

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Composition of Napier grass

The concentration of cell wall components was measured by using the Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Digestible Lignin (ADL) procedures (Van Soest *et al.*, 1991). The chemical composition was analyzed by the following Equations:

$$\text{NDF} = \text{Cellulose} + \text{Hemicellulose} + \text{Lignin} \quad (4.1)$$

$$\text{ADF} = \text{Cellulose} + \text{Lignin} \quad (4.2)$$

$$\text{ADL} = \text{Lignin} \quad (4.3)$$

Napier grass (*Pennisetum purpureum*) in this research consists of two types: Pakchong 1 (*Pennisetum purpureum* x *P. glaucum* Pakchong1) from Kanchanaburi province (Kan) and Saraburi province (Sa) and Jakkapat (*Pennisetum purpureum* x *P. alopecuroides*). The main chemical component of Napier grass is shown in Table 4.1.

Table 4.1 The chemical composition of untreated Napier grass

Composition	Percentage					
	Pakchong1 Kan (Leaf)	Pakchong1 Kan (Stem)	Jakkapat (Leaf)	Jakkapat (Stem)	Pakchong1 Sa (Leaf)	Pakchong1 Sa (Stem)
Cellulose	40.94	39.28	37.11	42.33	31.84	31.38
Hemicellulose	24.37	20.24	24.50	17.11	19.16	20.55
Acid detergent lignin	4.96	8.90	5.44	11.11	5.05	6.17
Others	29.73	31.58	32.95	29.45	43.95	41.90

Note : Others include extractive, acid soluble lignin, protein and ash.

Cellulose and hemicellulose are important for producing bioethanol and biobutanol because they contain a lot of sugar type such as glucose and xylose. In

addition, they can be used to investigate the theoretical maximum of total sugar yield from Equation as shown below:

$$\text{The theoretical maximum of total sugar yield (\%)} = (1.111 \times C) + (1.136 \times H) \quad (4.4)$$

Where 1.111 = the factor that converts cellulose to equivalent glucose

1.136 = the factor that converts hemicellulose to equivalent xylose

C = cellulose content

H = hemicellulose content (Janu *et al.*, 2011)

From Table 4.1 and Equation 4.4, 73.24% of Pakchong 1 Kan (Leaf) can be converted to sugar and followed by Jakkapat (Leaf) 69.13%, Pakchong 1 Kan (Stem) 66.69%, Jakkapat (Stem) 66.52%, Pakchong 1 Sa (Leaf) 57.20% and Pakchong 1 Sa (Stem) 58.27%, respectively. When using 20 g/l of substrate solution which was analyzed by the ratio of Napier grass (g) and volume of sodium citrate buffer (L) found that The theoretical maximum of total sugar concentration of Pakchong 1 Kan (Leaf), Jakkapat (Leaf), Pakchong 1 Kan (Stem), Jakkapat (Stem), Pakchong 1 Sa (Leaf) and Pakchong 1 Sa (Stem) were 14.65 g/l, 13.83 g/l, 13.34 g/l, 13.30 g/l, 11.44 g/l and 11.65 g/l, respectively. From the experimental results, the highest total sugar concentration was obtained from Pakchong 1 Kan. In addition, leaf gave higher total sugar concentration than stem. The sugar concentration obtained from Pakchong 1 Kan was higher result than those obtained Jakkapat and Pakchong 1 Sa.

4.2 Effect of Acid Type on Total Sugar Yield

To improve the total sugar concentration of Napier grass, adding acid as a catalyst was the first choice. Recently, acid can be used as a catalyst to the degradation of biomass in ionic liquid (Ogura *et al.*, 2014). Zhang *et al.*, 2012 reported that the HCl in aqueous-[Bmim][Cl] gave the higher yield of glucose concentration compared to [Bmim][Cl] only because it probably increases chloride ion in HCl aqueous-[Bmim][Cl]. To investigate the suitable acid type for [Emim][Ac], the optimal conditions from previous work was used to study the effect of acid type on total sugar yield. Trisinsub, 2013 found that the optimal conditions

for aqueous-50 volume% ionic liquid [Emim][Ac] pretreated rice straw samples was 162 °C for 48 min.

4.2.1 Total Sugar Concentration

In the present study, two acids (HCl and CH₃COOH) were used as an acid catalyst in the IL pretreatment. As shown in Figure 4.1, the napier pretreated with 1 volume% HCl-aqueous [Emim][Ac] solution provided equivalent sugar yield to aqueous [Emim][Ac] solution. However, the sugar yields became lower at higher hydrochloric acid (HCl) concentrations as a result of small ionic-size of chloride anion (Cl⁻) compared to (CH₃COO⁻). It can suitably interact with [Emim]⁺. Nevertheless, at 4 volume% HCl-aqueous [Emim][Ac] gave higher total sugar concentration than 3 volume% HCl-aqueous [Emim][Ac]. It is possibly due to reverse mechanism or acid catalyst reaction. Firstly, reverse mechanism, hydrochloric acid (HCl) enhanced the amount of chloride anion (Cl⁻) resulting in chloride anion (Cl⁻) more interacted with [Emim]⁺. Then, [Emim][Cl] could dissolve cellulose. Secondly, acid catalyst reaction, hydrochloric acid (HCl) acted as acid catalyst. When increasing acid concentration, total sugar concentration increased. Besides, at any acetic acid (CH₃COOH) concentration, CH₃COOH aqueous-[Emim][Ac] solution had higher total sugar concentration than aqueous [Emim][Ac] solution. But, the sugar yields obtained from CH₃COOH aqueous-[Emim][Ac] had the same tendency of using hydrochloric acid (HCl) as a catalyst; total sugar concentration decreased at higher acetic acid (CH₃COOH) concentrations. It is possibly because increasing acetate anion (CH₃COO⁻) concentration by adding acetic acid (CH₃COOH) can support acetate anion (CH₃COO⁻) in ionic liquid ([Emim][Ac]) system to improve pretreatment efficiency (Zhang *et al.*, 2012), as shown in Figure D1.

Moreover, the size of chloride anion (Cl⁻) in hydrochloric acid (HCl) is smaller than of acetate anion (CH₃COO⁻) and interact with [Emim]⁺. In addition, it could be explained by H-bonding basicity. Generally, hydroxyl group of cellulose interacts with the anion of ionic liquid which serves as H-bonding acceptor. The ability of ionic liquids to act as H-bonding acceptors is measured by H-bonding basicity (β). H-bonding basicity of acetate anion is 1.09, which is greater than of

chloride anion (0.87) (Ha *et al.*, 2011). From these reasons, acetate anion (CH_3COO^-) can interact with hydrogen atom in hydroxyl group better than chloride anion (Cl^-). Therefore, adding acetic acid (CH_3COOH) as a catalyst increases highertotal sugar concentration and it is more effective than hydrochloric acid (HCl).

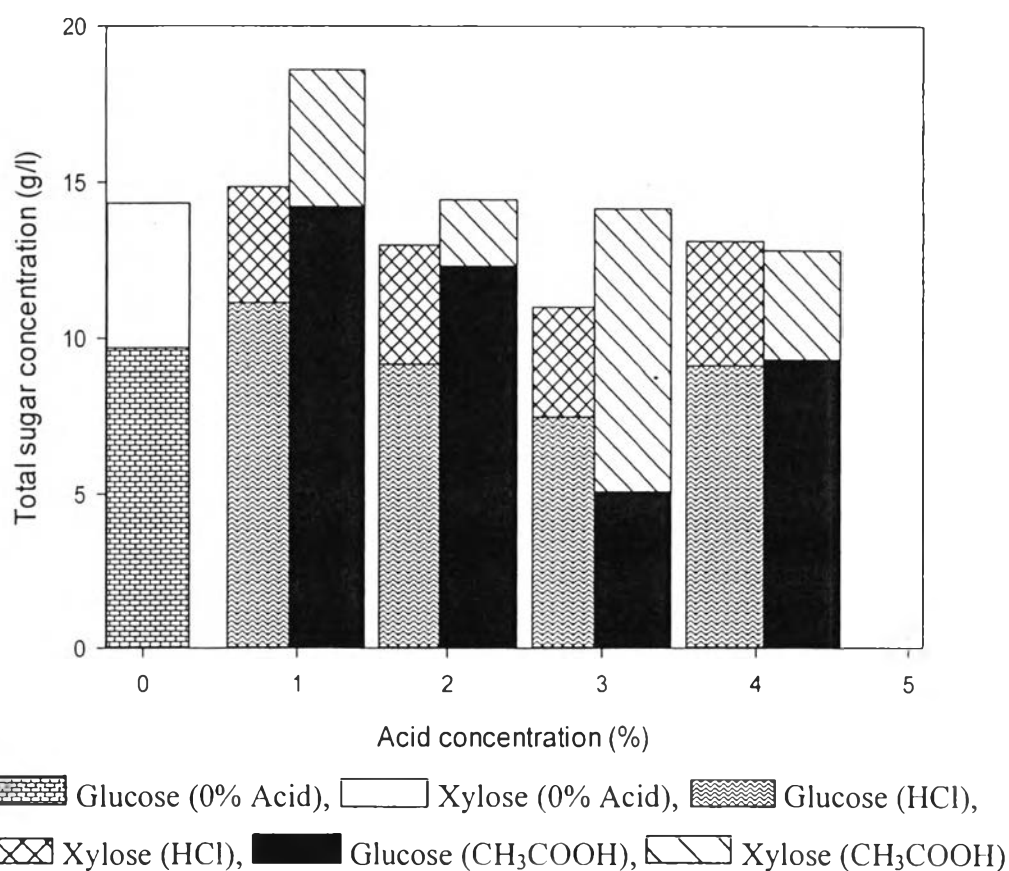


Figure 4.1 Comparison of total sugar concentration and acid type with different acid concentrations by using Pakchong1 from Saraburi province as a raw material. Pretreatment conditions: 50 volume% [Emim][Ac] and 162 °C for 48 min.

