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Research Article

Alkali Pretreatment and Analysis Of Biomass Content Of *Narlısaray* Population and *Vezir* Type Cannabis Plant

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ABSTRACT

The cell wall of the hemp plant consists of cellulose, hemicellulose, and lignin cross-linked to these components. In such a structure, lignin is considered an undesirable byproduct in the production of textiles, paper, and biofuels from hemp. Therefore, the removal of lignin is essential for the industrial utilization of cellulose from hemp. In this study, lignin removal processes were conducted for the first time on the (native to Anatolia) *Narlısaray* population and the registered *Vezir* hemp. Alkaline (NaOH) treatment was preferred for pre-treatment due to its relatively low cost, lower energy requirements, and reduced risk factors. Structural changes before and after alkaline pre-treatment were compared using FT-IR spectra, SEM, and EDX analyses of the biomass. Examination of elemental trace values revealed that the O:C ratios of *Narlısaray* and *Vezir* fibers increased to 0.84 and 0.85, respectively. The increase in the O:C ratio indicated the removal of lignin, while the nearly identical ratios suggested that the lignin cross-linking energies in both local hemp fibers were almost the same. Additionally, SEM images provided clear information regarding the structural changes in *Narlısaray* and *Vezir* fibers before and after the lignin removal process.

Keywords: Industrial hemp, lignocellulosic biomass, pre-treatment.

1. INTRODUCTION

Cannabis (*Cannabis sativa* L.), originating from Central and East Asia, has spread from the Caspian region and the Himalayas to China and Siberia.[1] Historically, hemp has been one of the five major crops, extensively utilized in the textile,[2] yarn,[3] paper,[4] and energy sectors.[5] However, the high labor demands for cultivating the *cannabis* plant, the challenges posed by mechanization, and the availability of cheap and soft cotton diminished its value. This decline was further exacerbated by the global prohibition of *cannabis* cultivation in the 1960s, primarily due to the narcotic properties of the *Cannabis* indica species. Since the 1990s, the cultivation of *cannabis* has been legalized in many countries, marking a significant shift. Consequently, the 20th century witnessed a substantial decline in the economic significance of *cannabis*.[6,7] In recent times, with governmental support, the production of industrial hemp and hemp-based products is on the rise. The increased interest in hemp is attributed to its ability to grow with minimal water requirements, its near immunity to pesticides, its status as a carbon dioxide-neutral material, its renewable and sustainable nature, and its capacity to generate substantial biomass rapidly. Notably, research on industrial hemp has accelerated significantly since 2017.[8]

Cannabis plants are composed of roots, stems, leaves, flowers, and seeds, with each part utilized in a variety of applications. The stalks of *cannabis* exhibit a herbaceous, rigid structure, with fibers located in the bark. These hemp fibers are renowned for their high strength, durability, and absorbency, and they form

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through the amalgamation of numerous elementary fibers. The primary structural components of these fibers include cellulose, hemicellulose, and lignin. Specifically, hemp fibers consist of approximately 70-74% cellulose, 15-20% hemicellulose, 4-6% lignin, and 0.8% pectin distributed along the entire length of the plant stem.[9] Within the *cannabis* stem, fibers are encased. Once the fibers are extracted, the remaining material, known as tow, is processed and utilized.[10] Both the fibers and tow from *cannabis* stalks possess a woody, cellulosic structure that is well-suited for composite production. Notably, hemp tows are among the most extensively studied natural materials for the creation of sustainable building materials.[11]

