Chapter 3

Isolation and characterization of CMF from *H. sabdariffa*

3.1 INTRODUCTION

Natural fibers consist of the main component cellulose and different amounts of subsidiary or non-cellulosic components such as hemicelluloses, lignin, pectin, wax, etc (Scheme 3.1). The removal of these non-cellulosic components gives rise to a new class of fibers, cellulose microfibers (CMF). Microfibrils are packed together to form the fibril bundles or to produce a single strand fiber (Lu et al (2008); Montanari et al (2005); (Andresen et al (2006); Lu et al (2008)). The critical constituent responsible for natural fiber strength and stiffness are cellulose microfibrils. These microfibrils are highly crystalline materials formed by the aggregation of long thread like bundles of molecules stabilized laterally by hydrogen bonds between hydroxyl groups and oxygens of adjacent molecules (Helbert et al (1996); Kentaro et al (2007)). The resultant stable structure has outstanding mechanical properties including a high Young’s modulus (138 GPa) in the crystal region along the longitudinal direction, tensile strength (1.7 GPa), and a very low coefficient of thermal expansion (10^{-1} K^{-1}) along the longitudinal direction.

Microfibrillated celluloses were first isolated by Turbak and coworkers in the 80s. The preparation of cellulose nano/micro fibers from natural fibers such as coir fiber (Rosa et al (2010)), banana fiber (Deepa et al (2011)), bagasse (Teixeira et al (2009)), wheat straw (Kaushik and Singh (2011)), jute (Wang et al (2009)), hemp (Wang et al (2007)), soybean (Wang and Sain (2007)) have been studied. Selective degradation of fibers results in the formation of micro/nano fibers and their properties depends on their origin, processing techniques adopted etc.

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Two different kinds of approaches have been used to extract highly-purified microfibrils from various sources of natural fibers. One is the chemical method mainly by strong hydrolysis and alkaline treatment. Chemical treatments with alkaline solutions at different concentrations were used to isolate microfibrils from sugar beet (Dufresne et al. (1997)), potato tuber cell (Dufresne et al. (2000)), soya bean (Wang and Sain (2007)), lemon and maize (Rondeau-Mouro et al. (2003)). Liu and Hu (2008) extracted cellulose from bamboo fibers by using sodium hydroxide. Eromosele et al. (1999) treated four different types of natural fibers such as roselle, okra, baobab and kenaf with four different percentage of sodium hydroxide. The effect of alkaline treatment on structural and morphological features of CMF obtained from banana rachis was investigated by Zuluaga et al. (2009). Isolated CMF were characterized using TEM, FTIR and solid $^{13}$CNMR. Potassium hydroxide showed better treatment performance compared to other alkaline treatment.

Cellulose microfibers obtained by strong acid hydrolysis from bagasse and rice straw were reported by Ambuji and Dhake (2008). The characteristic of the obtained microcrystalline cellulose have been compared with Indian standards and commercial

Figure. 3.1: Schematic representation of plant fiber structure
microcrystalline cellulose. The result showed that the lignocellulose waste materials could be converted into valuable microcrystalline cellulose. The isolation and characterization of cellulose microfibers from bagasse were carried out in three distinct steps: conventional pulping, mechanical separation and acid hydrolysis (Bhattacharya et al (2008)). The CMF thus obtained were characterized by SEM, AFM and solid state NMR technique. SEM and AFM studies indicated that the particle size of CMF varied between 200 nm to few microns. Silviya et al (2010) extracted cellulose whisker from banana fibers and studied the effect of reaction time and acid concentration on the properties of the resultant fibers. Zuluaga et al (2007) worked on the isolation of CMF from banana rachis, using a combination of chemical and mechanical treatments. The chemical treatment involved treatment of bleached banana rachis residues with a mixture of 80% acetic acid solution and 70% nitric acid solution at 120 °C for 15 minutes. The washed and purified cellulose was sonicated for 15 minutes. In the mechanical process, a bleached residue was suspended in water and homogenized. It has been noted that, acid treated products resulted in aggregation of cellulose microcrystallites, whereas individualized microfibrils were obtained by homogenization. Cellulose nanospheres were synthesized from cotton fibers by using acid treatment (6:1:3 = water: HCl: H₂SO₄) (Zhang et al (2007)). The product consisted of two different particle size species, averaging approximately 500 nm and 70–200 nm. The study revealed that cellulose nanoparticles of smaller sizes could be obtained by a further acidic sonication of the initially sonicated cellulose fibers. The authors pointed out that there was a linear relationship between the size of the cellulose nanoparticles and the treatment time. Another observation was that the initial cellulose sample was cellulose I, whereas the obtained cellulose spherical particles were of polymorphic character cellulose II.

