IMIDAZOLIUM IONIC LIQUIDS AS DISSOLVING SOLVENTS FOR CHEMICAL-GRADE CELLULOSE IN THE DETERMINATION OF FATTY ACIDS USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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A quick, simple, and environmentally friendly sample preparation method for fatty acids analysis from chemical-grade cellulose was developed employing imidazolium based ionic liquids as solvents. A variety of imidazolium based ionic liquids were screened for their ability to dissolve chemical cellulose at different temperatures. Dissolution of chemical cellulose was observed to be dependent on the ionic liquids' anions as well as temperature. The effect of ionic liquids on chemical cellulose was examined using FT-IR and TGA techniques, which showed no difference between the original and the regenerated cellulose except for the percentage mass residues in TGA profile which was high for regenerated cellulose, 15% compared to 8% of the original at 600 °C. Fatty acids extracted from cellulose were found to be predominant in the samples from different bleaching stages, with high levels in the oxygen delignification and low levels were observed in hypochlorite bleached samples. The number and levels of the identified fatty acids were observed to decrease with the bleaching sequence. The recoveries obtained using this method ranged from 90 to 107%.

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INTRODUCTION

Industrial grade chemical cellulose (a special grade of bleached sulfite pulp) refers to the cellulose that contains more than 90% pure cellulose (Jahan et al. 2008) obtained from both soft and hard woody plants. The preparation of chemical-grade cellulose involves the cooking of woods in a chemical solution called 'cooking liquor' at an appropriate temperature to remove all non-cellulosic compounds in the fibers. Apart from cellulose, wood contains other compounds such as lignin, hemicelluloses and a small fraction of organic extractives (wood extractives) (3 to 5 %) (Horvath 2006). During the pulping process, non-cellulosic compounds such as lignin and extractives are removed. However, due to the differences in chemical structures of these compounds and their location in the plant cells, they commonly survive the pulping process. This results in the presence of extractives in the final product of pulping (cellulose fibers). The types

of extractives that survive the pulping process are governed by pulping process, the chemical structures, and the bleaching chemicals used (Gutiérrez et al. 2008). For instance, fatty acids, glycerides and sterols are reported to be predominant in sulphite pulping compared to alkaline pulping processes because acidic condition is not able to break down these extractives into soluble sodium soaps; hence, sulphite pulps contain higher amounts of extractives compared to kraft pulps (Sithole et al. 2010). Extractives are located in different plant cells; some of these plant cells such as ray parenchyma have tiny holes (Horvath 2006; Challinor 1996), which make it difficult for the cooking liquor to penetrate during cooking stage of pulping and hence cause them to survive the process to the final product due to the low accessibility of these compounds in the fibre structure (Freire et al. 2006)

Wood extractives are a heterogeneous group of organic compounds obtained from wood by either organic solvent or water extraction. They constitute a small fraction of compounds in a plant but they cause very serious problems in the process of chemical cellulose production and the quality of the final products (Silvério et al. 2008). It is this fraction of compounds that cause high consumption of bleaching chemicals as well as pitch in the pulping machines, which often lead to the suspension of production for servicing. They also cause black spots in the final product of pulp sheet and are responsible for the low quality of chemical cellulose, as they form colloidal particles which coalesce into droplets that from deposits in pulp (Gutierrez et al. 2001). Extractives enriched on the fiber surfaces tend to decrease the fiber-fiber bonding ability (Asikainen et al. 2010), and they also affect reactivity during viscose production. Organic extractives that remain in chemical cellulose are mainly comprised of sterols, fatty acids, steryl esters, triglycerides, hydrocarbons, steroid hydrocarbons, fatty alcohols and ketone (Gutierrez et al.1998; Dorado et al. 2000; Sun and Tomkinson 2003)

Different analytical methods have been reported for the extraction of organic extractives from chemical cellulose prior to the gas chromatography mass spectrometry analysis. However, most of the extraction methods either take too long and are too laborious or they use large amounts of volatile organic solvents that are environmentally unfriendly and hazardous. Extraction methods, such as soxhlet extraction, are widely used. Gutiérrez et al (2008) used soxhlet extraction for 8 h per sample (Gutiérrez et al. 2008). The same extraction procedure was also reported by Freire et al. (2005), where they used 750 mL of dichloromethane for 16 h followed by ethyl acetate for another 16 h of extraction. Freire et al. (2006) also reported on the use of soxhlet extraction for 6 h per sample using acetone. Silvério, et al. (2008) also reported the use of soxhlet extraction using acetone for 6 h per sample.

With all the disadvantages of using large amounts, volatile solvents are still not able to extract all of the organic compounds in the chemical cellulose, because most of the organic extractives that survive the pulping process are trapped in cellulose fibers. Organic solvents extractions depend on their penetration capability to capture the trapped compounds. It is therefore imperative to develop an alternative analytical method with the strengths lacking in the previous developed methods to determine the fatty acids that survive pulping processes. This will enable a smooth monitoring of wood extractives throughout the pulping process so as to predict and control the effects caused by them. In

this study we report the use of imidazolium ionic liquids as solvents for chemical cellulose.

Imidazolium ionic liquids, just like all other ionic liquids, are organic salts consisting of cations and anions, and most of them are liquids at room temperature (Kubisa 2004; Kiefer et al. 2008). They have high solvent capacity and dissolve polymers as well as a wide range of organic compounds (Kiefer et al. 2008; Kline et al. 2010). Their solvating power is due to the interaction in the biopolymer matrix, such as formation of hydrogen bonding from the hydroxyl function to the anions of the solvent (Swatloski et al. 2002). Ionic liquids have recently been reported as 'green solvents' and considered to be environmentally friendly due to their negligible vapour pressure (Fort et al. 2007); hence, they cannot emit volatile organic compounds and they are also non-flammable (Earle and Seddon 2000; Zhu et al. 2006; Kline et al. 2010). Thus, they can eliminate or reduce the chemical release to the environment. Due to the strength of imidazolium ionic liquids in the dissolution of chemical cellulose, the extraction of the trapped organic compounds (extractives) becomes more efficient. The extractives are released into the solution, and hence pure cellulose is regenerated by the addition of precipitation solvent such as water, methanol, acetone, acetonitrile, etc. Extraction of analytes can therefore be easily achieved by the use of a small amount of organic solvents to the ionic liquid-aqueous filtrate. This approach is advantageous due to the fact that it is essentially one process; it regenerates pure cellulose from the cellulose-ionic liquid solution which is difficult for a normal organic solvent.

EXPERIMENTAL

Samples

Samples were obtained from the chemical cellulose industry in South Africa. They were collected from different bleaching stages namely; oxygen delignification (a stage just after cooking, sometimes referred to as an extended cooking stage, in which oxygen is added to oxidize most of the organic compounds so that they can be removed during the next washing stage), first washing (D1) stage, post caustic oxidation (E) stage, and hypochlorite bleaching (H) stage (final bleaching stage).

