CHARACTERIZATION OF ALKALINE AND ENZIMATICALLY TREATED HEMP FIBRES BY FT-IR ATR SPECTROSCOPY

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Abstract: *The FT-IR ATR spectra of the alkaline and enzymatically treated hemp fibres (1%, 2%, 3% o.w.f. enzyme, 15, 35, 55 minute enzyme action time) were recorded aiming to evaluate the pectin elimination. The relative absorbance values of the 1732 cm^{-1} band which might be attributed to the presence of the carboxylic groups (C=O) in pectin were evaluated. The results showed a diminishing of the pectin amount with increasing concentration (% o.w.f.) and action time (minutes) of the enzyme mixture. So that, the absorbance recorded at 1732 cm^{-1} with FT-IR ATR spectroscopy can quantify removed pectin from the material, thus enabling characterization of proposed biotechnology and monitoring the process.*

Keywords: hemp fibres, alkaline treatment, enzyme treatments, pectin elimination, FT-IR ATR

INTRODUCTION

Biocomposites are becoming strategic materials with various applicative purposes such as: building materials, automotive components, etc. Bast fibres such hemp, flax, or jute are the most used of plant fibres as bio based fillers or reinforcements constituents in polymer – based composites due to the fact that are biodegradable and have good mechanical, thermal and acoustic properties (Väisänen et al. 2018, Iucolano et al. 2018). Due to its strength and stiffness, hemp fibre has good potential as a composite reinforcement. The main constituent of hemp fibre is cellulose (~74 %). In addition to cellulose, other constituents like pectins (~1%), hemicellulose (~14 %), lignin (~5%), waxes, extractable substances, minerals, etc. (~6%) are present in the fibres structure (Väisänen et al. 2018, Shahzad, 2012). The homogeneity, fineness and effectiveness of fibres can be improved by removing the pectin and hemicellulose that bond the fibres bundle together (Kozlowski et al. 2006). For this purpose, chemical, physical or enzymatic treatments can be used. Recently, the use of enzymes is an ecological alternative to classical treatments, with a lower environmental impact. Enzymatic treatments provide numerous benefits over chemical and physical methods due to their high selectivity. Many studies have shown that the use of pectinases is very efficient for the removing of pectins which leads to the improvement of hydrophilicity without affecting the mechanical properties of the material (Iucolano et al. 2018, Thygesen et. al. 2013, Li et al. 2008).

The FT-IR ATR spectroscopy has proven to be a simple and rapid technique for obtaining information on the structure of the fibres constituents and chemical changes taking place in hemp fibres after different treatments (Dochia et al. 2018a, Wang et al. 2006, Terpáková et al. 2012, Choe et al. 2018, Ouajai et al. 2005).

Continuing our previous studies (Dochia et al. 2018a, 2018b), in this paper are presented the results obtained by FT-IR ATR investigation in order to quantify pectin elimination from hemp fibres after alkaline treatment and enzymatic treatments (conducted under different experimental conditions). For this purpose, the 1732 cm^{-1} intensity band, which might be attributed to the presence of the carboxylic groups (C=O) in pectin was evaluated.

MATERIALS AND METHODS

Materials

The following samples were analysed: raw hemp fibres denoted as RWH sample; alkaline treated hemp fibres denoted as ATH sample (raw sample treated at 95 ^0C with 10 $g.L^{-1}$ sodium hydroxide for 60 minutes); enzymatically treated hemp

fibres denoted as ETHx-y (were x is the concentration of enzyme, % over weight fibre - o.w.f, and y- minutes of the enzyme action time) –ETH1-35, ETH2-15, ETH2-35, ETH2-55, ETH3-35.

Enzymatic treatments were performed as follows: distilled water reaction media; 1:20 fibres to liquid ratio; commercial enzyme Beisol PRO from CHT Bezema Company (a mixture of Polygalacturonases - which act on pectin polygalacturonic chains and Pectinesterases enzymes - which act on pectin ester groups); pH 8.5 (assured by buffer CAS:7732-18-5); 55^0C treatment temperatures; 2 g.L^{-1} (~10 mmol.L^{-1}) sodium citrate (monosodium citrate, CAS: 18996-35-5) as complexing agent from Sigma-Aldrich; 0.5% surfactant Denimcol Wash-RGN detergent (from CHT Bezema Company).

Method

The FT-IR ATR experiments were performed on samples taken from conditioned raw and treated hemp fibres (up to 105 0C on Sartorius MA 100 system). The spectra were acquired using the Bruker Vertex 70 spectrophotometer equipped with the ATR cell, on the 600-3000cm^{-1} wavelength range with a resolution of 4cm^{-1} and 36 scans. The OPUS software was used for the acquisition and processing of experimental data (spectra normalization and baseline correction). The background calibration was made before each measurement.