4.2.2 Crystallinity Index by X-ray Diffraction

After pretreatment, the structure of cellulose will be changed from crystal to amorphous structure by disrupting inter- and intra- hydrogen bonding of cellulose (Li *et al.*, 2010). Therefore, X-ray diffraction was used to analyze structure of biomass. The changes in the crystallinity of aqueous-[Emim][Ac], HCl aqueous-[Emim][Ac], and CH_3COOH aqueous-[Emim][Ac] are presented in Figure 4.2 and

the crystallinity index (CrI) are shown in Table 4.2. The crystallinity index was used to examine the amount of cellulose I in biomass (Cheng *et al.*, 2011). It could be calculated from Equation 4.5.

$$CrI = \frac{I_{002} - I_{amorphous}}{I_{002}} \quad (4.5)$$

Where I_{002} is the intensity of crystalline portion (i.e., cellulose) at about $2\theta = 22.5^\circ$ and $I_{amorphous}$ is the minimum intensity between the 101 and 002 lattice plane (i.e., cellulose, hemicellulose and lignin) at about $2\theta = 18.7^\circ$ (Kumar *et al.*, 2009; Qing *et al.*, 2014). However, in this study, the amorphous peak occurred at $2\theta = 18^\circ$ and the crystalline peak occurred at $2\theta = 22.2^\circ$.

Cellulose has four polymorphic crystalline structure, including cellulose I, II, III and IV. However, cellulose I and II have been most studies form of cellulose. Cellulose I is the native cellulose structure which consist of amorphous and crystalline regions. In the crystalline regions, cellulose chains contain two phases of cellulose I; cellulose I_α and I_β (Yue, 2011). Cellulose I_α has a triclinic unit cell containing one chain and cellulose I_β has a monoclinic unit cell containing two parallel chains. The amount of cellulose I_α and I_β depends on the original cellulose (Nishiyama *et al.*, 2002). Cellulose I is converted to cellulose II via regeneration or mercerization (Cheng *et al.*, 2011). It has higher chemical reactivity than cellulose I, then it is useful fibers and used in many applications in chemical industry. Moreover, cellulose II is more stable form than cellulose I (Yue, 2011). The structure of cellulose I and II is shown in Figure 4.2.

From Figure 4.3, crystalline peak of untreated was board, compared to pretreated samples. After pretreatment, the peak of crystalline cellulose I (002) had higher intensity $2\theta = 22.2^\circ$ and changed a little bit at any acid concentration. However, the crystallinity index of treated biomass increased. As shown in Table 4.2, the crystallinity index of untreated Pakchong 1 from Saraburi province (CrI 0.25) was lower than aqueous-[Emim][Ac], CH_3COOH aqueous-[Emim][Ac] and HCl aqueous-[Emim][Ac]. When adding acid in aqueous-[Emim][Ac], the CrI increased because amorphous cellulose, hemicellulose, and lignin in pretreated biomass was broken down. (Li *et al.*, 2010). Our result agrees with Zhang *et al.*,

2012; however, the CrI decreased at high acid loadings. This may be due to the loss of amorphous hemicellulose (Qing *et al.*, 2014). When focused on the effect of acid type on CrI, almost all acid concentrations, CH₃COOH aqueous-[Emim][Ac] gave higher crystallinity index than HCl aqueous-[Emim][Ac]. Therefore, acetic acid (CH₃COOH) had more effective than hydrochloric acid (HCl).

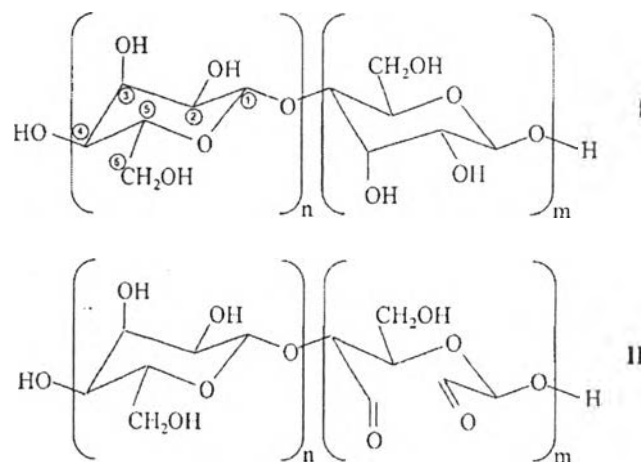


Figure 4.2 The structure of cellulose I and cellulose II

(http://patentimages.storage.googleapis.com/WO2012119229A1/imgf000023_0001.png).

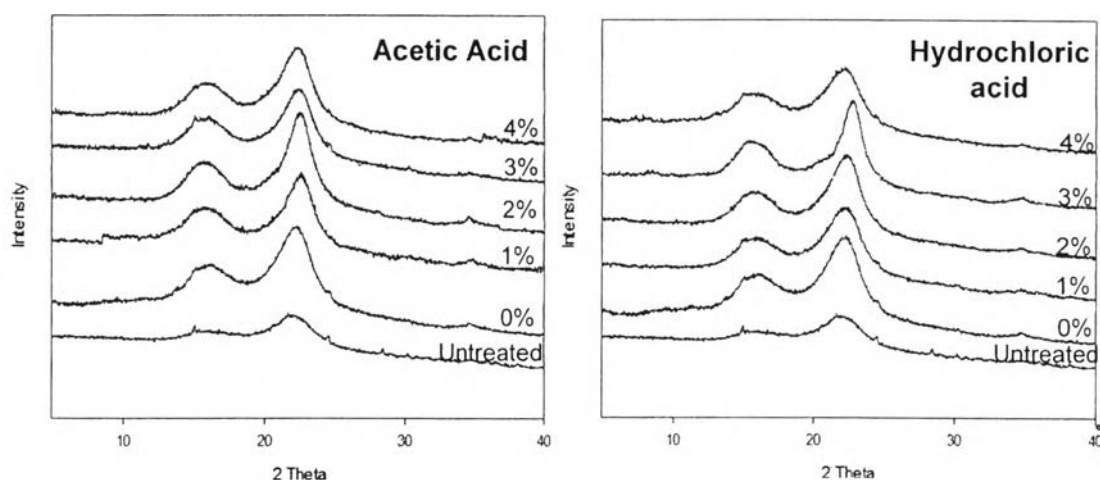


Figure 4.3 X-ray diffraction patterns of untreated Pakchong 1 from Saraburi province, aqueous-[Emim][Ac], (a) 1-4 volume% CH₃COOH in aqueous [Emim][Ac] and (b) 1-4 volume% HCl in aqueous [Emim][Ac], respectively. Pretreatment conditions: 50 volume% [Emim][Ac] and 162 °C for 48 min.

Table 4.2 Crystallinity index of aqueous-[Emim][Ac], CH₃COOH aqueous-[Emim][Ac], and HCl aqueous-[Emim][Ac]

Acid Concentration (volume%)	Crystallinity Index (CrI)	
	CH ₃ COOH	HCl
Untreated	0.25	
0	0.40	
1	0.41	0.38
2	0.50	0.46
3	0.41	0.52
4	0.44	0.36

4.2.3 Crystallinity Index and Functional Group by Fourier Transform

Infrared Spectroscopy (FTIR)

The FTIR spectra of untreated and pretreated Napier grass of Pakchong 1 from Saraburi province at any acid concentration are shown in Figure 4.4. The cellulose structure was estimated by TCI, LOI and lignin/cellulose ratio. Nelson and O'Connor introduced the total crystalline index (TCI, $A_{1372}/A_{2900\text{cm}^{-1}}$) which measured the crystallinity of cellulose I and II. The absorption band at 1372 cm^{-1} is assigned to C-H bending in cellulose and hemicellulose and 2900 cm^{-1} is assigned to C-H and CH₂ stretching of cellulose (Nelson and O'connor, 1964). Furthermore, Hurtubise and Krassig proposed the ratio of two absorption band between $A_{1430\text{cm}^{-1}}$ and $A_{897\text{cm}^{-1}}$. It is known as lateral order index (LOI) (Hurtubise and Krassig, 1960) which investigated cellulose I fraction in the cellulose structure (Oh *et al.*, 2005). The band at 1430 cm^{-1} is associated with the amount of crystalline structure of the cellulose I (CH₂ scissoring motion) while the adsorption band 897 cm^{-1} was assigned as C-O-C stretching at the β -(1,4)-glycosidic linkage which was strong and sharp in cellulose II and amorphous cellulose. (Ha *et al.*, 2011; Poletto *et al.*, 2012). The last, the ratio between the peak area at 1510 and 897 cm^{-1} was used to

estimate the amount of lignin and cellulose. The peak at 1510 cm^{-1} refers to aromatic skeleton from lignin (Li *et al.*, 2010).

