Characterisation of Laccase Modified Mukwa (Pterocarpus Angolensis) Wood Flour

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***Abstract*—**Characteristics of natural fibres like low density, biodegradability and low cost have led to their adoption in manufacturing of composites. Regardless of these good features, they exhibit poor compatibility with polymers. Therefore, this paper investigated the effect of laccase modification on mukwa wood flour chemical and morphological properties. The influence of the laccase treatment was study using weight loss analysis. Characterisation such as crystallinity index, surface morphology and chemical composition of untreated and treated flours were determined by X-ray diffraction (XRD), scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR) respectively. It was found through weight loss analysis that laccase treatment decreases the overall weight of the treated flour as impurities were extracted. It was also found that laccase modification improved flour characteristics namely; surface appearance and crystalline packing. Hence the present work inaugurates a base for future developments of laccase modification on mukwa wood flour as an effective approach to possible manufacturing of environmental friendly composites.

***Index Terms*—** Characterisation, laccase, morphology

I. Introduction

Mukwa (Pterocarpus angolensis) is a valuable hardwood species belonging to the leguminous Fabaceae family of about 10-20m in size. Recent, unsustainable harvesting of mukwa for medicine, furniture making, construction and wood-carving has been reported, with a few for research purposes [1]-[4].

Interests on surface modification of hydrophilic natural fiber have grown worldwide for the purpose of manufacturing enhanced wood plastic composites (WPCs), by adopting a variety of treatments. Different modifications have been reported, with thermalisation aiding cellulose hydrolysis of fiber [5]. Alkalisation is known for reducing the water absorption and improving mechanical properties of WPCs by extracting hemicellulose, lignin, surface oils and dewaxing fibre surface [6].

Chemical treatments such as alkalisation [7]-[9], silane [10], benzylation [11], [12], and acetylation [13] have been reported to successfully modify the surface of natural fibers in the production of WPCs having desirable functional characteristics, However these chemical treatments have a major setback of degrading the environment due to their handling and disposal at larger amounts [14]. In addition, analysis of the literature dwelling on the chemical treatments of natural fibers indicates that each of these treatments cannot on their own impart the most desirable functional performance into the natural fibres.

In addressing these concerns, enzymatic treatment of natural fibers has been employed to improve the functional properties of natural fiber [15]-[17]. Laccase is an oxidoredactase enzyme known for modfiying lignin structure as well on its constituent compounds (phenols, polyphenols, methoxy-substituted phenols, anilines, hydroxyindols, aryldiamines and benzenethiols. It aids in either the chemical or physcial bonding of fibres by generating free radicals [18]. Mamum & Bledzki (2013)[16] analysed the effect of lacasse treatment on natural fiber. They reported that enzymatic modification extracted hemicellulose, lignin, fat and protein from wheat husk surface, thereby improving fiber functional properties. Moreover, modification did not damage fiber but rather exposed micro fibers. Laccase enzyme modification exposes more surface –OH group to hydrogen bonding with polymer matrix [19].

Therefore, these present work aimed at investigating the effect of enzyme (laccase) modification on untreated and NaOH pre-treated mukwa wood flours. Weight loss assessment was carried out to determine the overall effect of laccase. Surface morphology through SEM was performed. X-ray diffractometer (XRD) and Fourier transform infrared spectroscope (FTIR) were used to investigate the effect of the treatments on the crystallinity and identification of the functional groups present.

II. Experimental

A. Materials

Waste mukwa wood flour was obtained from a Terry Cooney furniture manufacturing company, based in Gaborone, Botswana. Sodium hydroxide pellets of 99% concentration were obtained from Rochelle Chemicals, South Africa. *Trametes hirsuta* laccase enzyme was a kind gift from Department Biological Sciences and Biotechnology.

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B. Methods

1) Chemical treatment

Waste mukwa wood flour was thoroughly washed with tape water and dried in the sun for a week. The flour was sieved followed by oven drying (to reduce moisture content) at 115 ^{0}C for 24 hours. Thermalised flours were soaked in beakers containing 1%, 3% and 5% aqueous sodium hydroxide for 2.5hrs at room temperature. The alkalised flours were then washed and rinsed with distilled water to remove any excess NaOH prior oven drying at 100 ^{0}C for 24hrs. The samples were stored in airtight containers for laccase treatment.

2) Laccase enzyme treatment

An appropriate amount of alkalised and untreated flours were immersed in a 50 mM sodium acetate buffer of pH 4.5. 300µL laccase and 700µL catechol indicator were added, the mixtures were then incubated in a water bath at 37 ^{0}C and 150 rpm for 24hrs.