Chemicals and Reagents

1-butyl-3-methylimidazolium chloride [BMIM]Cl (98.0% purity), 1-ethyl-3-methylimidazolium chloride [EMIM]Cl (98.0% purity), 1-butyl-3-imidazolium acetate [BMIM]Ac (97.0% purity), 1-ethyl-3-imidazolium acetate [EMIM]Ac (97.0% purity), 1-butyl-3-imidazolium methylsulphate [BMIM]MESO$_4$, 1-butyl-3-imidazolium hexafluoro phosphate [BMIM]FP$_6$ (97.0% purity), HPLC grade methanol ($\geq$ 99.9% purity), HPLC grade acetone ($\geq$ 99.8% purity), hexane (85.0% purity), ethyl acetate (99.7% purity), hydrochloric acid (37.0% purity), hexadecanoic acid (99.0% purity), undecanoic acid (99.0% purity), and sodium sulphate anhydrous (99 to 100.5% purity), were all purchased from Sigma Aldrich (Steinheim, Germany).

Experimental Procedure

During dissolution of cellulose, 0.3 g of chemical cellulose samples, which were initially ground in a blender, were dissolved in 5.7 g of molten 1-butyl-3-methylimidazolium chloride [BMIM]Cl to obtain a 5% solution of cellulose. Samples were made in two sets in duplicate; one set was spiked with 0.001 g of a mixture of hexadecanoic and undecanoic acids. Dissolution of cellulose was performed at 90°C in an oil bath. After forming the solution of cellulose, 25 mL of distilled water was added to regenerate cellulose, followed by filtering using a 0.2 μm syringe filter. Filtered cellulose was washed using 5 mL of acetone, which made the total volume of the filtrate 30 mL. Thus the concentration of spiked compounds was 33.33 μg/mL. The pH values of the filtrates were recorded and adjusted to 2 using 1 M HCl and/or ammonia solution before extraction. The filtrates were then extracted for 20 minutes using 10 mL of hexane followed by hexane: ethyl acetate, 2:1% v/v, 2 x 10 mL. The extracts were combined and evaporated to dryness using a rotary evaporator at 40°C. The dried extracts were then dissolved in 1 mL of acetone and derivatized.

Derivatization

A portion of each of these extracts was derivatized using methanolic acid (3 M HCl) at a ratio of 1:2 v/v to the sample extracts, then heated for 1 h at 60°C in an oil bath. Derivatized extracts were analysed by gas chromatography mass spectrometry (GC-MS).

GC-MS Conditions

The GC-MS analysis was performed on an Agilent GC-MS, 7890A GC System with a triple-axis detector (5975C MSD) using an HP-5MS column (30m x 0.25 um x 0.25 um). The MS conditions of MS were as follows: Helium was used as the carrier gas. The samples were injected with an autoinjector (Agilent Technologies 7693 Auto-sampler). The temperature of the injector was set at 250°C, and the oven column was temperature-programmed from 80°C (1 min.) to 290°C at a rate of 15°C/min holding for 12 min. The column flow was 0.7 mL/min. The temperature of the transfer line and MS source was set at 280°C and 230°C, respectively. Compounds were identified by comparison of the mass spectra with those in NIST libraries, by mass fragmentation, and by comparison with standard compounds for those standards which were available.

FTIR and TGA Analysis of Original and Regenerated Cellulose

Both the original as well as the regenerated cellulose samples were thoroughly washed using acetone followed by de-ionized water then dried in oven at 90°C to make them ready for FTIR and TGA measurements.

FTIR spectroscopic measurements were performed using an FTIR spectrometer (Spectrum 100) from Perkin Elmer (Shelton, USA) with attached diamond attenuated total reflectance (ATR). The spectra were recorded from 4000 to 650 cm^{-1} in the transmittance mode with 4 scans per spectrum at a resolution of 4 cm^{-1}.

TGA measurements on the other hand were performed on a thermal gravimetric analyzer (TGA 4000) from Perkin Elmer (Shelton, USA). About 10 mg of the sample was placed in a platinum sample holder pan for analysis. TGA curves were recorded from 80°C to 700°C temperatures at a rate of 10 °C/min under nitrogen flow of 20 mL/min.

RESULTS AND DISCUSSION

Dissolution of Chemical Cellulose

Before performing the analysis of fatty acids, the dissolution capacity of chemical cellulose in different ionic liquids was investigated at different temperatures, ranging from 50 to 90°C (Table 1). The time needed for complete dissolution was observed to be between 7 and 45 min, depending on the temperature of the oil bath. Cellulose was found to be more soluble in imidazolium-based ionic liquids with acetate and chloride anions, while those with methylsulphate and hexafluoro phosphate anions could not dissolve cellulose; instead, the cellulose remained suspended. This can be explained by the nature of the ionic liquid used, i.e., the type of cation or anion of ionic liquids. It has been reported that ionic liquids that incorporate anions with strong hydrogen bonding acceptors, such as Cl^- or acetate, are the most effective solvents for chemical cellulose due to their ability to disrupt the extensive hydrogen bonding network of cellulose and lead to its dissolution, while those which contain 'non coordinating' anions such as PF_6^- are unsuitable solvents (Swatloski et al. 2002; Feng 2008; Spiridon et al. 2010). Spiridon et al. (2010) reported evidence of the interactions of chloride anions with hydroxyl groups of cellulose by NMR method in a solution of cellulose in [BMIM]Cl. The viscosity of ionic liquids also plays a crucial role in the dissolution of cellulose a function of structure and basicity of anion (Kilpeläinen et al. 2007).

Table 1. Dissolution of chemical cellulose (cellulose) in different ionic liquids

| Ionic Liquid	Percentage of cellulose (%)	Temperature / °C	Solubility
[BMIM]Cl	5	70	soluble
[BMIM]Ac	5	70	soluble
[EMIM]Cl	5	70	soluble
[EMIM]Ac	5	70	soluble
[BMIM]FP$_6$	5	70	insoluble
[BMIM]MESO$_4$	5	70	insoluble
[BMIM]Cl	5	90	soluble
[BMIM]Ac	5	90	soluble
[EMIM]Cl	5	90	soluble
[EMIM]Ac	5	90	soluble
[BMIM]FP$_6$	5	90	insoluble
[BMIM]MESO$_4$	5	90	insoluble

The influence of viscosity in this study was evident due to the fact that the two ionic liquids that could not dissolve cellulose are less viscous compared to the acetate and chloride ionic liquid solvents. Apart from the effect of the type of anion and viscosity in dissolution, high temperatures also increase the interaction of ionic liquid anions to the hydroxyl of cellulose, due to the reduction of viscosity (Vitz et al. 2009). However, very high temperatures and/or a large amount of cellulose used during dissolution may result in degradation of cellulose by pyrolysis (Vitz et al. 2009; Spiridon et al. 2010). Degradation of cellulose in ionic liquids can easily be observed by a deep coloration of the solution (Vitz et al. 2009). In this study therefore, higher temperatures were avoided

by maintaining the dissolution temperature at 90°C. In general, chlorides and acetate ionic liquids showed good capability of dissolving cellulose. These results were in agreement with what was reported previously about the capacity of imidazolium based ionic liquids to dissolve biomass components at different levels (Kline et al. 2010). For instance, 1-butyl-3-imidazolium acetate and 1-ethyl-3-imidazolium acetate could dissolve 5% w/w of cellulose at 70°C for 7 min. while 1-butyl-3-methylimidazolium chloride could dissolve 5% w/w of cellulose for 9 min.

