Effect of enzymatic treatments on mechanical, structural and thermal properties of alfa fibers

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Abstract

Previously, we developed a series of processes that involved chemical and enzymatic treatments to extract and separate the fibers from alfa leaves, while also preserving their native mechanical properties, by optimizing the implementation parameters. In this study, we describe the morphology of the alfa leaf, characterize the alfa fibers, and study the influence of different treatments on their crystalline structure, their colorimetric and thermal properties. This work mentions the application of Weibull statistics to analyze the mechanical properties of alfa fibers, which helps in understanding the probability of failure under different stress conditions. Additionally, we also examined the difference in mechanical properties between a fiber bundle and a single fiber. The results show that the cellulose has not been attacked after different treatments, and the thermal stability of the fibers has improved. X-ray analysis shows that the crystallinity index of the fibers increased by about 16.4% for the alkaline and pectinase treatments. Also, we noticed that the enzymatic treatments led to reduction in the dispersion of mechanical properties. Moreover, we observed that the bundle with a smaller diameter is stronger than the one with a larger diameter. Under tensile loading, it behaves similarly to a unitary fiber, demonstrating a single break.

Keywords: Enzymatic treatments, alkaline treatment, thermal stability, Weilbull approach, mechanical properties, single fiber.

1. Introduction

The valorization of natural resources for the protection of the environment is a major challenge for researchers. Indeed, the development of materials with natural fibers has been a topic of interest for the past decade. In fact, natural fibers derived from vegetable sources are tending to replace synthetic fibers thanks to their enormous advantages [1-3] such as their biodegradability, respect for the environment, renewableness, low cost, high performance, and availability [4-9]. But natural fibers have also some drawbacks, including low interaction with most polymers especially for the polyolefin such as the polypropylene, where it is necessary to add an accounting agent, limited resistance to moisture due to their hydrophilic character, which reduces the adhesion between fiber and polymer and decreases the mechanical properties of composites. This incompatibility with some polymers is due to the use of fibers in the raw state [5, 10-11] This hydrophilicity is due to the presence of hydroxyl groups related to the cellulosic amorphous components such as hemicelluloses [4, 12-13]. This feature persists in all fibrous plants, such as hemp, flax, kenaf, and alfa. Alfa, or Stipa tenacissima L, is a perennial plant distributed in North Africa and Spain [14]. In Tunisia, it is located in the center, more specifically in the Kasserin region, and covers about 400 000 ha. Alfa is known as one of the most attractive plants for the manufacturing of polymer composites [15] and for the generation of paper [16]. Alfa plant collected in Tunisia contains generally approximately 43.9% cellulose, 27.67% hemicelluloses, and 17.96% lignine [17].

Various treatments have been used to improve the surface qualities of natural fibers, including alfa, and to overcome the feature of adhesion with polymers. Alkaline treatment is one of these treatments, known for its potential to eliminate non-cellulosic components from the surface of fibers [18-19]. Rokbi et al., [20] focused their study on the optimization of the conditions of treatment of alfa fiber in order to incorporate it into polyester composite, and the results show that good adhesion without damage was obtained at a concentration of 10% NaOH. Mudoi et al., [3] optimized alkaline concentration for the surface treatment of Himalayan fiber. Hanana et al., [17] optimized the concentration of NaOH to modify the surface of alfa fibers, and the best is 0.5 M. In fact, this is the ideal concentration for removing impurities and the amorphous part of the alfa leaf without destroying the fiber. So, alkaline treatment is an essential step that precedes other gentle treatments using enzymes such as xylanase, pectinase, and laccase, whose role is to remove the remaining impurities without attacking the cellulose. So, enzymatic treatments based on the use of bacteria are eco-friendly and ensure better mechanical and thermal properties of fiber and matrix [21-23]. In

fact, xylanase was produced by the fungus Talaromyces, and pectinase was produced by the mutant Penicillium occinatis [17].

Some researchers studied the effectiveness of enzymatic treatments to enhance the properties of fibers. For example, Thakir et al., [24] focused their study on the treatment of ramie fibers by cellulase enzymes. The results show that the biological treatment removes the polysaccharides from the surface of the fiber. George et al., [25] treated the flax fiber with xylanase and pectinase treatments, and the results show a reduction of hemicellulosic compounds and amelioration of the thermal properties of the fiber. Nagula et al., [26] used laccase for the delignification of napier grass. This enzyme removed 50% of the lignin. Three enzymes, such as xylanase, pectinase, and laccase, were investigated by Hanana et al., [17] to separate alfa fibers. The results show an improvement in the mechanical properties of the fibers. In this study, our aim is to advance the understanding of the effects of various treatments on alfa fibers, focusing on enhancing their compatibility with polymeric matrices and improving their overall performance in composite materials. The novelty of our approach lies in the comprehensive investigation of enzymatic treatments specifically using xylanase, pectinase, and laccase to modify alfa fibers, aiming to optimize their thermal stability, color, crystallinity, and mechanical properties. Our contribution expands upon previous research by conducting a detailed analysis of the morphology of alfa fibers at the single fiber level, alongside evaluating the statistical significance of observed differences in tensile behavior and other mechanical properties. By elucidating these aspects, our study not only enhances the scientific understanding of natural fiber treatments but also provides valuable insights for developing sustainable and high-performance composite materials.

2. Materials and methods

2.1. Materials

Alfa plants were collected from the locality of Faj El Hdid, Hassi El Farid delegation in the governorate of Kasserine (Tunisia). Xylanase and pectinase enzymes were produced locally from the Laboratory of Biomass Valorization and Protein Production in Eukaryotes (CBS Sfax, Tunisia), and laccase was purchased from Sigma Aldrich (France).

2.2. Process of extraction of alfa fibers

The process of extraction of alfa fibers is that described by Hanana et al., [17]. The alfa fibers extraction process after optimization of implemented parameters is described briefly below.