From Table 4.3 shows that the trend obtained from CH_3COOH aqueous-[Emim][Ac] and HCl aqueous-[Emim][Ac] had different results. The TCI of the untreated sample is lower than those of aqueous-[Emim][Ac] and acidic aqueous-[Emim][Ac]. Moreover, CH_3COOH aqueous- [Emim][Ac] and HCl aqueous-[Emim][Ac] were contrary trend. On the other hand, LOI values and the ratio of lignin and cellulose continuously increased after the samples were pre-treated with CH_3COOH aqueous-[Emim][Ac] while it decreased in HCl aqueous-[Emim][Ac] pretreatment. Besides, low concentration of acetic acid (CH_3COOH) gave low LOI and lignin/cellulose ratio which implied that cellulose crystalline structure decreased and transformed to amorphous form. In addition, it provided high surface accessibility for enzyme (Spiridon *et al.*, 2010; Ha *et al.*, 2011). Normally, many reports did not show the correspond result of TCI (Fan *et al.*, 2012). According to the LOI and the ratio of lignin and cellulose, acetic acid (CH_3COOH) is the appropriate chemical as acid catalyst. These results agree with XRD results. It could be explained that after ionic liquid pretreatment, it removed lignin and reduced hemicellulose and cellulose crystallinity. Furthermore, it increased cellulose accessibility.

Table 4.3 TCI, LOI and the ratio of lignin and cellulose of the untreated and pretreated samples

Sample	TCI		LOI		Lignin/Cellulose	
	CH_3COOH	HCl	CH_3COOH	HCl	CH_3COOH	HCl
Untreated	0.90		3.63		3.07	
0%	0.96		2.16		1.24	
1%	1.56	1.02	1.10	1.73	0.59	1.23
2%	1.29	1.16	1.72	1.93	0.85	1.25
3%	0.87	1.21	2.25	1.65	1.30	0.74
4%	0.92	1.52	1.90	1.24	1.15	0.82

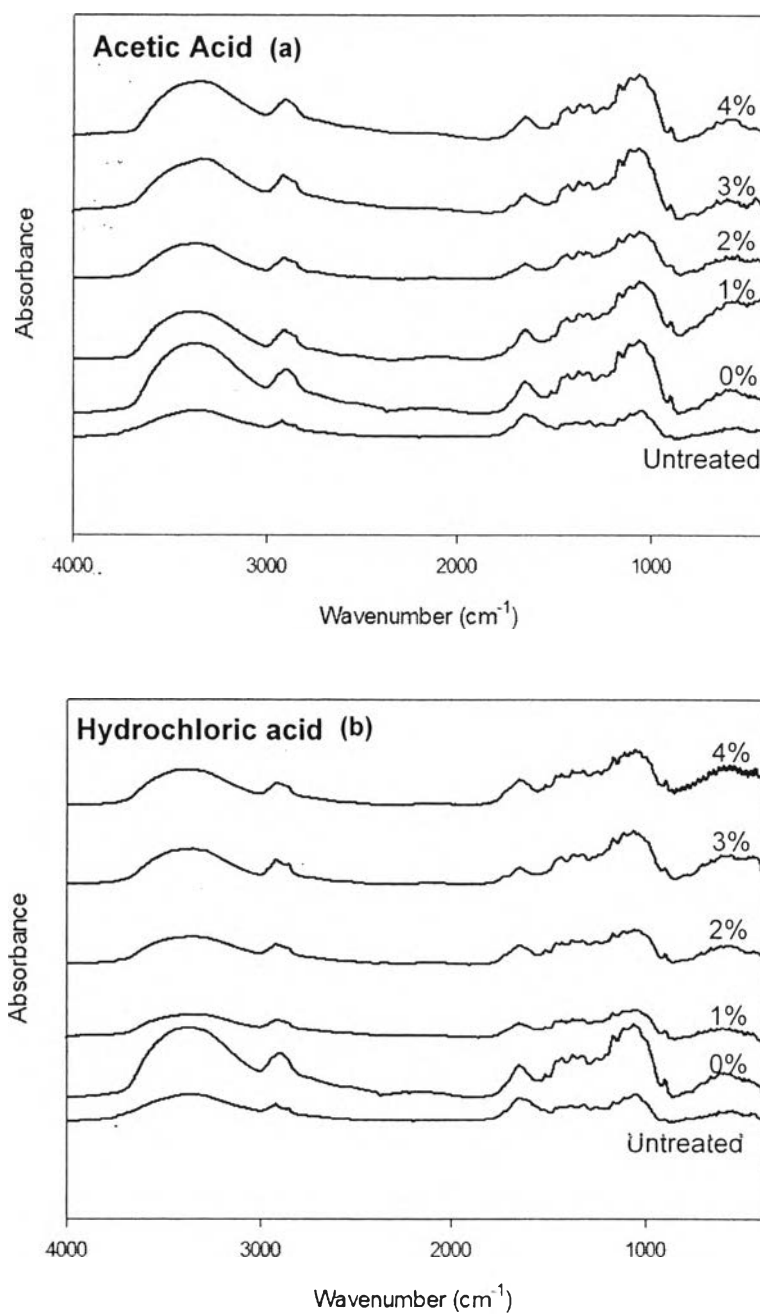


Figure 4.4 FTIR spectra of untreated Pakchong 1 from Saraburi province, aqueous-[Emim][Ac], (a) 1-4 volume% CH_3COOH in aqueous [Emim][Ac] and (b) 1-4 volume% HCl in aqueous [Emim][Ac], respectively. At pretreatment conditions: 50 volume% [Emim][Ac] and 162°C for 48 min.

4.3 Optimization of the Total Sugar Concentration using Response Surface

Methodology (RSM)

To enhance the efficiency of pretreatment, response surface methodology (RSM) with a full five factorial central composite design (CCD) was selected to investigate the effect of temperature, time, biomass loading, and acid concentration by using microwave irradiation as heat source and the Napier grass Pakchong 1 from Kanchanaburi province as raw material. The independent variables and total sugar concentration results are depicted in Table 4.4. The regression coefficient in Table 4.5 is the polynomial equation of total sugar concentration in terms of temperature, time, biomass loading, and acid concentration, as shown in Equation 4.6.

$$Y_1 = 4.58727 + 2.41059x_1 + 1.20252x_2 - 0.88586x_3 - 0.30140x_4 + 0.63041x_1x_2 - 1.51673x_1x_3 - 0.05115x_1x_4 - 1.10256x_2x_3 - 0.25568x_2x_4 + 0.33290x_3x_4 + 0.67467x_1^2 + 0.16244x_2^2 - 0.12454x_3^2 - 0.43529x_4^2 \quad (4.6)$$

Where Y_1 is the total sugar concentration (g/l), x_1 is the temperature ($^{\circ}\text{C}$), x_2 is the time (min), x_3 is the biomass loading (g/l) and x_4 is the acid concentration (volume%).

Table 4.4 Experimental design and results of the central composite design of CH_3COOH aqueous-[EMIM][Ac]/Microwave pretreatment

Run	Temp ($^{\circ}\text{C}$)	Time (min)	Biomass loading (g/l)	Acid Conc. (volume%)	Glucose (g/l)	Xylose (g/l)	Total sugar (g/l)
1	120	20	20	1	1.51	0.78	2.29 \pm 0.04
2	120	20	20	3	1.52	0.72	2.24 \pm 0.10
3	120	20	40	1	1.43	0.84	2.26 \pm 0.14
4	120	20	40	3	1.41	0.64	2.06 \pm 0.57
5	120	50	20	1	2.17	1.27	3.44 \pm 0.08

Table 4.4 Experimental design and results of the central composite design of CH₃COOH aqueous-[EMIM][Ac]/Microwave pretreatment (cont.)

Run	Temp (°C)	Time (min)	Biomass loading (g/l)	Acid Conc. (volume%)	Glucose (g/l)	Xylose (g/l)	Total sugar (g/l)
6	120	50	20	3	1.78	0.76	2.54 ± 0.06
7	120	50	40	1	1.8	0.9	2.70 ± 0.24
8	120	50	40	3	1.66	0.75	2.41 ± 0.48
9	160	20	20	1	3.77	3.18	6.94 ± 0.30
10	160	20	20	3	2.98	2.25	5.22 ± 0.13
11	160	20	40	3	3.01	2.06	5.08 ± 2.46
12	160	20	40	1	1.93	0.98	2.91 ± 0.33
13	160	50	20	1	9.43	4.9	14.33 ± 1.30
14	160	50	20	3	7.98	4.51	12.48 ± 0.18
15	160	50	40	1	2.2	1.06	3.25 ± 0.30
16	160	50	40	3	1.64	0.75	2.39 ± 0.65
17	100	35	30	2	1.21	0.44	1.65 ± 0.001
18	180	35	30	2	9.94	4.3	14.24 ± 0.89
19	140	5	30	2	1.63	0.69	2.32 ± 0.18
20	140	65	30	2	5.2	4.28	9.48 ± 0.48
21	140	35	10	2	2.22	1.23	3.45 ± 0.49
22	140	35	50	2	7.09	4.42	11.52 ± 1.36
23	140	35	30	0	2.58	1.81	4.39 ± 0.66
24	140	35	30	4	1.76	0.87	2.63 ± 0.79
25	140	35	30	2	1.98	1.21	3.19 ± 0.21
26-30	140	35	30	2	2.75	1.84	4.59 ± 0.03

Table 4.5 shows the results from ANOVA. The P-value are used to analyse the significance of coefficient and indicate the interaction between variables. If P-value had low value, the coefficient was more significance (Sasikumar and Viruthagiri, 2008). In this research, values of P-value less than 0.05 indicate that the term constants are significant. Therefore, temperature, time, and biomass loading had a significant effect on this model. The interaction between temperature and biomass loading and interaction between time and biomass loading were also significant.

