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Characterization and conditioning of *Hibiscus sabdariffa* biofibers by FTIR

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Abstract

Natural fibers have gained great relevance in recent years as an alternative to artificial fibers and plastics, its extraction is mainly concentrated in hemp, cotton and jute. However, the fibers obtained from *Hibiscus sabdariffa* have a high potential for use at industrial level because it is a plant with a global presence and low nutrient requirements. The most commonly used fiber extraction methods are chemical and biological, although in general terms, chemical treatments are better than biological treatments, duo to the efficiently eliminate non-essential compounds from the fibers, in addition to having defined times in the retting process, nevertheless, a negative point of chemical treatment is that they degrade the fibers. In the sense, the implementation of *Pleurotus ostreatus* is a novel biological treatment that shows similar performance to chemical treatments, without degrading the fibers. The samples with chemical and biological treatments were subjected to Fourier Transform Infrared Spectroscopy, to be compared and determine the performance of the fibers treated with *P. ostreatus*, mercerized fibers have good strength for manufacturing and clearer sweeping.

Keywords: fiber, cellulose, FTIR, *Hibiscus sabdariffa*, *Pleurotus ostreatus*

Introduction

Natural fibers or biofibers are materials obtained from plants, animals or minerals. These natural fibers, as well as materials composed or reinforced with them, are acquiring great importance in the industrial area (Abou-Sreea et al., 2022). The three main compounds of natural fibers are cellulose, hemicellulose and lignin. Cellulose is the most important and abundant organic material in fibers, which is why they are also known as cellulosic fibers due to their high content (Amoasah et al., 2018), cellulose is also responsible for their mechanical properties. On the other hand, lignin is a polymeric material that acts as a transport medium (Achebe et al. 2019). In addition, other important secondary compounds are also found, such as pectins, waxes and fats (Meftahizadeh et al., 2021).

Biofibers can be found practically all over the world, present in shrubs, vegetables, stems, seeds, woody crops and flora. Using natural fibers in industry brings important advantages such as biocompatibility, low density, and reduced energy consumption during production, low costs, biodegradability, abundant availability, and good mechanics (Li Tan & Sulaiman 2019). However, biofibers also present some specific disadvantages such as poor adhesion with hydrophobic matrices, low resistance to chemicals and high water absorption, which has considerably limited their use (Alara & Abdurahman, 2019).

Hibiscus sabdariffa “”is a flower that can be found throughout Mexico and much of South America, with great potential (although very little studied) for the extraction of fibers for industrial use, from its fibrous bark (Ilyas et al., 2021). This work aims to give usefulness and added value to the stems obtained from *Hibiscus* extracting fibers through an innovative retting method, using a fungus (*Pleurotus ostreatus*).

Agregar información respecto a la aplicació/uso de la flor de jamaica como agua de día…

Materials and Methods

Obtaining raw materials: The raw material used for the retting and conditioning procedure, to obtain fibers for textile use, were *Hibiscus sabdariffa* stems. The *Hibiscus* were collected from Tecoanapa, Guerrero, México CP. 39270.

Physical characterization of the raw material: vernier.The stems of the *Hibiscus* were prepared, discarding the parts that are not necessary for the work such as flowers, branches and roots. Subsequently, the physical characteristics of the stem were evaluated; the length of the complete stems (end to end), using a flexometer, the individual weight of each stem, with an analytical

balance and the diameter was recorded by measuring transversely at the midpoint of each stem section, using a vernier.

