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-RESEARCH ARTICLE-

Isolation of cellulose and hemicellulose by using alkaline peroxide treatment at room temperature from wasted fall leaves

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Abstract
Fall leaves are biodegraded and composted naturally in forests but they are wastes for urban areas. Moreover, they are widely available cellulose sources but have limited applications. Alkaline peroxide treatment of bioresources is one of the most widely studied clean methods for both delignification and hemicellulose removal but there is no study about application of that method on fall leaves at room temperature. In this study, the effect of alkaline peroxide treatment of fall leaves at room temperature on hemicellulose recovery and cellulose delignification were investigated. Fall leaves (FL) were treated with 0.3-3.0 M NaOH + 0-3 M H_2O_2 at room temperature. Hemicellulose recovery and cellulose delignification values were analyzed. Hemicellulose recovery and cellulose delignification increased and yield decreased by increasing NaOH and H_2O_2 concentrations. Hemicellulose recovery and cellulose delignification reached to the maximum levels, 99.5% and 81.6% respectively, at 3M NaOH + 3M H_2O_2 treatment condition. The end products were confirmed by analytically, spectrally and morphologically. Wasted fall leaves were turned into useful hemicellulose and cellulose products by using clean alkaline peroxide treatment at room temperature. The products can be further processed by known methods into other industrial products.

Keywords:
Waste management, fall leaves, alkaline peroxide treatment, hemicellulose recovery, delignification.

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Introduction

Fractionalization of plants into cellulose, hemicellulose and lignin presents many industrial opportunities. Especially, cellulose and hemicellulose are very important natural raw materials used in many areas of our life, from textiles to food, from construction to paint industries (R. Liu et al., 2005). These raw materials can also be composited with other materials to make new products (Ünlü et al., 2009).

Alkaline peroxide treatment is one of the cleanest fractionalization methods using clean, odorless and recyclable chemicals (Su et al., 2015). Because of decomposition of H_2O_2 to O_2 and H_2O, it doesn't leave residues harmful to further industrial processing of cellulose such as enzymatic hydrolysis for bioethanol production (Rabelo et al., 2014). Furthermore, operation at moderate temperature and pressure made hydrogen peroxide treatment one of the most widely studied analytical methods for fractionalization of lignocellulosic bioresources.

Fall leaves are one of the most common products of autumn months. Like other vegetal bioresources, they are composed of cellulose, hemicellulose and lignin. However, fall leaves emerge as waste that must be collected especially in urban areas due to the inadequate industrial processing methods (Kim et al., 2016). Alkaline peroxide treatment was applied on many vegetal resources such as corncob (Su et al., 2015), sugarcane bagasse (Rabelo et al., 2014) and ray straw (Fang et al., 2000). However, alkaline peroxide treatment of fall leaves has not been studied before. Therefore, the purpose of the study is to obtain hemicellulose and cellulose from wasted fall leaves by treating them with alkaline hydrogen peroxide at room temperature.

Material and methods

Wasted fall leaves were collected from Istanbul Technicak University Ayazaga Campus, Turkey. The collected samples were cleaned from stones and other contaminants, washed and dried at 40°C. Then, they were ground to powder and the final products were coded "FL".

Sodium hydroxide, potassium permanganate, hexadecyltrimethylammonium bromide, sodium lauryl sulfate, hydrochloric acid and sulfuric acid were of analytical grade and used as received. Isopropyl alcohol (IPA) was of technical grade and used after distillation.

FTIR spectra of the samples were taken using a Perkin-Elmer BXII model spectrometer via 1% w/w sample containing KBr pellets by averaging 16 scans in the scan range of 400-4000 cm^{-1}.

Surface morphology was examined using scanning electron microscopy. The samples were mounted on stub with double-sided adhesive tape and coated with a thin layer (4 nm) of gold–palladium alloy. Images were taken using a JEOL JSM-6510LV low-vacuum scanning electron microscope using an accelerating voltage of 10 kV.