C. Weight loss assessment

After laccase treatment, the flours were centrifuged and liquid drained, followed by thorough washed, pH checking was done after every wash until around pH 8 was obtained. An oven at 60 ^{0}C for 24 h was used to dry the flours, followed by weight loss assessment that was calculated as follows:

$$Weightloss(\%) = \left[W_0 - W_1/W_0\right] \times 100$$

Where W_0 was the initial weight of mukwa wood flour before treatment and W_1 the residual weight of mukwa wood flour after treatments.

D. Scanning electron microscopy

The surface morphology of carbon coated untreated and treated mukwa flours were examined using scanning electron microscopy, model JSM-7100F. This was carried out in order to verify the integrity of the mukwa.

E. X--ray diffractometer analysis

Crystallinity index (CrI) of untreated and treated mukwa wood flours was assessed using Bruker D8 advance X-ray diffractometer. The samples were scanned in the 2θ range of 5^0 to 50^0. The CrI of the flours was determined using Segal empirical method [20]:

$$CrI(\%) = \left[I_{002} - I_{am}/I_{002}\right] \times 100$$

Where I_{002} is the crystallographic counter reading and I_{am} is the amorphous phase counter reading at 2θ in the samples

F. Fourier transform infrared spectroscopy

As Fourier transform infrared spectroscopy assessments on untreated and laccase treated were carried out using Perkin Elmer spectrum two ATR-FTIR. About 32 scans for each spectrum at a resolution of 4 cm^{-1} were obtained in the region of 4000-500cm^{-1}.

III. Results and Discussion

A. Weight loss assessment

It is of no doubt that lignocellulosic materials consists of lignin, hemicellulose, cellulose, waxes and fats, which eventually make up the total mass of mukwa wood flour. The weight loss assessment of treated flours depict in Table1.

TABLE I
WEIGHT LOSS ASSESSMENT OF MUKWA WOOD FLOUR

Parameter (%)	Laccase	Alkali(%)+Laccase		
		1	3	5
Weight loss	6.11	-1.66	-9.70	-6.97

The results shown a significant weight loss in laccase treated wood flour without NaOH pre-treatment as indicated by 6.11% (Table1). This can be attributed to the degradation of lignin or phenolic extractives in the wood by the action of laccase enzymes, which adds to the overall weight of the flours, hence exposing more -OH group. Thus in agreement with the work reported by [19]. A similar observation was made by [15] who found that enzymatic treatment on bamboo fibres increased fibre surface thereby opening more pores for enzymatic penetration (increasing weight loss 25 times for autoclave pretreated than un-pretreated bundles) However, a weight gain was observed from all NaOH pre-treated samples, with 3%NaOH+Laccase recording the highest value followed by 5%NaOH+laccase. The weight gain could be due to the addition of catechol as it oxidizes wood thereby depositing radicals on fibre surface.

B. Scanning electron microscopy

The morphological changes due to NaOH pre-treatment and laccase treatment are shown in Fig.1. The images clearly show major differences between untreated and treated samples. As indicated by the SEM image of untreated mukwa flour (Fig.1a), noticeable impurities such as fats, oils and waxes can be observed. It is thought that laccase modification influences both non cellulosic (lignin and hemicellulose) and cellulosic (cellulose) components and surface impurities as well. Micrograph resulting from laccase treatment (Fig.1b) shows a smoother surface in comparison to untreated one. However, surface particles are still visible at a lesser degree. This can be credited to a fact that none of the treatments (on their own) can impart desired qualities, as fibre cleaning was not fully achieved. A review made by [18] found that laccase treatment improved inter bonding strength of fibres in numerous ways like surface smoothness/roughness that encourages mechanical interlocking between fibres