Retting: Preliminary retting tests were carried out with five different samples to verify the most appropriate treatment to be applied to final fibers. Samples P250A and P350A were immersed in distilled water at a controlled temperature of 50°C for seven days. Sample P53TA was also immersed in distilled water, however, it was kept at room temperature for ten days. Sample P55TM was subjected to a 0.044 M $Mg(OH)_2$ solution at room temperature for seven days. The fifth sample was immersed in a 0.05 M NaOH solution for seven days. Mostrar Referencias…

Retted: The most suitable stems for the retting procedure were selected and cut evenly. The stems were distributed equally to obtain three repetitions, and each repetition was weighed on an analytical balance. Then, in a pot, a solution of $Mg(OH)_2$ was prepared, the solution was heated in a water bath, then the repetitions were introduced (individually). After the established time, the repetitions were extracted from the solution and placed in beakers at room temperature. Working under aseptic conditions, the three repetitions were placed in different polyethylene bags, where they were inoculated with *Pleurotus ostreatus*. Finally, the bags were sealed. Sample F14R1 was intended to remain in contact with the fungus for two weeks, sample F21R2 for three weeks, and samples F28R1, F28R2 and F28R3 for one month.

Fourier Transform Infrared Spectroscopy (FTIR): The first FTIR was carried out using a spectrophotometer (UV 765), prior to conditioning the fibers to be used as a comparison measure and test the effectiveness of retting. Work was done with computing equipment that included the storage device for the spectrophotometer. Before taking the reading, the analysis software was started and a scan was performed to prepare the equipment. To obtain the sample that was subjected to the spectrum, 1 g of fiber was weighed on an analytical balance and macerated in a mortar with a pestle until a fine powder was obtained, with which a tablet was created using hand tweezers. This tablet was introduced into the spectrophotometer to obtain the first results. Spectroscopy showed the intensity level of the functional groups belonging to cellulose, hemicellulose and lignin. The conditioning the fibers obtained (mercerized, bleached and neutralized), finally tests will be carried out. Performance, as well as ftir, to compare the fibers and check the efficiency of the experimental method.

Fiber conditioning: Once the retting process was finished, the stems were washed with distilled water to eliminate residues, and manual extraction of the fibers was carried out using a card. Subsequently, the fibers were subjected to mercerization, bleaching and neutralization, using solutions with NaOH, H_2O_2 and CH_3COOH to eliminate natural dyes, provide shine to the fibers and to achieve a neutral pH.

Mercerized: Initially, mercerization process were carried to obtain fibers with an alkaline pH and thus promote whitening. A NaOH solution was prepared, 300 mL of water with 33 g of NaOH, were added to a 500 mL beaker, subsequently the solution was separated into three 100 mL beakers, the solutions were heated in a laboratory oven. Once the solutions reached the desired temperature the fibers were introduced. At the end of the time, the fibers were extracted and washed with plenty of water, to avoid a reaction with H_2O_2. .Ver Referencias….

Whitening: Bleaching will allow the elimination of natural dyes from the fibers, for which a H_2O_2 solution was prepared, 190 mL of water and 110 mL of H_2O_2 were added to a 500 mL beaker, then the solution was separated into three 100 mL beakers. mL. The solution was heated and the fibers were subsequently introduced (at a constant temperature). At the end of the time, the fibers were extracted and washed again with plenty of water.

Neutralized: Finally, the neutralizer will maintain the pH of the fibers neutral to avoid considerable damage and decomposition. The acetic acid (CH_3COOH) solution was prepared in a 500 mL beaker, 297 mL of water and 3 mL of CH_3COOH were added, the solution was divided into three 100 mL beakers. The fibers were soaked in the solution, subsequently the fibers were placed in a 100 mL beaker with water, and using a potentiometer the pH was obtained. Ver Referencias…

Fourier Transform Infrared Spectroscopy (FTIR)

The second FT-IR was carried out, after conditioning the fibers to compare them and confirm the effectiveness of the treatment carried out, using a spectrophotometer (UV 765). We worked with a computer that had the storage device for the spectrophotometer. Before taking the reading, the analysis software was started and a scan was performed to prepare the equipment. To obtain the sample that was subjected to the spectrum, 1 g of fiber was weighed on an analytical balance and macerated in a mortar with a pestile until a fine powder was obtained, with which a tablet was created using hand tweezers. This pellet was introduced into the spectrophotometer to obtain the final results. The resulting spectrum was analyzed to check the peaks that denote the presence of hemicellulose, lignin and natural dyes of the fibers.

