alkali-treated samples and the untreated samples are presented in Table 1. As the concentration of alkali increases, the moisture content (%) decreases because lignin and hemicellulose are progressively removed from the fibers. Alkali treatment also breaks intermolecular hydrogen bonds that are formed by the hydroxyl groups (-OH) found in cellulose, hemicellulose, and lignin. This disruption results in smaller fiber segments, leading to an increased surface area. The moisture content values obtained in this study are 16.48% & 11.17% for untreated paddy straw and wheat straw, and 44.83% & 56.40 % for 10% alkali treatment of samples. In the alkali treatment sample reduction of moisture content was 30.02% and 16.66% is, due to free hydroxyl and other polar groups that exist in untreated fibers, which can adsorb moisture over hydrogen bonding. Similar reported in (Ejikeme 2008) There are significant differences observed between all the treated and control samples.

3.1.2. Color values

The color of the paddy straw and wheat straw values was obtained for alkali-treated and untreated samples. The values are shown in Table 1. For alkali-treated samples, as the concentration increases the L^* value increases because the straw gets bleached and increases its lightness value. For a^* and b^* values it tends to decrease, and the total color difference (ΔE) of paddy and wheat straw increases, measured as $(13.87 \pm 0.005, 13.93 \pm 0.011, \text{ and } 14.53 \pm 0.015)$ and $(17.32 \pm 0.110, 17.26 \pm 0.002, \text{ and } 17.35 \pm 0.002)$, respectively. The degree of variance among color characteristics might be a factor and largely dependent on the concentration and time of

the alkali treatment hence, there were significant differences observed between treatment and untreated samples (Rao et al. 2021).

3.1.3. Density and porosity

The densities and porosities of paddy straw and wheat straw for alkali-treated samples, and untreated samples have been studied. The values are presented in Table 1. For alkali-treated samples, when the alkali concentration increased the straw became fragile and lighter in color. The bulk density of paddy straw and wheat straw after increasing alkali concentrations was found to fall in the range of (0.14 ± 0.02 , 0.11 ± 0.08 , and 0.08 ± 0.05 g/mL) and (0.09 ± 0.07 , 0.08 ± 0.02 , and 0.07 ± 0.02 g/mL), respectively. This is due to NaOH treatment removing some of the lignin and hemicellulose from straw. When the concentration increases there will be degradation of hemicellulose which affects the structural properties and leads to swelling, so, that the bulk density value tends to decrease as alkali concentration increases. For control samples of paddy straw and wheat straw obtained values are (0.06 ± 0.01 and 0.09 ± 0.05) The pretreated straws can enhance the bulk density which can be accommodated in a lesser space, storing huge amounts of paddy straw in a limited space investigated (K. Kaur and Phutela 2018) Similarly, the tapped densities of paddy straw and wheat straw after alkali treatment fall in the range of (0.21 ± 0.02 , 0.18 ± 0.01 and 0.13 ± 0.07 g/mL) and (0.22 ± 0.05 , 0.18 ± 0.02 , and 0.16 ± 0.01 g/mL), respectively. As the alkali concentration increases the tapped density values tend to decrease due to degradation and swelling action; the fibers are less able to compact tightly. For untreated samples of paddy and wheat straw, the obtained values are (0.19 ± 0.05 and 0.24 ± 0.03 g/mL). Similarly, the true density of the paddy straw and wheat straw decreases as alkali concentration increases because alkali treatment affects amorphous components like hemicellulose and lignin without affecting the cellulose content the obtained values are (0.63 ± 0.03 , 0.57 ± 0.02 , and 0.53 ± 0.01 g/mL) and (0.41 ± 0.08 , 0.34 ± 0.05 , 0.29 ± 0.03 g/mL).

The porosity values obtained in this study for alkali-treated paddy and wheat straw (75.76 ± 1.32 , 82.14 ± 0.73 , 85.89 ± 0.62) % and (73.76 ± 0.42 , 77.42 ± 0.87 , 83.76 ± 0.60) %. The porosity values increase as the alkali concentration increases, due to the breakdown of hemicellulose and lignin. These components serve as a binding agent within the straw, maintaining its structural integrity. Removing these components leads to fibers swelling and decomposing, which creates more void space within the straw. For control samples of paddy and wheat straws, the values are (87.81 ± 1.183 and 88.36 ± 1.338)% (Jurado-Contreras 2022). There are significant differences observed between all the treated and untreated samples.

3.2. Effect of alkali pretreatment on Chemical Properties

The compositional analysis of paddy straw and wheat straw was conducted to examine the effect of the pretreatment method on different properties, including holocellulose, alpha-cellulose, hemicellulose, lignin, cellulose, and ash. The results are shown in Table 2. By evaluating the yield of these different components, the effectiveness of the alkali pretreatment method can be determined. The alkali-pretreated paddy straw and wheat straw exhibited a reduction in holocellulose content. As the concentration increases (2%, 5%, and 10%) the holocellulose content tends to decrease due to the solubilization of hemicellulose and the degradation of cellulose at higher concentrations of alkali. There is no significant difference between 2% and 5% of paddy straw. Treated samples have significant differences ($P \leq 0.05$). For alpha-cellulose, as the concentration increases it tends to increase. There are significant differences observed between treated and untreated samples. In another study, (Sharma 2023), it was observed that pretreating paddy straw with several concentrations of NaOH (2%, 5%, 8%,

and 12%) enhanced the amount of alpha-cellulose. The 12% alkali concentration was used to attain the highest yield of cellulose content, followed by delignification and hemicellulose content, with a notable recovery of cellulose. The extraction of cellulose of both paddy and wheat straw was illustrated in Fig.1. In this study, the cellulose content of alkali-treated paddy straw and wheat straw increased noticeably from 47.01 to 58.89%, and from 44.61 to 52.96% respectively. There was a significant difference between the treatment and the samples.