The three-dimensional response surface and two-dimensional contour plots for the total sugar concentration in Figures 4.5-4.10 described the effects of interactions of each independent variable on total sugar concentration.

Table 4.5 Statistics regression for the optimization of total sugar concentration

Term constant	Regression coefficient	Standard error	P-value
Intercept	4.58727	0.8413	6.67×10^{-5}
x_1^a	2.41059	0.42065	3.98×10^{-5}
x_2^b	1.20252	0.42065	0.0120
x_3^c	-0.88586	0.42065	0.0525
x_4^d	-0.3014	0.42065	0.4847
x_1x_2	0.63041	0.51519	0.2400
x_1x_3	-1.51673	0.51519	0.0101
x_1x_4	-0.05115	0.51519	0.9222
x_2x_3	-1.10256	0.51519	0.0492
x_2x_4	-0.25568	0.51519	0.6269
x_3x_4	0.33290	0.51519	0.5279
x_1^2	0.67467	0.39348	0.1070
x_2^2	0.16244	0.39348	0.6856
x_3^2	-0.12454	0.39348	0.7560
x_4^2	-0.43529	0.39348	0.2860

^aTemperature (°C)

^bTime (min)

^cBiomass loading (g/l)

^dAcid concentration (volume%)

Figure 4.5 shows the interaction between time and temperature. The total sugar concentration increased with time and temperature. The effect of biomass loading and temperature on the total sugar concentration is presented in Figure 4.6. At high temperature and low biomass loading, the total sugar concentration reached to maximum. The interaction between biomass loading and time gave the same effect

on biomass loading and temperature, as shown in Figure 4.7. The maximum sugar concentration increased with high temperature and low biomass loading. From Figure 4.8, acid concentration were little effective to the total sugar concentration. At any acid concentration, total sugar concentration increased with temperature. The next interaction is presented in the Figure 4.9, the effect of biomass loading and acid concentration on total sugar concentration. At low acid concentration and biomass loading, they gave the optimum point, but the total sugar concentration was very low. The last effect on total sugar concentration were time and acid concentration, as shown in Figure 4.10. At low acid concentration and high pretreatment time gave the highest total sugar concentration.

The optimal conditions from RSM are 147 °C, 76 min, 20 g/l biomass loading, and 1.287 volume% acetic acid. The extent of lignin dissolution in the ionic liquid decreases with increasing in biomass loading. These conditions gave sugar concentration of 15.72 g/l while the actual total sugar concentration at the same condition was 14.38 g/l. Although the total sugar concentration obtained from actual pretreatment experiment was lower than the model, the error of model was less than 10% (8.52%) and these conditions gave the high theoretical yield (98.17%; actual total sugar concentration/theoretical maximum total sugar concentration). In addition, the P-value of this model was 0.0024 and multiple R-square was 0.8159. Therefore, this was confirmed that this model could be used to predict the optimal conditions.

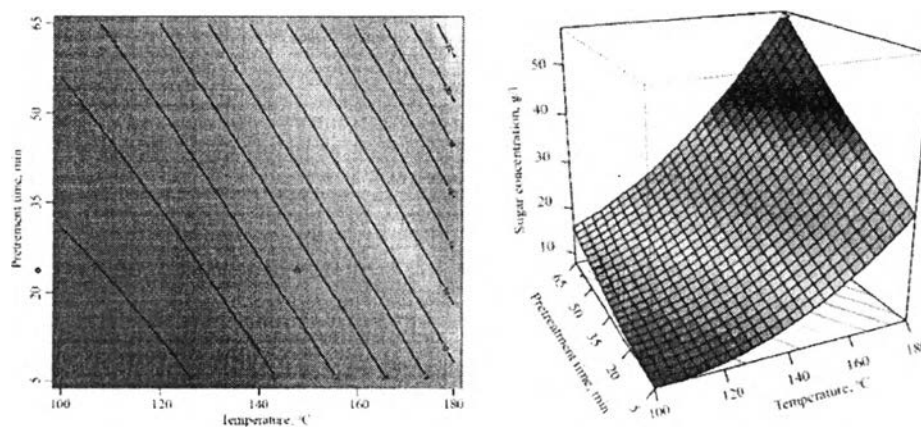


Figure 4.5 (a) Response surface and (b) contour plots of central composite design for optimization condition between pretreatment time and temperature of acidic aqueous ionic liquid pretreatment.

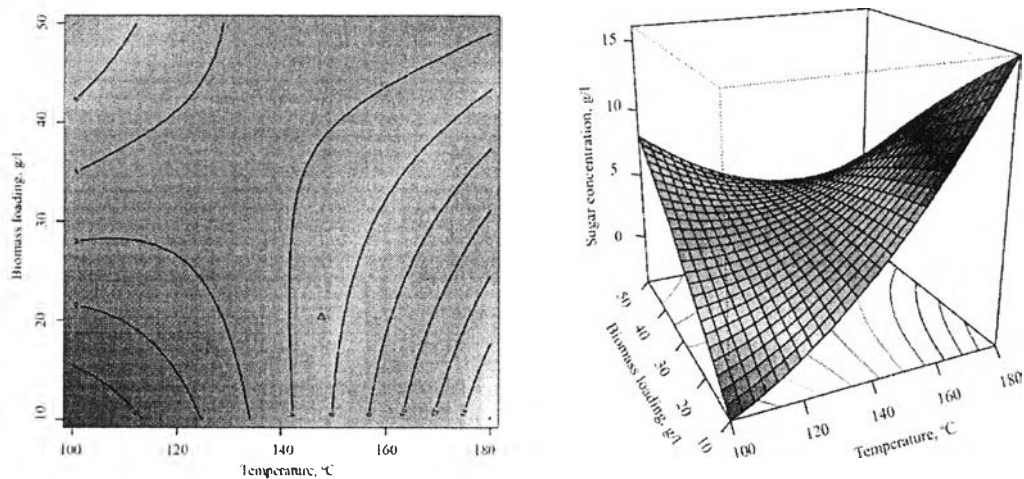


Figure 4.6 (a) Response surface and (b) contour plots of central composite design for optimization condition between biomass loading and temperature of acidic aqueous ionic liquid pretreatment.

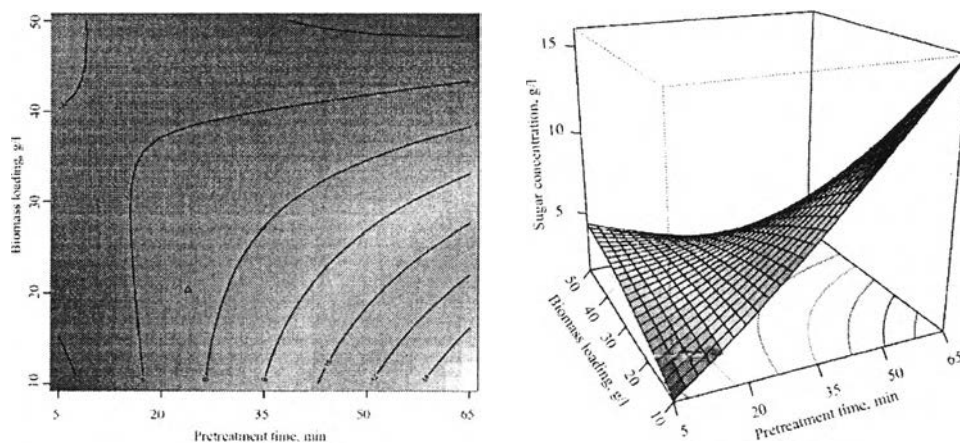


Figure 4.7 (a) Response surface and (b) contour plots of central composite design for optimization condition between biomass loading and pretreatment time of acidic aqueous ionic liquid pretreatment.