Analytical Methods

Cellulose and hemicellulose contents of the samples were determined via acid detergent fibre (ADF) and neutral detergent fibre (NDF) calculations (Huang et al., 2010). ADF was calculated by measuring the residue after refluxing the biomaterials with acidified detergent solution (2% hexadecyltrimethylammonium bromide + 0.5 M H_2SO_4). Then, the biomaterials were treated with 72% H_2SO_4 solution and the residue amounts were used for calculation of cellulose contents **(1)** (Huang et al., 2010). NDF was calculated by measuring the residue after refluxing the biomaterials with 3% sodium lauryl sulfate solution. Then, hemicellulose contents were calculated via NDF (%) and ADF (%) values **(2)** (Huang et al., 2010).

Cellulose (%) = ADF (%) - Residue of 72% H_2SO_4 treatment (%) (1)

Hemicellulose (%) = NDF (%) – ADF (%) (2)

Lignin contents of samples were calculated from consumption of 0.02 M potassium permanganate of samples as described at TAPPI Standard T236 cm-85 (1993). Finally, delignification ratios were calculated from the initial and final lignin contents of the samples (3).

$$\text{Delignification Ratio (\%)} = \left[\frac{\text{Initial Lignin (\%)} - \text{Final Lignin(\%)}}{\text{Initial Lignin(\%)}}\right] \text{x } 100 \quad (3)$$

Room temperature alkaline peroxide treatment

Alkaline peroxide treatment studies found at literature were adapted to wasted fall leaves (Fang et al., 2000). Shortly, 25 ml of solution containing 0.3-3 M NaOH + 0-3 M H_2O_2 was added per g of FL. The system was dipped in water bath at room temperature and magnetically stirred for 18 hours. Later, the mixture was centrifuged and the pellet, cellulosic fraction, was isolated. Then, pH of the filtrate was set to 5.5 with 12% HCl and hemicellulose was precipitated from the filtrate by 3 volume of isopropanol. Finally, the cellulose and hemicellulose fractions were washed with distilled water and isopropyl alcohol (IPA) respectively and dried at vacuum oven.