Results

Fourier Transform Infrared Spectroscopy of preliminary samples

cm^{-1} Figure 1 shows the IR spectra of fibers with the different preliminary treatments after retting. The spectral bands demonstrate that the most effective treatment was using magnesium hydroxide (sample P55TM), since the absorbance levels obtained show a high concentration of functional groups and mainly cellulose. On the other hand, sample P53TA showed significantly lower absorbance levels in the 3500-3200 region. Samples P250A and P27TS obtained very similar results in general terms. Finally, sample P350A presented the lowest level of absorbance, which denotes the considerable loss of cellulose, which indicates that it is the least effective treatment.

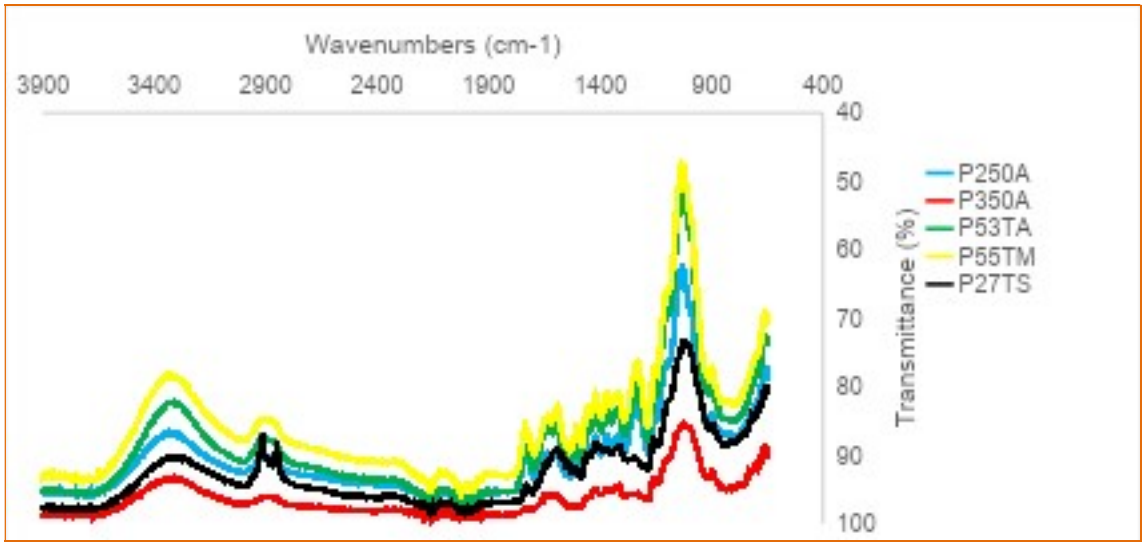

Figure 1. FTIR spectrum of preliminary fibers, after retting.

1 cm^{-1} In Table 1, the characteristic regions of the fibers in the IR spectra can be seen. The region 3500-3200 cm-1 represents the transmittance of the hydroxyl group (O-H), within this group there are the bonds of alcohols and phenols, in this region there are also the nitrogen-hydrogen bonds (N-H) of the amines and bonds carbon hydrogen (C-H) from methyl stretching. In this sense, sample P55TM obtained the highest transmittance (78.39%), while sample P350A recorded the lowest amount of the hydroxyl group (93.47%). The 3000-2800 cm^{-1} region is characteristic of the absorption of different C-H bonds such as: stretching of the C-H group in alkenes (=CH), carbon hydrogen stretching in alkanes (-CH) or C-H stretching in aldehydes (O=CH). In our spectra, sample P55TM recorded the highest absorbance (84.78%) and sample P350A the lowest absorbance. The 2200-2000 region belongs to the absorption bands of unsaturated compounds such as amides (C=N) and unsaturated carbon-carbon bonds (C=C) in alkenes. Sample P55TM obtained 92.40% absorbance, and sample P350A did not register values in the IR spectrum. The 1800-800 cm^{-1} region represents the absorption bands of five- and six-carbon ring ketones, aliphatic ketones, α, β unsaturated ketones, esters, carboxylic acids and nitrites, with sample P55TM being the one that obtained the highest levels of absorbance of these bands.