Firstly, alfa fibers were rusted for one week in salt water. Then, the fibers have been combed in order to be parallelized and to keep only the longest fibers. Secondly, alkaline treatment (NaOH 0.5M) was implemented to modify the surface, remove the amorphous phase as hemicelluloses and lignin, and improve the adhesion between fiber and matrix. Finally, enzymes such as xylanase, pectinase, and laccase were implemented to further improve the extraction and division of bundles. For laccase activities, the maximum of phenolic compounds was released at 50 °C and the enzyme concentration was 230 U/g, at pH 5 using sodium acetate buffer at 50 mM. For xylanase activities, the highest concentration of enzyme was 70 U/g at 50 °C using phosphate buffer at pH 7 at 50 mM. For pectinase activities, the concentration of enzyme was 5620 U/gat 50 °C using citrate buffer at pH 4.8.

2.3. Microscopic analysis

To characterize the fibers present in alfa leaves, a microscopic analysis was conducted. This includes measuring the average diameter of the fibers and detecting any potential defects using a polarized light microscope. The aim of this analysis is to understand the structure and quality of the fibers, which is crucial for various scientific and industrial applications.

The leaves were embedded in an epoxy resin, and the cross-section of the leaves was polished. A Nikon Eclipse Lv150 polarized light microscope was used to determine the average diameter of the separated fibers and to detect the presence of defects. Microscopic analyses were carried out using an optical microscope on sections obtained with a microtome or prepared using a fine razor. Staining of the sections is obtained by impregnating the microscope slide with a solution of safranin or gentian violet (1%) for 5 minutes.

2.4. Microstructure analysis

X-ray diffraction evaluates the crystallinity of alfa fibers before and after enzymatic treatments by identifying and quantifying the crystalline phases within the sample. Mechanical properties of the fibers were impacted by their crystalline structure. The X-ray diffraction analyses were carried out using a Philips X'Pert MDP diffractometer set at 45 kV and 40 mA. The samples were analyzed from an angular $2\theta = 5°$ range-up to 60° with steps of 0.02° using monochromatic copper ($K\alpha = 1.5418A^{\circ}$) ($\lambda = 0.154$ nm). The alfa samples were dried and ground in a ceramic mortar to a size less than 125 µm. The crystallinity index of the fibers was determined based in Segal method using the following equation [27]:

$$\mathbf{I_{cr} = I_{200} - I_{am} / I_{200} \times 100 \ (1)}$$

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Where I_{200} is the maximum peak intensity of the crystalline phase located at 2θ = 22.8°, which corresponds to the crystallographic plane of cellulose (200), and I_{am} is the minimum intensity of the amorphous phase of the sample at 2θ = 18°.

2.5. Colorimetric analysis

The colorimetric analysis of enzyme-treated alfa fibers is crucial for assessing the effects of enzymatic treatments on fiber color, ensuring the quality of end products, optimizing manufacturing processes, and enhancing understanding of enzymatic interactions in this specific context. Colorimetric properties were carried out using the CIELAB 1976 color system according to ISO 11664 (observation angle = 10°, illumination D65). The color parameters such as L* (Lightness), a* (redness) and b*(bluness) were measured using OpenRGB software version 2.01.80406 to visualize color changes. The color difference between untreated and treated samples was calculated based on this equation:

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

The untreated and treated fibers were collected and placed in a square plastic box. Sufficient quantities of fibers were used to prevent light transmission through the fibers inside the plastic box (incident light is reflected from the sample surface without passing through the sample). The color measurement was carried out three times for each sample in order to average the color data obtained.

2.6. Thermogravimetric analysis

Thermogravimetric analysis (TGA) was performed using a Perkin Elmer Pyris-1 TGA instrument. All characteristics were measured on fiber samples for a temperature range of 25 °C to 600 °C at a heating rate of 10 °C/min under nitrogen.

2.7. Statistics study: Weibull model

In previous work, Hanana et al. [17] studied the effect of enzymatic treatments on mechanical properties of alfa fiber. A large dispersion in the ultimate stress was noticed. This was attributed to the pre-existing defects. The data collected by Hanana et al. [17] were analyzed in the present study using Weibull statistics. According to Weibull statistics, the probability of failure for a fiber of length L and applied stress σ is given by:

$$P_f = 1 - \exp\left(-\frac{l}{l_0}\left(\frac{\sigma}{\sigma_0}\right)^m\right)S \quad (3)$$

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where P_f is the cumulative probability of failure of a fiber at applied stress σ, m is the shape parameter, and σ_0 is the scale parameter (the characteristic resistance, at which the probability of failure is 0.63).

3. Result and discussions

3.1. Morphology

A schematic and microscopic observation of the cross-section of alfa leaf is shown in Fig. 1. The cross-section shows the internal structure of the alfa leave, with a non-rectilinear hole inside the leave. Microscopic observations show the structure and organization of the cells and cell walls throughout the leaf surface. Fig. 1b shows observations under an optical microscope of a section obtained by impregnation with a safran in solution. A fiber bundle consists of several fibers bonded together by a polysaccharide-based interphase called the middle lamella (Fig. 1c). An elementary fiber is a multilayer material made up of concentric cylindrical walls. Its cross-section is approximately polygonal [28]. At its center, it has a cavity called the lumen. This lumen contributes to the circulation of water [29]. The outer wall of the fiber, also known as the primary wall, is approximately 0.2 µm thick. The inner wall, or secondary wall, makes up the majority of the fiber's volume, with a thickness of around 0.8 µm [30]. The alfa leaf is made up of two types of fiber, one located on the outer wall and the other on the ventral epidermis. A statistical analysis of fiber cross-sections was developed to provide access to the perimeter and surface of the fibers. The various results are presented below and discussed as a function of fiber position. The equivalent surface areas, perimeters, and diameters of the cells and their lumens were calculated from the image analysis results. In fact, the cross-sectional area of the fibers is an influential parameter when determining the mechanical parameters of the alfa fibers [31]. A total of 100 fibers were analyzed. The average values obtained are presented in Table 1. For fibers located at the outer wall, the equivalent diameter measures 11 µm, while those located in the ventral epidermis it is 8.3 µm. Similarly for the lumen; the equivalent diameter is 3.6 µm at the outer wall and 2.5 µm in the ventral epidermis. We can therefore conclude that the fibers in the outer wall are the largest and most porous [32]. The difference between the total surface area and the surface area of the lumen corresponds to the surface area of the cell walls. It would appear that the fibers in the ventral epidermis are significantly thinner than those in the outer wall. This thinness may be a natural result of the genetic material specific to this variety or due to maturity of leaf. This study included morphological characterization of alfa fibers to predict

an in depth understanding of the relationship between the structure of alfa fibers and their mechanical properties. Further details were mentioned in section 3.~~7~~6.