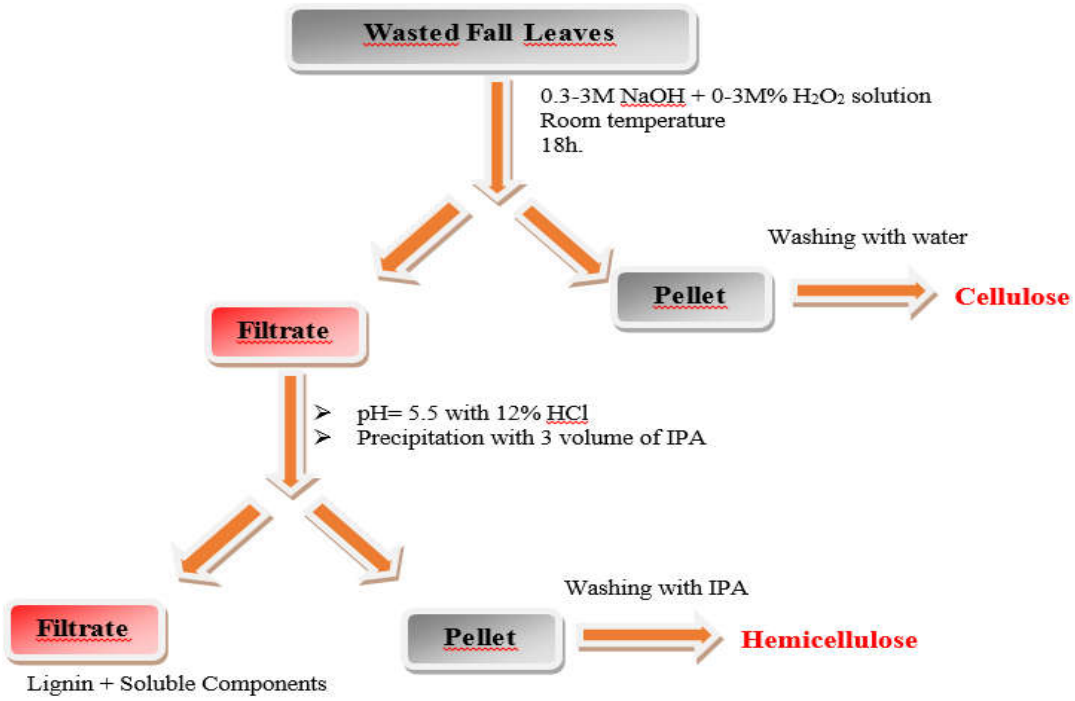


Figure 1. The scheme of alkaline peroxide treatment

Results

Cellulose, hemicellulose and lignin contents of fall leaves (FL) were determined as 30.1%, 29.5% and 26.9%, respectively. FL were treated with 0.3-3.0 M NaOH + 0-3 M H_2O_2 at room temperature for isolation of cellulose and hemicellulose. The yield, hemicellulose recovery and delignification ratio values were recorded (Table 1) and were graphed by arranging on constant H_2O_2 concentration groups (Figure 2) and constant NaOH concentration groups (Figure 3) separately.

Table 1. Initial conditions and results of the experiments arranged in constant concentration groups of H_2O_2.

Code	NaOH (M)	Yield (%)[a]	Hemicellulose Recovery (%)	Cellulose Delignification (%)[b]	Code	NaOH (M)	Yield (%)[a]	Hemicellulose Recovery (%)	Cellulose Delignification (%)[b]
No H_2O_2 Group					**1 M H_2O_2 Group**				
FL1	0.3	56.1	53.6	56.1	**FL16**	0.3	48.4	60.0	73.7
FL2	0.6	55.8	59.6	68.2	**FL17**	0.6	43.8	62.7	69.4
FL3	1.0	50.0	69.2	70.1	**FL18**	1.0	37.9	83.3	73.2
FL4	2.0	49.3	62.2	71.5	**FL19**	2.0	37.3	75.3	80.9
FL5	3.0	47.3	60.9	71.7	**FL20**	3.0	36.8	78.4	77.0
0.3 M H_2O_2 Group					**2 M H_2O_2 Group**				
FL6	0.3	55.3	49.9	64.4	**FL21**	0.3	45.2	54.4	75.2
FL7	0.6	48.5	83.7	65.4	**FL22**	0.6	45.1	60.8	70.7
FL8	1.0	47.9	70.0	76.5	**FL23**	1.0	37.0	84.3	76.9
FL9	2.0	40.0	69.8	76.9	**FL24**	2.0	36.5	89.3	77.1
FL10	3.0	39.6	69.2	74.7	**FL25**	3.0	36.1	94.1	79.6
0.6 M H_2O_2 Group					**3 M H_2O_2 Group**				
FL11	0.3	50.3	57.1	66.0	**FL26**	0.3	44.0	51.5	76.8
FL12	0.6	44.8	70.5	74.6	**FL27**	0.6	43.6	54.1	80.2
FL13	1.0	39.0	72.5	81.0	**FL28**	1.0	36.4	96.4	76.8
FL14	2.0	38.0	74.0	75.8	**FL29**	2.0	36.1	97.2	79.3
FL15	3.0	37.3	76.2	76.0	**FL30**	3.0	35.9	99.5	81.6

[a] Yield (%) = 100 × (weight of the remaining samples after the reactions/weight of initial FL)

[b] Cellulose delignification (%) = 100 × [Initial lignin(%) – Final lignin (%)]/Initial lignin (%).