(Mukherjee 2018) reported that sawdust treated in 10% NaOH increased the concentration of cellulose from 41% to 51.8%, yet other components including xylan and acid-insoluble lignin, only slightly increased. In this study observed that the hemicellulose, lignin, and ash contents were reduced from (9.74 to 2.85% and 15.55 to 5.38%), (13.77 to 9.24%, and 18.70 to 8.75%), and (10.73 to 7.96% and 9.52 to 4.76%), respectively. For hemicellulose, the 5% and 10% of treated paddy straw had no significant difference. However other samples showed significant variations within treatments.

In the case of lignin, the 2% and 5% of paddy straw had no significant difference, but other treatments had significant differences. This is due to a decline in the relative hemicellulose concentration since the alkali pretreatment saponifies the intermolecular ester linkages (which include uronic and acetyl acid substitutions) and solubilizes the xylan hemicellulose. Furthermore, the breaking of glycosidic linkages and β and α -aryl ethers leads to delignification. A similar study was reported by (P. Kaur 2023) when wheat straw was treated with alkali it showed a reduction in lignin and hemicellulose contents, from 19.8 to 2.2% and 19.1 to 11.1%, respectively.

The ash content decreases as the alkali concentration increases. Initially, at a lower concentration, the ash content of the straw increases due to the addition of alkali residues and the removal of organic matter. They had a significant difference between treated and untreated samples. For paddy straw not much differences were observed among the samples. As the subsequent concentration of alkali increases, the removal of organic residues continues. The initial increase in ash content might stabilize and the ash content slightly starts to decrease because the proportion of inorganic residues becomes higher compared to other organic matter studied by (Mukherjee, Banerjee, and Halder 2018).

3.3. Impact of alkali treatment on Functional Properties

3.3.1. Effect of water-holding capacity, and oil-holding capacity

The effect of alkali treatment on the water-holding and oil-holding capacity of paddy and wheat straw, and the untreated results, are depicted in Fig.2. For alkali-treated samples, the ability of water absorption is influenced by the concentration of alkali and the duration used during the treatment process. As alkali treatment increases, it reduces the water-holding capacity because of the drop in hemicellulose and lignin content. As these components are hydrophilic when removed, the straw becomes less prone to water absorption. They also alter the structure of straw fibers, making them less porous and reducing their capacity to retain water. (Madival 2022) reported the same result as alkali-treated straw has better mechanical properties and absorbs less moisture than untreated straw. It implies that chemical treatment has increased hydrophobicity. Similarly, for the oil holding capacity of paddy and wheat straw, the alkali-treated sample decreases due to the removal of hydrophilic components, modification of structural changes, and an increase in hydrophobicity. The cellulose becomes more hydrophobic due to the loss of polar groups associated with hemicellulose and lignin this hydrophobic nature further diminishes the oil-holding and water-holding capacity. (Viswanathan & Panneerselvam 2019) observed similar trends in oil holding and water holding capacity.

3.3.2. Swelling capacity and solubility index

The Swelling capacity and solubility index are used to examine the accessible surface area. The swelling capacity was found to be increased for the alkali-treated samples of paddy and wheat straw, are presented in Fig.3. As alkali concentration increases the swelling capacity increases; the obtained values are $(15.83 \pm 0.53, 18.16 \pm 0.24, 20.37 \pm 1.01)$ g/g, and $(10.18 \pm 0.35, 11.55 \pm 0.71, \text{ and } 13.02 \pm 0.58)$ g/g, respectively. The swelling capacity of alkali-treated samples was found higher than the untreated samples $(12.42 \pm 0.38 \text{ and } 9.35 \pm 0.23)$ g/g. (Bay 2021) reported that alkaline pretreatment of rice straw at 93°C higher temperature exhibits the highest water swelling capacity. Similarly, for the solubility index, the alkali-treated straw increases as the concentration of paddy and wheat straw increases $(22.99 \pm 0.68, 26.22 \pm 0.76, \text{ and } 28.44 \pm 0.94)$ and $8.77 \pm 0.73, 11.41 \pm 0.87, \text{ and } 19.99 \pm 0.56)$, whereas for the untreated sample $(17.94 \pm 0.62 \text{ and } 7.78 \pm 0.22)$. It is due to a higher concentration of alkali it effectively dissolves hemicelluloses and lignin from straw, and most of the straw material is soluble in alkali solution. Treatment modifies the structural integrity and leads to more solubilization. When the fiber disrupts the overall solubility increases.

3.4. Effect of Nonthermal Treatment on Extraction of Cellulose from Biomass

Treatment was optimized based on the physical and chemical properties of the hemicellulose, lignin, and cellulose content and functional properties. The alkali-treated 10% was effective as it had a higher cellulose yield. (Sharma 2023) reported the same trend that the agro waste of pretreated rice straw was examined with different Proportions of alkali (2,5,8, and 12%), the maximum amount of cellulose yield was attained in the case of 12%.