The other way by which CMF can be obtained is the mechanical method by super grinder treatment (Chakraborty et al (2005); Iwamoto et al (2005); Taniguchi (1996); Yano et al (2005)), microfluidizer (Zimmermann (2004)), high-pressure homogenizer treatment (Dufresne et al (1997); Herrick (1983); Nakagaito and Yano (2005); Yano et al (2005a)). Microfibrillated celluloses from wheat straw and soy hulls have been extracted using cryocrushing by Alemdar and Sain (2009). The obtained microfibrillated celluloses have a diameter in the range of 30 - 40 μm. Iwamoto et al (2008) obtained fibrillated celluloses from Pinus radiata by passing two or three times through the grinder. Spence et al (2011) made a comparative study on the energy consuming and physical properties of MFC obtained by different mechanical treatment such as homogenizer, microfluidizer and grinder from unbleached and bleached krafts hard wood pulps. The report shows that the homogenizer process is the best mechanical treatment to extract MFC with less energy consumption and high surface area.
Steam explosion is an excellent alternative for the conventional methods such as CMP (chemical mechanical pulps) and chemical thermo mechanical process. The principles of steam explosion technique are the steam under pressure with increased temperature penetrates through the space between the fibers, thus the middle lamella and the fiber adherent substance becomes soft and water soluble (Kessler et al (1998)); Excoffier et al (1991)). Marchessault (1988) described steam explosion process as an auto-hydrolysis and the function of the process based on biomass has been described by them as, i) the cleavage of glycosidic bond, β-ether linkage and lignin-carbohydrate complex bond ii) some chemical modification in lignin and carbohydrate. The steam explosion process was first introduced by Mason in 1927 to defibrillate wood fiber for board production. Steam explosion is a high yield pulping process based on vapor phase at temperature in the range 80 °C – 210 °C.

The present chapter discusses the properties of CMF obtained from H. sabdariffa. The CMF were obtained as described in chapter 2 (2.2.1). The structural and morphology of fibers thus extracted were analyzed in detail using SEM, optical microscopy, FTIR and XRD. TGA of fibers were carried out to study the thermal stability of the extracted fibers.

3.2 RESULTS AND DISCUSSION

3.2.1 CHEMICAL COMPOSITION OF FIBERS

<table>
<thead>
<tr>
<th>Materials</th>
<th>Cellulose (%)</th>
<th>Hemicellulose (%)</th>
<th>Holocellulose (%)</th>
<th>Lignin (%)</th>
<th>Moisture content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw fibers</td>
<td>63.5</td>
<td>17.5</td>
<td>46</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>CMF</td>
<td>81.5</td>
<td>4</td>
<td>77</td>
<td>1.5</td>
<td>9</td>
</tr>
</tbody>
</table>

The chemical compositions of the fibers are given in Table. 3.2.1. The treated fibers shows lower percentage of hemicellulose, lignin and higher percentage of α-cellulose when compared with untreated fibers. When the raw fiber was subjected to steam explosion process, the 1-4 glycosidic linkages between hemicellulose and lignin gets hydrolyzed, leading to depolymerisation of lignin and hydrolysis of hemicellulose. The process dissolves hemicellulose and lignin and paves way for the formation of water soluble sugar and phenolic content. High pressure steaming followed by rapid decompression is called steam explosion. The steam explosion process includes saturating the dry material with steam at elevated pressure and temperature followed by sudden release of pressure, during which the flash evaporation of water exerts a thermo -mechanical force causing the material to rupture. Steam explosion in alkaline medium results in the hydrolysis of hemicellulose within the fiber. The resulting sugars can be subsequently washed out in water, leaving a residue of cellulose and
lignin. It also leads to the cleavage of hemicellulose–lignin bonds. The reaction results in an increased water solubilisation of hemicellulose and in an increased solubility of lignin in alkaline solvent, leaving the cellulose as a solid residue with a reduced degree of polymerization. Yamashiki et al. (1990) proposed an explanation for the solubility of steam exploded cellulose in NaOH solution, suggesting that during steam explosion there is a partial break down of the intramolecular hydrogen bond at C-3 and C-6 position of the glucopyranose unit. This results in significant variation in the network and strength of the cellulose. However, the complete removal of these components does not take place during steam explosion; it results in the changes in the arrangements of macromolecular chain.