The trend of dissolution trend was observed as follows: 1-butyl-3-imidazolium acetate > 1-ethyl-3-imidazolium acetate > 1-butyl-3-methylimidazolium chloride > 1-ethyl-3-methylimidazolium chloride, whereas those ionic liquids with methylsulphate and hexafluoro phosphate anions were found to be non-solvents. Dissolution at temperatures below 70°C was also investigated. At 50°C the dissolution was carried out for 45 min but no complete dissolution was formed, even with ionic liquid that had shown high ability of chemical cellulose at relatively higher temperature.

Characterization of Original and Regenerated Cellulose

Cellulose regenerated from prepared sample solutions by the addition of water was washed, dried, and characterized by Fourier transform infrared (FTIR) spectroscopy for comparison with the spectra of the original cellulose to investigate the presence of any degradation or reaction of cellulose caused by ionic liquids. Original and regenerated cellulose from ionic liquid solutions were as well analysed for thermal stability using thermal gravimetric analyzer (TGA). The spectra obtained from FTIR are shown in Fig. 1, whereas TGA curves are depicted in Fig. 2.
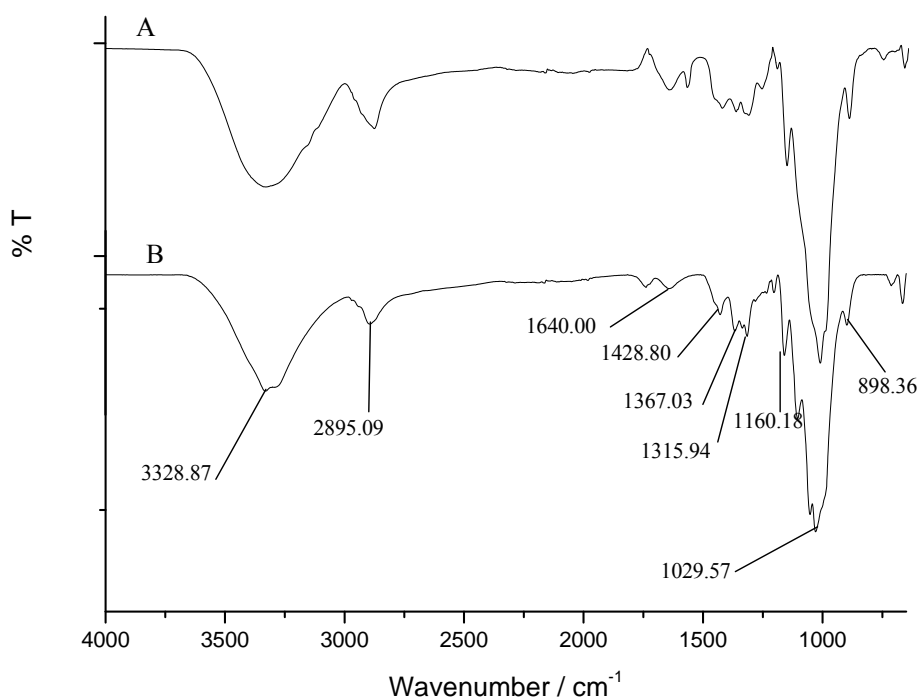
Fig. 1. FTIR spectra of regenerated cellulose from ionic liquid (A) and original cellulose (B)
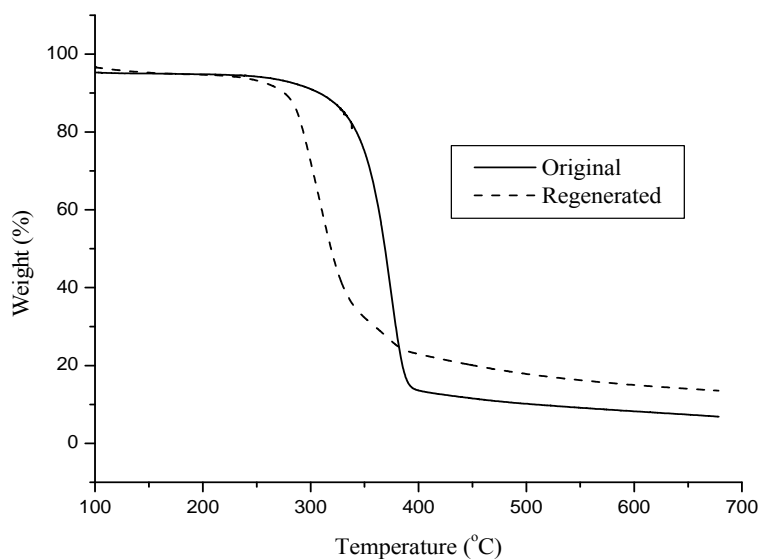
Fig. 2. TGA analysis of original and regenerated chemical cellulose as analysed from 80°C to 700°C temperatures at a rate of 10 °C/min

FT-IR analysis showed that the spectra of the original and regenerated cellulose were similar, indicating the absence of chemical reaction during dissolution and regeneration steps of chemical cellulose. This result was similar to what was reported previously (Bonet et al. 2004; Liu et al. 2007; Zhang et al. 2005). The absorbance observed on the spectrum of the original cellulose at 3328.87 cm^{-1} is due to stretching of O-H groups and that at 2895.09 cm^{-1} is due to the C-H stretching, while the peak at 1640.00 cm^{-1} is because of the bending mode of the absorbed water (Bonet et al. 2004; Liu et al. 2007). The peak at 1428.80 cm^{-1} is due to CH_2 scissoring motion (Zhang et al. 2005), while the one at 1315.94 cm^{-1} is due to the O-H bending. The band at 1160.18 cm^{-1} is for C-O stretching, while that at 1029.57 cm^{-1} is due to C-O-C vibration (Liu et al. 2007). The peak observed at 898.36 cm^{-1} is due to C-O stretching in the amorphous region (Zhang et al. 2005). The similarity of FTIR spectra (except the shift of some of the peaks) of the original and regenerated cellulose shows that there was no effect of ionic liquid on cellulose structure. It has been reported previously that, at relatively low temperatures [BMIM]Cl can dissolve biomass with no significant structural chemical changes to its components (Kline et al. 2010) and that cellulose is the major component of biomass (Spiridon et al. 2010). The observed shift of O-H stretch for example, from low frequency of the original cellulose to high frequency of the regenerated cellulose is due to the splitting of hydrogen bonding in the cellulose (Zhang et al. 2005). Additionally, the FTIR spectrum of regenerated cellulose showed that this cellulose was free of imidazolium traces, as the imidazolium modes were not observed in the spectrum (Fig. 1, spectrum A). Kiefer et al. (2009), reported that imidazolium modes are observed at 1005 cm^{-1}, 1047 cm^{-1}, and 1089 cm^{-1}, which correspond to symmetric C-N-C stretching, CCH scissoring, and C-C stretching modes, respectively (Kiefer et al. 2009).