Hemp fiber has historically been utilized in the production of textiles, rope, and paper. Presently, research is being conducted on the production of bioethanol from hemp fiber. For the fiber to be used in these applications, it must first be extracted from the plant, the bast fibers separated, and structures such as lignin and pectin removed.[12] Lignin, which provides structural strength to the plant, is an undesirable by-product in most applications because it forms cross-links with hemicellulose and cellulose. These cross-links in hemp biomass exhibit high resistance to enzymatic degradation and microbial digestion. Therefore, pre-treatment is essential to disrupt these bonds. In bioethanol production, the presence of lignin hinders sugar formation during the hydrolysis and fermentation stages, thereby reducing ethanol yield. Similarly, in paper and rope production, lignin is an undesirable by-product because it imparts a yellowish color to paper and excessive rigidity to rope. Consequently, many studies have focused on lignin removal. Lignocellulose pre-treatment methods are classified into physical, chemical, physico-chemical, and biological categories.[13] A review of the literature indicates that chemical methods, particularly those involving alkali and acid, are prominently used for lignin removal from hemp fiber. Additionally, other methods such as steam explosion, liquid hot water, microwave, and ionic liquids are also studied.[14-16] Among the chemical methods, the alkali treatment stands out for its advantages, such as producing fewer by-products, generating more sugars, and neutralizing released acids.[17] The value of hemp is increasing due to its high cellulose content and low lignin content compared to many other plants. For instance, in one study, a 1% NaOH pre-treatment was applied to four different hemp species in a sand bath for bioethanol production. The study reported that the lignin structure of the hemp fibers was successfully removed, and there was no significant difference in bioethanol production among the four different hemp species.[17]

This study aimed to remove undesirable structures, such as lignin, through alkaline pre-treatment of *Narlısaray* population and *Vezir* variety hemp plants. The research utilized NaOH, the most preferred and least disadvantageous substance for pre-treatment, to achieve lignin removal. The raw and pre-treated fibers were analyzed and compared using Scanning Electron Microscopy (SEM), Energy Dispersive X-ray Spectroscopy (EDX), and Fourier Transform Infrared Spectroscopy (FT-IR).

2. MATERIALS AND METHODS

2.1. Reagents

The HCl (ChemistryLab) and NaOH (Merck) utilized in this study were commercially procured.

2.2. Devices Used

The characterization of the fibers was conducted using a FIE Quanta FEG 450 model SEM equipped with an EDX analyzer. FT-IR spectra were recorded in the range of 400–4000 cm^{-1} on a Jasco FT-IR 4700 spectrometer. The spectra were analyzed with Jasco Spectra Manager version 2.14.05 software, JASCO Co., Tokyo, Japan. The grinding of the fibers was carried out with a Fritsch Pulverisette 9 model device. Pretreatment was carried out with a Hiriyama HG-90 model autoclave.

2.3. Obtaining Hemp

This study was conducted at Yozgat Bozok University (YOBU), which specializes in the field of "Industrial Hemp". The hemp material required for the project was cultivated by YOBU, utilizing the *Narlısaray* population and *Vezir* type hemp in specially designated cultivation areas in May 2021, under field conditions in Yozgat. The physiological maturation of the plant was completed between August and September, and the plants were harvested in October 2021. The stems were dried in an appropriate environment to equalize the moisture levels of the harvested plants for use in the experiments.

2.4.Obtaining Hemp Fiber

The mechanical separation method was employed at the Faculty of Agriculture of YOBU to extract fibers from the stems. This process resulted in the removal of the woody (tump) parts of the stems, thereby exposing the fibers (Figure 1).

Figure 1. The production of *Narlısaray* and *Vezir* hemp fibers.

2.5. Preparation of Hemp Fibers

Two separate experiments were planned for the fibers intended for the study. Initially, following the hemp harvest, the fibers were left untreated, cleaned of any impurities, and subsequently ground into a fine powder using ball milling at the YOBU Science and Technology Application and Research Center. The obtained powder sample was characterized by SEM, EDX and FT-IR analyzes and the lignin structure of the fibers was observed morphologically and elementally (Figures 2, 3, 4, 5).