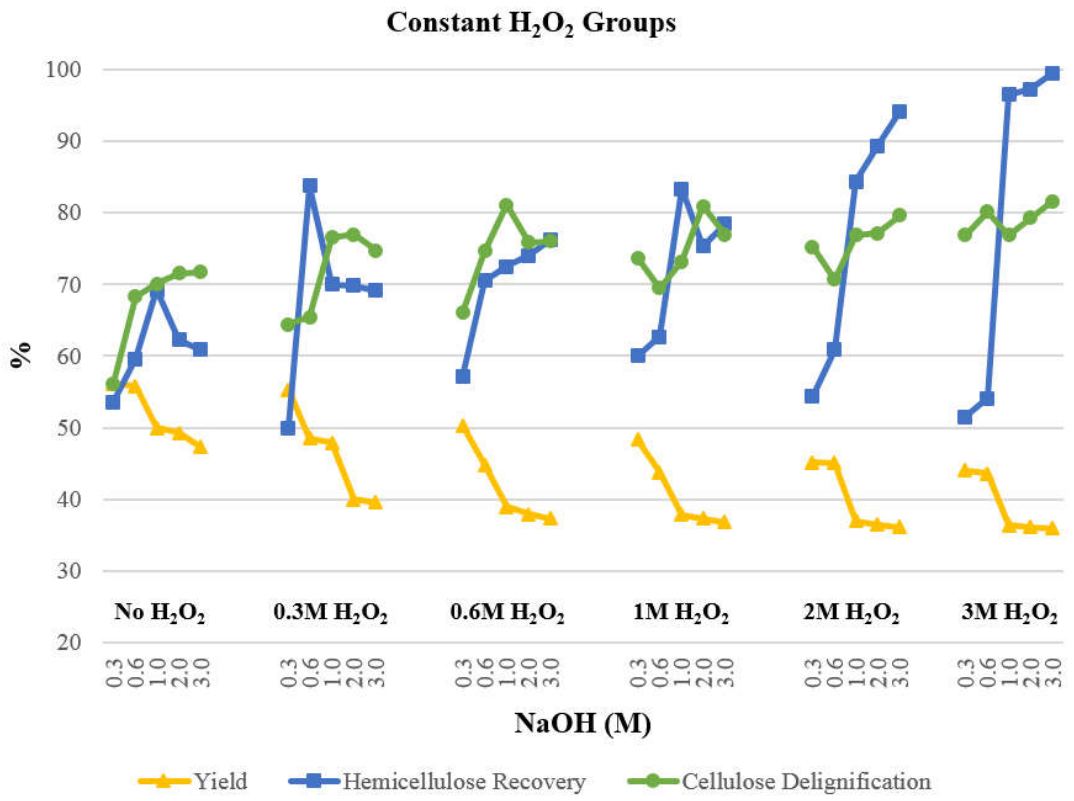


Figure 2. The effects of NaOH and H_2O_2 concentrations on yield, hemicellulose recovery and cellulose delignification. It was grouped under 6 constant H_2O_2 concentration groups.

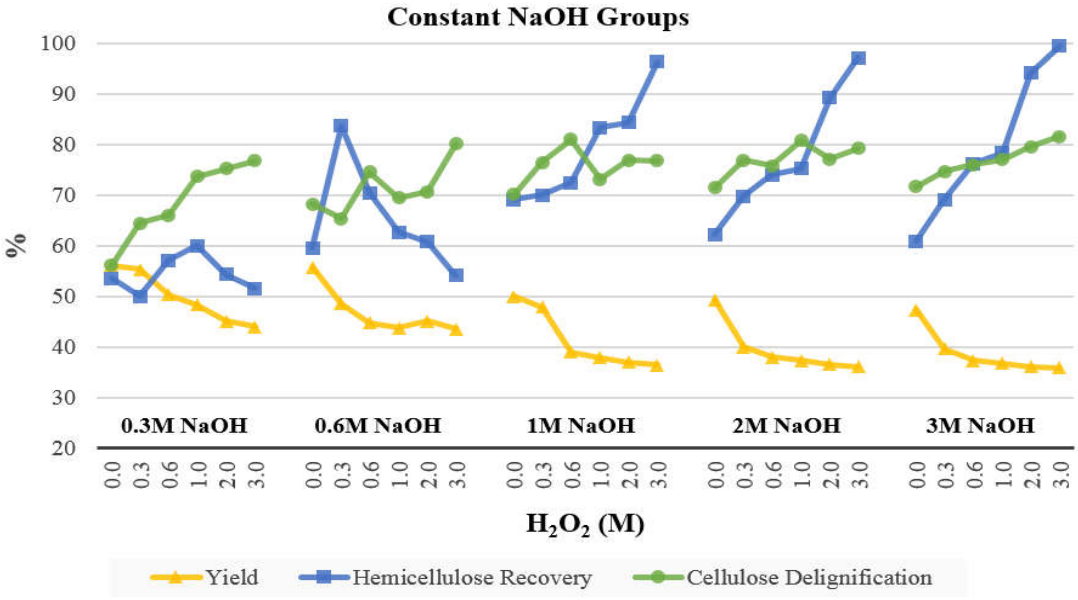


Figure 3. The effects of NaOH and H_2O_2 concentrations on yield, hemicellulose recovery and cellulose delignification. It was grouped under 5 constant NaOH concentration groups.