RESULTS AND DISCUSSIONS

Lignocellulosic fibres are usually characterized by several absorption bands as can be notice in Scheme 1 which shows the FT-IR ATR spectra of the RWH, ATH and ETH3-35 samples recorded on 600 – 4000 cm^{-1}.

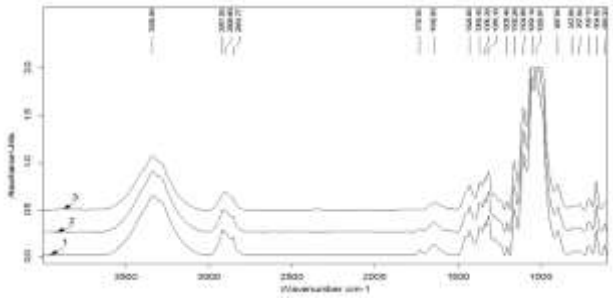

Figure 1. FT-IR ATR spectra of: 1- RWH, 2 - ETH3-35 and 3 – ATH samples

The peak recorded at 3000cm^{-1}-3600cm^{-1} can be assign to the free -OH stretching vibration and to the intra- and intermolecular hydrogen bond related to cellulose structure (Wang et al. 2006, Perincek et. al. 2016, Le Troedec et al. 2008). The peak located at 2800-3000cm^{-1} is due by CH symmetrical stretching of cellulose and the two extra bands at 2917cm^{-1} and 2851 cm^{-1}, can be related to the CH2 and CH groups stretching vibration from pectin and waxes (Wang et al. 2006, Choe et al. 2018, Subramanian et al. 2005). The bands observed at around 1732cm^{-1} and 1642cm^{-1} are specifics for pectin and can be attributed to the COOH and COOCH3 groups of polygalacturonic acid and to symmetrical/asymmetrical oscillations of ionized carboxyl groups COO-, respectively (Wang et al. 2006, Dai et al. 2010) Due to the superposition of the OH bending of absorbed water (1642 cm^{-1}) with the carboxyl ion band recorded around 1550cm^{-1}-1700cm^{-1}, the quantification of the pectin elimination in this area is quite difficult. (Dai et al. 2010, Ouajai et al. 2005).

The fingerprint area of the cellulose located between 600cm^{-1}-1400cm^{-1} contains specific and common bands (Dai et al. 2010). The 1428 cm^{-1} band was attributed to HCH and OCH in-plane bending vibration of cellulose and is known as "crystalline" band (Ouajai et al. 2005, Ciolacu et al. 2011).

The band from 1368cm^{-1} is given by in-the-plane CH bending of cellulose and hemicellulose. Band due to the deformation of OH group of cellulose was recorded at 1335cm^{-1}. CH2 rocking vibration from cellulose was observed at 1315 cm^{-1} (Dai et al. 2010).

The bands to 1160cm^{-1} and 1203cm^{-1} are for C-O-C symmetric and asymmetric stretching from cellulose and hemicellulose (Dai et al. 2010). The bands at 1029cm^{-1}, 1053 cm^{-1} and 1104 cm^{-1} indicate the C-OH, C-C, C-H ring and side group vibrations in cellulose and hemicellulose (Dai et al. 2010).

The observed band at 897cm^{-1} can be attributed to COC, CCO and CCH deformation and stretching from cellulose being known as "*amorphous*" band (Ciolacu et al. 2011, Ouajai et al. 2005).

The band at 812 cm^{-1} is specific for lignin (Ouajai et al. 2005) and that to 661 cm^{-1} to the OH

out-of-plane bending (Le Troedec et al. 2008, Dai et al. 2010).

In order to analyse the influence of the enzymatically treatments, which aims the elimination of a large amount of pectin from cellulosic and lignocellulosic materials, different authors recommend the investigation of the changes occurring in the absorbance intensity of the bands located at 2900cm^{-1}- 2919cm^{-1}, 2850cm^{-1}- 2860cm^{-1} and around 1630-1640cm^{-1} but especially of those at 1730cm^{-1}- 1734cm^{-1}, which is specific for the carboxylic group of polygalacturonic acid and COOCH3 (Wang et al. 2006, Abdel-Halim et al. 2008, Ouajai et al. 2005, Le Troedec et al. 2008).

From Figure 1 it can be seen that the intensity of the two extra bands located at 2917cm^{-1} and 2851cm^{-1} decrease from RWH to enzymatically treated sample due to the elimination of waxes and of a pectin fraction by breaking of the 1-4 carbohydrate bonds from D-galacturonic acid under Polygalacturonases action, and disappear for ATH sample.

In the alkaline treatment the CH and CH2 stretching vibration of cellulose was recorded at 2899 cm^{-1}.

The intensity of the specific band at 1732 cm^{-1} which indicate the existence of pectin it's diminishing for ETH3-35 sample and is not found in the FT-IR spectrum of the ATH sample. This behaviour suggests that enzymatically treatment eliminates hemp fibre pectin only partially comparing to alkaline conditions.