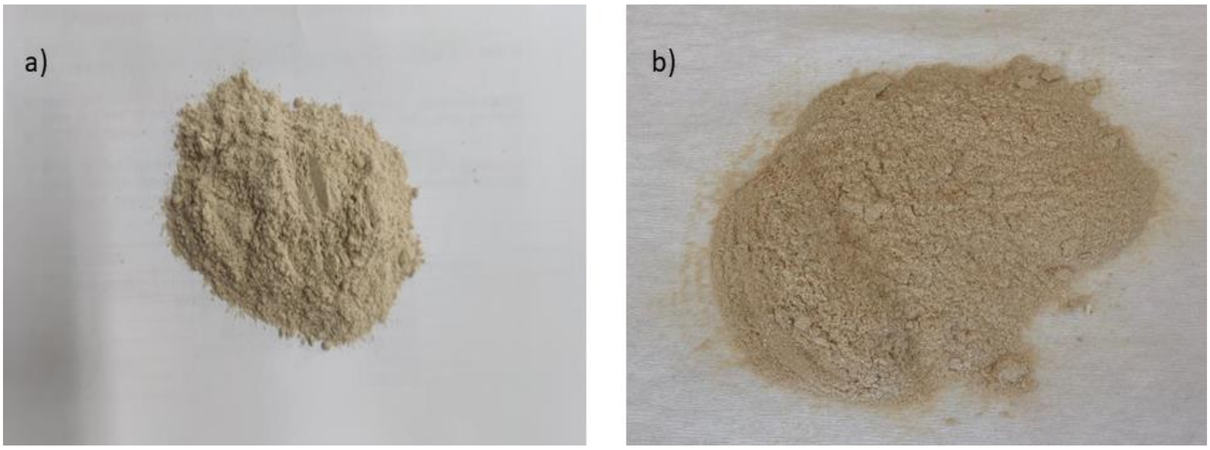

Figure 2. Milled raw fiber: a) *Narlısaray* b) *Vezir*.

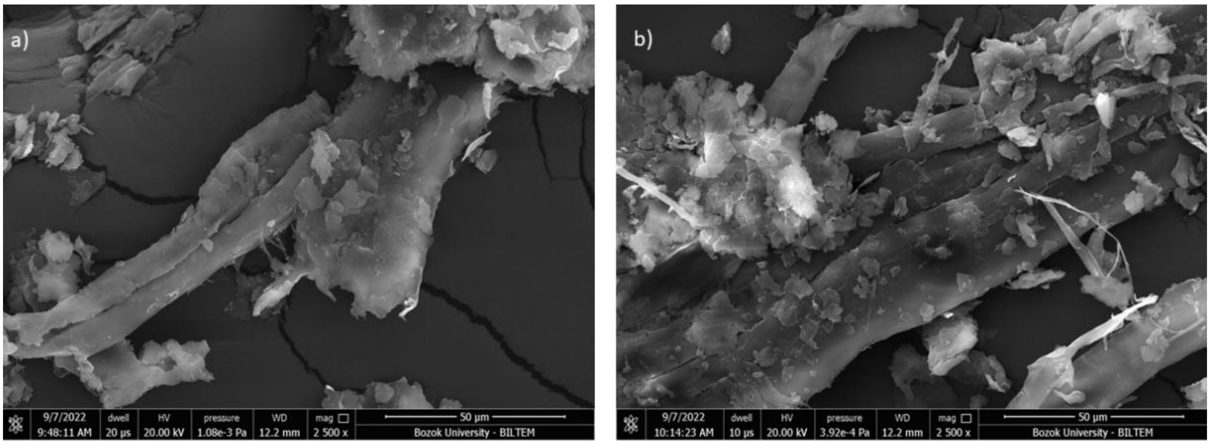

Figure 3. Raw fiber SEM image: a) *Narlısaray* b) *Vezir*.

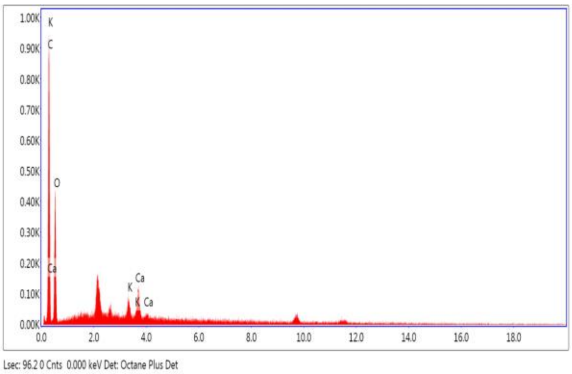

Figure 4. *Narlısaray* raw fiber EDX analysis results.