Bleaching process carried out using sodium hypochlorite helps to remove majority of lignin content of fibers. This resulted in further solubilisation of lignin and hemicelluloses. Also, it resulted in the formation of pits on the fiber surface which in turn increases the surface area of the fibers (Mohanty et al. (2001)). The bleaching treatment was performed to break down phenolic compounds or molecules having chromophoric groups present in lignin and to remove the by-products of such breakdown. During bleaching, lignin was oxidized and became soluble in the alkaline medium. Also, steam explosion at this stage resulted in the removal of other additives and impurities from the external and internal surface of the fiber. It has been reported that cementing materials like lignin and hemicellulose gets dissolved predominantly during the bleaching process (Yaqoob et al. (2011)).

The impacts of acid treatment on natural fibers were investigated previously by many researchers (Dong et al. (1998); Araki et al. (2001); Viet et al. (2007)). Xiao et al. (2010) proposed that during steam explosion, the hydrolysis of glycosidic linkages in hemicellulose and ether linkages in lignin were catalyzed by acetic acid. In the present case the acid hydrolysis was carried using oxalic acid. At the end of the process, the steam was suddenly released providing additional mechanical defibrillation. The hydrolysis with acid results in the complete dispersion of the microfibers.

The moisture content of cellulose microfibers thus obtained is found to be higher than that of the raw fibers (Table. 3.2.1). Moisture content is an important parameter that determines the properties and end uses of the fibers. During alkali treatment process, some alkali labile linkages (ether and ester linkages) between lignin monomers or between lignin and polysaccharide may be broken. In addition, steam explosion and bleaching treatments acted to remove some loose substance from the fiber surface, leaving a hard structure that was difficult to dissolve in the caustic solution. Therefore, the percentage of cellulose content increases. As the percentage of the cellulose content increase the moisture absorption rate also increased. This is because, the chemical structure of cellulose contains three hydroxyl groups,
which in the macromolecular cellulose structure forms hydrogen bonds. When the fibers were exposed to alkaline medium, swelling of the fibers as well as development of strongly hydrophilic ionic groups on the fiber surface were induced, promoting absorption of moisture. Chemical treatment results in the removal of surface materials thereby opening up the fiber with more scooped individual cells (Ajayi et al (2000)). The removal of surface impurities exposes the hydroxyl groups (Eromosele et al (1999)). The removal of surface impurities and exposure of hydroxyl groups on the microfibers cause increased moisture absorption.

3.2.2 FTIR STUDIES

Figure. 3.2.2 shows the FTIR spectrum of raw fiber and fibers at each stage of treatment. The peak at 1729 cm$^{-1}$ in raw fibers is due to either the acetal and uronic ester groups of the hemicellulose or the ester linkage of the carboxylic group of the ferulic and p-coumeric acid of lignin and hemicelluloses (Cherian et al (2008); Sun et al (2005)). The change in intensity of the peak at 1729 cm$^{-1}$ is more evident from stage II to III indicating that the removal of non-cellulosic part is mostly in the bleaching stage. The C=C stretch from aromatic ring of lignin gives two peaks at 1504 cm$^{-1}$ and 1428 cm$^{-1}$ as observed in untreated fibers. The peak at 2917 cm$^{-1}$ is due to the aliphatic C-H stretching vibration which has shown some changes in absorbance after treatment, due to the changes in chemical composition during removal of lignin from the fibers. The hydrophilic tendency of raw fibers and chemically treated fibers is reflected in the broad absorption band in the 3700 – 3100 cm$^{-1}$ region which is related to the $–$OH groups present in the main component. The peak at 3288 cm$^{-1}$ in the spectra of treated fibers shows the stretching vibration of hydrogen bonding of the hydroxyl groups. In the FTIR spectrum of fibers in stage I, the absorbance at 3700 to 3100 cm$^{-1}$ of $–$OH stretching is not clearly visible, due to interfering contributions from a variety of stretching modes in the amorphous regions (Liang and Marchessault, (1959)). The band at 1633 cm$^{-1}$ is due to the carboxylate group and also due to the bending mode of the absorbed water in the treated fibers (Sun et al (2005)). The band at 1504 cm$^{-1}$ has almost disappeared and also the intensity of the 1428 cm$^{-1}$ has been decreased after the treatment. It indicates the removal of lignin from the fibers. The band at 1372 cm$^{-1}$ is due to the asymmetric C-H deformation. The sharp peak at 1245 cm$^{-1}$ has sharply decreased after chemical treatment indicating the removal of hemicellulose.
The band region between 1202 cm\(^{-1}\) to 1024 cm\(^{-1}\) represents the C-O stretch band and deformation bands in cellulose, lignin and residual hemicellulose (Sun et al. (2005)). The increase in the intensity of the peak at 896 cm\(^{-1}\) indicates the typical structure of cellulose (due to β-glycosidic 1-4 linkage of glucose ring in cellulose (Ganan et al. (2004))).
XRD STUDIES

