The influence of structure of lignocellulosic fibres on enzymatic grafting of functional phenolics

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Abstract

In this study, the efficiency of laccase-induced grafting of two phenolic compounds, guaiacolsulfonic acid (GSA) and 2,6-dimethoxyphenol (DMP), on jute and sisal fibres containing different amounts and types of lignin were studied using ATR-FTIR and colour determination. The results indicate that enzymatic grafting depends on the combination of phenolic molecule grafted and lignino-based fibres used. In the case of DMP an increased anti-flammable effect was observed.

INTRODUCTION

The use of lignocellulosic fibres for technical applications is gradually increasing during the last decade. The main reason for enlarged application for technical purposes lies in the amplified ecological awareness which emphasizes the use of biodegradable, biocompatible and in general environmental friendly materials. Lignocellulosic fibres are used both for textile technical applications (e.g. as composites in automotive industry or as geo-textiles) and also in pulp & paper industry[1][2]. Besides itemized characteristics, natural fibres also overcome several good mechanical properties like tensile strength, e-modulus and elongation properties that are comparable to some synthetic fibres and posses a quality which is not specific for technical synthetic fibres, i.e. moisture absorption [1].

The main difference between cellulosic and lignocellulosic fibres is in formation of fibres during their growing. A cellulose molecule is formed by polymerisation of glucose as basic building unit which forms bundles bonded together by hydrogen bonds. During this stage, bundles are twisting and forming rope-like structures which are joined together and form the fibres. Lignocellulosic fibres are formed by a similar way, although before twisting together cellulose bundles are embedded in lignin which cements them to each other and forms matrix. The formatted structure results in increased stiffness and strength. Lignin in cellulosic fibres is present in traces or it is completely absent. The difference among lignocellulosic-based fibres is in the content and chemical structure of the lignin that depends on the fibre originality. Consequently fibres possess different physical-mechanical characteristics that also affect a commercial usage of individual type of fibres [1].

Fibre surface modification or functionalization is of great interest recently since it can bring an added value to the final fibre-based material. Among the processes used,

[1] Bast and other plant fibres, edited by Franck R.R. Woodhead, March 2005 (Ch. 6)
[2] From http://www.fao.org/; Sisal Fibre: Market Opportunities in the Pulp and Paper Industry

the development of new environmentally friendly technologies is an additional advantage [2]. Enzymatic approaches represent an innovative and ecologically friendly way of lignocellulosic materials functionalisation. It was established recently, that the lignin presence in the flax fibres can be used as a substrate for enzymatic graft-polymerisation of different phenolics, resulting in new fibre functionality, like colouration, antibacterial activity and reduced flammability, depending on the nature of the fibres as well as the phenolic molecule used [3][4][5][6].
Laccases are blue copper containing polyphenol oxidases which play an important role both in lignin biosynthesis and lignin biodegradation. Laccases are reported as rather unspecific and able to oxidize several substrates e.g. *o*- and *p*-diphenols, aminophenols, polyamines, lignin, and aryl diamines [3]. In this study two different lignocellulose-based fibres containing different amounts and types of lignin were used in order to investigate the laccase induced grafting of two ortho-substituted phenolics, guaiacolsulfonic acid and 2,4-dimethoxyphenol (Figure 1), for their potential to impart fibre colouration and flame retardancy.

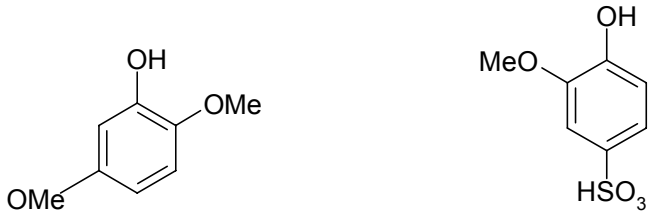

Figure 1: Chemical structure of guaiacolsulfonic acid (GSA, *left*) and 2,4-dimethoxyphenol (DMP, *right*)

MATERIALS AND METHODS

Materials

Sisal and jute fibres, as typical representative of fibres obtained from leaves and steam, were used. The fibres physical and chemical characteristics are presented in tables 1 and 2 [1] and figure 2). Jute as a representative of angiosperms is mainly formed by guaiacylpropane (G) and syringylpropane (S) units, and consequently referred as GS-lignin, while sisal as representative of monocotyledons is formed additionally from -hydroxyphenylpropane (H) units and consequently referred as HGS-lignins. In addition, HGS-lignin type is a subgroup of GS-lignin type. [7]
Phenolic components (GSA and DMP) and laccase *T. versicolor* used in this study were obtained from Sigma. Tanaterge LFN as non-ionic surfactant used as washing agent was purchased from Sybron Chemie. All other chemicals used were of analytical grade.