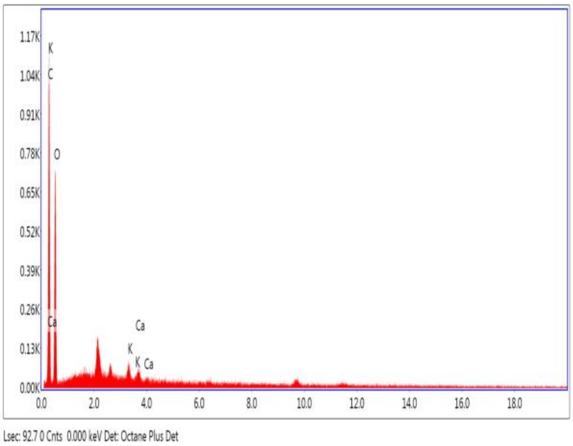

Figure 5. EDX analysis results of *Vezir* untreated raw fiber.

2.6. Chemical Treatment of Hemp Fibers

The hemp fibers underwent chemical pre-treatment to remove structures such as lignin and pectin from their composition, resulting in two sets of samples: the first comprised raw fibers, while the second consisted of chemically treated fibers. Various chemicals, including ethylene, oxalic acid, sulfuric acid, sodium hydroxide, acid, sodium carbonate, oxygenated water, and sodium sulfite, have been employed in pre-treatment processes to eliminate lignin and pectin from fiber plants, as reported in the literature.[18, 19] Among these, treatment with NaOH has been identified as yielding the most effective purification.[18] Accordingly,[20] a 2% NaOH solution was prepared, and the fiber-solution mixture was subsequently autoclaved at 121°C for 1 hour. Following neutralization with 37% HCl, the mixture underwent three wash cycles with distilled water and filtration. The upper portion of the resulting mixture, after reaching room temperature, was decanted, and the precipitated fiber was dried and subsequently maintained in an oven at 50°C for 1 day. As shown in the figure, the dried fibers were preserved after pre-treatment (Figure 6). Subsequently, SEM, EDX and FT-IR analyzes were performed to characterize the samples, and post-pretreatment lignin removal was observed morphologically and elementally (Figures 7, 8, 9).

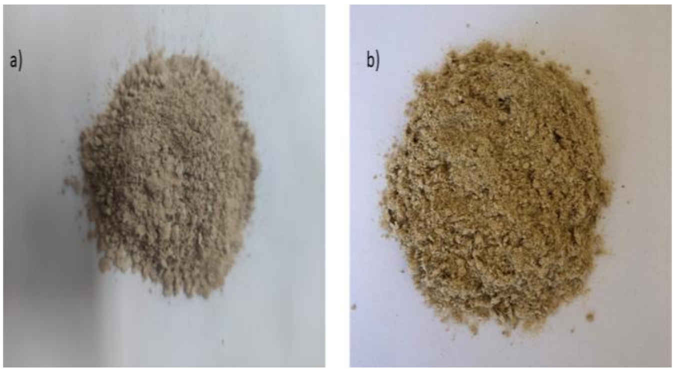

Figure 6. Pre-treated fiber image a) *Narlısaray* b) *Vezir*.

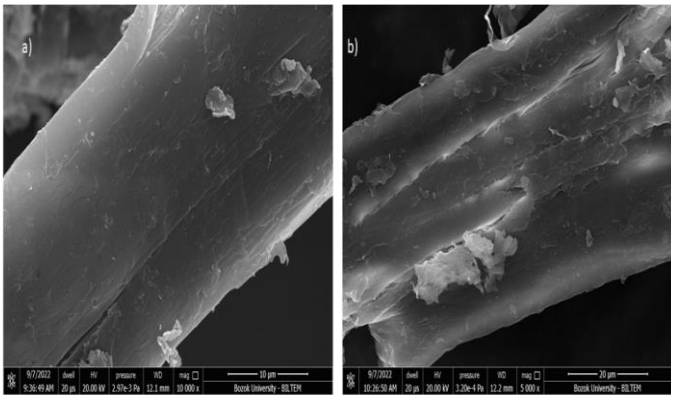

Figure 7. Pre-treated fiber SEM image a) *Narlısaray* b) *Vezir*.