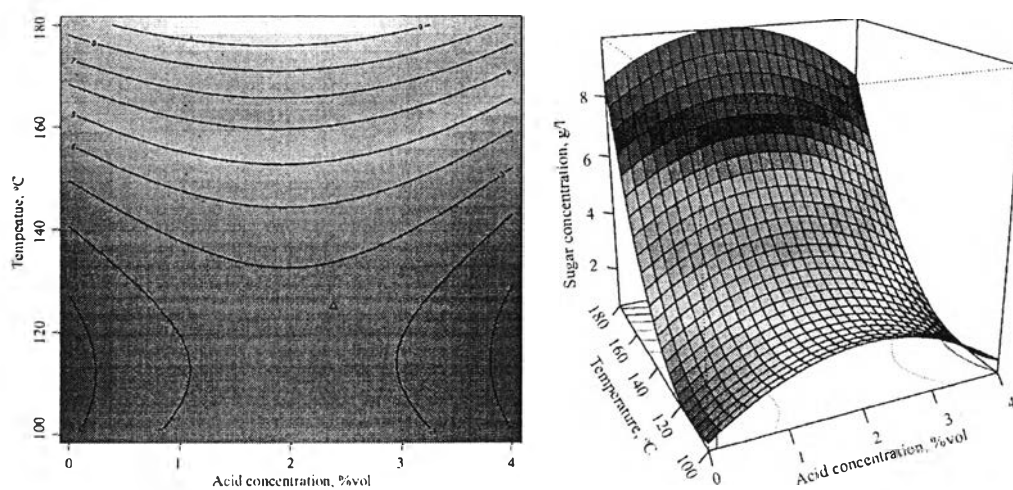


Figure 4.8 (a) Response surface and (b) contour plots of central composite design for optimization condition between temperature and acid concentration of acidic aqueous ionic liquid pretreatment.

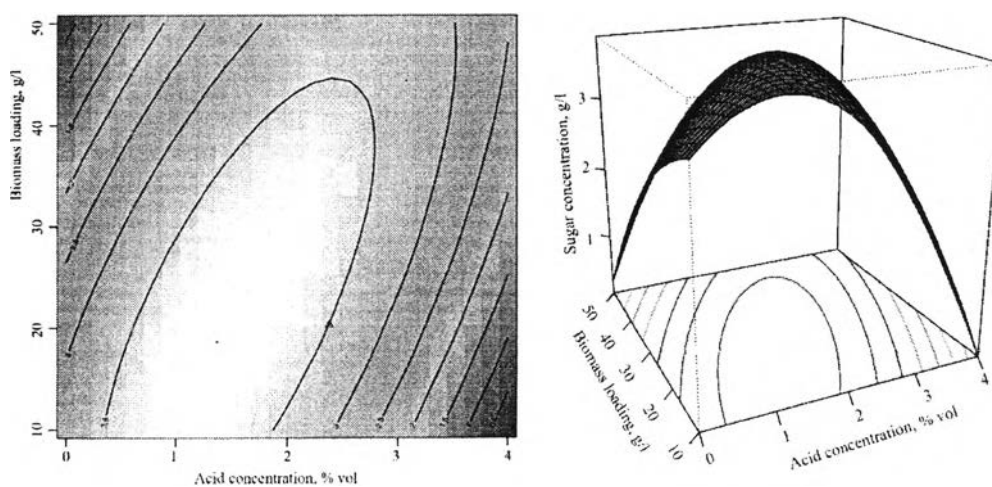


Figure 4.9 (a) Response surface and (b) contour plots of central composite design for optimization condition between biomass loading and acid concentration of acidic aqueous ionic liquid pretreatment.

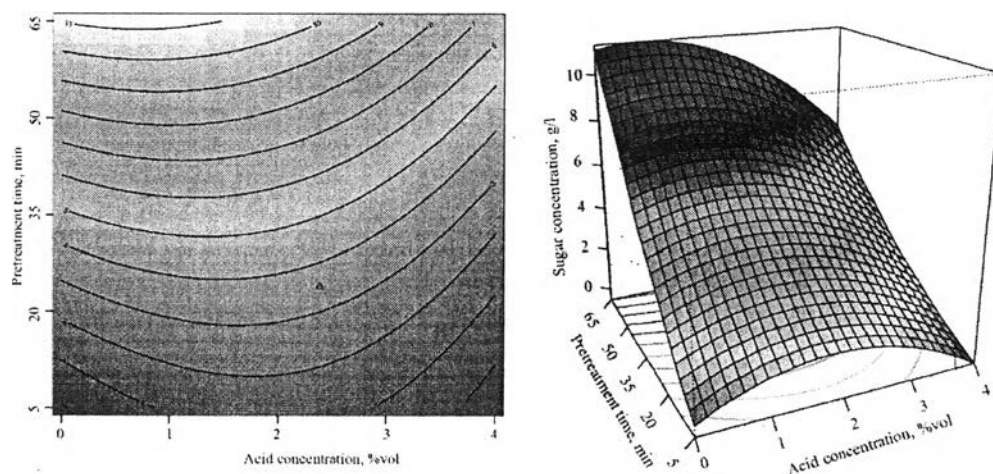


Figure 4.10 (a) Response surface and (b) contour plots of central composite design for optimization condition between pretreatment time and acid concentration of acidic aqueous ionic liquid pretreatment.

4.3.1 Crystallinity Measurement and Functional Group Analysis by XRD and FTIR

Figure 4.11 and Table 4.6 illustrate the lower crystalline structure and crystalline index (CrI) after pretreatment due to the disruption of inter- and intra-hydrogen bonding in cellulose by ionic liquid and increase more efficiency in enzymatic hydrolysis (Li *et al.*, 2010; Qing *et al.*, 2014). These results were confirmed by FTIR results. As mentioned before, TCI (total crystallinity index; $A_{1372}/A_{2900\text{cm}^{-1}}$) is used to measure the crystallinity of cellulose material (Nelson and O'Connor, 1964). LOI (lateral order index; $A_{1430}/A_{897\text{cm}^{-1}}$) (Hurtubise and Krassig, 1960) is indicated the cellulose I fraction in the cellulose structure (Oh *et al.*, 2005). Lastly, the ratio between lignin and cellulose ($A_{1510}/A_{897\text{cm}^{-1}}$) are used to estimate the amount of lignin and cellulose (Li *et al.*, 2010).

For the untreated samples, the strong peak at 1732, 1600, and 1510 cm^{-1} correspond to the presence of unjugated carbonyl, acetyl group of hemicellulose and aromatic skeleton vibrations, respectively (Sun *et al.*, 2009; Hosseinaei *et al.*, 2011). When the samples were pretreated, the intensity of the strong peaks was dramatically decreased, suggesting the removal of the lignin in the samples.

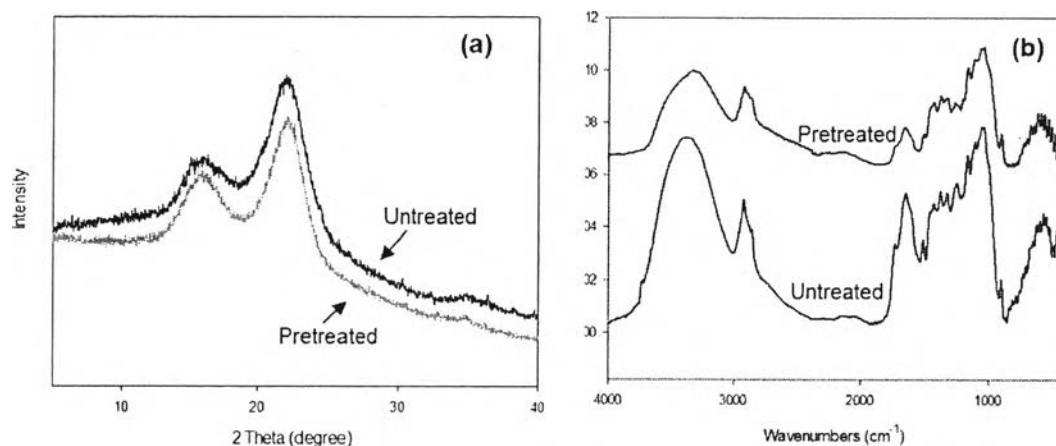


Figure 4.11 (a) XRD patterns and (b) FTIR spectra of untreated and pretreated Napier grass of Pakchong1 from Kanchanaburi province. Reaction conditions: at 147 °C, 76 min, 20 g/l biomass loading, and 1.287 volume% acetic acid.

Table 4.6 The crystalline index of untreated and pretreated samples of Pakchong1 from Kanchanaburi province by FTIR results

	Untreated	Pretreated
XRD	0.37	0.34
FTIR TCI ($A_{1372}/A_{2900} \text{ cm}^{-1}$)	8.99	0.93
LOI ($A_{1430}/A_{897} \text{ cm}^{-1}$)	2.41	1.47
Lignin/Cellulose ($A_{1510}/A_{897} \text{ cm}^{-1}$)	1.72	0.99

4.3.2 Thermal Analysis

The STA (simultaneous thermal analysis) was used to measure weight losses (TG) and derivative thermogravimetric (DTG) of biomass structure at specific temperature. Generally, the different two peaks in the DTG of pretreated samples was the degradation of hemicellulose and cellulose, respectively. In addition, lignin was decomposed in a wide range that overlaps partially of hemicellulose and cellulose (Zhang *et al.*, 2014). Hemicellulose started

decomposition at 220-315 °C and cellulose occurred at 315-400 °C while the degradation of lignin occurred in a wide temperature range (100-900 °C) (Yang *et al.*, 2007).

Figure 4.12 depicts the first derivative of untreated and pretreated of Napier grass. At 220-315 °C, the DTG peak of pretreated Napier grass appeared the gradually peak of hemicellulose but this peak disappeared in the untreated Napier grass. Thus, hemicellulose was reduced after pretreatment. In addition, at 315-400 °C, the peak of cellulose dropped sharply and reached to higher temperature, compared to the untreated, suggesting more thermal stability. The results described that the cellulose changed from cellulose I to cellulose II due to thermally more stable than native cellulose (cellulose I) (Zhang *et al.*, 2014).