The thermal stability analysis performed by TGA showed that both original and regenerated cellulose started decomposing at about 250°C followed by the rapid decomposition to 600°C, where the original cellulose retained 8 wt%, while the

regenerated cellulose retained 15 wt %. Thus the regenerated samples gave a higher char yield (non-volatile carbonaceous materials) on pyrolysis (Swatloski et al. 2002), which was exhibited by the higher mass residual after decomposition compared to the original cellulose. These results were in agreement with that reported previously (Swatloski et al. 2002; Fort et al. 2007). Thus, this TGA profile shows that thermal variations in celluloses reconstituted from ionic liquid solution are similar with those of pure cellulose.

GC-MS Analysis

In GC-MS analyses, long chain fatty acids ranging between C_{12} to C_{26} were identified. Fatty acids, which were identified as their methyl esters, were eluted in order of the increase in their molecular masses, as can be observed on the selected chromatogram given in Fig. 3.
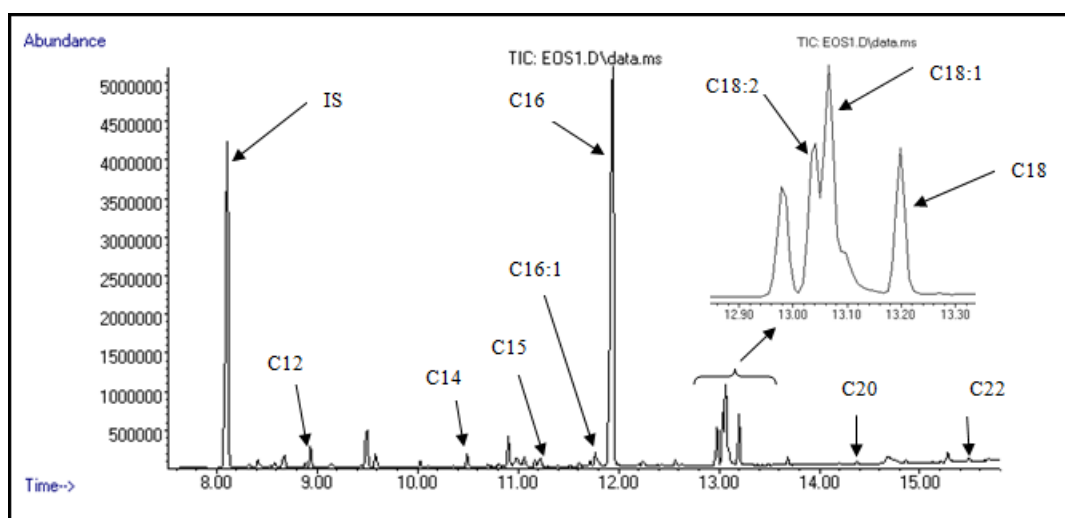

Fig. 3. GC-MS chromatograms showing the identified fatty acids from oxygen delignification (O) bleaching stage sample

Identification was done by the comparison of the mass spectra with those in NIST libraries and also by the identity of the mass fragments to confirm a compound suggested by the library. Standards were also used to compare with the spectra of compounds from the sample. These fatty acids methyl esters were easily earmarked from the spectra by observing their base peak at m/z = 74 (100%), which is formed by β – cleavage in the McLafferty rearrangement mechanism (Scheme 1).
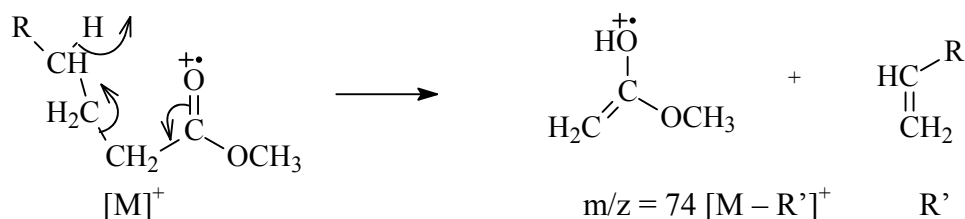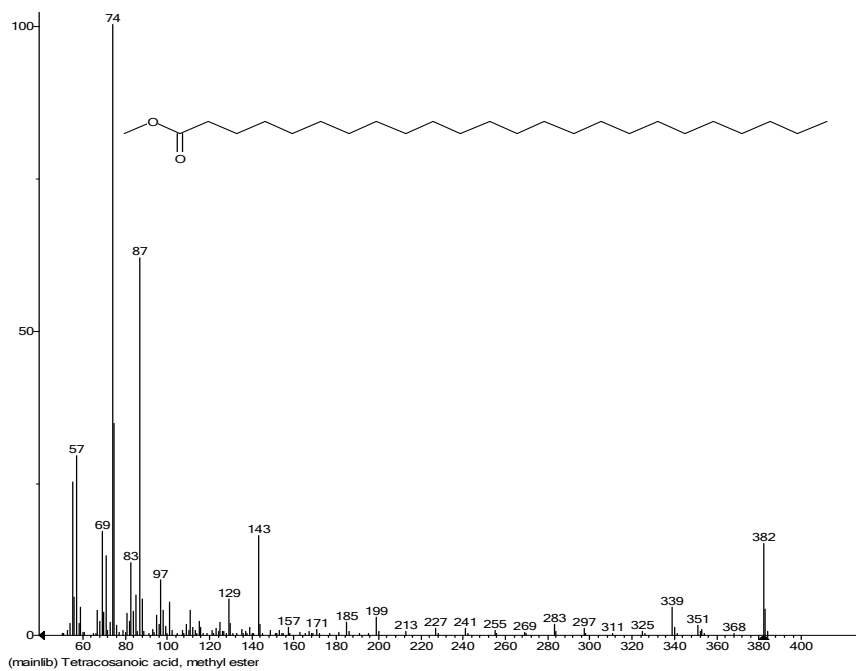