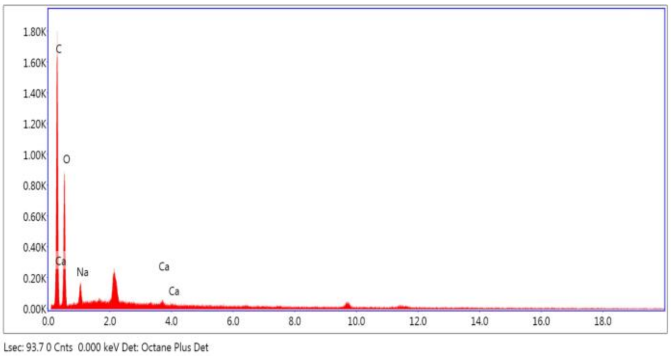

Figure 8. *Narlısaray* pre-treated fiber EDX analysis results.

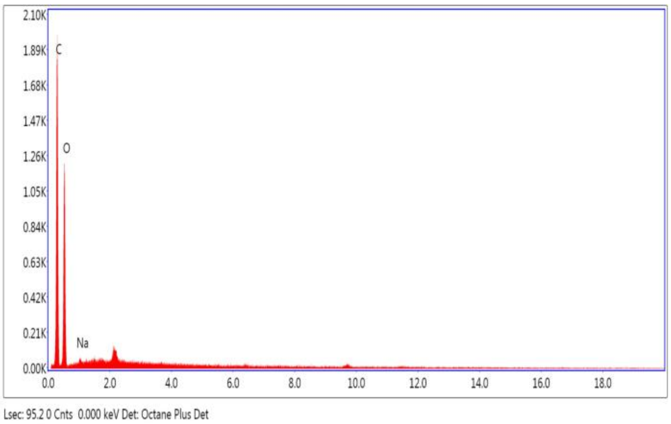

Figure 9. EDX analysis results of *Vezir* pre-treated fiber.

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3.RESULT AND DISCUSSION

3.1.Comparison of Samples

As depicted in Figure 10, a noticeable disparity in physical color is evident between the treated and untreated fiber samples. Specifically, the treated sample exhibits a darker hue compared to the lighter color of the raw fiber. While the sole visually discernible data pertains to color variation, more comprehensive insights are provided by microscope images obtained from EDX and FT-IR analyses.

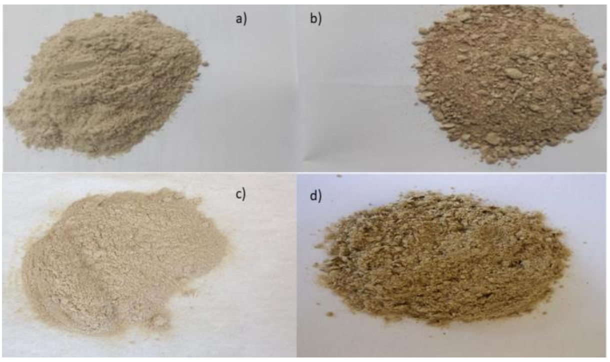

Figure 10. Raw fiber and pre-treated fiber: a) *Narlısaray* raw fiber image b) *Narlısaray* pre-treated fiber image c) *Vezir* raw fiber image d) *Vezir* pre-treated fiber image.