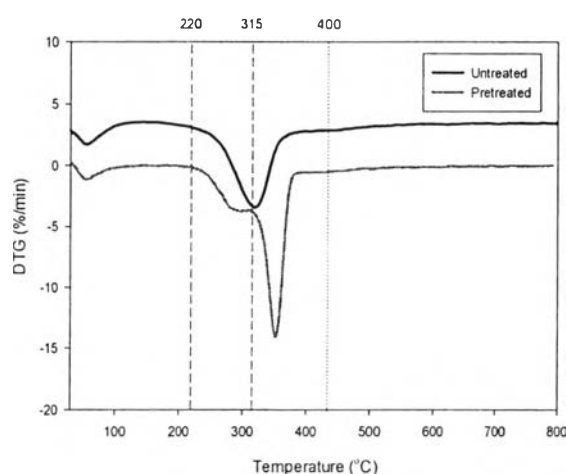


Figure 4.12 The first derivative of untreated and pretreated samples of Pakchong1 from Kanchanaburi province.

4.3.3 BET Surface Area and Pore Size Diameter

The surface area of pretreated Napier grass reduced from 3.98 to 3.27 m²/g and pore size diameter increased from 59.91 to 72.13 Å, as shown in Table 4.7. It was because of the swelling behavior (Manaso *et al.*, 2013). A decrease in surface area and cleavage of the pore was observed. These results were in accordance with SEM images. The pore size of the Napier sample after pretreatment was larger than that of untreated samples.

Table 4.7 Surface area and pore size diameter of untreated and pretreated samples of Pakchong1 from Kanchanaburi province

Sample	Surface area (m ² /g)	Pore size diameter (Å)	Total pore volume (cc/g)
Untreated	3.98	59.91	5.96×10^{-3}
Pretreated	3.27	72.13	5.90×10^{-3}

4.3.4 Scanning Electron Microscopy (SEM)

SEM images of untreated and pretreated samples of Pakchong1 from Kanchanaburi province are illustrated in Figure 4.13. As expected, compared to untreated samples, the pretreated samples show swelling surface and large pore which is presumably caused by removal of lignin. These images could support the crystallinity index obtained from the XRD and FTIR results, suggesting hemicellulose and lignin was removed after pretreatment. Moreover, the high pore size diameter and a decrease in crystallinity of XRD and FTIR results which indicated that the efficiency of enzymatic hydrolysis increased (Qiu *et al.*, 2012; Mood *et al.*, 2013).

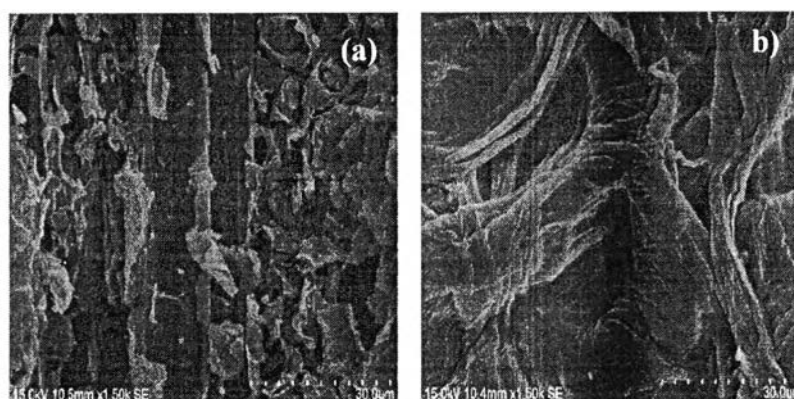


Figure 4.13 (a) SEM images of untreated and (b) pretreated samples of Pakchong1 from Kanchanaburi province.

4.4 Comparison of Total Sugar Concentration of Napier Grasses

In this research studied the effect of type (Pakchong 1 from Kanchanaburi and Saraburi province and Jakkapat Grass) and composition (leaf and stem) of Napier grass on total sugar concentration by using the optimal condition from the previous section. Pakchong 1 from Kanchanaburi province is the same subspecies of Pakchong 1 from Saraburi province, but they grow at different locations. Whereas Jakkpat grass is different subspecies from Pakchong 1 from Kanchanaburi province, but they grow at the same location.

As shown in Figure 4.14, both untreated and pretreated Napier grass, leaf provided higher total sugar concentration than stem and Pakchong 1 from Kanchanaburi province had better results than Jakkapat and Pakchong 1 from Saraburi province. It was possibly due to the recalcitrance of cell wall and chemical composition. Generally, the amount of cellulose and hemicellulose have an impact on the amount of reducing sugar after enzymatic hydrolysis. From Table 4.1, stem had more lignin and leaf provided higher total of cellulose and hemicellulose than stem. Although, Pakchong 1 leaf and stem from Saraburi province gave equivalent total of cellulose and hemicellulose, stem gave lower total sugar concentration. After pretreatment, the sugar concentration was increased. These values are about 4 to 5 fold higher than those obtained from untreated biomass. Thus, CH₃COOH aqueous-[Emim][Ac] could be used to reduce the amount of amorphous hemicellulose and lignin. Furthermore, it increased the accessibility of enzyme.

4.4.1 Crystallinity Measurement and Functional Group Analysis by XRD and FTIR

Figure 4.15 illustrated the XRD patterns of Napier grasses. It was found that the CrI in Table 4.8 increased after pretreatment. But, CrI of Pakchong 1 from Kanchanaburi province decreased. It might be determine from two factors: (1) swell behavior and dissolution of cellulose (decrease CrI); (2) removal of lignin and hemicellulose (increase CrI)(Shi *et al.*, 2014). Hence, other Napier grasses increased in CrI because removal of lignin and hemicellulose mechanism were dominant. Besides, CrI of Pakchong 1 from Kanchanaburi province depended on swell behavior

and dissolution of cellulose mechanism. The total sugar concentration and yield of other Napier grasses are shown in Table 4.9.

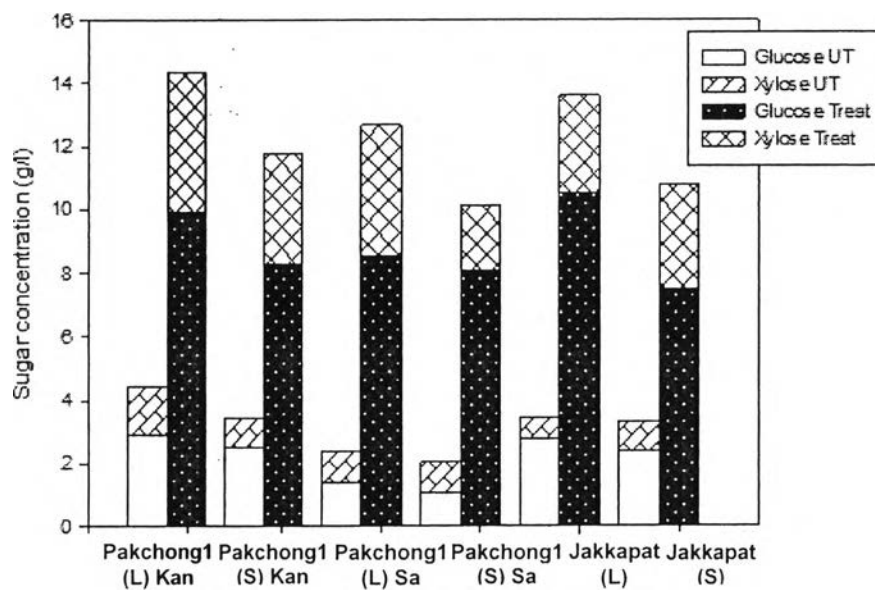


Figure 4.14 Total sugar concentration of untreated and pretreated of Napier grasses (leaf and stem).