Scheme 1. McLafferty rearrangement of fatty acid methyl esters, where R and R' are alkyl groups
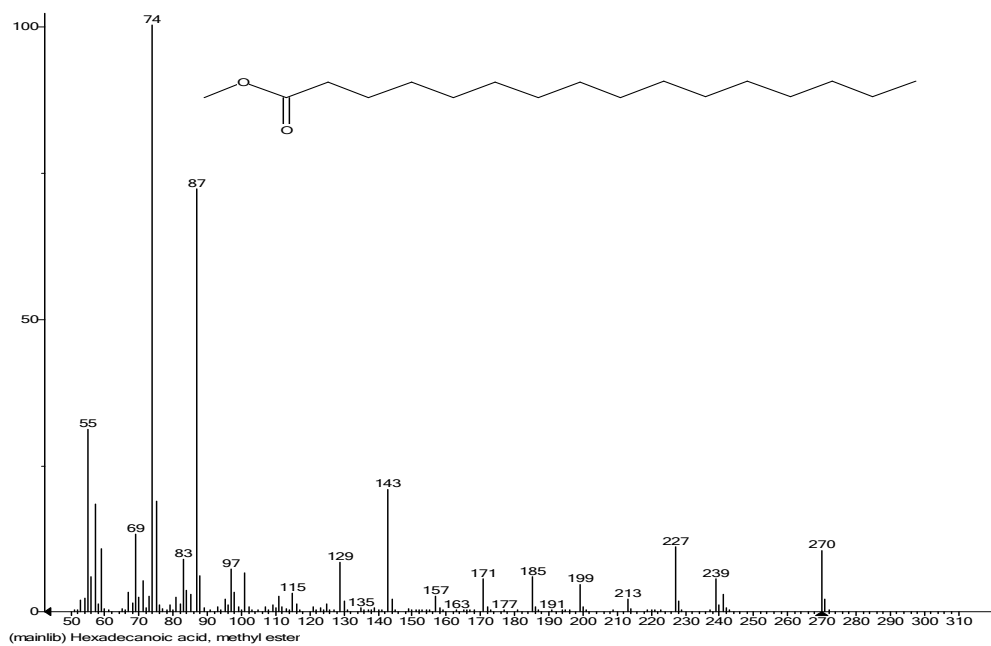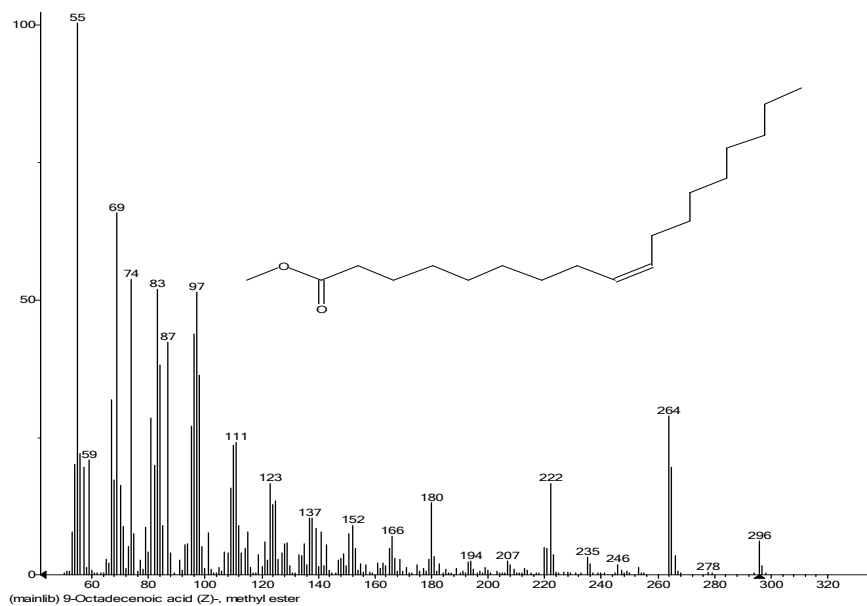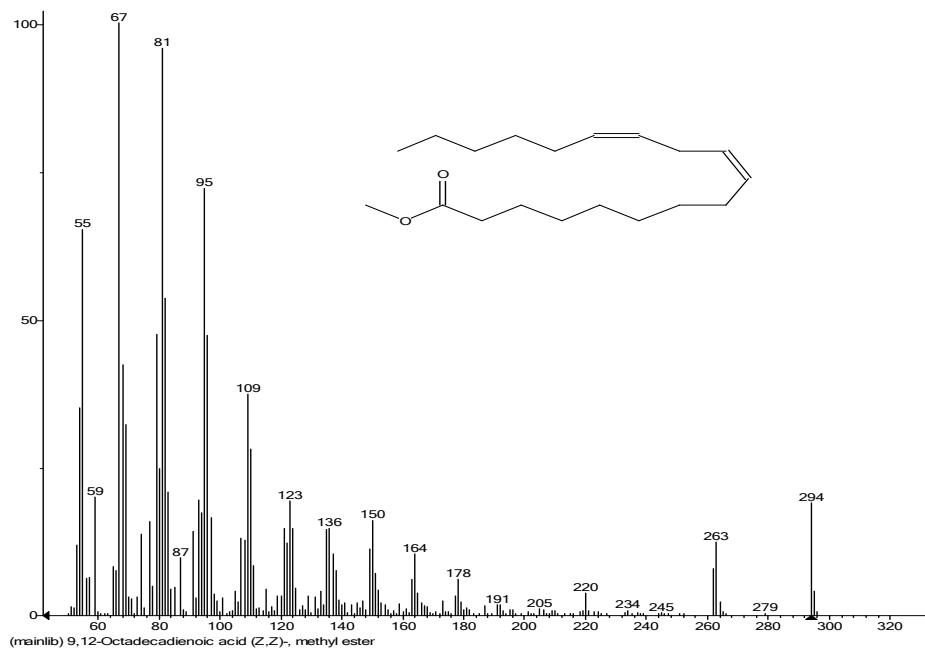
Fig. 4. Mass spectra of hexadecanoic acid methyl ester showing the peaks identified from oxygen delignification sample

Fig. 5. Mass spectra of tetracosanoic acid methyl ester showing the peaks identified from hypochlorite (H) bleached sample

Fig. 6. Mass spectra of 9-octadecenoic acid methyl ester showing the peaks identified from hypochlorite (H) bleached sample


Fig. 7. Mass spectra of 9, 12-octadecadienoic acid methyl ester showing the peaks identified from oxygen delignification sample

Other observed strong peaks that were found can be attributed to the cleavage of C-C bonds, giving alkyl ions. Oxygen-containing ions are also formed in the mass spectra of fatty acid methyl esters, which result in the formation of high abundance peaks due to the formation of hydrocarbon clusters at an interval of fourteen mass units given by $C_nH_{2n-1}O_2$ (Burdi et al. 2007). A strong peak at m/z = 87 is due to the oxygen-containing ion $(C_4H_7O_2)^+$. The peaks at m/z 74 and 87 were not the most abundant peaks in the mass spectrum of unsaturated fatty acid methyl esters, as can be evidenced in the mass spectra of 9-octadecanoic acid methyl ester and 9,12-octadecadienoic acid methyl ester (Figs. 6 and 7).