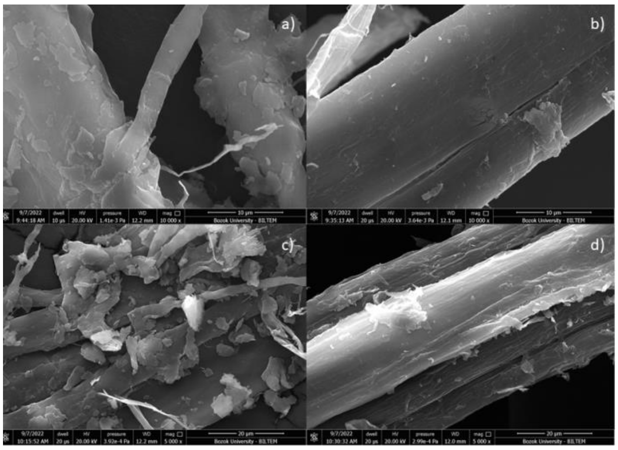

Figure 11. Raw fiber and pre-treated fiber SEM: a) *Narlısaray* raw fiber SEM b) *Narlısaray* pre-treated fiber SEM c) *Vezir* raw fiber SEM d) *Vezir* pre-treated fiber SEM.

Observations revealed that both the *Narlısaray* population and the *Vezir* type untreated raw hemp biomass exhibited a sedimentary layer on their surface areas (see Figure 10a, 10c). Subsequent SEM imaging of the hemp biomass post-pre-treatment indicated partial purification of the surface area, characterized by the disappearance of network-type features and a resulting cleaner, smoother appearance (see Figure 11b, 11d). Morphological alterations suggestive of structural damage to the biomass were observed. It can be inferred that the application of 2% NaOH disrupts the connection between lignin and hemicellulose, leading to increased surface area and a cleaner surface topography. An EDX analysis was conducted to observe the changes in carbon content and the presence of oxygen in the fiber samples. The oxygen-to-carbon ratio (O:C) is indicative of the lignin and hemicellulose content in natural fibers. The EDX analysis results for both raw and processed fibers are presented in Figure 4, 4, 8, and 9. A higher O:C ratio signifies lower lignin and hemicellulose content in the fiber. For alkali-treated fibers, the O:C ratio increases.[21] Upon examination of the elemental trace values in Table 1, it was found that the O:C ratio of untreated *Narlısaray* raw fiber was 0.75, whereas the O:C ratio of processed *Narlısaray* fiber was 0.84. Similarly, in Table 2, the O:C ratio of untreated *Vezir* raw fiber was determined to be 0.62, while the O:C ratio of pre-treated *Vezir* fiber was 0.85. These findings indicate an increase in the O:C ratio of alkali-treated fibers, suggesting the removal of lignins.

Table 1. Comparison of *Narlısaray* raw fiber and *Narlısaray* pre-treated fiber EDX element.

***Narlısaray* raw fiber EDX data**			***Narlısaray* processed fiber EDX data**		
Element	**Weight %**	**Atomic %**	**Element**	**Weight %**	**Atomic %**
C	55.95	63.44	**C**	52.76	60.31
O	42.19	35.92	**O**	44.26	37.98

Table 2. Comparison of *Vezir* raw fiber and *Vezir* pre-treated fiber EDX element.

***Vezir* raw fiber EDX data**			***Vezir* processed fiber EDX data**		
Element	**Weight %**	**Atomic %**	**Element**	**Weight %**	**Atomic %**
C	61.23	67.97	**C**	53.46	60.62
O	38.21	31.84	**O**	45.65	38.85

3.2.FT-IR Analysis

The FT-IR analysis results are depicted in Figures 12, and 13, and a corresponding table was generated based on the findings (Table 3). The characteristic peak at 1024 cm^{-1} corresponds to aromatic C-H in-plane deformation and C-O stretching in primary alcohols of lignin (guaiacyl). Following alkali pre-treatment, these peaks exhibited an increase of 10 cm^{-1} and 8 cm^{-1} for *Narlısaray* and *Vezir*, respectively, indicating the removal of lignin from the processed fibers. The peak detected at 1214 cm^{-1} is associated with the aromatic ring vibration coupled with C-O and C=O stretching within the condensed guaiacyl units of lignin. Subsequent to alkaline pre-treatment, this spectral feature underwent a downward shift in wavenumbers for both *Narlısaray* and *Vezir* fibers, indicating a degree of lignin degradation as a result of the treatments. Additionally, the peak observed at 1713 cm^{-1} is associated with conjugated carbonyl stretching resulting from hydroxycinnamic acids linked to the alcoholic polysaccharides of lignin. A decrease in these peaks was noted in the alkali-treated fibers for both *Narlısaray* and *Vezir*, indicating lignin removal. The untreated fibers