3.4.1. Effect of Ultrasound

The alkali-treated sample (10%) and an untreated sample of paddy and wheat straw were subjected to ultrasound at different power levels, 350 W, 450 W, and 550 W for 30 min, and the cellulose extraction was estimated and presented in Table 3. Ultrasound-treated sample has a high impact on cellulose extraction because of its cavitation effect. This enhances the surface area available for solvent interaction, as high-intensity ultrasound promotes the disintegration of cellulose fibers. As a result, the fibers become shorter and their overall surface area increases (El khayat driaa 2025). Compared with the untreated sample, the alkali-treated sample with ultrasound has a higher cellulose yield. Similar trends were reported in (Dinh Vu 2017) the alkaline solution NaOH with a higher concentration, treated with high-temperature ultrasound, was used to separate lignin efficiently and increase the cellulose yield. So, alkali-treated ultrasonic assistance cleaves the bonds between lignin and hemicelluloses from the paddy and wheat straw cell walls than alkali treatment without ultrasound assistance. The alkali-treated sample of a higher power level of 550 W extracted more cellulose than a lower power level of 350 W. A similar observation was obtained by (Chagas 2023), where cellulose nanocrystals (CNC) were treated using three sonication power levels 90%, 70%, and 50%. The results showed that higher ultrasonic power improved extraction efficiency, highlighting power intensity as a key factor in enhancing CNC productivity. A study by (Chen 2023) emphasizes the structure of cellulose nanofibrils from windmill palm fibres by alkali-ultrasound treatments. With NaOH of 6% and ultrasound of 800 W parameters resulted in the diameter <90 nm of nanocellulose. (Ardila A. 2024) examined that the combination of 30% NaOH with conventional ultrasound (300 W for 1 hr) proved to be the most effective for obtaining high-purity and high-yield cellulose, reaching up to 99.5%. In another study, the ultrasound treatment (750 W at 3 hr) emerges as an optimal strategy for reducing the microfiber diameter in sugarcane bagasse (Foroushani, Foroushani, and Yarahmadi 2025).

3.4.2. *Effect of Pulsed Electric Field*

The alkali-treated sample (10%) and an untreated sample of paddy straw and wheat straw were exposed to PEF at different power levels of 20 kV/cm, 30 kV/cm, and 40 kV/cm for 15 min and the cellulose extracted is depicted in Table 3. By applying a high electric field strength creates electroporation and cavitation in cell membranes, and the structural changes lead to the degradation of hemicellulose and lignin components. This disruption makes it convenient to extract. Compared to the untreated sample, the alkali-assisted PEF results in a higher cellulose yield. (Suryanto 2018) examined Medong fiber, where PEF-assisted alkali treatment enhanced cellulose extraction, increasing the cellulose content from 72.1% to 92.1%. Similarly, in another study (Putranto 2021) investigated that corn cob biomass by using PEF (9 kV/cm of electric field strength) and NaOH pretreatment resulted in the enhancement of the cellulose content by 40.59%. In this study, the alkali-treated sample with a high electric strength of 40 kV/cm extracted more cellulose than the 20 kV/cm.

Since alkali-treated samples assisted with the non-thermal treatment of PEF and ultrasound are more effective than the untreated sample. However, in the extraction of cellulose, the alkali-treated with a higher power level of ultrasound, 550 W, and high voltage of PEF, 40 kV/cm, has a greater yield and better extraction than the control. The treatment was optimized based on these parameters.

3.4.3. *Scanning Electron Microscopy (SEM)*

The morphological features of cellulose extracted from paddy and wheat straw were examined using Scanning Electron Microscopy (SEM), as illustrated in Fig.4. This figure shows fiber surface morphology at 2500 x magnification for paddy straw. Figure (a) depicts that in the untreated sample, the presence of non-cellulosic components leads to a rough surface, compact structure, and intact. Figure (b) shows that the ultrasound-treated sample has less breakage and loosening of fibril structure and a smooth surface can be seen, which indicates the breakdown of non-cellulosic components and delignification. Figure (c) observed that in PEF sample, the fiber strands are visible and the fiber bundles are together compared to the ultrasound sample. While using the extraction of NaOH, it dissolves some of the constituents, such as lignin and hemicelluloses and it leads to rougher debris. A similar trend was found in (Sharma 2023) alkali-treated samples the loosening of fiber structure and disruption of smooth surface are observed. The wheat straw of the SEM images shows the fibers surface morphology at 1500 x magnification. From Figure (d) it can be seen that the surface has a highly rigid, compact, and irregular surface and also has debris because of the existence of amorphous components lignin and hemicellulose, whereas the Pretreated sample of ultrasound and PEF has a disrupted surface, hard, and disordered. Figure (e) shows that the fibers bundles are together and there is no debris whereas from Figure (f) it was observed that the fibers are single-stranded and some pores occur due to the presence of cellulosic components. A similar observation was found in (Suryanto, Fikri, Permanasari, Yanuhar, et al. 2018) alkali-assisted PEF has a single cellulosic fiber due to the degradation of hemicellulose and lignin content; some fibers may have bundle forms. A similar study, (Sharma 2023), reported that the untreated samples of wheat straw were highly ordered, and the occurrence of rigid structure and ultrasound-treated samples was found to have more cracks and larger pores due to its cavitation effect.