Quantitation

For quantification of fatty acid methyl esters, a calibration curve was obtained from a mixture of two fatty acid methyl esters (hexadecanoic acid methyl ester and octadecanoic acid methyl ester) at a concentration ranging from 2 to 50 mg/L. The correlation coefficient for calibration curve was found to be higher than 0.997 (Fig. 8).
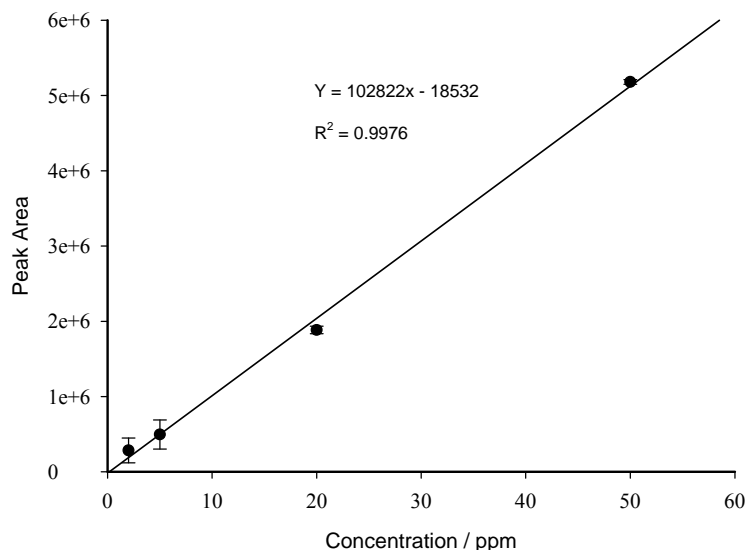
Fig. 8. Calibration curve used for the quantification of fatty acid methyl esters
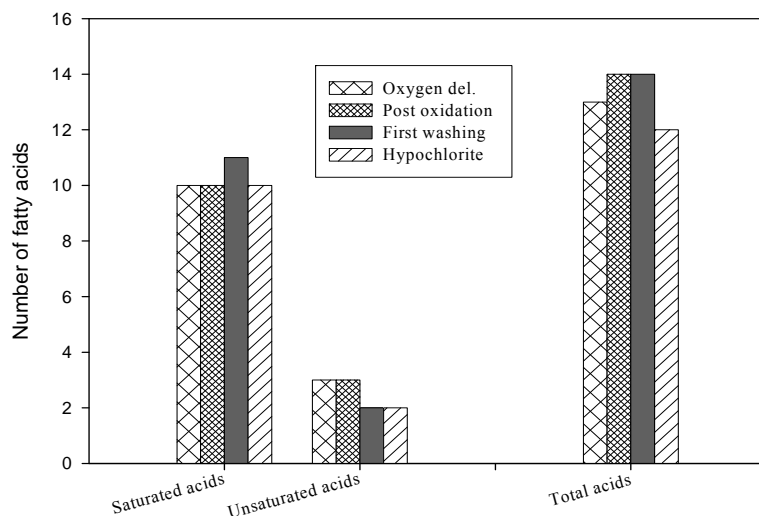
Fatty acids identified as their fatty acid methyl esters with their mass fragmentation patterns are listed in Table 2. Confirmation of identities by mass spectra revealed saturated, unsaturated, and branched-chain molecular species. Fatty acid methyl esters (FAMEs) from chemical cellulose obtained from different bleaching stages in sulphite pulping process are presented in Table 2.

Identified fatty acids ranged from dodecanoic (C_{12}) to hexacosanoic acid (C_{26}). Hexadecanoic acid and octadecanoic acid were the most abundant saturated fatty acids, while 9-octadecanoic acid was the most abundant unsaturated fatty acid in the analysed samples.

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Table 2. Weight of Detected Fatty Acids in mg/g Weight of Dry Pulp Sample

Compounds	M$^+$	Oxygen del (O)	Washing (D1)	Post-Oxid-ation	Hypo-chlor-ite (H)	m/z fragments
Methyl dodecanoate	214 = $[C_{13}H_{26}O_2]^+$	0.119	0.517	0.651	0.167	214/183/171/143/129/115/101/87/74
Methyl tetradecanoate	242 = $[C_{15}H_{30}O_2]^+$	0.127	0.119	0.115	0.067	242/224/199/182/165/143/87/74
Methyl pentadecanoate	256 = $[C_{16}H_{32}O_2]^+$	0.087	0.045	0.028	0.074	256/225/179/143/129/87/74
Methyl 7-hexadecenoate	268 = $[C_{17}H_{32}O_2]^+$	0.166	0.179	0.241	0.097	268/236/217/194/152/123/96/74/53
Methyl hexadecanoate	270 = $[C_{17}H_{34}O_2]^+$	0.017	0.882	0.869	0.210	270/239/227/213/199/143/129/87/74
Methyl heptadecanoate	284 = $[C_{18}H_{36}O_2]^+$	0.066	0.034	nd	nd	284/237/199/163/143/87/74
Methyl 9,12-octadecadienoate	294 = $[C_{19}H_{34}O_2]^+$	0.695	nd	0.129	nd	294/263/220/178/150/123/95/81/67/55
Methyl 9-octadecenoate	296 = $[C_{19}H_{36}O_2]^+$	3.354	0.606	0.518	0.343	296/264/222/180/97/83/74/69/55
Methyl octadecanoate	298 = $[C_{19}H_{38}O_2]^+$	1.115	0.232	0.301	0.165	298/255/199/175/143/129/87/74
Methyl eicosanoate	326 = $[C_{21}H_{42}O_2]^+$	0.204	0.020	0.027	0.038	326/283/237/213/185/143/87/74
Methyl docosanoate	354 = $[C_{23}H_{46}O_2]^+$	0.352	0.029	0.037	0.016	354/311/255/167/135/105/87/74/57/55
Methyl tricosanoate	368 = $[C_{24}H_{48}O_2]^+$	nd	0.012	0.016	nd	368/316/143/129/87/74/55
Methyl tetracosanoate	382 = $[C_{25}H_{50}O_2]^+$	0.090	0.040	0.072	nd	382/339/283/241/199/171/143/129/87/74
Methyl pentacosanoate	396 = $[C_{26}H_{50}O_2]^+$	nd	nd	0.028	nd	396/353/311/255/241/199/143/87/74/57
Methyl hexacosanoate	410 = $[C_{27}H_{54}O_2]^+$	0.548	0.010	nd	nd	410/367/341/311/297/255/143/129/87/74/57

The percentages of the identified components of fatty acids methyl esters (FAMEs) were relatively higher in oxygen delignification and post oxidation samples. Among the FAMEs components, the major fatty acid class was saturated fatty acids, with few unsaturated fatty acids. More acids were identified from oxygen delignification samples, first washing stage (D1), and post-caustic oxidation, compared to the hypochlorite bleached samples. The total number of these compounds was less in hypochlorite-bleached (H) samples compared to the amounts in the other four samples (Fig. 9).