Table 1. Characteristic bands of the FTIR spectra of the preliminary fibers with different treatments, after retting.

		P250A		P350A		P53TA		P55TM		P27TS	
Vibration	**Source**	**λ (cm^{-1})**	**Abs. (%)**	**λ (cm^{-1})**	**Abs. (%)**	**λ (cm^{-1})**	**Abs. (%)**	**Λ (cm^{-1})**	**Abs. (%)**	**λ (cm^{-1})**	**Abs. (%)**
Stretch O-H	Cellulose / Hemicellulose/ Lignin/ Pectins	3299	87.04	3333	93.47	3318	82.34	3335	78.39	3308	90.32
Symmetric Stretch C-H	Cellulose / Hemicellulose/ Lignin/ Pectins	2878	90.40	2895	96.05	2917	87.33	2896	84.78	2919-2848	87.29-88.45
Unsatured compounds	Cellulose / Hemicellulose/ Lignin/ Pectins	2097	95.47	---	---	2104	95.14	2117	92.40	2106	96.91
Stretch C=O	Cellulose / Hemicellulose/ Lignin/ Pectins	1733	89.70	1743	97.83	1735	88.83	1735	85.77	1733	94.46
Bending water abs O-H	Water	1592	89.13	1592	95.79	1597	85.80	1590	82.87	1592	89.00
Symmetric Stretch C=C	Lignin	1508	89.55	---	---	1500	88.66	1506	85.93	---	---
Bending H-C-H	Cellulose	1459	88.79	---	---	1459	86.33	1459	83.23	1461	89.99
Bending O-C-H	Cellulose	1426	87.90	1426	95.05	1424	84.12	1422	81.08	1420	88.32
C-H	Cellulose/ Hemicellulose	1373	87.40	1372	94.90	1370	83.08	1366	80.98	---	---
Wagging CH_2	Cellulose	1312	87.22	1318	94.40	1321	83.24	1325	80.96	1319	88.57
Ring stretch / C=O	Lignin	1234	82.80	---	---	1237	79.81	1234	76.32	---	---

Symmetric Stretch C-O-C	Cellulose/ Hemicellulose	1159	84.01	1159	93.28	1155	79.18	1157	77.06	1159	87.72
Stretch C-C / C-H	Cellulose/ Hemicellulose	1107	76.96	---	---	1101	69.15	1101	66.07	---	---
Stretch C-OH	Cellulose/ Hemicellulose	1032	62.57	1027	85.37	1029	51.19	1030	47.35	1021	73.36
Twisting	Cellulose	900	84.30	894	92.38	894	79.76	894	77.52	894	85.50

Fourier Transform Infrared Spectroscopy of final samples (Retting)

Figure 2 shows the IR spectra of fibers inoculated with *Pleurotus ostreatus*, after retting. According to what was observed in the spectra, the differences compared to the preliminary fibers are notable, mainly because a greater decrease in the concentration of functional groups is observed in them. Sample F28R2 recorded the best levels of absorbance, that is, it maintained the greatest amount of functional groups (mainly cellulose), samples F14R1, F21R2 and F28R1 obtained similar results, while sample F28R3 recorded the lowest levels of absorbance, which which indicates the loss of most of the functional groups.