3.4.4. *FTIR analysis*

The functional components extracted cellulose were analyzed using FTIR, 'Origin 6.0' software, and the spectra details are shown in Fig.5. FTIR spectra of the cellulose of paddy straw and wheat straw extracted by a

pulsed electric field (PEF), Ultrasound-assisted extraction, and the untreated sample are shown in the figure. The development of hydrogen bonds between and within molecules affects the physical characteristics of cellulose, including such characteristics as solubility, the reactivity of its hydroxyl groups, and its crystallinity (Medronho 2012). The broad absorption peak obtained between 3000-3500 cm^{-1} is ascribed to the hydrogen-bonded -OH group which was noted in all the rice straw and extracted cellulose variants (Sharma 2023). The peak at 2916 cm^{-1} represented the C-H stretch in alkane groups. The peak at 2901 observed stretching of the C-H group and deformation vibration in the glucose unit (Dinh Vu 2017). The C=C bond found in the aromatic structure of lignin within the fiber was identified as the origin of the band observed at 1430–1630 cm^{-1} . In cellulose samples, the band at 1161 cm^{-1} is linked with the -C-O-C- stretching of the β -1,4-glycosidic linkage, which is notable. The peak at 890-900 cm^{-1} was a cellulose-contributed feature of the β -glycosidic bond (Freitas, González-Martínez, and Chiralt 2022). The 615 and 441 cm^{-1} represent the removal of silica Si-o-Si stretch, isolating cellulose (Yusefi 2020), where the peaks observed at PEF-treated, ultrasound-treated, and untreated samples did not have much difference. For ultrasound treatment, the ultrasonic vibrations do not alter the structure of cellulose, similar to PEF treatment. Overall, no significant differences were observed between the treated and untreated samples, indicating that the functional group of cellulose remains unaffected.

4. Conclusion

This Study explored the effect of alkali treatment and non-thermal extraction methods on paddy and wheat straw. The results indicated that increasing alkali concentration significantly affects the reduction of moisture content, color, and porosity. Higher alkali concentrations improved the lightness (L^* value) and increased the total color difference (ΔE) due to the bleaching effect. The alkali treatment also resulted in a decreased moisture content by breaking intermolecular hydrogen bonds, leading to smaller fiber segments with an increased surface area. It also significantly enhanced physicochemical properties and functional properties (WHC, OHC, WSC, and SP) of straw ($p < 0.05$). The non-thermal methods, including ultrasound-assisted and pulsed electric field (PEF)-assisted alkaline extraction, showed promising results in cellulose extraction, improving the efficiency and yield. The morphological analysis using SEM and FTIR confirmed the structural changes and functional group modifications in the treated samples. Overall, a study demonstrates that alkali-treated and non-thermal extraction methods can effectively modify the properties of paddy and wheat straw, making them suitable for various industrial applications.

Author Contributions

Rahini P: conducting the experiments, acquisition of data, analysis and interpretation of data, and writing -original draft; K Nithyadevi: design of methodology, data analysis, review, and edit the draft ; S. Anandakumar: conceptualization, development, or design of methodology, supervision, project administration, review, and edit the draft; Ashish Rawson: development, or design of methodology, acquisition of data and analysis, review, and edit the draft; R. Jagan Mohan: conceptualization, design of methodology, review, and edit the draft. All authors read and approved the manuscript.

Ethical Statement

No human or animal subjects were involved in this research.

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495 Declaration of Competing Interest

496 The authors declare that they have no known competing financial interests or personal relationships that
497 could have appeared to influence the work reported in this paper.

498 Data Availability

499 The data obtained during the study are available from the corresponding author upon reasonable request.

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642 **Table 1.** Estimation of Moisture, Color, Bulk Density, Tapped Density, True Density, And Porosity

Sample	Treatment	Moisture (%)	Bulk density (g/mL)	Tapped density (g/mL)	True density (g/mL)	Porosity (%)	L*	a*	b*	ΔE
Paddy straw	T1	16.48 ± 2.14 ^a	0.06 ± 0.01 ^a	0.19 ± 0.05 ^c	0.61 ± 0.06 ^c	87.81 ± 1.18 ^c	59.35 ± 0.54 ^c	6.33 ± 0.75 ^c	24.31 ± 0.43 ^c	-
	T2	22.77 ± 0.55 ^b	0.12 ± 0.05 ^d	0.21 ± 0.08 ^d	0.53 ± 0.01 ^a	81.47 ± 0.53 ^b	49.27 ± 0.18 ^a	4.21 ± 0.55 ^d	30.37 ± 0.86 ^e	7.15 ± 0.03 ^a
	T3	75.03 ± 0.80 ^c	0.14 ± 0.02 ^c	0.21 ± 0.02 ^d	0.63 ± 0.03 ^d	75.76 ± 1.32 ^a	51.28 ± 0.14 ^b	3.60 ± 0.48 ^c	25.74 ± 0.58 ^d	13.87 ± 0.00 ^b
	T4	67.98 ± 0.65 ^d	0.11 ± 0.08 ^c	0.18 ± 0.01 ^b	0.57 ± 0.02 ^b	82.14 ± 0.73 ^c	55.26 ± 0.78 ^c	2.73 ± 0.68 ^b	23.42 ± 0.73 ^b	13.93 ± 0.01 ^c
	T5	44.83 ± 0.53 ^c	0.08 ± 0.05 ^b	0.13 ± 0.07 ^a	0.53 ± 0.01 ^a	85.89 ± 0.62 ^d	56.88 ± 0.95 ^d	2.48 ± 0.84 ^a	21.83 ± 0.90 ^a	14.53 ± 0.01 ^d
Wheat straw	T6	11.17 ± 1.03 ^a	0.09 ± 0.05 ^b	0.24 ± 0.03 ^d	0.55 ± 0.04 ^d	88.36 ± 1.33 ^c	49.16 ± 1.02 ^a	4.56 ± 1.05 ^a	32.64 ± 1.25 ^b	-
	T7	20.96 ± 0.51 ^b	0.09 ± 0.06 ^d	0.25 ± 0.02 ^c	0.62 ± 0.04 ^c	79.96 ± 0.65 ^c	60.41 ± 0.15 ^c	5.97 ± 0.50 ^d	25.62 ± 1.50 ^a	11.12 ± 0.00 ^a
	T8	73.07 ± 0.74 ^c	0.09 ± 0.07 ^c	0.22 ± 0.05 ^c	0.41 ± 0.08 ^c	73.76 ± 0.42 ^a	50.16 ± 0.95 ^b	7.33 ± 0.85 ^c	36.41 ± 0.75 ^c	17.32 ± 0.11 ^b
	T9	69.05 ± 0.71 ^d	0.08 ± 0.02 ^b	0.18 ± 0.02 ^b	0.34 ± 0.05 ^b	77.42 ± 0.87 ^b	53.96 ± 0.65 ^c	5.12 ± 1.25 ^c	34.82 ± 0.48 ^d	17.26 ± 0.00 ^b
	T10	56.40 ± 0.10 ^c	0.07 ± 0.02 ^a	0.16 ± 0.01 ^a	0.29 ± 0.03 ^a	83.76 ± 0.60 ^d	55.15 ± 0.30 ^d	4.81 ± 1.12 ^b	33.13 ± 0.56 ^c	17.35 ± 0.00 ^b