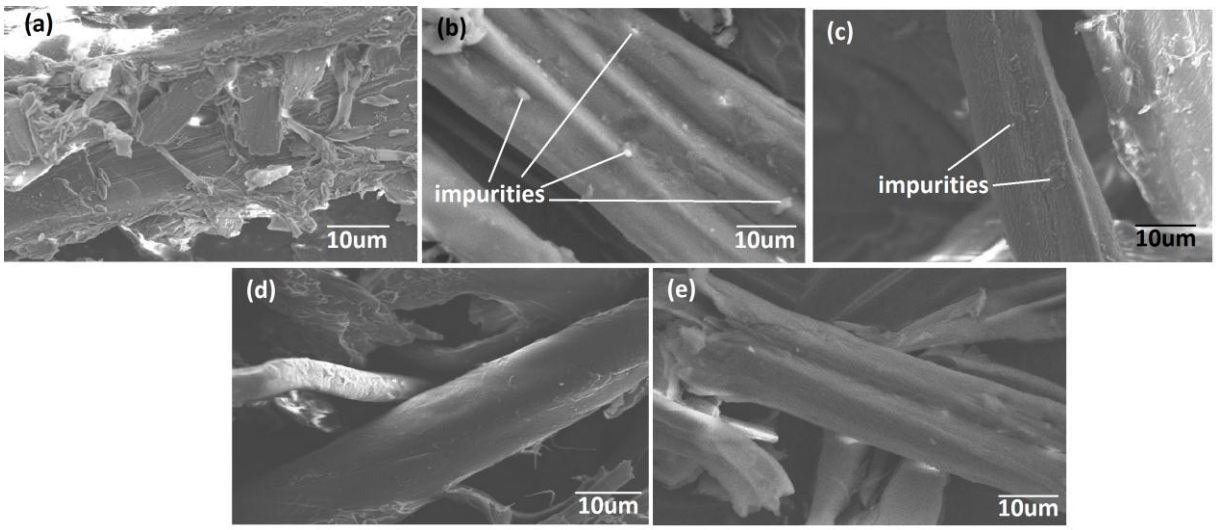

Fig. 1 SEM micrographs of (a) untreated, (b) Laccase treated, (c) 1%NaOH+Laccase, (d) 3%NaOH+Laccase and (e) 5%NaOH+Laccase treated mukwa flours

It was observed that NaOH pre-treatment on mukwa flours had an impact on cleaning and removal of surface impurities as seen on Figs 1(c, d and e). It can be seen that Fig 1d has fewer surface impurities when compared to Fig 1c, suggesting that a combination of laccase and higher concentration of NaOH is more effective in refining fibre surface. Enzymatic treatment resulted in more defined structures as more non-cellulosic materials and surface impurities were extracted. Fig 1e depicts the morphology of 5%NaOH+Laccase. The image produced the best cleaning as more fats, waxes and surface impurities were removed. Also show more pronounced fibre structure with a smoother surface when compared to other SEM micrographs.

The effect of laccase, alkali and alkali/laccase treatments have been reported by [21] who found a decrease in surface lignin concentration with alkali/laccase recording the lowest (18%) when compared to 35, 24 and 20% of untreated, laccase and alkali treatments respectively.

A. X-ray diffractometer analysis

Crystallinity index is one of the principal factors to consider when determining the mechanical and physical behavior of cellulose fibres [22]. Figs 2 and 3 show the X-ray diffractograms and crystallinity index (CI) results of untreated and treated mukwa flours. All spectrums have two major peaks at around 2θ 22.1^0 and 34.4^0. It can be observed that both untreated and treated spectra have a broader amorphous shoulder at around 16^0 2θ. Crystallinity index (CrI) was calculated according to the method described in section II part E, with results presented in Fig 3. Crystallinity index increased by 6.3% as a result of laccase treatment. A similar observation was made by [23] and [22] who studied the effect of laccase on cellulosic fibre and crystallinity index increased up to 22% and 10% respectively.