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[3]. Rittstieg, A. Suurnakki, T. Suortti, K. Kruus, G. Guebitz, J. Buchert. Investigations on the laccase-catalyzed polymerization of lignin model compounds using size-exclusion HPLC. *Enzyme Microb Technol*, 2002, 31, 403–410.
[4] Schroeder M., Krajnc H., Kokol V., Guebitz G.M. Enzymatic finishing and functionalization of lignocellulose based technical fibres. 8th ILI Forum, Rome, Italy 2007.
[5] Schroeder M., Aichernig N., Guebitz G.M., Kokol V. Enzymatic coating of lignocellulosic surfaces with polyphenols. Biotechnol. J. 2007, 2, 334-341.
[6] Schroeder M., Aichernig N., Guebitz G.M., Kokol V. Laccase induced coupling of functional phenolics on lignocellulosic materials, INTB 4, Seoul, Korea, 2006.
[7] Faix O. Classification of Lignins from Different Botanical Origins by FTIR spectroscopy. Holzforschung, 1991, 45, 21-27.

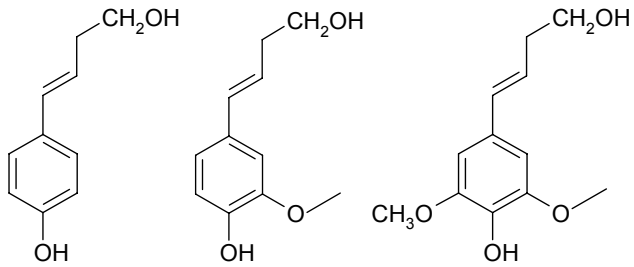

Figure 2: Chemical structure of (*from left*) 4-hydroxyphenylpropane, guaiacylpropane and syringylpropane units formed lignin structure

Table 1: Physical characteristics of bast fibres [1]				
Fibre	Length of fibres [mm]	Length of ultimate fibres [mm]	Diameter [microns]	Density [g/cm^3]
Jute	150-360	0.8-6	5-25	1.4
Sisal	600-1000	0.8-8	100-400	1.2-1.45

Table 2: Chemical composition of bast fibres [%] [1]						
Fibre	Cellulose	Hemicellulose	Pectin	Lignin	Water solubles	Fat and wax
Jute	64.4	12	0.2	11.9	1.1	0.5
Sisal	65.8	12	0.8	9.9	1.2	0.3

Fibre pre-treatment

Fibre pre-treatment was implemented in the process in order to remove impurities and other alloys on fibre surface which could affects their further enzymatic grafting. Namely, after retting and extracting fibres from the stem include moieties like epidermis and phloem residues, surface waxes and pectin (Figure 3). In order to investigate influence of pre-treatment on enzymatic grafting of phenolics, fibres were pre-treated using two different methods. According to the first method fibres were washed in demineralised water at 50°C for 60 min, while in the second method the washing was performed on the same way, but with the addition of non-ionic surfactant. After treatments the fibres were air-dried.

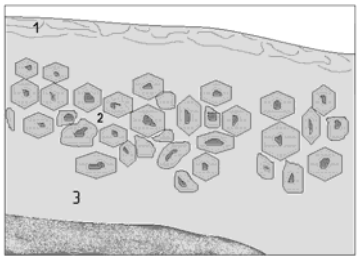

Figure 3: A typical stem cross-section of bast fibres formed by a bundle of ultimate fibres linked together with lignin and pectin as well as some phloem and epidermis residues. Legend: no. 1 epidermis, no. 2 bast fibres, and no. 3 phloem. [8]

[8] Soure: Day A., Ruel K., Neutelings G., Crônier D., David H., Hawkins S., Chabbert B. Lignification in the flax stem: evidence for an unusual lignin in bast fibres. Planta (2005), 222, 234-245.

Enzymatic treatment of fibres

1 g of selected fibres was incubated in 100 ml of citrate phosphate buffer (25 mM, pH 5.0) and in addition of 10mL of different phenolics (12.5 mM), e.g. GSA or DMP, and laccase from *T. versicolor* (10 nkat) for 2 hours at 50°C. After treatment the fibres were rinsed under tab and distilled water and air-dried.