Table 4.8 Crystalline index of untreated and pre-treated of Napier grasses

Sample	XRD		FTIR					
	CrI		TCI ($A_{1372}/A_{2900} \text{ cm}^{-1}$)		LOI ($A_{1430}/A_{897} \text{ cm}^{-1}$)		Lignin/Cellulose ($A_{1510}/A_{897} \text{ cm}^{-1}$)	
	Untreated	Pretreated	Untreated	Pretreated	Untreated	Pretreated	Untreated	Pretreated
Pakchong 1 (Stem) Kan	0.48	0.51	1.05	0.88	1.38	1.18	1.78	1.81
Pakchong 1 (Leaf) Sa	0.30	0.42	0.90	1.06	3.63	2.01	3.07	0.97
Pakchong 1 (Stem) Sa	0.26	0.35	1.36	0.94	3.83	1.41	1.36	0.94
Jakkapat (Leaf)	0.37	0.45	0.90	0.97	2.68	1.84	2.04	1.14
Jakkapat (Stem)	0.47	0.50	0.95	0.89	2.10	1.59	1.62	0.91

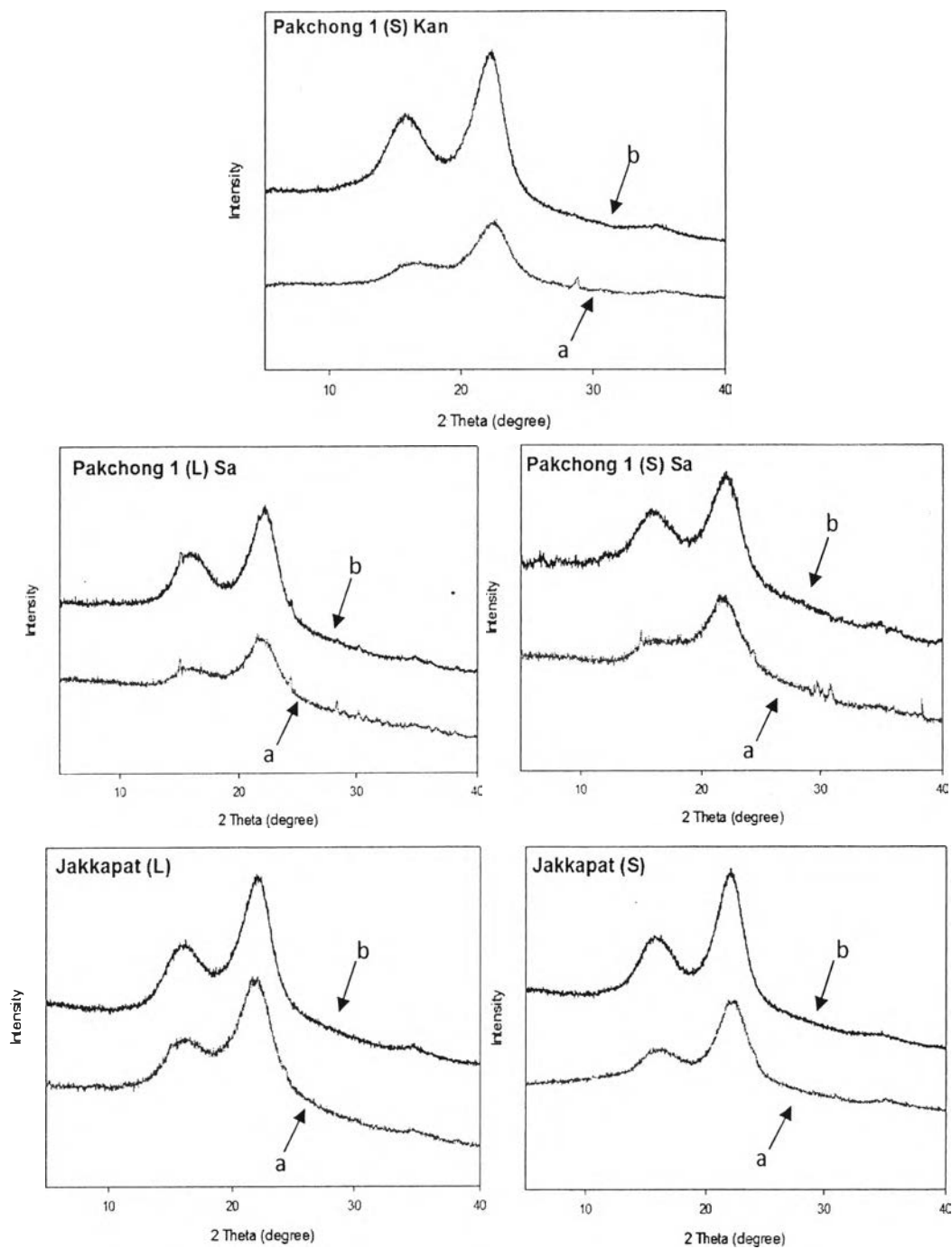


Figure 4.15 XRD patterns of a) untreated and b) pretreated samples of Pakchong 1 Napier grasses. Reaction conditions: at 147 °C, 76 min, 20 g/l biomass loading, and 1.287 volume% acetic acid.

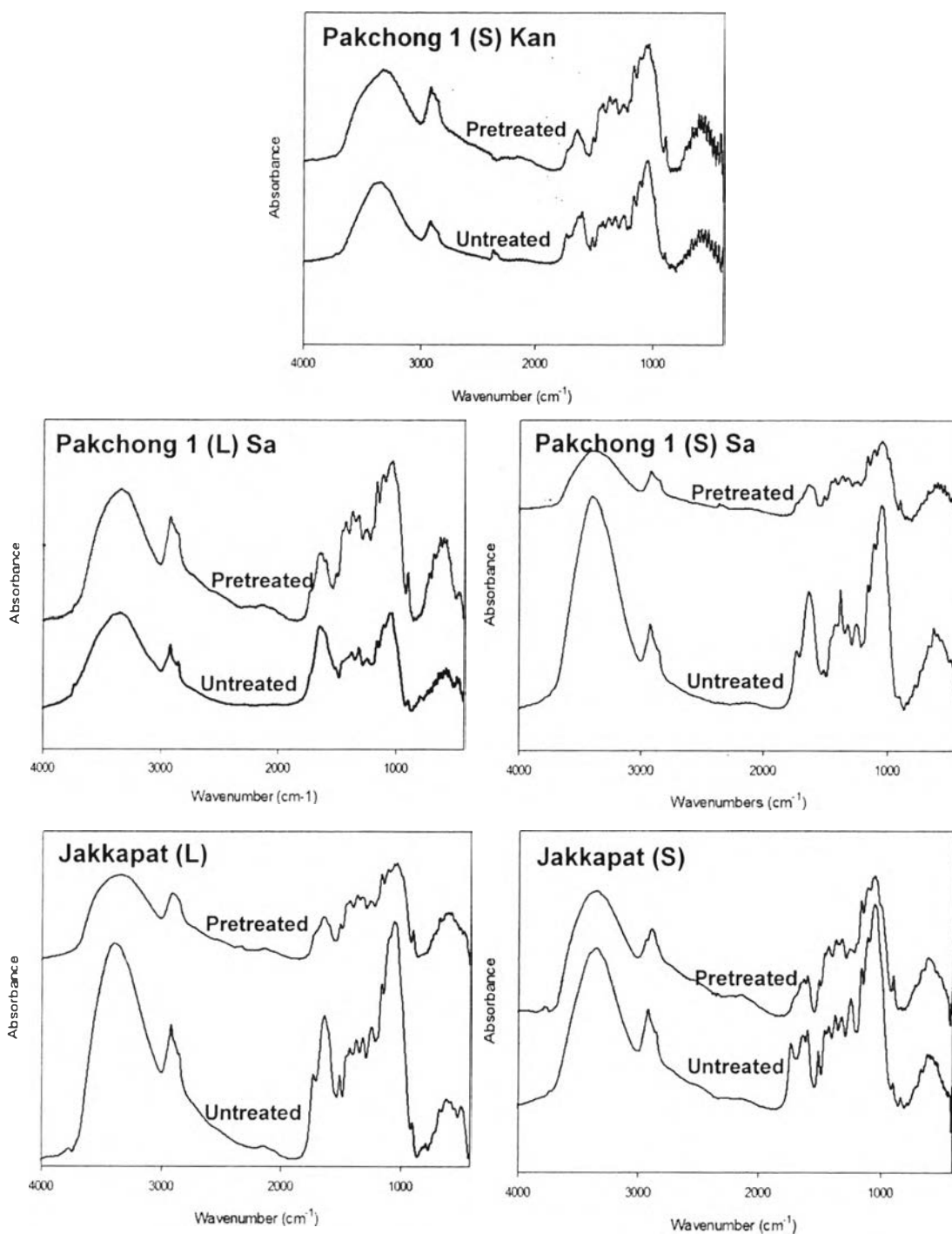


Figure 4.16 FTIR spectra of untreated and pretreated samples of Pakchong1 Napier grasses. Reaction conditions: at 147 °C, 76 min, 20 g/l biomass loading, and 1.287 volume% acetic acid.