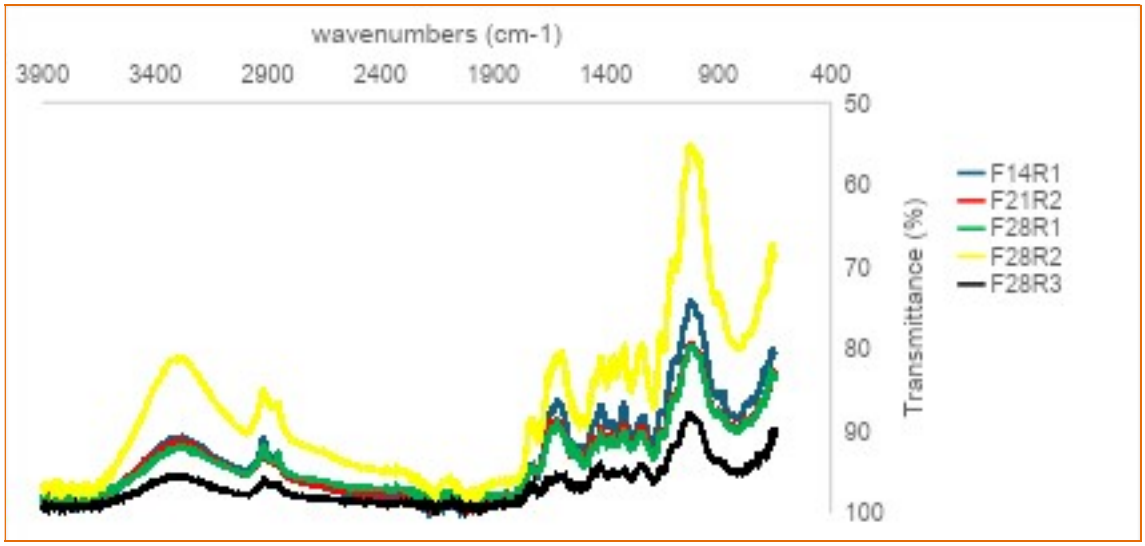

Figure 2. FTIR spectrum of final fibers, after retting.

1015 Table 2 shows the characteristic bands of the final fibers, where the decrease in transmittance levels is confirmed, compared to the preliminary fibers. Sample F28R2 recorded the highest transmittances in the four characteristic regions of the functional groups, followed by sample F14R1, the third highest transmittance was recorded by sample F21R2, followed by sample F28R1 and finally the sample with the lowest transmittance F28R3. The region 3500-3200 cm^{-1} represents the tension of the O-H group, the region 3000-2800 cm^{-1} frames the tension of the C-H group, and the region 2200-2000 cm^{-1} identifies the C=C group of the unsaturated compounds, these three regions indicate the amount of cellulose, hemicellulose, lignin and pectins present in the fibers, however, with these readings it is difficult to determine precisely which specific compound it belongs to. The fourth region 1800-800 cm^{-1} represents the fingerprint of the samples, therefore, it is the most important region of the IR spectrum.

Table 2. Characteristic bands of the FTIR spectra of the final fibers after retting, $Mg(OH)_2$ / *Pleurotus ostreatus*.