Determination of weight loss

The efficiency of the pre-treatment was evaluated by determining the weight change (ΔW) of dried and conditioned (T=20ºC, RH=65%, t=24 hours) fibres. The weight changing was determined gravimetrically as $\Delta W(\%)=[(W_t-W_i)/W_i]x100$, where W_i is the initial and W_t the final fabric weight.

Determination of colour

Enzymatic treatment resulted in visually obtained colour differences and consequently, colour evaluation using CIELAB colour values (C* - chroma or intensity, L* - lightness takes values from 0 (absolute black) to 100 (absolute white), +a*/ -a* position on red/green axis, +b*/-b* position on yellow/blue axis) was performed. The source of light was a halogen lamp with xenon lightning that gives standardized daylight D65. Colour measuring was performed in a spectral area of 400-700 nm wavelengths using a two-ray SF 600+ spectrophotometer (Datacolor) with an Ulbricht sphere and measuring geometry of d/8°. All samples were measured three times at different areas and the average value was calculated.

FTIR analysis

A fourier-transform infrared spectrophotometer (Perkin – Elmer) with Golden Gate attenuated total reflection (ATR), attachment with a diamante crystal (Specac), was used to confirm the coupling of phenolics on lignocellulosics. The spectra were obtained by accumulating 16 scans within the range 4000 cm^{-1} to 650 cm^{-1} with a resolution of 4 cm^{-1} and intervals of 1 cm^{-1}. All samples were additionally dried at 60°C for 60 min before IR analysis in order to decrease the influence of water OH-groups which could affect on increase of significant lignin peaks [5].

Testing of fibres flammability

Fibres were formed into ropes by twisting and taking into consideration that equal mass and length of the ropes must be achieved before testing. Flammability was expressed as time needed for complete combustion, i.e. from flame initiation to flame termination.

RESULTS AND DISCUSION

Weight loss determination

In order to evaluate the effectiveness of pre-treatment methods on further grafting, the weight-loss of fibres was determined. The weight-losses presented in Table 3 for both types of fibres after different washing procedure confirms the importance of the non-ionic agent in removing the impurities, fats and waxes, which results in a relatively high weight loss. Nevertheless, sisal fibres contain more water soluble substances than jute. Consequently after both pre-treatment methods the weight-loss of sisal fibres was higher.

Table 3: Weight-loss after fibre treatment [%]		
Fibre	Washing with demineralized water	Washing with addition of non-ionic surfactant
Jute	6. 05 +/- 0.2,	7.11+/- 0.3
Sisal	7.12+/- 0.1	8.10+/- 0.5

Colour evaluation

In Tables 4 and 5 CIELAB colour values of untreated, pre-treated and enzymatically phenolics-grafted fibres are collected. The positions of treated fibres on +a* and +b* axis (red/yellow), which indicate colour values in the first quadrant of CIELAB colour space are represented in Figure 4.

It can be seen form the data that the lightness of both fibres was increased after the pre-treatments resulting in more intensive colour. The colour of jute fibres changed from yellowish to slightly reddish but the colour of sisal fibres changed to more yellow; this effect was stronger when the non-ionic surfactant was used in the pre-treatment.

Jute fibres treated with GSA resulted in a lighter, but orange shade in comparison to the pre-treated fibres; the chroma was also increased. In contrast, the polymerization of GSA on sisal resulted in a darker and more intense reddish colour.

In the case of using DMP as a grafting compound, jute fibres resulted in a darker reddish shade compare to untreated and differently pre-treated fibres, while on sisal the coating with DMP yielded less colouration effect, i.e. a bit lighter and more reddish colour with intensity similar to untreated or pre-treated fibres is observed.

Table 4: CIELAB colour values of jute fibres				
	L*	a*	b*	C*
JUTE-US	**53.54**	**5.07**	**18.24**	**18.93**
JUTE-DW	**55.73**	**4.73**	**16.13**	**16.81**
JUTE-NA	**55.08**	**5.74**	**15.27**	**16.32**
JUTE-DW-GSA	**62.31**	**7.14**	**21.67**	**27.50**
JUTE-NA-GSA	**63.98**	**8.28**	**22.85**	**28.04**
JUTE-DW-DMP	**55.01**	**13.38**	**24.03**	**22.81**
JUTE-NA-DMP	**46.91**	**15.28**	**23.51**	**24.31**