643 Results were expressed as mean ± SD (n = 3); different superscripts of lowercase letters along the samples in each parameter represent significantly different according

644 to Duncan's test (p<0.05).

645 *T1&T6-Control; T2&T7- Water Soaked; T3&T8- 2% alkali; T4& T9- 5% alkali; T5&T10- 10% alkali

Table 2. Estimation of Holocellulose, Alpha-Cellulose, Hemicellulose, Lignin, Cellulose & Ash

Sample	Treatment	Holocellulose (%)	Alpha-cellulose (%)	Hemicellulose (%)	Lignin (%)	Cellulose (%)	Ash (%)
Paddy straw	T1	88.37 ± 1.20 ^c	58.34 ± 2.01 ^b	27.28 ± 1.86 ^c	20.49 ± 1.44 ^d	34.09 ± 1.19 ^a	9.03 ± 0.11 ^b
	T2	92.26 ± 0.96 ^d	45.97 ± 0.71 ^a	37.54 ± 0.94 ^d	16.65 ± 0.99 ^c	38.80 ± 0.67 ^b	4.76 ± 0.51 ^a
	T3	86.90 ± 0.88 ^b	78.07 ± 0.73 ^c	9.74 ± 0.61 ^b	13.77 ± 0.43 ^b	47.01 ± 1.59 ^c	10.73 ± 0.62 ^b
	T4	86.06 ± 0.24 ^b	82.38 ± 0.80 ^d	4.22 ± 0.52 ^a	12.12 ± 0.76 ^b	52.24 ± 2.00 ^d	9.74 ± 0.42 ^b
	T5	83.99 ± 0.64 ^a	85.91 ± 1.17 ^c	2.85 ± 1.86 ^a	9.24 ± 0.71 ^a	58.89 ± 0.63 ^c	7.96 ± 0.49 ^a
Wheat straw	T6	86.60 ± 1.53 ^a	60.48 ± 1.85 ^b	26.15 ± 1.90 ^d	25.70 ± 0.57 ^d	36.88 ± 0.57 ^a	11.20 ± 0.33 ^c
	T7	93.96 ± 1.23 ^c	55.52 ± 1.19 ^a	38.44 ± 0.75 ^c	19.32 ± 1.09 ^c	39.97 ± 0.30 ^b	12.98 ± 0.60 ^d
	T8	95.00 ± 0.68 ^c	79.48 ± 0.80 ^c	15.55 ± 0.17 ^c	18.70 ± 0.64 ^c	44.61 ± 0.56 ^c	9.52 ± 0.04 ^b
	T9	90.79 ± 0.58 ^b	81.60 ± 0.32 ^d	9.18 ± 0.30 ^b	12.16 ± 0.86 ^b	49.57 ± 0.54 ^d	9.03 ± 0.11 ^b
	T10	89.06 ± 0.54 ^b	83.68 ± 0.50 ^c	5.38 ± 1.04 ^a	8.75 ± 0.78 ^a	52.96 ± 0.65 ^c	4.76 ± 0.51 ^a

Results were expressed as mean ± SD (n = 3); different superscripts of lowercase letters along the samples in each parameter represent significantly different according to Duncan's test (p<0.05)

*T1&T6-Control; T2&T7- Water Soaked; T3&T8- 2% alkali; T4& T9- 5% alkali; T5&T10- 10% alkali