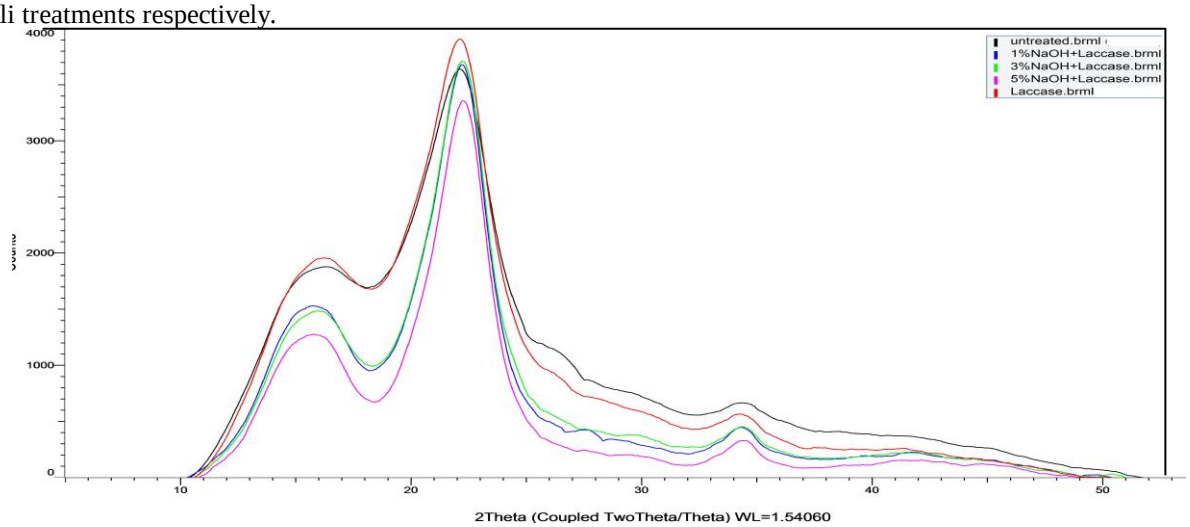

Fig. 2 X-ray diffractograms of untreated; Laccase treated; (1, 3 and 5) %NaOH+Laccase treated mukwa wood flours

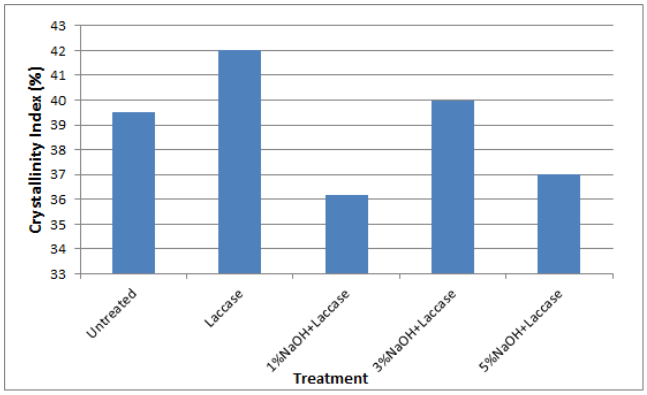

Fig. 3 Crystallinity index (%) of untreated and treated mukwa wood flour

Crystallinity index of flours is shown in Fig3; it can be observed that CI for laccase treated flour recorded the highest value (increase). It is supposed that a rise in crystallinity index is due to the extraction of amorphous lignin loosely attached to the cellulose fibre. The same cannot be said for NaOH pre-treated flours, though alkali treatment is known for increasing CI as observed by other researchers [24] and [25] who found that realignment of cellulose molecules due to loss of amorphous lignin and hemicellulose resulted in an increase of crystallinity. However, 1% and 5 %NaOH+Laccase treated flours have lower crystallinity index than untreated. While 3%NaOH + laccase produced a slight increase in crystallinity index when compared to untreated flour. It is thought that mercerisation increasing the overall crystallinity index thereby improving the order of crystallites and thickening cell walls upon treatment [26]. Laccase oxidizes lignin without affecting the structure of cellulose [18]. Therefore, it can be deduced that both mercerisation and laccase treatment improve crystallites packing order rather than increasing crystallinity index. These results are in agreement with FTIR and surface topography of SEM.

A. Fourier transform infrared microscopy

Fig. 4 shows the changes in FTIR spectra of untreated, laccase and NaOH pretreated laccase modified mukwa wood flours. Both untreated and treated spectrums show a typical pattern of cellulosic compounds as of all wood samples.