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exhibited a C-H stretch peak at 2896 cm^{-1} and 2896 cm^{-1} in *Narlısaray* and *Vezir* fibers, respectively, indicative of C-H stretching in lignin aromatic hydrocarbon, methoxyl, and methylene groups. Following alkali treatment, these peaks shifted to 2895 cm^{-1} and 2894 cm^{-1} compared to untreated fibers.[22]

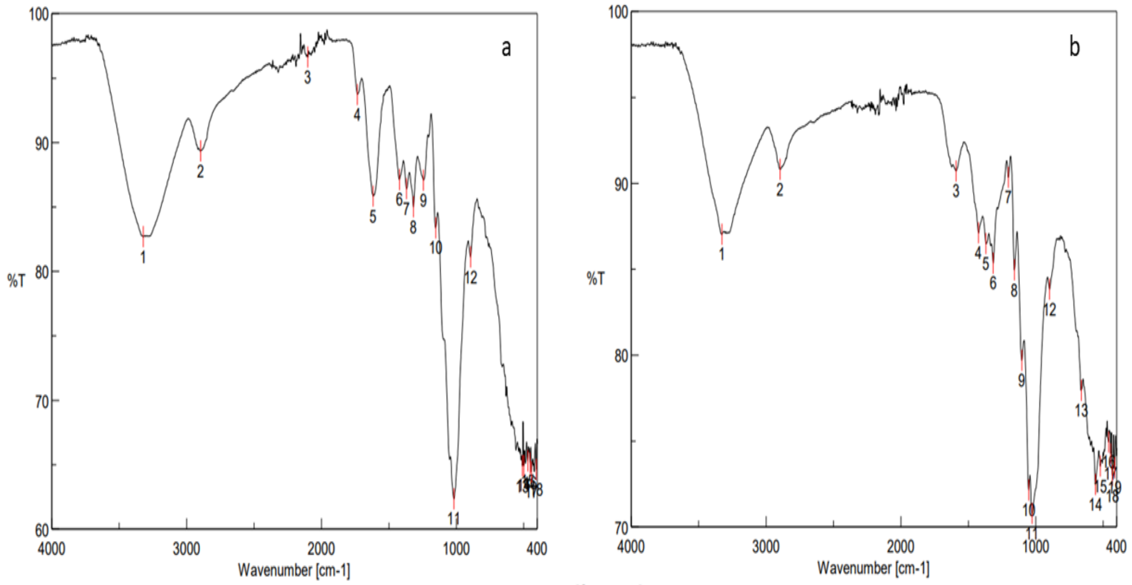

Figure 12. FT-IR analysis: a) *Narlısaray* raw fiber b) *Narlısaray* pre-treated fiber.