Table 3. Extraction of cellulose using Ultrasound and PEF treatment

Sample	Ultrasound Treatment	Cellulose (%)	PEF Treatment	Cellulose (%)
Paddy straw (10%)	350 W	64.66± 1.527 ^c	20 kV/cm	62.53±2.636 ^c
	450 W	76.66±1.527 ^d	30 kV/cm	72.52±1.656 ^d
	550 W	82.33±2.516 ^c	40 kV/cm	78.97±0.665 ^c
Untreated Paddy straw	350 W	57.30±1.558 ^a	20 kV/cm	47.33±1.154 ^a
	450 W	61.65±1.212 ^b	30 kV/cm	50.03±0.468 ^b
	550 W	63.53±0.606 ^c	40 kV/cm	52.23±1.202 ^b
Wheat straw (10%)	350 W	67.50±1.322 ^c	20 kV/cm	65.66±1.527 ^d
	450 W	75.33±1.154 ^d	30 kV/cm	75.66±0.577 ^c
	550 W	84.33±1.154 ^e	40 kV/cm	82.33±0.577 ^f
Untreated Wheat straw	350 W	58.94±0.711 ^a	20 kV/cm	51.27±1.005 ^a
	450 W	64.17±0.817 ^b	30 kV/cm	54.91±1.157 ^b
	550 W	67.49±1.980 ^c	40 kV/cm	58.10±0.718 ^c

Results were expressed as mean ± SD (n = 3); different superscripts of lowercase letters along the samples in each parameter represent significantly different according to Duncan's test (p<0.05)