As pectin is eliminated the band located at 812 cm^{-1}, assign to lignin, becomes more intense compared to the RWH sample. This band disappears totally for ATH sample, suggesting that alkaline treatment removes all non-cellulosic attendants.

The changes occurring in the FT-IR spectra of the ATH sample observed at 3000-3500 cm^{-1}, 2851-2917 cm^{-1}, 1428 cm^{-1} and 897 cm^{-1} suggest that NaOH affected the crystalline cellulose structure and diminished the number of OH bonds present in cellulose chains with the formation of –O^-Na^+ groups.

In Figure 2 are presented the FT-IR spectra recorded around 1732 cm^{-1} (band specific for pectin) for raw hemp fibres and enzymatically treated samples at different enzyme concentrations.

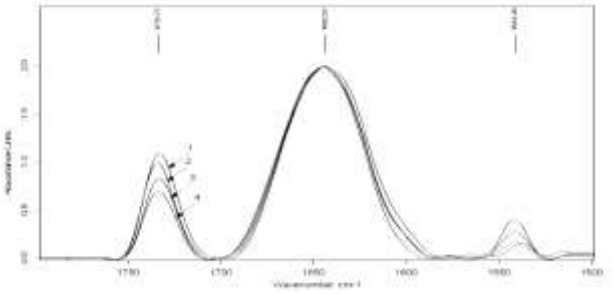

Figure 2. FT-IR ATR spectra of: 1- RWH, 2 – ETH1 -35, 3 – ETH2 -35, 4 – ETH3 -35 samples on 1500 – 1800 cm^{-1} range

With the increasing of the enzymes concentration, the absorbance intensity values of the 1732 cm^{-1} band (A_{1732}) presented in Table 1 decreases.

Table 1. The relative absorbance values, A_{1732} (a.u.) of hemp samples for the same enzyme action time at different concentration

Samples	A_{1732} (a.u.)
RWH	1.22
ETH1 -35	1.05
ETH2 -35	0.75
ETH3 -35	0.60

At the same enzyme action time, a more noticeable decrease in relative absorbance values can be observed by increasing the concentration from 1% to 2% than from 2% to 3%.

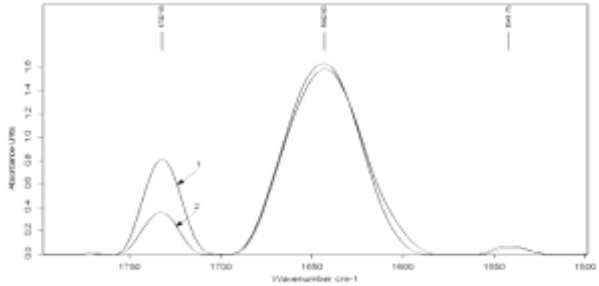

Figure 3. FT-IR ATR spectra of: 1- ETH2 - 15, 2 – ETH2 - 55 samples on 1500 – 1800 cm^{-1} range

Table 2 shows the influence of enzyme action time on the absorbance intensity values of the 1732 cm^{-1} band (A_{1732}).

Table 2. The relative absorbance values, A_{1732} (a.u.) of hemp samples for the same enzyme concentration at different action time

Samples	A_{1732} (a.u.)
ETH2 -15	0.83
ETH2 -55	0.37

Prolonging the enzyme action time from 15 to 55 minutes leads to an advanced decrease of the absorbance intensity value suggesting that a larger amount of pectin was removed. Similar behaviour was reported in the literature by Wang Q. et al. for the characterization of bioscoured cotton fabrics using FT-IR ATR spectroscopy (Wang et al. 2006).

Even that a long enzyme action time favours a more advanced elimination of pectin from the lignocellulosic substrate, the results reported in the literature do not recommend uses of prolonged treatments because this can lead to the degradation of the crystalline cellulose structure with negative effects on the mechanical properties of the fibres.

CONCLUSIONS

The influence of the alkaline and enzymatic treatments on the pectin elimination from hemp fibres were investigated by FT-IR ATR spectroscopy. For enzymatically treated samples, the relative absorbance values of the pectin specific band located at 1732 cm^{-1} showed a diminishing of the amount of pectin with increasing concentration (% o.w.f.) and action time (minutes) of the enzyme mixture. The obtained results suggest that, the input variables (concentration of enzyme and action time) are factors that consistently influence dependent variable of interest (% of eliminated pectin). So that, the absorbance recorded at 1732 cm^{-1} with FT-IR ATR spectroscopy can quantify removed pectin from the material, thus enabling characterization of proposed biotechnology and monitoring the process.

Even that the alkaline treatment has led to a complete removal of non-cellulosic components, enzyme treatment is recommended because it has the advantage of being eco-friendly with good results in pectin removal without affecting the properties of hemp fibres.

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