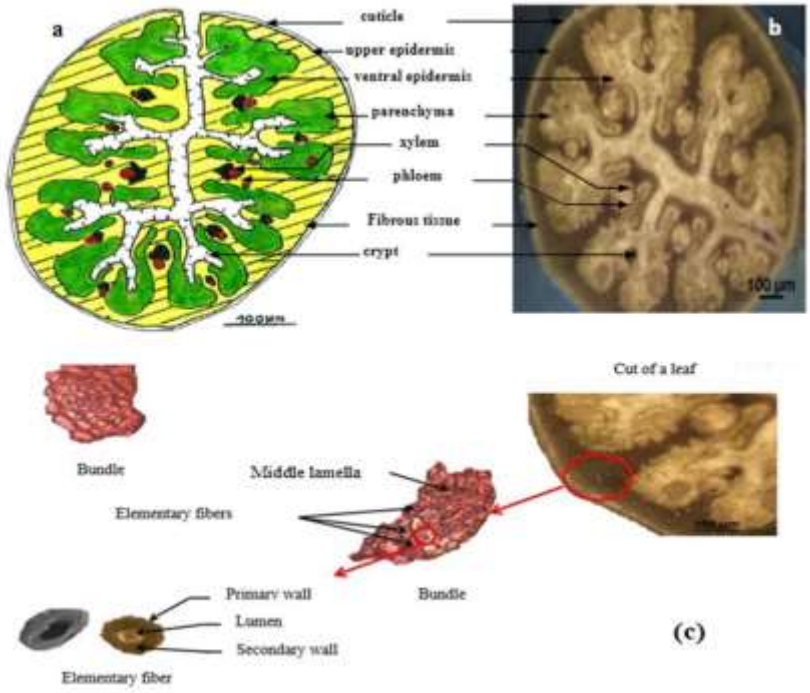

Fig. 1

Table. 1. Average dimensions of alfa fibers located on the outer wall and ventral

		Area (µm^2)	**Perimeter (µm)**	**Apparent diameter (µm)**
Fiber	Outside wall	84±27	34±7	11±2
	Ventral epidermis	49.06±19	26.13±6	8.32±3
Lumen	Outside wall	10.7±6	11.5±3	3.6±1
	Ventral epidermis	4.57±3	8.1±3	2.57±0.8
Wall	Outside wall	7.3±24		
	Ventral	44.5±17		

	epidermis	

3.2. X- ray diffraction

X-ray diffraction was performed to evaluate the crystallinity index of alfa fibers after different treatments. The different diffractograms of the raw and treated fibers are shown in Fig. 2. The results show the presence of four peaks located around 2θ = 15°, 16.4°, 22.7°, and 34.7°, which respectively represent the crystallographic planes (101), ($10\bar{1}$), (002), and (040) characteristic of native cellulose I–[33]. The major crystalline peak located at 2θ = 22.7° corresponds to the crystallographic plane (002) of cellulose. The low-intensity crystal peak at 2θ = 34.7° corresponds to the crystallographic plane (004) in the two-chain monoclinic lattice. The two other peaks of planes (101) and ($10\bar{1}$) correspond, respectively, to diffraction angles of 15° and 16.4°. For raw fibers, it is difficult to distinguish the peak at 15° from that at 16.4°. This is due to a high percentage of amorphous materials such as lignins, pectins, hemicelluloses, and amorphous cellulose. In contrast, when cellulose I content is high, these two peaks are separated, as in treated fibers [34]. The crystallinity indices calculated by the Segal method are represented in Table 2. We can notice that the crystal structure of the alfa fibers is influenced by the different treatments. The X-ray analysis carried out on alfa fibers treated with NaOH (Fig. 2) reflects an increase in crystallinity (increase in the main peak of cellulose). For untreated fibers, the crystallinity is around 64.4%. After alkaline treatment, the crystallinity index increases up to 80.76%. This increase of 16.4% is the consequence of the degradation of the amorphous materials of the fiber in the alkaline environment (separation of the two peaks at 15° and 16.4°).This result agrees with previous studies [35]. A decrease in the degree of crystallinity was observed with the enzymatic treatments (laccase and xylanase) compared to that of fibers treated with NaOH. We can therefore conclude that these two enzymes dissolve crystallized cellulose. After treatment with the pectinase enzyme, the crystallinity index remains constant. This proves that crystallinity is not altered by the action of this enzyme. Based on these results, it can be concluded that the crystallinity of the fibers was increased by the alkaline and enzyme treatments, which reduce the moisture absorption of natural fiber and improve the adhesion with polymer. Similar results were reported by Banvillet et al., [36]. It is essential to notice that the mechanical properties of the fibers were influenced by their crystalline structure [18]. This conclusion will be discussed in the section 3.5.

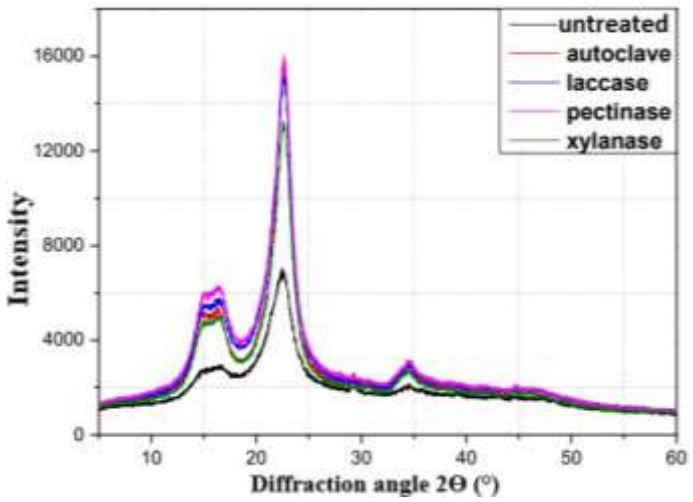

Fig. 2

Table. 2. Crystallinity indices of untreated and treated alfa fibers

Treatments	Crystallinityindex (%)
Untreated	64.4
Alcalin autoclave	80.7
Laccase	75.4
Pectinase	80.4
Xylanase	76.4