		F14R1		F21R2		F28R1		F28R2		F28R3		
Vibration	**Source**	**λ (cm^{-1})**	**Abs. (%)**	**Λ (cm^{-1})**	**Abs. (%)**	**λ (cm^{-1})**	**Abs. (%)**	**λ (cm^{-1})**	**Abs. (%)**	**λ (cm^{-1})**		**Abs. (%)**
Stretch O-H	Cellulose / Hemicellulose/ Lignin/ Pectins	3277	90.87	3290	91.39	3294	92.02	3320	81.31		1325	95.54
Symmetric Stretch C-H	Cellulose / Hemicellulose/ Lignin/ Pectins	2919-2852	91.00-92.60	2917-2850	92.27-9342	2917-2850	91.82-92.88	2919-2852	84.99-86.63		2919-2850	95.92-96.36
Unsatured compounds	Cellulose / Hemicellulose/ Lignin/ Pectins	2108	98.80	2091	98.19	2114	97.65	2097	96.09		---	---
Stretch C=O	Pectins	1733	94.47	1735	94.17	1735	94.33	1735	88.24		1735	96.19
Bending water abs O-H	Water	1616	86.57	1636	88.73	1618	89.30	1603	80.59		1595	95.40
Bending H-C-H / O-C-H	Cellulose	1418	87.01	1420	89.77	1420	90.53	1420	80.75		1420	94.24
Bending C-H	Cellulose / Hemicellulose	1366	88.86	1370	90.49	1372	90.97	1364	81.11		1364	94.74
Wagging CH_2	Cellulose	1321	86.83	1321	89.20	1319	89.81	1318	79.78		1314	94.62
Ring stretch / C=O	Lignin	1237	88.18	1235	89.40	1241	90.17	1239	79.43		1241	94.09
Symmetric Stretch C-O-C	Cellulose / Hemicellulose	1155	87.46	1155	90.06	1159	90.30	1155	78.37		1157	94.53
Stretch C-C / C-H	Cellulose / Hemicellulose	1098	81.40	1098	85.62	1101	86.06	1101	69.27		1103	91.83
Stretch C-OH	Cellulose / Hemicellulose	1021	74.27	1021	79.34	1025	79.72	1025	55.17		1029	87.86

Figure 3 shows the comparison of IR spectra between the fibers that were only subjected to retting with *Pleurotus ostreatus* (F28R1) and the fibers that underwent the conditioning, mercerizing, bleaching and neutralization process (A28R1). A considerable increase in the percentage of absorbance of the conditioned sample can be observed with respect to the retted sample, indicating the ability of the conditioning process to increase the expression of functional groups.

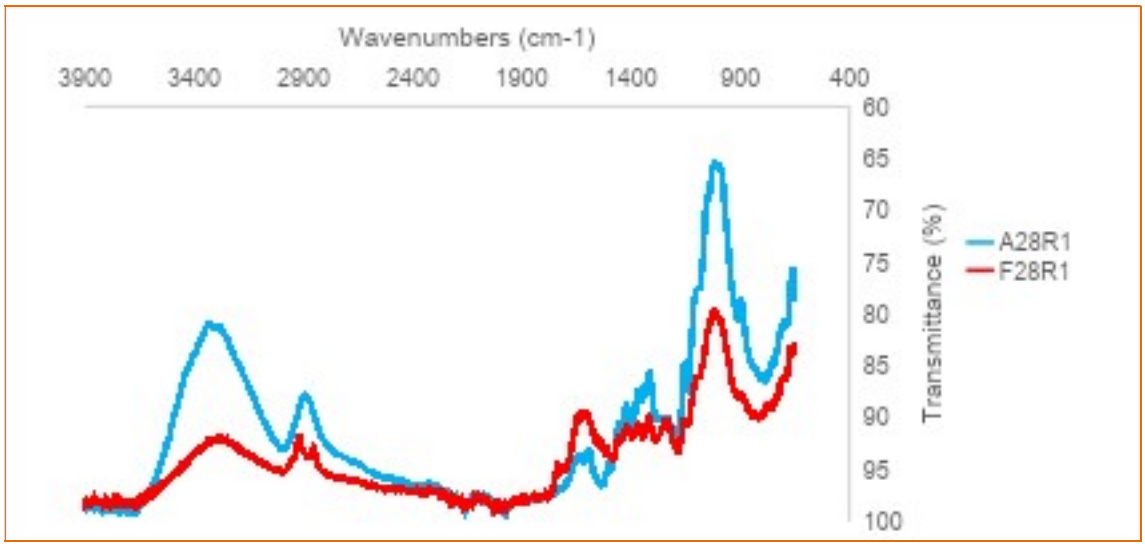

Figure 3. Comparison of IR spectra, fibers before and after conditioning.