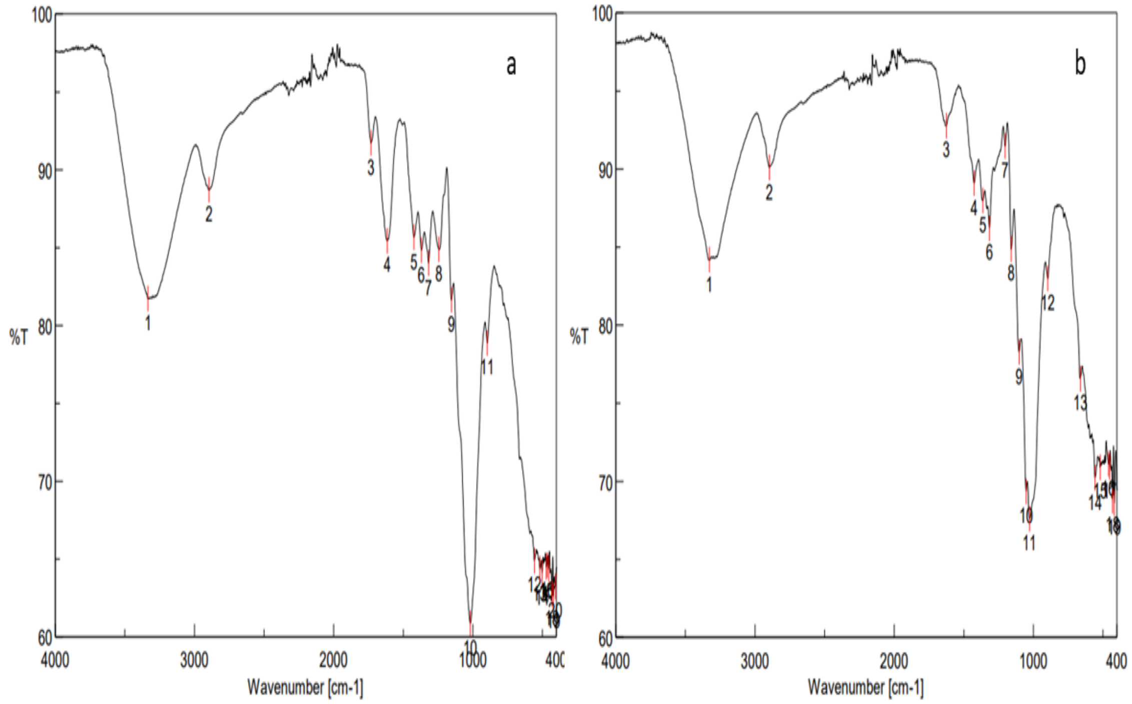

Figure 13. FT-IR analysis: a) *Vezir* raw fiber b) *Vezir* pre-treated fiber.

Table 3. Comparison of the FT-IR analysis of *Narlısaray* and *Vezir* fibers after pre-treatment.[22]

Structure of the League	cm^{-1}	*Narlısaray*	*Vezir*
Aromatic C-H in plane deformation and C-O in lignin stress	1024	Increased	Increased
Aromatic ring respiration by C-O and C=O stress of lignin	1214	Decreased	Decreased
Conjugated carbonyl groups of hydroxycinnamic acids	1713	Decreased	Decreased
C-H stretching in the aromatic methoxyl group and methylene group	2920	Decreased	Decreased

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4.CONCLUSION

The study investigated various alternative pre-treatment methods and successfully achieved lignin removal from *Narlısaray* and *Vezir* fibers using NaOH, a widely employed method. Analysis results revealed no significant differences in lignin removal between the *Narlısaray* and *Vezir* populations. Morphological, elemental trace, and spectroscopic analyses indicate partial lignin removal in both fiber types. The elimination of unwanted structures such as lignin from hemp fibers renders them more suitable for applications in the textile industry, where their high strength is particularly advantageous. Moreover, lignin removal is crucial in the paper industry to mitigate the yellow coloration imparted by lignin. Simultaneously, fiber pre-treatment is essential for the bioethanol production process, offering an alternative fuel source amid the depletion of non-renewable petroleum resources. Lignin presence impedes sugar formation by hindering cellulose accessibility, potentially lowering sugar yield during enzymatic hydrolysis of pre-treated industrial hemp. Hence, lignin removal is imperative to maintain fiber structure integrity for efficient and sustainable bioethanol production. Here this study underscores the potential of hemp fibers across various sectors and suggests avenues for developing new, cost-effective pre-treatment processes to enhance their utility. Further research involving diverse pre-treatment approaches can deepen our understanding of hemp fiber chemistry and inform sustainable industrial applications.

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Conflict of interest

Authors declare that there is no a conflict of interest with any person, institute, company, etc.

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