Discussion

Cellulose delignification and yield

Cellulose delignification is a measure of cellulose quality. As it approaches to 100%, lignin remained on cellulose approaches to 0%. Mostly, delignification increased at higher NaOH and higher H_2O_2 concentrations (Table 1). The highest delignification (81.6%) was reached at the highest concentrations (FL30: 3 M NaOH + 3 M H_2O_2). However, 1 M NaOH + 0.6 M H_2O_2 treatment condition (FL13) resulted in reasonable delignification (81%), so it may be more feasible to operate at that condition if only delignification is aimed.

Yield was the remaining matter after treatments of wasted fall leaves. At both constant H_2O_2 (Figure 2) and constant NaOH (Figure 3) experiments, increase of NaOH or H_2O_2 concentrations removed more hemicellulose and lignin (Table 1). Therefore, yield reached the minimum (35.9%) at the highest concentrations (FL30). As more hemicellulose and lignin were removed, cellulose in the remaining matter was concentrated more. At 3 M NaOH + 3 M H_2O_2 treatment (FL30) removed 99.5% of hemicellulose and 81.6% of lignin, so the remaining matter composed mostly of cellulose. This situation was further confirmed by FTIR and SEM analyses (Figure 4 and Figure 5).

Hemicellulose recovery

Hemicellulose recovery was investigated at constant H_2O_2 concentration groups (Figure 2) and at constant NaOH concentration groups (Figure 3) separately, and some fluctuations (local maximum and minimum points) were observed. The fluctuations of no H_2O_2 group are because of hemicellulose degradation at higher NaOH concentrations. The fluctuations of H_2O_2 added groups are also due to reactions between H_2O_2 and NaOH at alkaline peroxide mechanism. H_2O_2 and NaOH form hydroperoxide anion (HOO^-, **(4)**), which preferentially attacks chromophore groups of lignin and bleaches it (R. Sun et al., 2000). If NaOH concentration is kept high, hydroxyl radicals ($HO^{\bullet}$) and superoxide anion radicals ($O_2^{-\bullet}$) are formed **(5)** by decomposition of H_2O_2 (R. Sun et al., 2000). These radicals are responsible for delignification and hemicellulose dissolution. However, they also react with each other to form oxygen and hydroxyl anions (**6)** causing H_2O_2 consumption (R. Sun et al., 2000). If H_2O_2 concentration is kept high, it occupies NaOH until degradation of H_2O_2. Therefore, the ratio between NaOH and H_2O_2 becomes especially important when either of NaOH or H_2O_2 concentrations was low. pH-delignification relationship at alkaline peroxide delignification reactions was investigated in literature for different biomaterials and it was found that pH must be 11.5 or more for ideal radical formation and stabilization (Gould, 1985). Therefore, all the reaction of this study was performed at pH ≥ 11.5.

$$H_2O_2 + OH^- \rightleftharpoons HOO^- + H_2O \quad \textbf{(4)}$$

$$H_2O_2 + HOO^- \longrightarrow HO^{\bullet} + {}^{\bullet}O_2^- + H_2O \quad \textbf{(5)}$$

$$HO^{\bullet} + {}^{\bullet}O_2^- \longrightarrow O_2 + HO^- \quad \textbf{(6)}$$

Firstly, hemicellulose recoveries were investigated at constant H_2O_2 groups (Figure 2). Hemicellulose recoveries increased up to 0.6 M – 1 M NaOH concentrations sharply. Increasing NaOH concentration further caused much slower increase or decrease of hemicellulose recovery.

When no H_2O_2 was added (FL1-FL5), hemicellulose recovery reached local maximum (69.2%) at 1 M NaOH concentration (FL3). Higher NaOH concentration decreased hemicellulose recovery possibly because of degradation of hemicellulose. H_2O_2 added groups (FL6-FL30) mostly achieved higher hemicellulose recovery than not added group (FL1-FL5). Hemicellulose recovery was the highest at 3 M H_2O_2 group (FL26-FL30) and it reached the maximum (99.5%) at 3 M NaOH + 3 M H_2O_2 (FL30).