Discussion

The IR spectra of the preliminary samples were analyzed (artisanal treatment and chemical treatments, figure 1), observing absorbance variations, this can be attributed to the different treatments used. The stems of the control sample P53TA (artisanal treatment) were immersed for ten days in water waiting for fermentation by microbial action of the bacteria belonging to the plant and the water (*Clostidia* and *Bacillus*) (Kazi & Ramasastry, 2022), due to the biological action of the treatment, the chemical composition of the fibers were not affected to a large extent, obtaining the second highest concentration of functional groups, which indicates the presence of cellulose, hemicellulose, lignin, among other compounds (Ralph, 2019). On the other hand, samples P250A and P350A were treated with the same procedure described above, only adding the temperature control at 50 °C for seven days, in order to accelerate and favor the bacterial growth process (Shaker & Nawab, 2022).

However, the absorbance levels of both samples turned out to be very different, this could be due to the lack of standardization of the process, because the samples were obtained from different *Hibiscus* flowers and with different drying and decomposition times, which represents a concentration of different compounds and functional groups (De la Rosa et al. 2020). With the P55TM sample, a chemical treatment was carried out ($Mg(OH)_2$ solution, at 0.044 M, at room temperature), obtaining the highest concentration of functional groups, this is attributed to the fact that $Mg(OH)_2$ is a base and It does not affect cellulose or hemicellulose, eliminating only lignin and pectins, compounds that are not very abundant in the fibers (Maceda et al., 2020). The P27TS sample was treated with a 0.05 M NaOH solution, at room temperature, being a strong base, it dissociates completely, and consequently, the fibers present greater damage and loss of functional groups, registering lower transmittances, in comparison with the magnesium hydroxide treatment which is a weak base (Pinos & Braulio, 2019).

The biological treatments showed a poor elimination of lignin, pectins and other non-essential compounds in the fibers, due to the difficulty in determining the moment at which the process has ended. However, there is an advantage to these treatments since the cellulose remains intact and does not cause considerable damage to the fibers (Jacome et al., 2023). On the other hand, chemical treatments allow greater control over times and the elimination of non-essential compounds, but cause considerable damage to the fibers (Jagadeesh et al., 2021).

In this sense, it was decided to consider the optimization of a biological process, implementing an inoculation treatment to the stems of *Hibiscus sabdariffa* using the *Pleurotus ostreatus* fungus.

In this way, the IR spectra of the final samples (Torres, 2019) showed a reduction in functional groups compared to the preliminary study, this is attributed to the action of *Pleurotus ostreatus* on the stems. The five samples were subjected to a fermentation process with the fungus, which developed favorably on the woody surface of the *Hibiscus* stems, favoring the elimination of lignin and pectins, by the action of *Laccase* enzymes, without damaging the cellulose and facilitating the degumming process in the fibers, providing greater resistance and eliminating natural stains (Serrano, 2022). The samples were extracted at different time periods to check the progress of retting. Similarities in transmittance were observed in samples F14R1, F21R2 and F28R1, however, in samples F28R2 and F28R3 greater variation was observed, this may be due to the ability of the fungus to penetrate the phloem and xylem of the stems and in turn generate changes in the chemical composition of the fibers (cellulose, hemicellulose, lignin, pectins, among others) (Vijay et al., 2020).

Conclusion

The fibers obtained from a biological treatment with *Pleurotus ostreatus* showed notable advantages compared to chemical treatments, eliminating non-essential compounds from the fibers such as lignin, pectins, waxes or fats, keeping the cellulose and hemicellulose relatively intact, in the same way in as would happen with chemical treatments. However, *P. ostreatus* does not cause significant damage to the fibers. In addition, it provides resistance and eliminates natural dyes. Together with the conditioning process, it represents a reliable, safe and sustainable alternative to artificial fibers or plastics.

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