3.3. Colorimetric analysis of treated fibers

The treated and untreated fibers were analyzed using a spectrophotometer to measure their color and gloss simultaneously. From Fig. 3, it's evident that the treatments affect ~~on~~ the color of the fibers, with noticeable differences observed. change in color indicates the removal of fiber components, a relationship strongly correlated with lignin content and discoloration degree [38]. However, detailed description of each chromatic coordinate is necessary to quantify the color difference. As shown in fig.4, the luminance of untreated and treated fibers is comparable, showing no significant variance among different treatments remains consistent, with fibers treated with xylanase appearing slightly brighter compared to untreated fibers, which exhibit the least luminosity. An increase in the red shade a* (Fig. 4b) is observed as a ~~the~~ result of color alteration, particularly prominent in retted fibers overone

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week, whereas alkaline treatment decreases the a* value, reflecting significant change in soda treated fibers. Enzymatic treatments generally reduce the red coloration, especially evident in fibers treated with pectinase. The yellow color observed (Fig. 4c) represented by the chromaticity coordinate b*, shows ytreated fibers tend to exhibit more yellow tones. Retted fibers exhibit a stronger yellow color due to higher b* values, while pectinase-treated fibers display less yellow compared to other treatments. Calculation of the ΔE* index is essential to characterize overall color change. The increase in the ΔE* for retted fibers compared to untreated fibers primarily results from changes in yellow and red hues, while maintaining initial brightness levels. Fibers treated with xylanase and laccase show relatively minor color changes despite their bleaching capability. Pectinase treatment results in the highest ΔE*, means the greatest color indicating the most significant color evolution (Table 3). Reduction in a* and b* values contribute to this evolution, signifying decreased yellow tones attributed to lignin removal.

Table 4 shows RGB values obtained from L, a, and b using Open RGB software to visualize color changes. The actual color of each fiber, as measured by the spectrocolorimeter, is shown in Fig. 5. All the results are confirmed. The color evolution is now more visible. It can be concluded that the removal of non-cellulose substances, particularly lignin, causes the color change of the alfa fibers [37].

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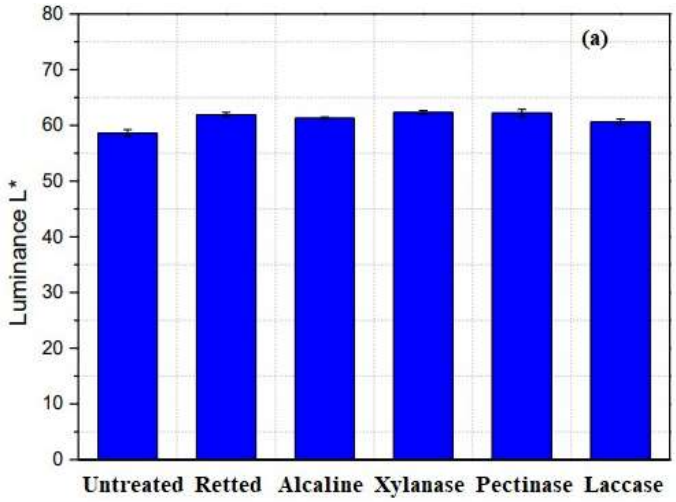
Fig. 3

Table. 3. Color evolution of fibers at different treatments

	Untreated	**Rettedfibers**	**Alcalin autoclave**	**Xylanase**	**Laccase**	**Pectinase**
L	58.60±0.81	61.91±0.18	61.28±1.19	62.32±1.53	60.62±2.52	62.23±1.16
a	0.34±0.16	2,15±0.11	1.04±0.22	2.11±0.18	1.93±0.29	1.5±0.23
b	11.30±0.59	16.19±0.80	15.11±0.05	13.95±0.53	12.80±0.46	9.85±0.27
ΔE*		6.17	4.71	1.89	2.56	7.31



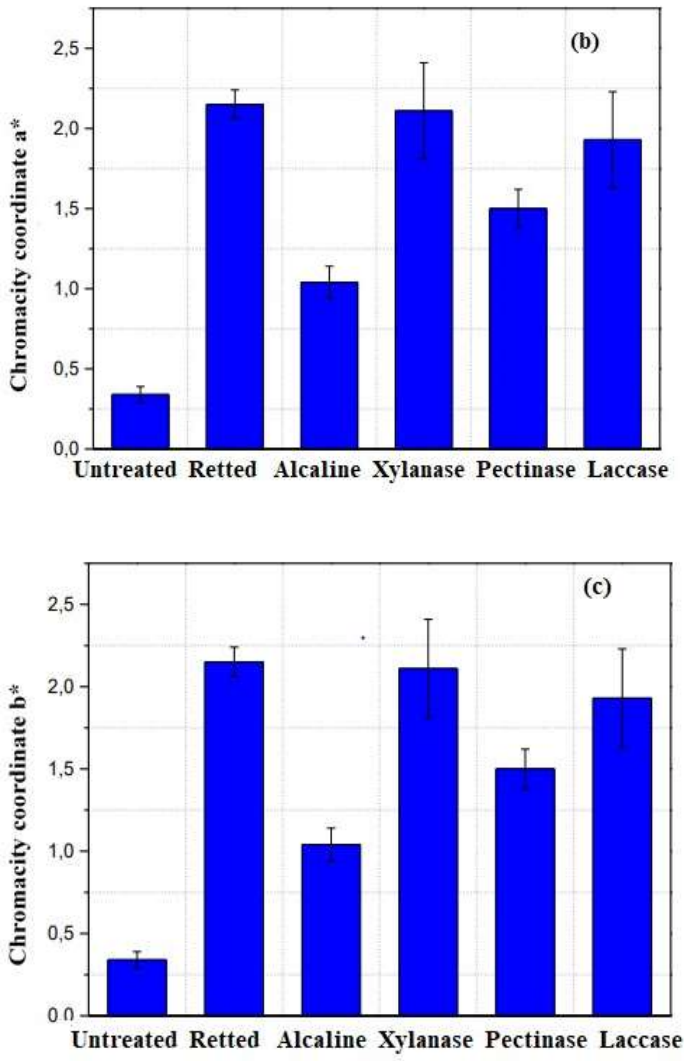


Fig. 4

Table. 4. RGB values of untreated and treated alfa fibers

	Untreated	**Retted**	**Alcalin autoclave**	**Xylanase**	**Laccase**	**Pectinase**
R	149.54	164.33	160.14	164.08	158.53	160.26
G	140.13	147.47	146.51	148.68	144.41	149.03