Table 5: CIELAB colour values of sisal fibres				
	L*	a*	b*	C*
SISAL-US	**73.25**	**2.66**	**18.87**	**19.06**
SISAL-DW	**75.47**	**1.70**	**15.76**	**15.85**
SISAL-NA	**76.34**	**2.18**	**16.99**	**17.13**
SISAL-DW-GSA	**60.38**	**6.52**	**15.57**	**16.88**
SISAL-NA-GSA	**58.34**	**7.79**	**18.37**	**19.95**
SISAL-DW-DMP	**74.76**	**3.82**	**17.74**	**18.14**
SISAL-NA-DMP	**75.74**	**3.35**	**18.75**	**19.05**

Legend: US – untreated sample, DW – washed with demineralised water, NA – washed with addition of non-ionic washing agent, DW-GSA - grafted with GSA, NA-GSA grafted with GSA, DW-DMP grafted with DMP, NA-DMP grafted with DMP

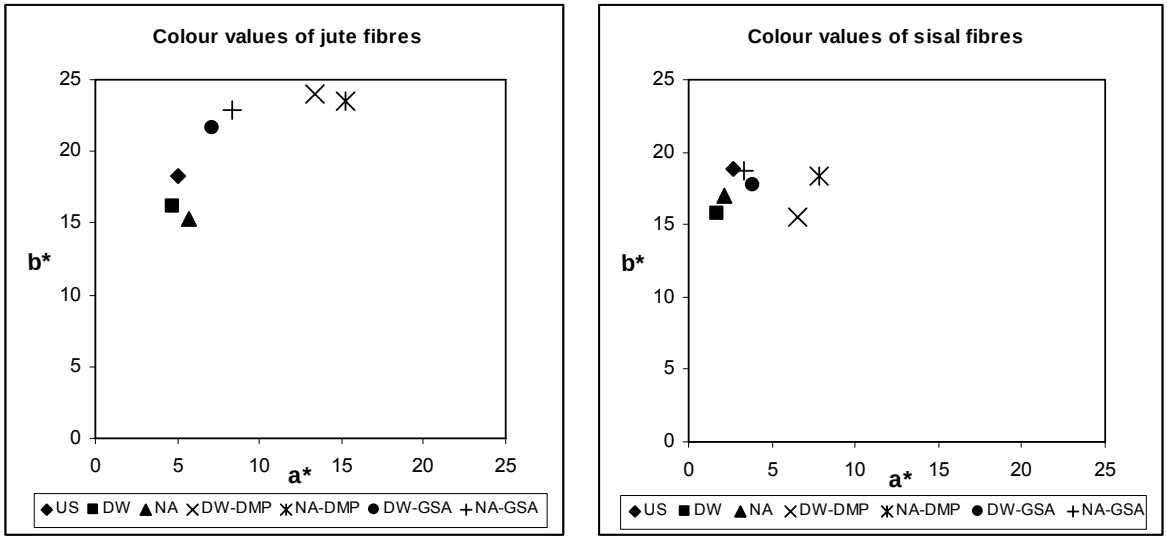

Figure 4: CIE a* and b* coordinates in CIELAB colour space of differently treated jute and sisal fibres

FTIR characterization of treated fibres

The emphasis of the FTIR fibres analysis was in the detection of enzymatic grafting of phenolics onto lignin moieties of fibres. Since laccases preferentially oxidize groups (hydroxyl or methoxyl) presented on ortho-position of an aromatic ring in the phenolic compound, the change in the absorbance of mentioned groups can be observed.

An exhaustive study of IR spectra characterization of different types of lignins was performed by Boeriu et al. (2004) [9] who estimated chemical composition and functional properties of various non-wood, hardwood and softwood lignins isolated by different processing technologies. Among non-wood lignins, IR characterization of wheat straw, sisal, abaca, hemp, jute and flax were performed. It was established a broad band at 3410-3460 cm^{-1} typical for hydroxyl groups on phenolic and aliphatic structures and also bands around 2938 and 2842 cm^{-1} attributed to C-H stretching in aromatic methoxyl groups, and methyl and methylene groups of side chains.