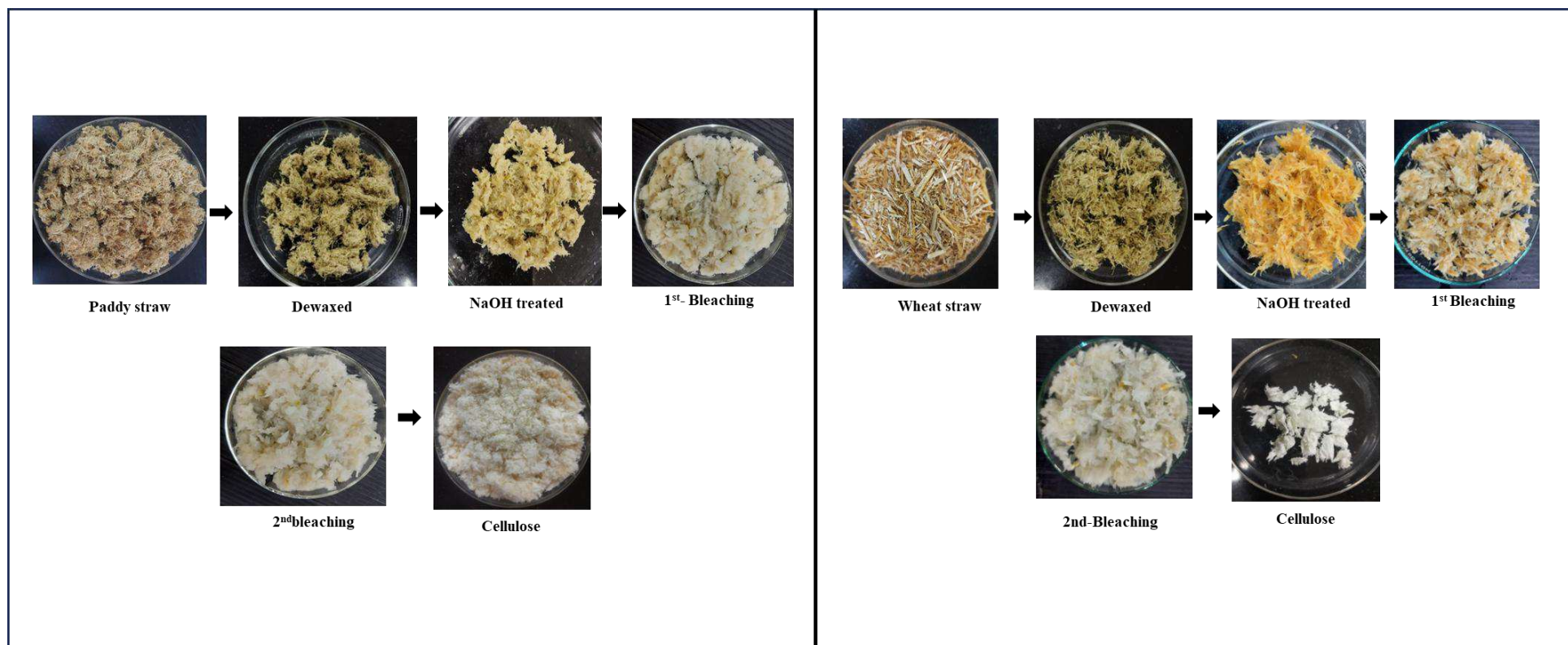


Fig.1. Extraction of cellulose by alkali treatment

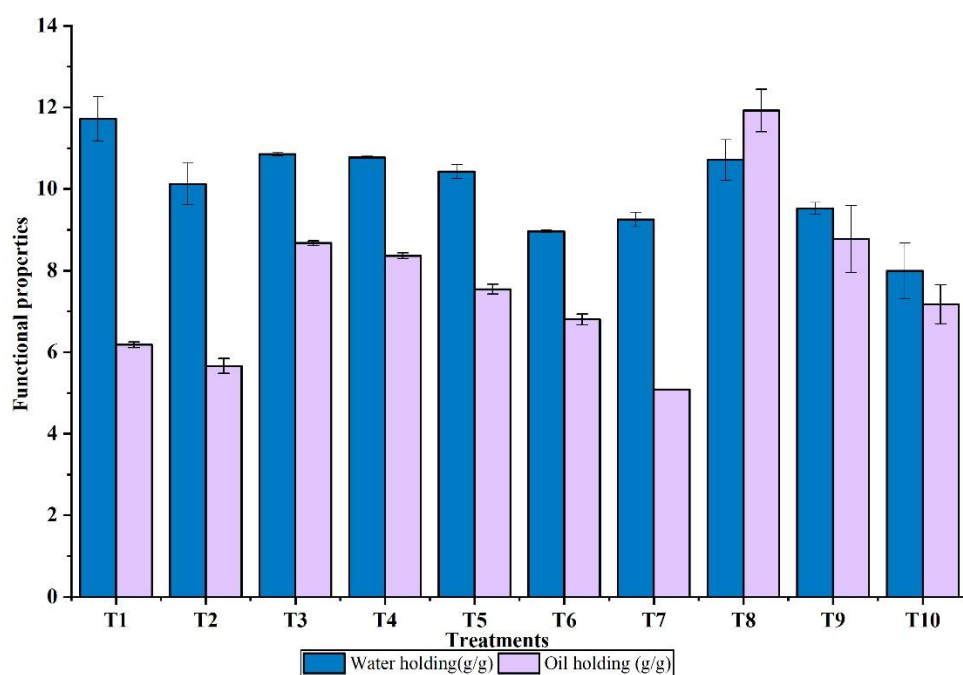


Fig.2. Water & Oil holding capacity of paddy & wheat straw

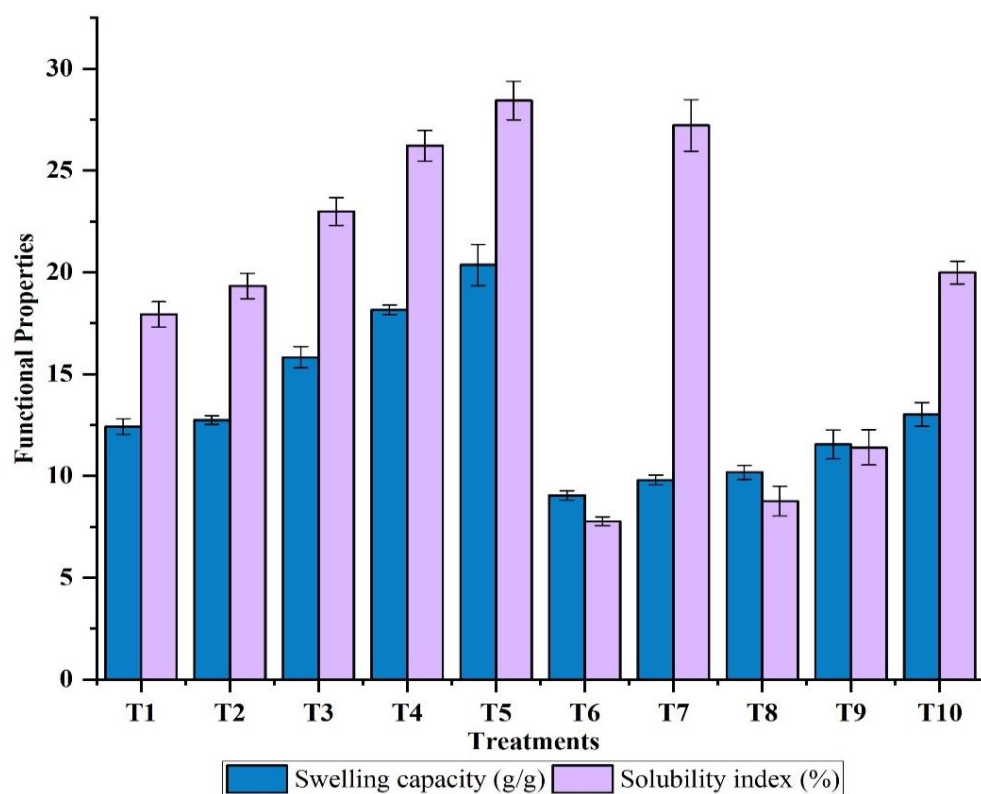


Fig.3. Swelling capacity and solubility index of paddy & wheat straw

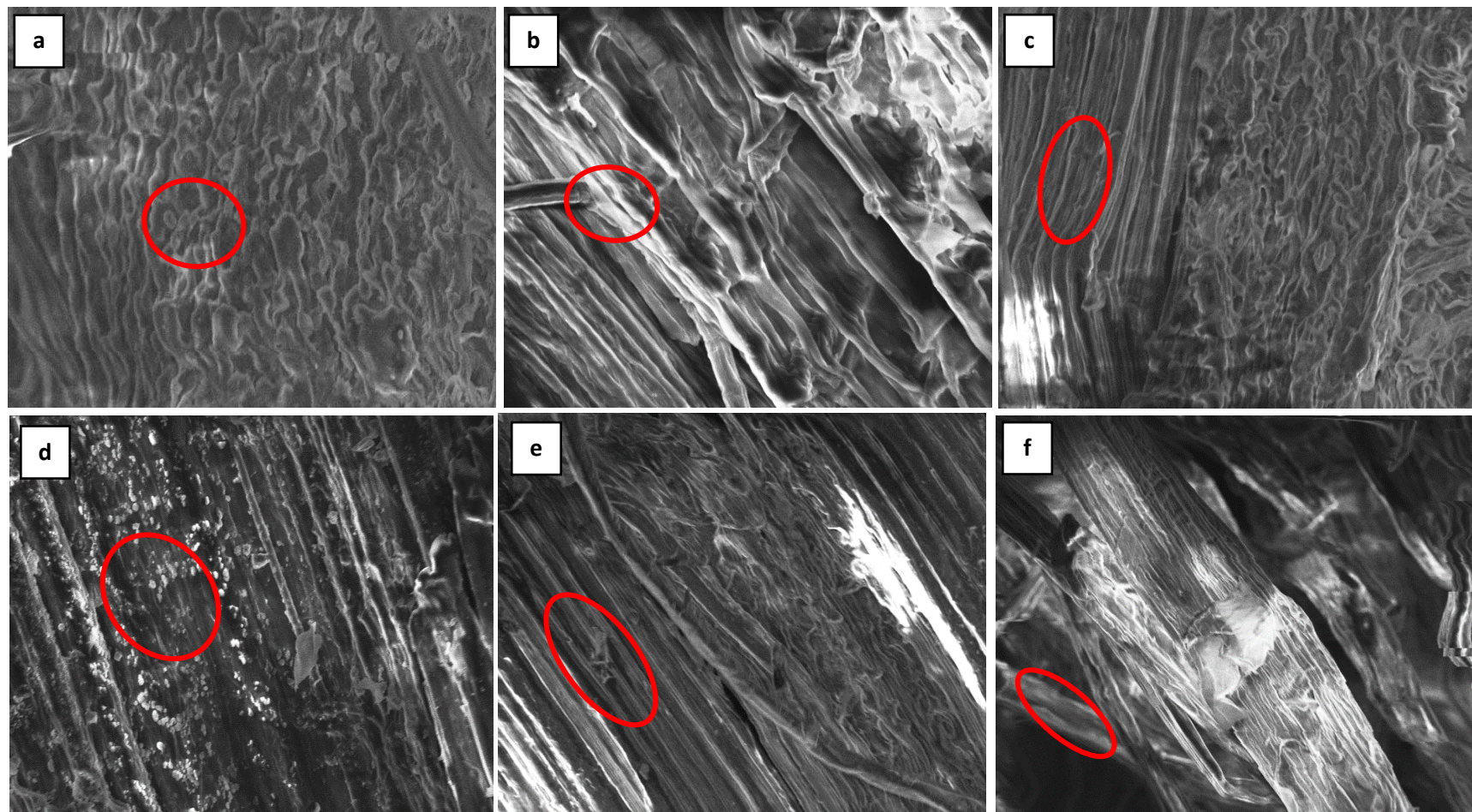