B	120.28	120.13	120.40	125.11	122.82	132.05

Fig. 5

3.4. Thermogravimetric analysis

To compare the influence of the different treatments on the stability of the fibers, thermogravimetric analyses were conducted. The structure and biochemical composition of plant fibers affect their thermal behavior. Fig. 6 shows that the curves of the treated fibers are shifted to the right, indicating that the fibers degrade at higher temperatures than the ~~raw~~ untreated fibers, meaning that the treated fibers are more thermally stable in the temperature range of ~~(~~200°C–400°C~~)~~.The thermogravimetric curves exhibit a similar pattern of mass loss with temperature and three main phases can be distinguished.

From ambient temperature up to 150 °C, the mass loss corresponds to the evaporation of free water fixed on the surface of the fiber [38]. Between 150 °C and 200 °C, the mass loss is practically negligible, indicating that the fibers are thermally stable in this range. The second phase is characterized by significant mass loss. Fig. 6a shows peaks and shoulders corresponding to the degradation of lignocellulosic. According to Ouajai et al., [33], the degradation of hemicelluloses and pectins occurs between 250 °C and 320 °C, while cellulose degrades between 390 °C and 400 °C. Tanobe et al., [39] report that hemicelluloses degrade between 200 °C and 260 °C, followed by cellulose between 240 °C and 350 °C, whereas lignin degrades between 280 °C and 500 °C. The third phase is characterized by a decrease in the rate of mass loss and corresponds to the thermal degradation of the remaining organic matter from the second phase [40]. It should be noted that some structural hemicellulose complexes may also degrade in this temperature range [41]. It is generally accepted that the initial thermal degradation of cellulosic materials occurs between 200 °C and 400 °C [42]. Initial degradation of cellulose components occurs mainly in the amorphous regions [43].

Table 5 presents the various degradation temperatures for each treated fiber. It can be observed that fibers treated with xylanase and pectinase exhibit good thermal stability compared to other fibers, showing no signs of initial degradation until around 77 °C and 85

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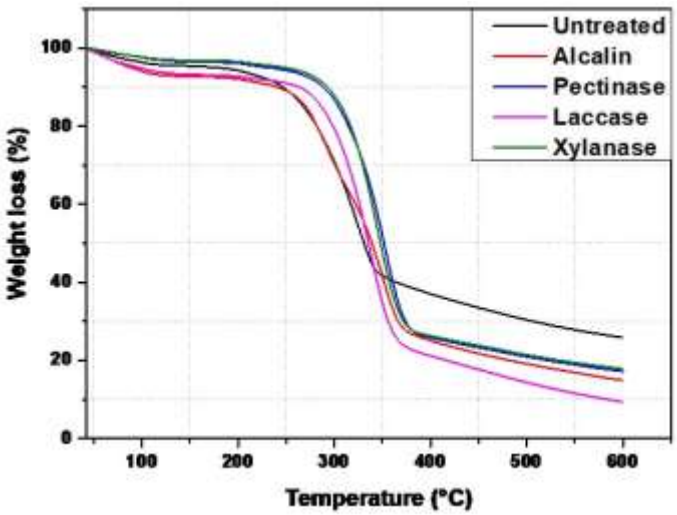
°C, respectively. In contrast, the initial degradation temperature for the laccase-treated fiber is around 70 °C. This temperature is lower than that of the untreated fibers, indicating that laccase treatment reduces the thermal stability of the alfa fibers. The TGA curve for fibers treated with NaOH shows a notable slope (Fig. 6b) which characterizes the release of volatile hydrocarbons from the thermal degradation of alfa fiber [44].

The amounts of residues remaining after heating to 500 °C differed among the treated fibers (Table 5). The results show that at 500 °C, the laccase-treated fiber has the lowest carbon residue. This could be due to the removal of hemicelluloses and lignin and the resulting improved accessibility of cellulose in the laccase- treated fibers [45]. It can be concluded that the alkaline and enzymatic treatments improved the thermal stability of the fibers. The thermal stability of alfa fibers increased due to the chemical degradation of hemicelluloses, lignin, and pectin during the various treatments. This is indicated by the higher degradation temperatures. The maximum transition temperature of untreated alfa fiber is 205 °C, whereas that of the alcaline-treated fiber is 289 °C. The untreated fiber degrades at a lower temperature because it contains thermally unstable components such as hemicelluloses, while the treated fiber is more stable due to the removal of these components [46]. It ~~is~~ has been reported that begin to degrade at around 200 °C, whereas other polysaccharides, such as cellulose, degrade at higher temperatures.



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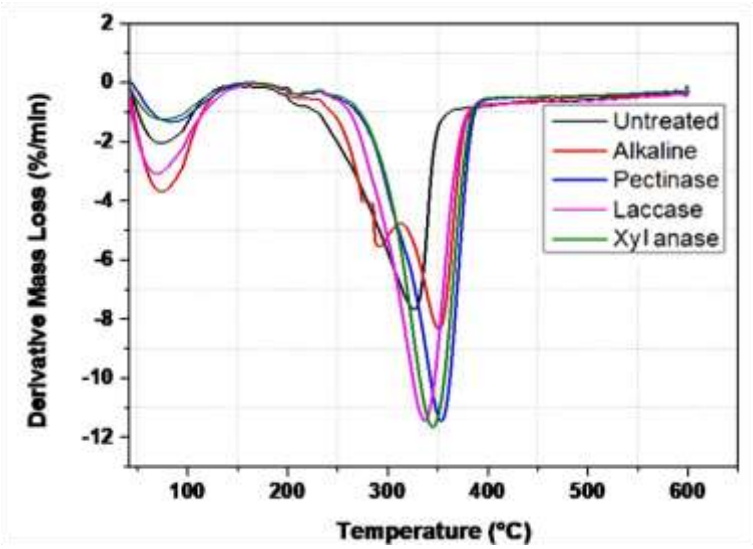