According to Faix (1991) [6] some typical lignin band assignments could be detected in the area from 1800 to 780 cm^{-1}, e.g. around 1030-1035 cm^{-1} aromatic C-H in-plane deformations, around 1166 cm^{-1} C=O in ester groups, which are typical for HGS lignins, and around 1266-1270 cm^{-1} a syringyl and guaiacyl condensed rings.
The above mentioned bands could also be emphasized in the case of the fibres investigated in this study. As it can be seen from the Table 6 the most characteristic peaks in the case of jute fibres were found to be situated at around 3340 cm^{-1} representing absorption bands of phenolic OH stretching. Additionally, characteristic absorption peaks were also found at 1325 cm^{-1} representing syringyl ring (S-ring) with condensed guaiacyl ring (G-ring), at 1265 cm^{-1} represented the G-ring plus C=O stretching and at 1030 cm^{-1} representing aromatic in-plane deformations where G-ring content is higher than S-ring. Table 7 represents some characteristic peaks of untreated and treated sisal fibres. Again, the absorption bands of phenolic OH stretching were found at 3340 cm^{-1} and at 1030 cm^{-1} aromatic in-plane deformations

[9] Boeriu C.G., Bravo D., Gosselink R.J.A., van Dam J.E.G. Characterization of structure-dependant functional properties of lignin with infrared spectroscopy. I*ndustrial Crops and Products*, 2004, 20, 205-218.

plus C=O stretching with additional C=O stretching in ester groups at 1160 cm^{-1}, typical for HGS lignins [6].
From the absorption bands of the most characteristics peaks in IR spectra it can be observed the decrease of absorbance of OH stretching at 3340 cm^{-1} which leads to the assumption that hydroxyl groups of the aromatics in lignin structure might have been coupled to phenolic compounds. In addition, in the case of jute the increase of absorbance of peaks at 1325 cm^{-1} and 1265 cm^{-1} for both phenolics used may be another indicate for phenolic grafting.

The effectiveness of jute fibre treated with GSA is presented also in Figure 5 indicating that peaks of grafted fibres are in good correlation with peaks of GSA.

Table 6: Absorption peaks of jute fibres at different ATR-FTIR spectra

Sample	3340 cm^{-1} (O-H stretch, phenols)	1325 cm^{-1} (S-ring plus G-ring condensed)	1265 cm^{-1} (G-ring plus C=O stretch)	1030 cm^{-1} (aromatic C-H in-plane deform., G>S, C=O deform.)
Jute-US	0.57	0.23	0.28	1.50
Jute-DW	0.53	0.21	0.26	1.49
Jute-NA	0.52	0.23	0.26	1.50
Jute-DW-DMP	0.49	0.25	0.34	1.49
Jute-NA-DMP	0.50	0.27	0.39	1.49
Jute-DW-GSA	0.51	0.26	0.36	1.49
Jute-NA-GSA	0.51	0.27	0.37	1.49

Table 7: Absorption peaks of sisal fibres at different ATR-FTIR spectra

Sample	3340 cm^{-1} (O-H stretch, phenols)	1160 cm^{-1} (C=O in ester groups, typical for HGS lignins)	1030 cm^{-1} (aromatic C-H in-plane deform., G>S, C=O deform.)
Sisal-US	0.64	0.36	1.49
Sisal -DW	0.55	0.35	1.49
Sisal -NA	0.57	0.33	1.49
Sisal -DW-DMP	0.51	0.33	1.49
Sisal -NA-DMP	0.54	0.34	1.49
Sisal -DW-GSA	0.50	0.32	1.49
Sisal -NA-GSA	0.50	0.32	1.49