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Fig. 9. The number of fatty acids identified from chemical cellulose samples obtained from different bleaching stages
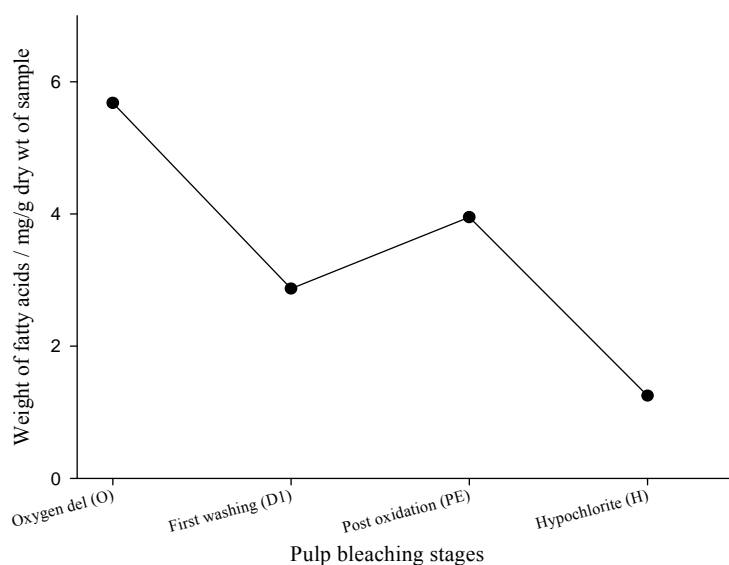
Fig. 10. Trend of weight of fatty acids in different bleached pulp samples

From Fig. 10 the trend shows a decrease of these fatty acids with bleaching sequence, but some do survive this process and remain in the final product, as it has been observed. Fatty acids that were identified in the hypochlorite bleached samples indicated their resistance to the bleaching chemicals and sequence. The quantity of fatty acids obtained per each bleaching stage shows the effectiveness of each stage but also indicates the resistance of fatty acids in these bleaching sequences, especially the saturated fatty acids. Dienoic (methyl 9,12- octadecadienoate) fatty acid was not detected in the final bleaching stage, indicating that it was fully eliminated in the bleaching process.

The effect of sample matrix on the extraction and analysis procedures were studied by the spiking of undecanoic (C_{13}) and hexadecanoic (C_{16}) acids (33.33 μg/mL) into the pulp samples. Recoveries ranged between 90% and 107%, as shown in Table 3

Table 3. Recoveries Obtained by Spiking Undecanoic and Hexadecanoic acids into the Samples

Sample spiked	Compounds	Percentage recovery
Oxygen del (O)	Undecanoic acid	106.17 ± 5.8
	Hexadecanoic acid	106.32 ± 3.6
Hypochlorite (H)	Undecanoic acid	107.09 ± 1.6
	Hexadecanoic acid	90.02 ± 1.4

Undecanoic acid was used as internal standard because it was not detected in the pulp samples analysed previously.

Table 4. Classes of Fatty Acids / mg / g of Dry Pulp Sample

	Saturated FA / mg / g	Unsaturated FA / mg / g
Oxygen del (O)	1.93 ± 1.19	3.75 ± 2.01
Washing stage (D1)	2.09 ± 0.26	0.78 ± 0.34
Post Oxidation (PE)	3.13 ± 0.51	0.82 ± 0.09
Hypochlorite (H)	0.82 ± 0.01	0.44 ± 0.14
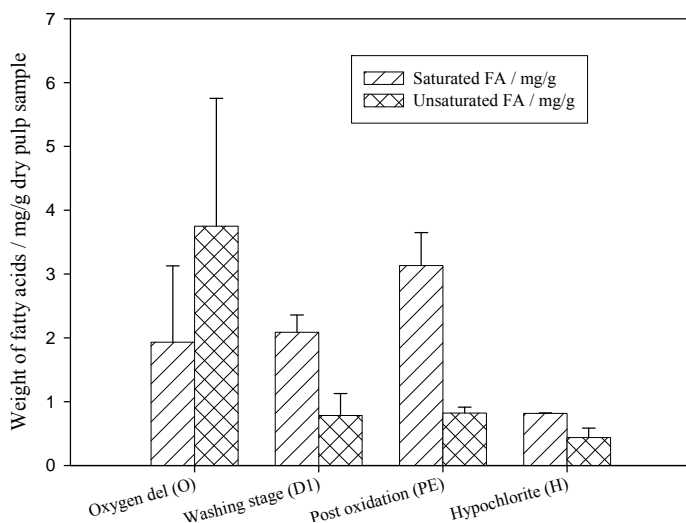
Fig. 11. Classes of fatty acids / mg / g of dry pulp sample

All pulp samples from different bleaching stages except oxygen delignification were determined to contain high amounts of saturated fatty acids compared to unsaturated fatty acids (Fig. 10). This is due to the fact that during the oxygen delignification stage is when most of the unsaturated acids are oxidized and in the next stages are washed out.

Fatty acids that survive pulping present different behaviour during bleaching, depending on their chemical structure and on the bleaching agent used (Gutiérrez et al. 2008). It was observed, for instance, that unsaturated fatty acids were higher in chemical cellulose samples obtained at oxygen delignification stage, and decreased across the bleaching sequence, as shown in Fig. 11.

CONCLUSIONS

The present work has reported the successful use of ionic liquids as solvents in the analysis of fatty acids from chemical-grade cellulose. It was observed that ionic liquids possess the ability to dissolve cellulose and reconstitute it upon addition of any precipitating solvents, as has been reported previously. In the course of dissolution and regeneration of cellulose, alien compounds are released into the aqueous-ionic liquid filtrate, from which fatty acids can be recovered using small amount of organic solvents. From this study it can be concluded that the employed procedures can be useful for the analysis of fatty acid contaminants in chemical cellulose samples. Furthermore, the use of ionic liquids in such analytical procedures as solvent promotes the reduction of environmental pollution and avoids use of large amounts of volatile organic solvents in such analyses.

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REFERENCES CITED

Asikainen, S., Fuhrmann, A., Ranua, M., and Robertseri, L. (2010). "Effect of birch kraft pulp primary fines on bleaching and sheet properties," *BioResources* 5, 2173- 2183.

Bonet, M., Quijada, C., and Cases, F. (2004). "Characterization of ethylcellulose with different degrees of substitution (DS): A diffuse-reflectance infrared study," *Canadian Journal of Analytical Sciences and Spectroscopy* 49(4), 234 -239.