Fig.4. Scanning Electron Microscopy (SEM) images of extracted cellulose

(a) Untreated paddy straw (b) ultrasound-treated paddy straw (c) PEF-treated paddy straw (d) Untreated wheat straw (e) ultrasound-treated wheat straw (f) PEF-treated wheat straw

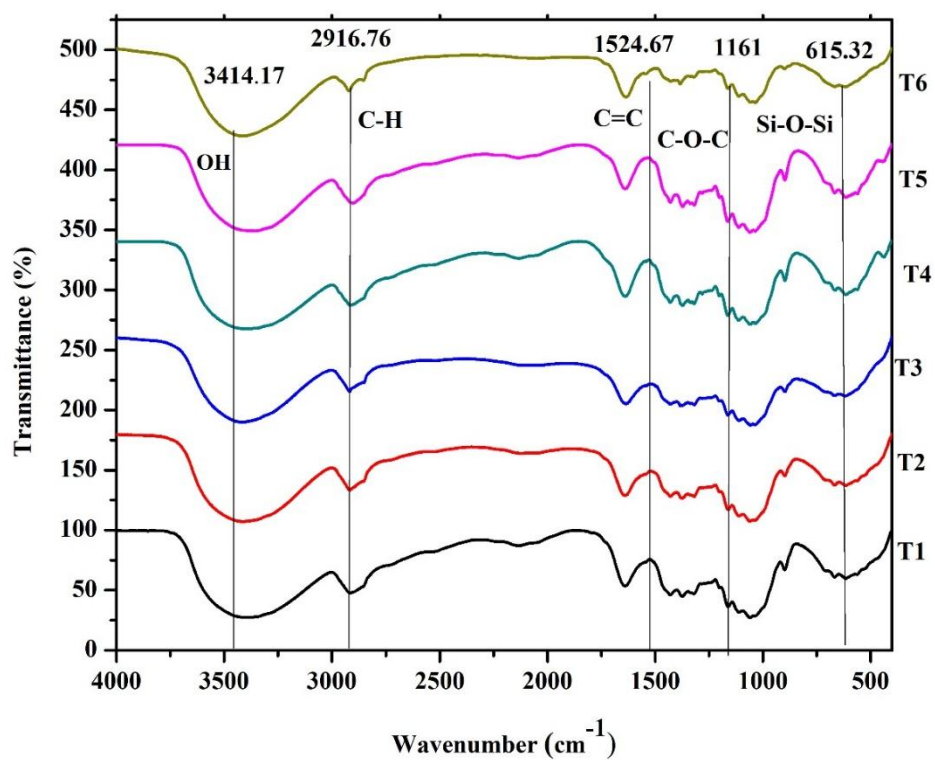


Fig.5. FTIR spectra of chemical and non-thermal treated paddy and wheat straw extracted cellulose