Figure 3.2.3: XRD pattern of raw fiber and CMF

XRD patterns of raw fiber and CMF are given in Figure 3.2.3. XRD analysis of the treated and untreated fibers has been done in order to determine the structural and chemical change of the treated fibers. Cellulosic fibers consist of three components namely lignin, hemicellulose and α-cellulose. Cellulose shows crystalline nature while lignin is amorphous in nature. As a result, the crystallinity of the fibers should increase after treatment. The cellulosic fibers are surrounded by non-cellulosic polysaccharides such as hemicellulose and lignin matrix. The increase in the overall order of the hydrolyzed fibers can be attributed to the removal of the hemicellulose and lignin during the chemical treatment. The diffractograms, probably representing typical cellulose diffractograms, shows a peak at 2θ = 22° and a shoulder in the region 2θ = 14°-17° (Bodor (1991)), which indicates presence of cellulose. The treated fibers shows strong crystalline nature due to its higher diffraction intensity at 2θ = 22° (Bondsen et al (2006)). CMF shows diffraction intensity at 22.5° and a shoulder in the region 2θ = 14°-17° and also exhibits peaks at some lower intensity such as 14.2°, 17.05° and 34.4°. The broad peaks of the raw fibers are due to the amorphous nature of the lignin where as α- celluloses are crystalline in nature. The diffraction intensity peak at 2θ
corresponds to the 002 lattice plane of the treated fibers. The higher peak intensity of the treated fibers indicates the removal of non-cellulose. The crystallinity index of the cellulose microfibers has been increased to 78.95% from 53.21%. The crystallinity order index has been increased to 81.05% from 58.41% after chemical treatment. The additional peaks observed in treated fibers may be due to the rearrangement of fibrils in the interfibrillar regions giving rise to new crystalline regions. Only the amorphous regions and crystal surfaces in the cellulose structure, that is, the cementing materials, can react with alkali and be removed. Thus, the interfibrillar regions are likely to be less dense and less rigid and thereby make the fibrils more capable of rearranging by themselves (Gassan and Bledzki (1999)).

3.2.4 MORPHOLOGY ANALYSIS

Figure. 3.2.4.1: Optical images of (a) raw fibers and (b) CMF

Figure. 3.2.4.2: SEM images of (a) raw fibers and (b) CMF

Optical images of raw fiber and CMF are shown in Figure. 3.2.4.1. The images showed excellent difference in both diameter and length of CMF from that of raw fibers. The
diameter and length of the fibers were reduced drastically with chemical treatment. This is further supported by SEM photographs as shown in Figure. 3.2.4.2. The raw fiber shows a diameter of 90 µm while the treated fiber shows an average diameter of 10.04 µm and length of 198.8 µm.

3.2.5 THERMAL CHARACTERIZATION OF TREATED FIBER

The thermogram of raw fiber and CMF are given in Figure. 3.2.5. CMF shows an onset degradation temperature at 78 °C, the onset degradation of untreated fibers is below 50 °C. The first step of weight loss noted between 60–250 °C in the thermogram corresponds to the heat required for the vaporization of moisture content in the fiber. The second-step weight loss is noted between 300–350 °C corresponding to the depolymerisation of hemicellulose and lignin. The degradation and decomposition of lignin content occurs in the temperature range of 80–150 °C. However, the cellulosic degradation starts at still higher temperature (Yang and Kokot (1996)). The thermal stability of the cellulose is higher at lower temperature due to the hydrogen bonding. A greater crystalline structure requires a higher degradation temperature (Ouajai et al (2005)). However, both non-cellulosic components and the crystalline order of cellulose plays an important role in thermal degradation of the fibers.

![Figure. 3.2.5: TGA graphs of raw fiber and CMF](image-url)
The structure and crystallinity of cellulose are related to the degradation of the sample. This indicates that the crystalline sample have higher thermal stability. Due to its higher cellulose content and lower percentage of other non-cellulosic content, CMF shows higher thermal stability at low temperature. After 380°C, the residual decomposition products maintain a slow degradation profile (Sakhawy and Hassan (2007)). Cellulose is responsible for the strength of the fiber which has been proved by the requirement of higher temperature for the degradation of treated fibers. Whereas lignin is responsible for the char formation of fibers. Thus the raw fiber shows higher ash content and CMF shows lesser ash content.