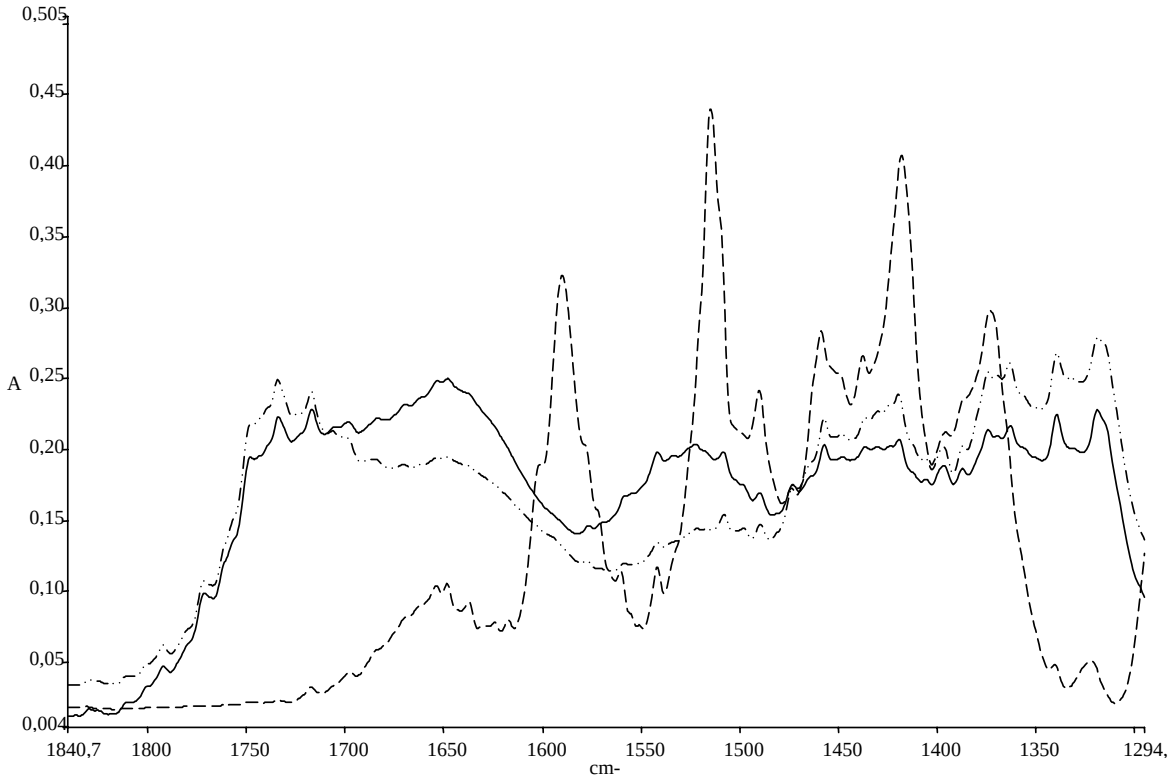

Figure 5: IR spectra of jute fibres grafted with GSA (— — fibres pre-washed with demineralised water, — ▪ ▪ — fibres washed with addition of non-ionic washing agent)

Testing of fibres flammability

In Figure 7 results of time needed for fibres combustion before their treatment and after the treatment with different phenolics are represented. From the results it can be concluded that fibres treated with GSA show no significant improvement in flame retardant properties (around 7.5%). Nevertheless, treatment with DMP improves flame retardant properties by up to 38%.

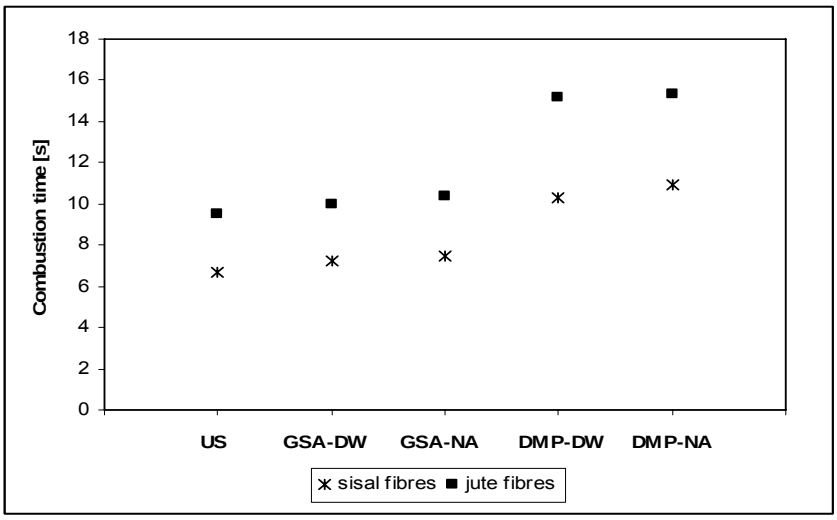

Figure 7: Comparison of combustion time of untreated fibres and fibres grafted with GSA and DMP. Legend: see Tables 4 and 5.

CONCLUSION

Enzymatic grafting of different phenolics (GSA and DMP) onto jute and sisal fibres was studied. The functionalization of different phenolics was increased by the fibres pre-treatment using non-ionic surfactant resulting to improved anti-flammable properties and to colouration, depending on the type of phenolic and the type of lignin used. The effectiveness of grafting of functional phenolics onto lignocellulosic fibres was examined also by ATR-IF indicated that parallel to the enzymatic graft-polymerization of phenolics on lignin surface, the lignin oxidation and its degradation may also occur. Future studies will focus on the elucidation of the grafting mechanism and optimization of reaction conditions.

Acknowledgement
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