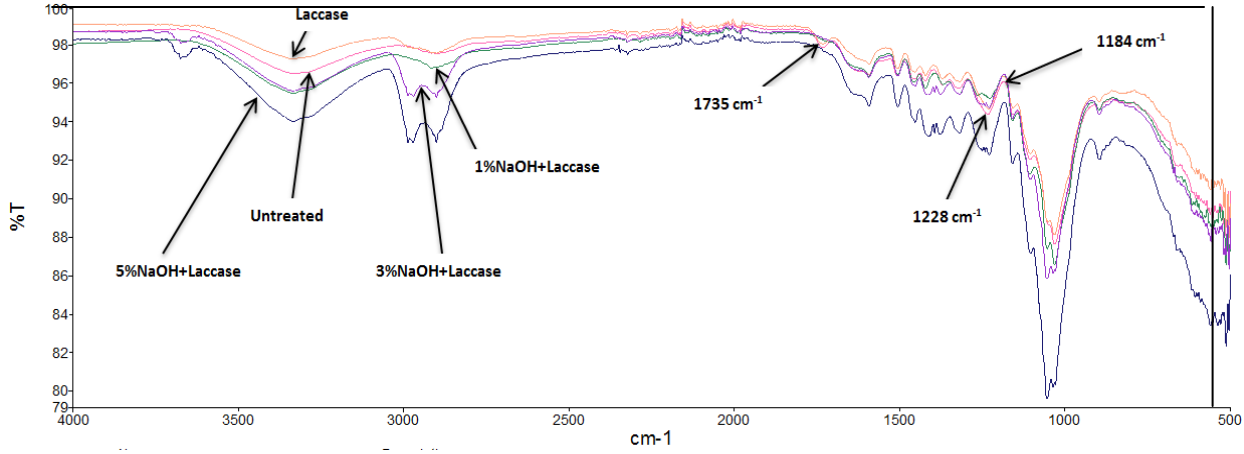

Fig. 4 FTIR spectra of untreated, laccase treated and NaOH pretreated laccase mukwa wood flours

About eight main absorption bands can be found in the FTIR spectra. This can be attributed to different functional groups which play crucial role to the characterisation of mukwa wood flour. Absorption bands between 3300 and 3600 cm^{-1} in FTIR spectra are due to O-H stretching as hydroxyl substitution is not high enough. The spectrum of untreated and laccase treated wood flours are almost similar whereas NaOH pretreatment shown a significant improvement. Strong broad bands of 3% and 5% NaOH pretreated at 3341 and 3334 cm^{-1} respectively can be attributed to aromatic and aliphatic OH functional groups [18]. From Fig. 4, absorbance in the regions 2894-2988 cm^{-1} can be observed, they correspond to C-H stretching, asymmetric and symmetric stretching of methylene (-CH_2-) groups. 3 and 5% NaOH pretreated flours show lower transmittance intensity on the regions of 2988-2900 cm^{-1}, that maybe due interaction of functional groups. The most visible spectrums were at 2988, 2900, 2159, 1735, 1592, 1506, 1228 and 1184 cm^{-1}. For lignin, absorption bands at 1593, 1512, 1450 and 1033 cm^{-1} relate to C=C stretching, C-O stretching, -CH_3 asymmetric and aromatic –CH in plane deformation respectively. For hemicelluloses, the absorption bands focused at 1735 and 1228 are assigned to C=O carbonyl stretching and C-O-C stretching vibrations of ester groups [25]. The absorption bands at 1600-1650 cm^{-1} correspond to bending of absorbed water molecules. A wider absorption band for NaOH+Laccase treated flours focused at 1228 cm^{-1}can be observed. 1735 cm^{-1} band disappeared for NaOH pretreated wood flours due to the NaOH+Laccase degradation of hemicellulose and lignin. The lower transmittance intensity at 1592 cm^{-1} in the 5%NaOH+Laccase treated mukwa wood flour may be due to increased lignin degradation. 1592 and 1506 $cm^{-1}$are assigned the aromatic skeletal vibrations [27]. For cellulose, absorption bands at 1428, 1322, 1155, 1110, 1055 cm^{-1} relate to –CH2 scissoring, -OH bending, C-O-C and C-O stretching vibrations respectively [28]. For hemicellulose and cellulose, strong peak at 1185cm^{-1} correspond to C-O-C

stretching vibration of ester groups while the absorption band at 897cm^{-1} corresponds to β-glucosidic linkages between the sugar units [28]. The intensity of the peak at 1184 cm^{-1} of 5%NaOH+Laccase was weaker suggesting that hemicellulose loss was greater than all other spectrums. Amorphous and crystallinity content of cellulose are assigned cellulose absorption bands 1335-1316 cm^{-1} [22].

Overall, there are strong links that exist between FTIR spectra, the XRD and microstructure (SEM) of laccase modified mukwa.

IV. CONCLUSION

This study investigated the feasibility of laccase treatment and NaOH pre-treatment of laccase modified mukwa wood flours. The following conclusions could be drawn:

1. Laccase treatment saw a 6.11% weight loss while NaOH pretreated flours had a weight gain with 3%NaOH+Laccase recording the highest of 9.70%.
2. The surface topography of NaOH pretreated flours shown smoother fibre surfaces, while 5%NaOH+laccase showing the best results when compared to untreated and laccase treated fibres.
3. Laccase modified flour revealed 6.3% increase in crystallinity index, while 1% and 5%NaOH pretreated shown a decrease of 8.4 and 6.3% respectively.

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