When constant NaOH groups were investigated (Figure 3), local maximum hemicellulose recoveries of 0.3 M NaOH and 0.6 M NaOH groups were observed at 1 M H_2O_2 and 0.3 M H_2O_2 respectively. At 1 M and higher NaOH concentration groups, hemicellulose recoveries increased as H_2O_2 concentrations increased. It reached the maximum at 3 M NaOH + 3 M H_2O_2 (99.5%).

Although 99.5% of hemicellulose was recovered at the highest NaOH + H_2O_2 concentrations (FL30), still 83.7% of hemicellulose could be recovered at 0.6 M NaOH + 0.3 M H_2O_2 treatment condition (FL7). That treatment condition can be economically significant when NaOH, H_2O_2 and hemicellulose prices were compared with hemicellulose recoveries. Therefore, this study may be a reference for future commercial applications.

Characterization

Conversion of wasted fall leaves to usable products, cellulose and hemicellulose, using alkaline peroxide treatment at room temperature had been measured analytically. All of the cellulose and hemicellulose fractions were further characterized using FTIR and SEM they were confirmed by comparing with literature.

FTIR spectra

FTIR spectroscopy was used to identify polysaccharides, to check their purity, and to determine structure by comparing with literature. Characteristic FTIR signals of cellulose are O-H stretching as a broad band at about 3400 cm^{-1} (C. Liu et al., 2006), aliphatic C-H stretching at about 2930-2840 cm^{-1} (J. Sun et al., 2004), H-O-H deformation due to adsorbed water at 1625-1639 cm^{-1} (J. Sun et al., 2004), CH_2 bending vibration at 1425-1465 cm^{-1} (C. Liu et al., 2006), C-H alkane bending vibrations at about 1260 and 1321 cm^{-1} (Fang et al., 2000), C-O, C-O-C stretching vibrations at 1180-1000 cm^{-1} (C. Liu et al., 2006), C-O-H bending vibration at 1041 cm^{-1}, and sharp b-glycosidic band at 898-900 cm^{-1} (C. Liu et al., 2006).

Hemicelluloses have similar functional groups with celluloses. Therefore, FTIR spectra of hemicelluloses have similar bands with celluloses. Hemicelluloses also have arabinosyl units which are detected via C-O-C vibrations with low intensity shoulder at 1158-1162 cm^{-1} (R. Sun et al., 2000) and 980-990 cm^{-1} (R. Sun et al., 1999).
Lignin is aromatic structures that are detected from aromatic skeleton vibrations at about 1510-1520 cm^{-1}. Its intensity is proportional to the lignin content associated in hemicellulose and cellulose (C. Liu et al., 2006). Also, lignin is detected from acetyl or uronic ester group signals at 1730-1745 cm^{-1} when associated to hemicellulose (Fang et al., 2000).

FTIR spectra of hemicellulosic and cellulosic fractions of all samples were taken and compared with each other. Lignin indicative peaks were observed at all of FTIR spectra of

hydrogen peroxide free samples (FL1-FL5). The strengths of lignin indicative peaks decreased as NaOH concentration increased but they did not disappear at the highest NaOH concentration (3 M NaOH, FL5). Cellulose fractions of no H_2O_2 group had hemicellulose indicative peaks showing hemicellulose impurity. Increase of NaOH concentration decreased the strength of arabinosyl shoulders at about 990 cm^{-1} and 1158 cm^{-1} up to 1 M NaOH concentration (FL3) where they were almost disappeared. Increasing NaOH concentration further caused to increase the strength of the shoulders further. These findings support the analytical hemicellulose measurements (Table 1) where hemicellulose recovery increased up to 1 M NaOH at no H_2O_2 group.