Burdi, D. K., Samejo, M. Q., Bhanger, M. I., and Khan, K. M. (2007). "Fatty acid composition of *abies pindrow* (*West Himalayan Fir*)," *Pak. J. Pharm. Sci.* 20, 15-19.

Challinor, J. M. (1996). "Characterisation of wood extractives by pyrolysis-gas chromatography/mass spectrometry of quaternary ammonium hydroxide extracts," *J. Anal. Appl. Pyrolysis* 37(1), 1-13.

Dorado, J., Claassen, F. W., Lenon, G., Van Beek, T. A., Wijnberg, J. B. P. A., and Sierra-Alvarez, R. (2000). "Degradation and detoxication of softwood extractives by sapstain fungi," *Biores. Technol.* 71, 13-20.

Earle, M. J., and Seddon, K. R. (2000). "Ionic liquids. Green solvents for the future," *Pure Appl. Chem.* 72(7), 1391-1398.

Feng, L., and Chen, C.- I. (2008). "Research progress on dissolution and functional modification of cellulose in ionic liquids," *J. Molecular Liquids* 142,1-5.

Fort, D. A., Remsing, R. C., Swatloski, R. P., Moyna, P., Moyna, G., and Rogers, R. D. (2007). "Can ionic liquids dissolve wood? Processing and analysis of lignocellulosic materials with 1-n-butyl-3-methylimidazolium chloride," *Green Chem.* 9, 63-69.

Freire, C. S. R., Silvestre, A. J. D., and Neto, C. P. (2005). "Lipophilic extractives in eucalyptus globulus kraft pulps. Behavior during ECF bleaching," *J. Wood Chem. Technol.* 25(1-2), 67-80.

Freire, C. S. R., Pinto, P. C. R., Santiago, A. S., Silvestre, A. J. D., Evtuguin, D. V., and Neto, C. P. (2006). "Comparative study of lipophilic extractives of hardwoods and corresponding ECF bleached kraft pulps," *BioResources* 1(1), 3-17.

Freire, C. S. R., Silvestre, A. J. D., Neto, C. P., and Evtiguin, D. V. (2006). "Effect of oxygen, ozone and hydrogen peroxide bleaching stages on the contents and composition of extractives of eucalyptus globules kraft pulps," *Biores. Technol.* 97(3), 420-428.

Gutierrez, A., Del Rio, J. C., Gunzalez-Vila, F. J., and Martin, F. (1998). "Analysis of lipohilic extractives from wood and pitch deposits by solid-phase extraction and gas chromatography," *J. Chromatogr. A* 823, 449-455.

Gutierrez, A., Del Rio, J. C., Martinez, M. J., and Martinez, A. T. (2001). "The biotechnological control of pitch in paper pulp manufacturing," *Trends Biotechnol.* 19(9), 340-348.

Gutiérrez, A., Rodríguez, I. M., and Del Río, J. C. (2008). "Chemical composition of lipophilic extractives from sisal (*Agave sisalana*) fibers," *Industrial Crops and Products* 28, 81-87.

Horvath, A. L., (2006). "Solubility of structurally complicated materials: I. Wood," *J. Phys. Chem. Ref. Data,* 35(1), 77-92.

Jahan, M. S., Ahsan, L., Noori, A., and Quaiyyum, M. A. (2008). "Process for the production of chemical cellulose from *Trema orientalis* (Nalita) by prehydrolysis kraft and soda-ethylenediamine process," *BioResources* 3(3), 816-828.

Kiefer, J., Obert, K., Fries, J., Bösmann, A., Wasserscheid, P., and Leipertz, A. (2009). "Determination of glucose and cellobiose dissolved in the ionic liquid 1-ethyl-3-methylimidazolium acetate using fourier transform infrared spectroscopy," *Applied Spectroscopy* 63(9), 1041-1049.

Kiefer, J., Obert, K., Bösmann, A., Seeger, T., Wasserscheid, P., and Leipertz, A. (2008). "Quantitative analysis of alpha-d-glucose in an ionic liquid by using infrared spectroscopy," *ChemPhysChem.* 9, 1317-1322.

Kline, L. M., Hayes, D. G., Womac, A. R., and Labbé, N. (2010). "Simplified determination of lignin content in hard and soft woods via uv-spectrophotometric analysis of biomass dissolved in ionic liquids," *BioResources* 5(3), 1366-1383.

Kilpeläinen, I., Xie, H., King, A., Granstrom, M., Heikkinen, S., and Argyropoulos, D. S. (2007). "Dissolution of wood in ionic liquids," *J. Agric. Food Chem.* 55, 9142-9148.

Kubisa, P. (2004). "Application of ionic liquids as solvents for polymerization processes," *Prog. Polym. Sci.* 29, 3-12.

Liu, C. F., Sun, R. C., Zhang, A. P., and Ren, J. L. (2007). "Preparation of sugarcane bagasse cellulosic phthalate using an ionic liquid as reaction medium," *Carbohydr. Polym.* 68, 17-25.

Silvério, F. O., Barbosa, L. C. A., Maltha, C. R. A., Fidêncio, P. H., Mariluze P., Cruz, M. P., Veloso, D. P., and Milanez, A. F. (2008). "Effect of storage time on the composition and content of wood extractives in eucalyptus cultivated in Brazil," *Biores. Technol.* 99, 4878-4886.

Spiridon, I., Teacă, C. A., and Bodîrlău, R. (2010). "Structural changes evidenced by FTIR spectroscopy in cellulosic materials after pre-treatment with ionic liquid and enzymatic hydrolysis," *BioResources* 6(1), 400-413.

Sithole, B., Shirin, S., Zhang, X., Lapierre, L., Pimentel, J., and Paice, M. (2010). "Deresination options in sulphite pulping," *BioResources* 5, 187-205.

Sun, R. C., and Tomkinson, J. (2003). "Comparative study of organic solvent and water-soluble lipophilic extractives from wheat straw I: Yield and chemical composition," *J. Wood Sci.* 49, 47-52.

Swatloski, R. P., Spear, S. K., Holbrey, J. D., and Rogers, R. D. (2002). "Dissolution of cellose with ionic liquids," *J. Am. Chem. Soc.* 124(18), 4974-4975.

Vitz, J., Erdmenger, T., Haensch, C., and Schubert, U. S. (2009). "Extended dissolution studies of cellulose in imidazolium based ionic liquids," *Green Chem.* 11, 417-424.

Zhang, H., Wu, J., Zhang, J., and He, J. (2005). "1-allyl-3-methylimidazolium chloride room temperature ionic liquid: A new and powerful nonderivatizing solvent for cellulose," *Macromolecules* 38(20), 8272-8277.

Zhu, S., Wu, Y., Chen, Q., Yu, Z., Wang, C., Jin, S., Ding, Y., and Wu, G. (2006). "Dissolution of cellulose with ionic liquids and its application: A mini-review," *Green Chem.* 8, 325-327.