Fig. 6

~~The amounts of treated fiber residues differed after heating to 500 °C (Table 5). The results show that at 500 °C, the lowest carbon residue was obtained for the laccase-treated fiber. The removal of hemicelluloses and lignin and the consequent improved accessibility of cellulose in the treated fine fibers [45] may explain the relatively low amount of residue in the laccase-treated fibers. It can be concluded that the alkaline and enzymatic treatments improved the thermal stability of the fibers. The thermal stability of alfa fibers was increased by the chemical degradation of hemicelluloses, lignin, and pectin during different treatments. Increasing degradation temperatures are indicative of this. The maximum transition temperature of the untreated alfa fiber is 205 °C, while that of the alcalin-treated fiber is 289 °C. The untreated fiber degrades at a lower temperature due to the presence of thermally unstable components such as hemicelluloses, whereas the treated fiber is more stable due to the removal of these components [46]. It is reported that the degradation of hemicelluloses starts at a temperature of around 200 °C, while other polysaccharides such as cellulose degrade at higher temperatures.~~

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Table. 5. Various degradation temperatures for treated fibers

	Transition temperatures (°C)			Weight loss at corresponding transition (%)	Residual weight (%) at 600 °C
	T_i°C)	T_m(°C)	T_f(°C)		
Untreated	72.35	205.74	325.78	59	25.76
Alcalin	74.44	289.41	349.59	70	15.01
Pectinase	82.36	209.56	353.16	73.20	16.91
Laccase	67.12	208.42	373.16	74.92	9.11
Xylanase	75.97	208.78	344.75	72.27	17.85

T_i: Initial temperature for transition, T_m: Maximum temperature for transition, T_f : Final temperature for transition

3.5. Statistical study of the dispersion of mechanical properties

The data collected by Hanana et al., [17] through mechanical testing revealed a significant effect of enzymatic treatments on mechanical properties, particularly for pectinase treatment. This treatment resulted in the highest tensile strength of 1327 ± 342 MPa and a Young's modulus of 58 ± 17.4 GPa, which are close to values reported by Bessadok et al., [47]. This improvement is mainly attributed to the removal of hemicelluloses and the enhancement of crystallinity and cellulose content in the alfa treated with pectinase enzyme (Section 3.2). Like all plant fibers, the mechanical properties of alfa fibers exhibit significant variation. The fiber diameter distribution of all tested fibers and their corresponding strengths is shown in Fig. 7. The data points revealed a logarithmic trend, with larger diameter fibers showing lower tensile strength. This inverse relationship between diameter and tensile strength has been highlighted in the literature. The breakage of plant fibers is often controlled by the presence of defects, which are more likely to occur in fibers with larger diameters. ~~C~~ritical flaws can also arise from imperfections during growth and defects introduced during extraction [48]. As illustrated in Fig. 1, the fiber diameters along the length of alfa fibers, as observed with an optical microscope, generally show higher variation. The decrease ~~of~~ in tensile strength with increasing fiber diameter has been previously described for flax fibers by Sparnins et al., [49],

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in terms of Weibull statistics: larger fibers have a higher probability of containing ~~a~~ defects, leading to premature failure. Fig. 8. shows the Weibull distribution for different stages of the defibration process. For raw and retted alfa fibers during both periods, the curves exhibit a non-linear trend, indicating significant dispersion in the results. In contrast, for alkaline and enzymatic treatments, the curves show a linear trend, suggesting that reduce result dispersion and consequently the number of defects compared to raw and retted fibers. The Weibull modulus and characteristic strengths for each treatment are determined using linear regression (Table 6). The influence of the treatment on the Weibull modulus is evident with values ranges from 1.63 to 4.04 (Fig. 8). A higher Weilbull modulus indicates that defects along the fiber are more uniform in size, while a lower value suggests more variability in defect dimensions. In all cases, the R^2 coefficient ~~is~~ are between 0.9 and 0.98, indicating a good fit for the linear model. However, it is linearity based solely on the R^2 values. The Weibull modulus of 3.9 and 4 for the alkaline autoclave treatment and pectinase treatment respectively, are higher than that of raw alfa fibers. This indicates that fibers treated with NaOH and pectinase have fewer defects and, consequently, less variability.

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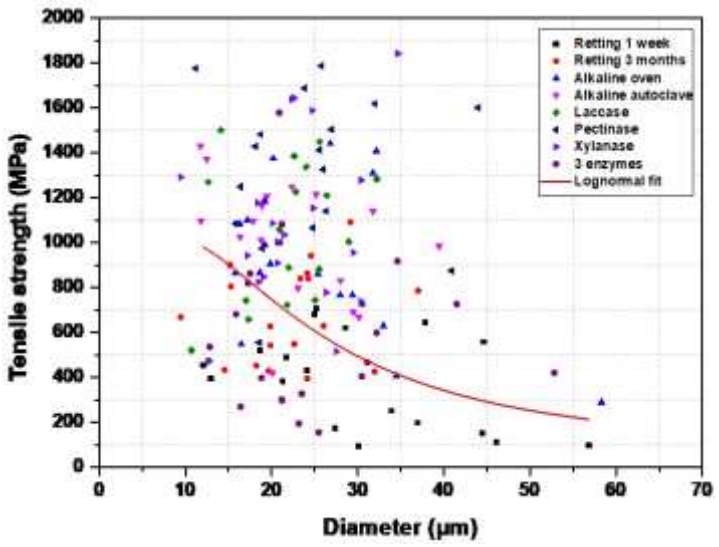

Fig. 7

~~Fig. 8. shows the Weibull distribution plotted for the different stages of the defibration process. For raw and retted alfa fibers during the two periods, the curves have a non-linear~~

~~trend that shows the wide dispersion of the results obtained. Whereas for alkaline treatments and enzymatic treatments, the curves have a linear trend, which means that after these treatments, the dispersion of the results has been reduced and, consequently, the treated fibers have fewer defects than the raw and retted fibers. The Weibull modulus and the characteristic constraints are determined for each treatment value using linear regression (Table 6). The influence of the treatment on the Weibull modulus is obvious; the Weibull modulus for alfa fiber ranges from 1.63 to 4.04 (Fig. 8). A high value of the parameter m indicates that the defects along the fiber are relatively similar in size. A low value of m indicates that the defects present on the fiber surface have very different dimensions. In all cases, the R^2 coefficient is between 0.9 and 0.98, indicating a good degree of linearity. However, it is not correct to conclude that the curves are linear from the values of the R^2 coefficients alone. The Weibull modulus of 3.9 and 4 were obtained from the slope of the best fit lines for the alkaline autoclave treatment and the treatment with pectinase. These values are higher than the Weibull modulus of the raw alfa fibers. This indicates that the fibers treated with NaOH and pectinase have fewer defects and, consequently, less variability.~~

In this part we have studied the influence of treatments on the mechanical properties of the fibers, in the next part we will compare the properties of single fibers and fiber bundles of the same treatment.