Hydrogen peroxide added groups usually had weaker lignin and hemicellulose indicative peaks at cellulose fractions. Hemicellulose and lignin indicative peaks usually weaken as increase of H_2O_2 or NaOH concentrations and they were disappeared at the highest H_2O_2 and NaOH concentrations (FL30). At that treatment condition, cellulose fraction was free of hemicellulose and lignin indicative peaks while hemicellulose fraction was almost free of lignin indicative peaks (Figure 4).

In short, the results of spectral analyses were in parallel to the analytical findings (Table 1). The strengths of lignin indicative bands of cellulosic fractions usually decreased as increase of H_2O_2 and NaOH concentrations and disappeared at the highest H_2O_2 and NaOH concentrations (FL30). Similar pattern was observed at hemicellulose fractions and lignin indicative bands were almost disappeared at the FL30 sample (Figure 4).

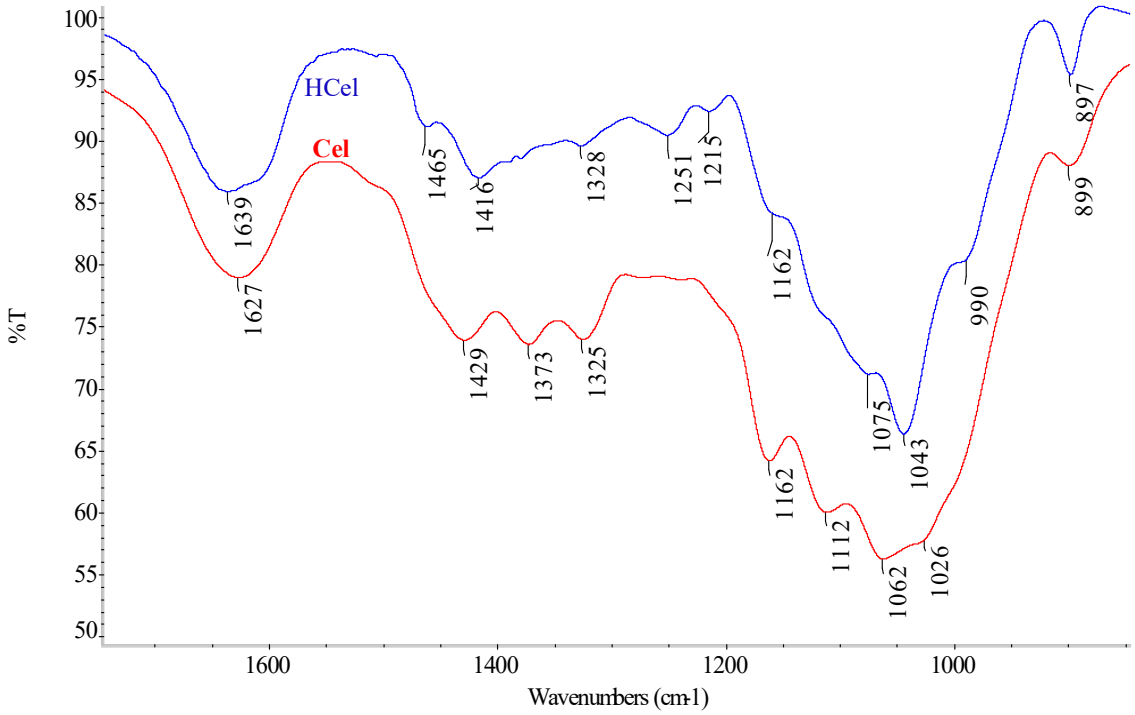
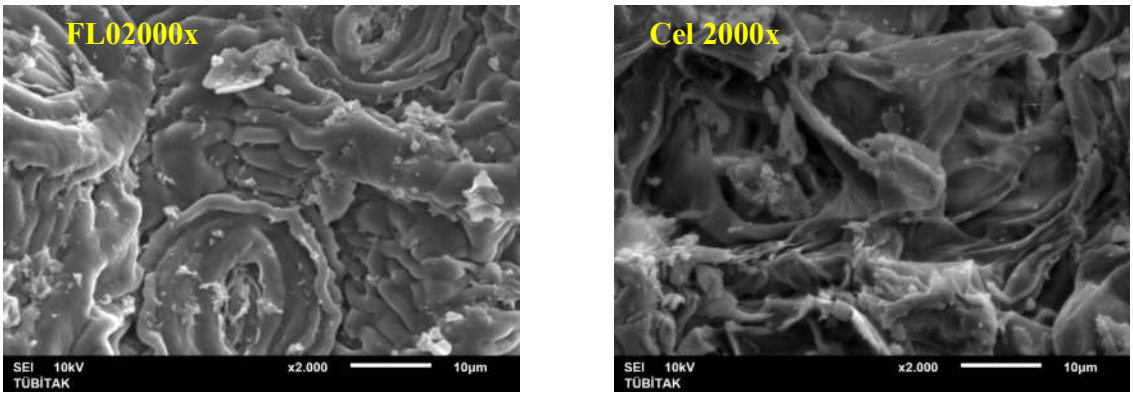


Figure 4. FTIR spectra of cellulose (Cel) and hemicellulose (HCel) fractions of FL30

SEM images

Untreated fall leaves (FL0) and the cellulose fraction of 3 M NaOH + 3 M H_2O_2 treatment condition (FL30-Cel) were also compared via SEM images (Figure 5). Cellulose is surrounded by hemicellulose and lignin naturally. When the biomaterials are processed to isolate cellulose, filamentous structure of cellulose can be identified at SEM images (Lu & Hsieh, 2010). In other words, observability of filamentous structures of cellulose microfibrils at SEM images are good indicators for cellulose purity.



a) FL0-2000X **b) Cel-2000X**

Figure 5. SEM images of untreated fall leaf litters (FL0) and cellulose fraction (Cel) of FL30 under 2000x magnification.

3 M NaOH + 3 M H_2O_2 treatment condition had removed 99.5% of hemicellulose and 81.6% of lignin, which prevents industrial evaluation of cellulose such as bioethanol production by inhibition of cellulase enzyme (Si et al., 2015; Talebnia et al., 2010). In other words, the alkaline peroxide treatment increased the purity of cellulose by removing most of hemicellulose and lignin, which was supported with FTIR spectra. Purification of cellulose was also supported with SEM images (Figure 5). Filamentous structure of cellulose was observed at the cellulose fraction of FL30 (Cel) but was not observed at untreated FL0 sample. Therefore, 3 M NaOH + 3 M H_2O_2 treatment condition (FL30) seems successful at removing hemicellulose and lignin surrounding cellulose microfibrils. Also, since the alkaline peroxide treatment condition released the cellulose microfibrils, they became reachable for further processes such as enzymatic modifications. Therefore, by using known methods, the cellulose end product can be converted to other products such as degradation to glucose for bioethanol production (Si et al., 2015; Talebnia et al., 2010), or modification to other cellulosic products such as cellulose ethers, cellulose esters (Klemm et al., 2005) and oxidized cellulose (Isobe et al., 2013).

Conclusion

Fall leaves are environmental problems to urban areas. Because of limited study of processing wasted fall leaves, they are collected and destroyed. Destroying of the fall leaves wastes the high cellulose and hemicellulose contents of them, which could be evaluated if isolated. For these reasons, this study aimed conversion of wasted fall leaves into precious hemicellulose and cellulose products by using clean alkaline peroxide treatment at room temperature and atmospheric pressure. In this concept, room temperature alkali peroxide treatment conditions were optimized for

hemicellulose recovery and cellulose delignification. Hemicellulose recovery and cellulose delignification increased mostly in parallel to NaOH and H_2O_2 concentrations. Optimum treatment condition was determined as 3 M NaOH + 3 M H_2O_2 concentrations where 99.5% hemicellulose recovery and 81.6% cellulose delignification were achieved. The cellulose and hemicellulose products were confirmed by analytical, spectral and morphological methods.

Since several parameters of alkaline peroxide were searched at this study, the optimum parameters for commercialization can be chosen by comparing the consumptions and prices of NaOH and H_2O_2 with yields and prices of hemicellulose and cellulose. Also, the optimizations were performed at room temperature and at atmospheric pressure, so scale up and commercialization studies can be performed by simple and cheap instruments. Therefore, in the light of this study, wasted fall leaves can be evaluated industrially as source of cellulose and hemicellulose in industry.

Acknowledgements

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