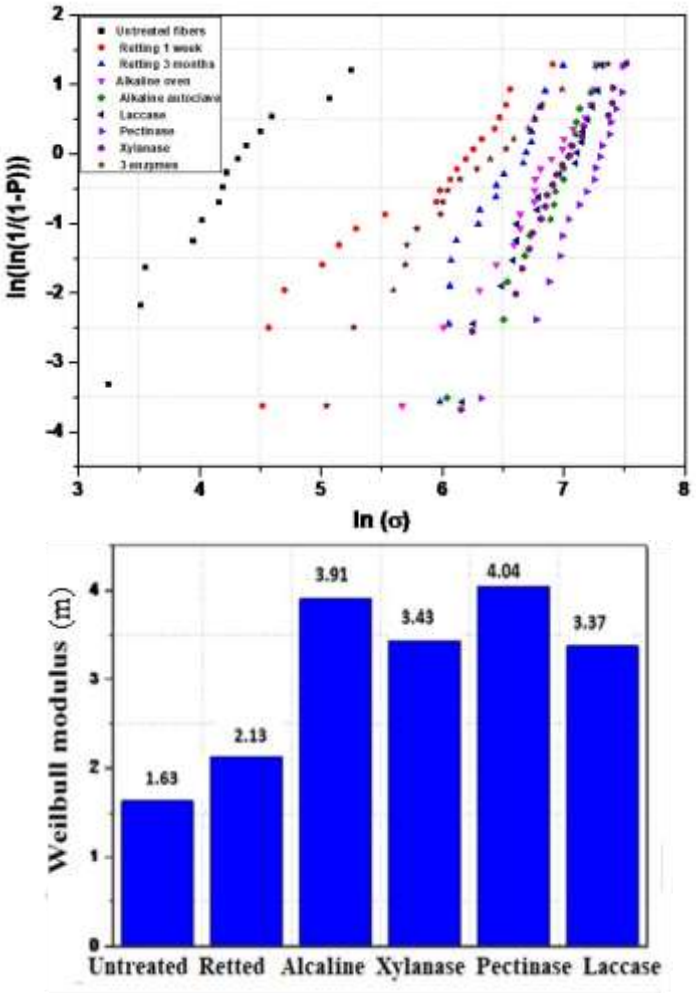

Fig. 8

Table. 6. Stress characteristic (scale parameter) and Weilbull modulus (shape parameter) as function of the types of treatment

Method of treatment	**σ_0 (MPa)**	**Weibull modulus (m)**	**r^2**
Untreated fibers	125.2	2.13	0.92

Retting 1 week	523.2	1.63	0.94
Retting 3 months	804.3	3.65	0.9
Alkaline oven	1085.7	2.9	0.98
Alcaline autoclave	1139.1	3.91	0.97
Laccase	1224.1	3.43	0.96
Pectinase	1495.2	4.04	0.97
Xylanase	1152.9	3.37	0.97

3.6. Comparison between fiber bundles and single fiber

Fig. 9. shows the comparison between the single fiber and the fiber bundles for the treatment of salt water for one week, alkaline treatment, and pectinase treatment using the Weibull statistical model to understand the effect of these treatments on the dispersion of the results. In the case of fibers retted for a week, the single fibers show a large dispersion, unlike the fiber bundles, where the points follow a very linear regression, showing a good dispersion of the results. In fact, fibers retted for a week are still well bonded due to the presence of a high percentage of non-cellulosic materials such as pectin and lignin. Thus, the removal of individual fibers with pliers is aggressive and may damage the fiber. For the alkaline treatment, single fibers present less dispersion than bundles of fibers. The alkaline treatment removed a high percentage of non-cellulosic materials, leading to good fiber separation, which shows that the extraction of unit fibers is easier and subsequently the dispersion of the unit fiber results has decreased.

Fibers treated with the pectinase enzyme have low percentages of non-cellulose materials and are well separated. Consequently, the mechanical results of fiber bundles have a high dispersion, while those of single fibers have a low dispersion.

Weibull parameters increased with decreasing diameter, revealing a more uniform distribution of defects in small diameters (Fig. 9). Except for fibers retted for one week, where single fibers were aggressively extracted from fiber bundles, since the fibers are still well bound together by pectins and lignin (Table7). The Weibull statistical analysis of the tensile strength of the alfa fibers shows that the highest values are obtained for individual fibers treated with pectinase. Conversely, a low Weibull modulus was observed in the case of fiber bundles.

To conclude, the variability of the measured tensile strength could be explained by the size of the fibers measured, the method of extracting the fibers, and then the distribution of defects or in homogeneities within the fibers.

A large bundle of alfa fibers, with more unit fibers, has a greater distribution of two types of fibers: the weaker and the stronger, due to the wide variation in properties of lignocellulosic fibers. Therefore, during tensile testing, a weaker fiber in a large bundle is more likely to break at lower stress than a weaker fiber in a small bundle. This is due to the dissolution of most of the cementing components, such as lignin, pectin, and hemicelluloses. Fiber bonding in a large bundle is therefore weak. As soon as the first fiber is broken, it causes a defect in the structure of the fiber bundle, which will propagate until it breaks completely [50].

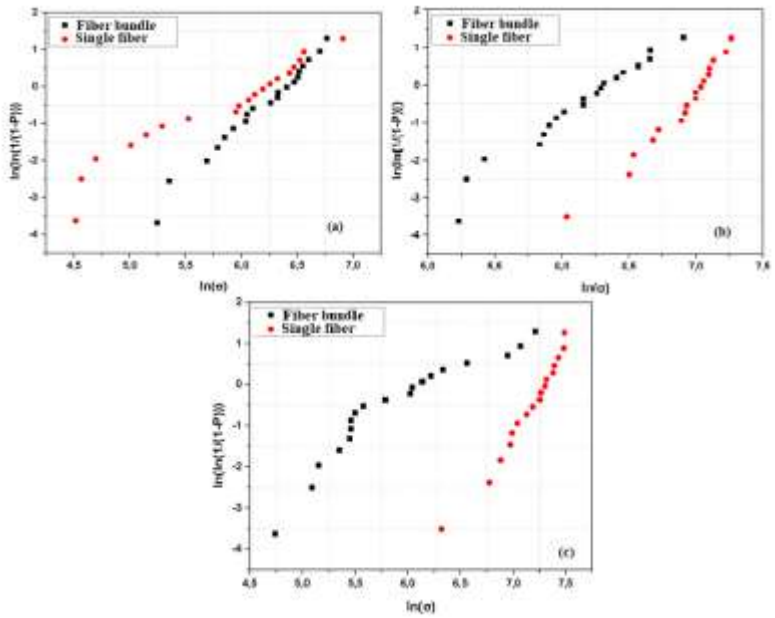

Fig. 9

Table. 7. Mechanical characteristics of bundles of alfa fibers at different processing

Treatments	Diameter (µm)	σ (MPa)	Strain (%)	E(GPa)
Retted one week (fiber)	29.93±11.9	419.69±251	1.94±0.54	21.24±12.921

Retted one week (bundle)	61.13±11.87	519±195.4	2.4±0.48	21.37±6.54
Autoclave (fiber)	22.18±7.69	1022.74±267.6	2.36±0.52	46.82±12.98
Autoclave (bundle)	61.9±12.55	502.27±215.9	2.4±0.91	18,63±6.44
Pectinase (fiber)	24.34±8.57	1327.4±341.8	2.44±0.64	58.3±17.41
Pectinase (bundle)	62.4±23.36	464.78±350.9	1.95±0.5	21.91±16.53

Fig. 10. shows that many fibers in a large bundle will tend to break more under tensile loading compared to a small bundle with fewer fibers. Therefore, the smaller-diameter beam is more resistant than the one with a larger diameter, and during a tensile test, it will behave as a single fiber with a single break.

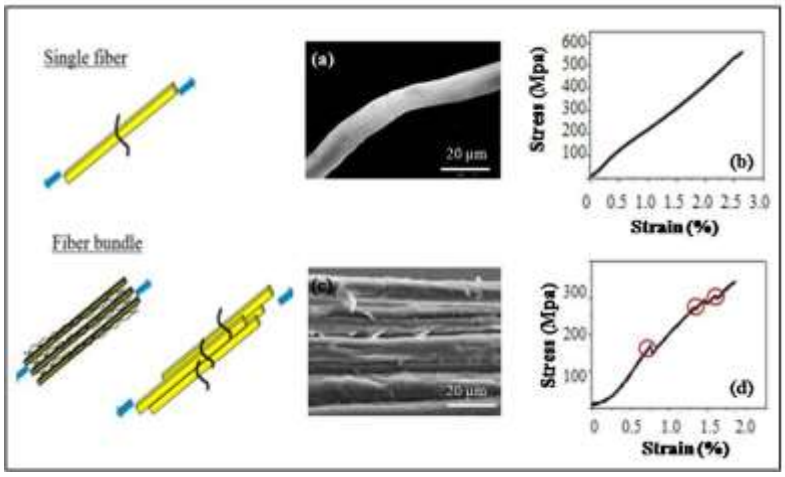

Fig. 10

Conclusion

In this work, the morphology, the crystallinity, colorimetric, thermal and mechanical properties of alfa fiber before and after treatments were investigated and discussed. The microscopic analysis reveals the morphology of alfa fibers, including their structure,

organization, and dimensions. Understanding the morphology of fibers is crucial for predicting their mechanical properties and behavior in composite materials. The enzymatic and alkaline treatments applied to alfa fibers result in significant improvements in their morphological, structural, and thermal properties, thereby enhancing their compatibility with polymer matrices and potentially leading to the development of high-performance biocomposites. It was also found that mechanical properties decrease as the fiber size increased. We used the Weibull statistical model to study the effect of the treatments used on the dispersion found. We concluded that enzymatic treatments reduced this dispersion and especially with the use of the enzyme pectinase.

Autor's contribution statement

Sabrine Hanana: Conceptualization, Investigation, Methodology, Writing - original draft**. Afef Fajraoui**: Methology and Experimental discussion. **Vincent Placet**: Review and Supervision, conceptualization, funding acquisition. **Ahmed Elloumi**: Review and Editing, ~~Conceptualization,~~ Supervision. **Chedly Bradai**: Supervision, Review and Editing. **Hafedh Belghith**: Supervision and Review.

Declaration of Competing Interest

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

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Figure captions

Fig.1 Schematic representation of cross section of alfa leaf (a), optical observations of cross section of alfa leaf (b), and (c) cut of leaf

Fig. 2 X-Ray diffraction spectrograms of untreated and treated fibers

Fig. 3 Pictures of the boxes of alfa fibers prepared for colorimetric analysis, (a): untreated fibers, (b): retted fibers, (c): fibers treated with NaOH, (d): fibers treated with xylanase, (e): fibers treated with laccase and (f): fibers treated with pectinase

Fig.4 Chromatic coordinates of the different types of fibers, (a) Luminance, (b) chromacity coordinate a* and (c) chromacity coordinate b*

Fig 5 RGB values of untreated and treated alfa fibers

Fig. 6 TGA (a) and DTG (b) curves of untreated and treated fibers

Fig. 7 Evolution of the tensile strength of single alfa fiber according to their mean diameter

Fig. 8 Weibull distribution for elementary alfa fibers to different treatments

Fig. 9 Weibull distribution for fiber bundles and single fibers, after retting for a week (a),after alkaline treatment (b), and after pectinase treatment (c)

Fig. 10 Comparison between single fiber and fiber bundle, (a) SEM image of single fiber,(b) mechanical test of single fiber, (c) SEM image of fiber bundle and (d) mechanical test of fiber bundle