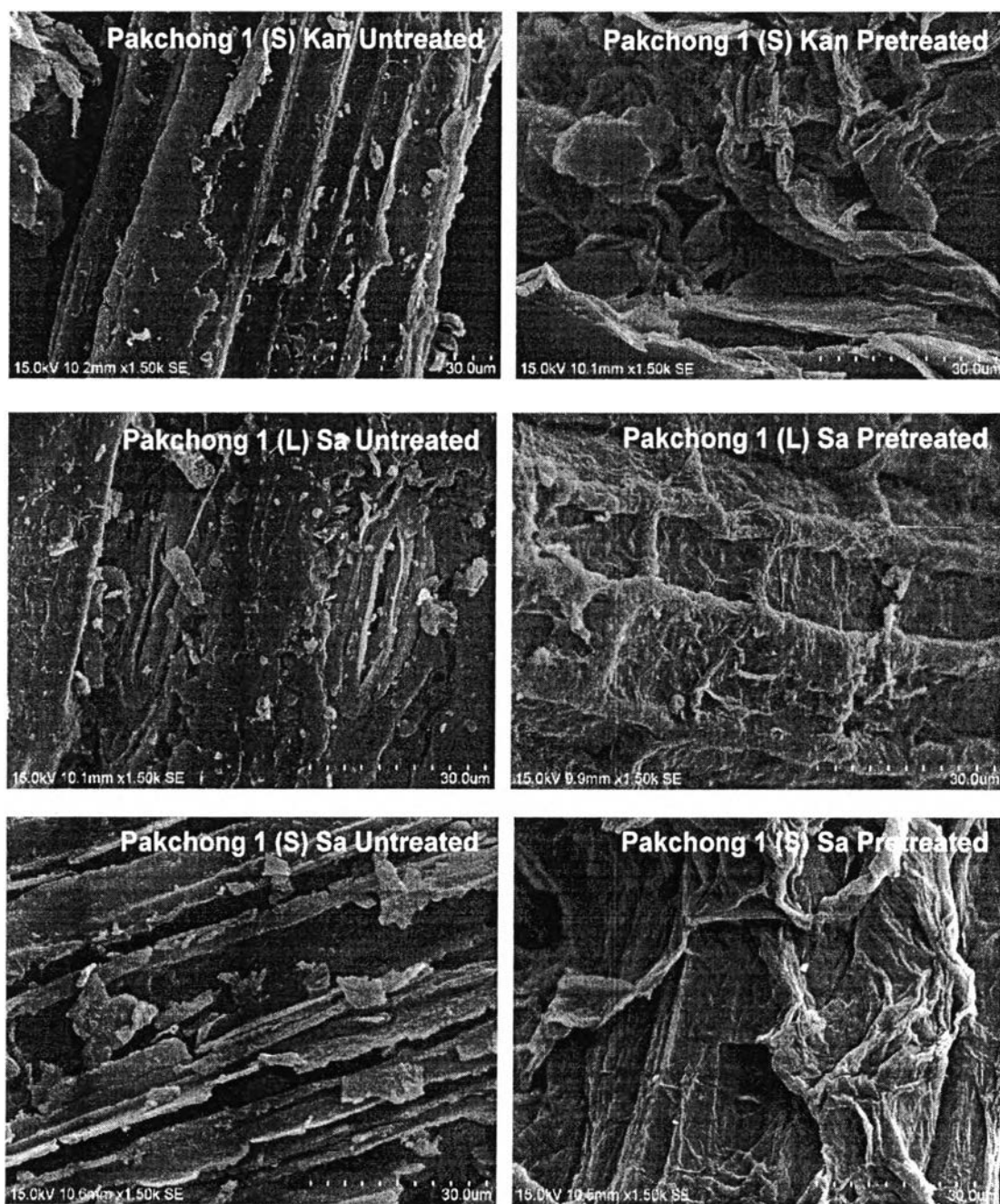


Figure 4.17 SEM images of untreated and pretreated samples of Pakchong 1 Napier grasses. Reaction conditions: at 147 °C, 76 min, 20 g/l biomass loading, and 1.287 volume% acetic acid.

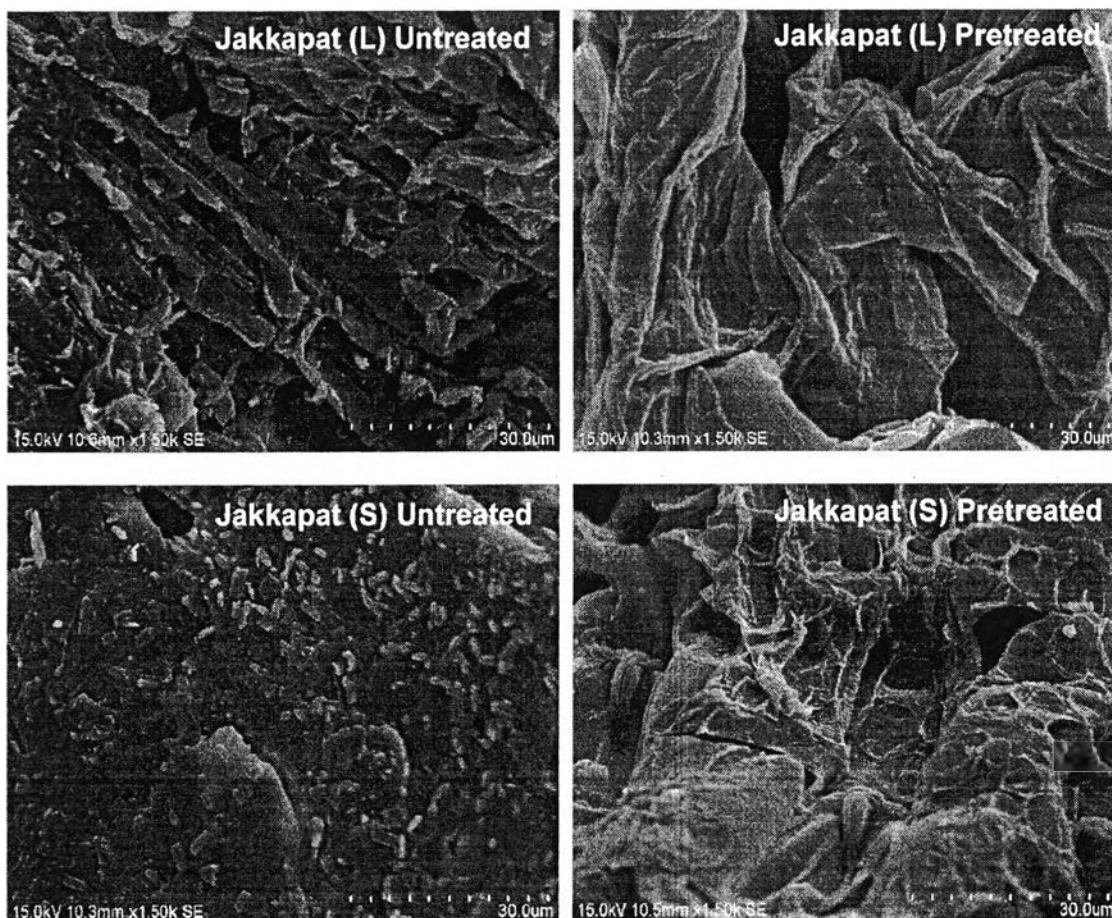


Figure 4.17 SEM images of untreated and pretreated samples of Pakchong1 Napier grasses. Reaction conditions: at 147 °C, 76 min, 20 g/l biomass loading, and 1.287 volume% acetic acid (cont.).

Table 4.9 The total sugar concentration and theoretical yield of Napier grasses

Type of raw material	Sugar concentration (g/l)		
	Untreated	Pretreated	Yield (%)
Pakchong1 (Leaf) Kan	4.45 ± 0.38	14.38 ± 2.04	98.16
Pakchong1 (Stem) Kan	3.45 ± 0.65	11.77 ± 2.68	88.23

Table 4.9 The total sugar concentration and theoretical yield of Napier grasses (Cont.)

Type of raw material	Sugar concentration (g/l)		
	Untreated	Pretreated	Yield (%)
Pakchong1 (Leaf) Sa	2.41 ± 0.22	12.68 ± 0.80	110.84
Pakchong1 (Stem) Sa	2.49 ± 0.14	10.16 ± 0.11	87.21
Jakkapat (Leaf)	3.48 ± 0.41	13.62 ± 0.30	98.48
Jakkapat (Stem)	3.34 ± 0.04	10.81 ± 1.00	81.28

FTIR results in Table 4.8 and Figure 4.16 had the same trend with Pakchong 1 from Kanchanaburi province. However, LOI and the ratio of lignin and cellulose of Pakchong 1 (Stem) from Kanchanaburi province have higher values after pretreatment. This is probably due to high lignin in structure of the samples.

4.4.2 Surface Area and Pore Size Diameter with Scanning Electron Microscope

From Table 4.10, pore size diameter of almost all samples increased but surface areas reduced after pretreatment. Because of the swell behavior, as depicted in Figure 4.17. After adding Napier grass in CH₃COOH aqueous-[Emim][Ac], Napier grass structure became balloons, as illustrated in Figure 4.18. Next, the balloons swollen until they reached to the maximal swelling ratio and then balloons exploded, cellulose inside balloon released and dissolved in ionic liquid (Navard and Cuissinat, 2006). While Pakchong 1 (Stem) from Saraburi province and Jakkapat (stem) had higher surface area after pretreatment due to the fragmentation of surface (Manaso *et al.*, 2013).

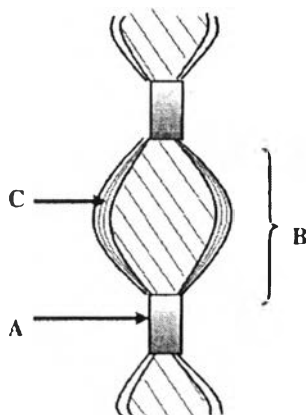


Figure 4.18 (a) Schematic drawing of a swollen fiber of native cellulose. A is an unswollen section, B is balloon, and C is cellulose swollen and dissolved in side the membrane (Navard and Cuissinat, 2006).

Table 4.10 Surface area and pore size diameter of untreated and pretreated of Napier grasses

Type of raw material	Surface area (m ² /g)		Pore diameter (Å)		Total pore volume (cc/g)	
	Untreated	Pretreated	Untreated	Pretreated	Untreated	Pretreated
	Pakchongl (Leaf) Kan	3.98	3.27	59.91	72.13	5.96*10 ⁻³
Pakchongl (Stem) Kan	4.49	3.03	68.66	81.12	7.70*10 ⁻³	6.15*10 ⁻³
Pakchongl (Leaf) Sa	3.57	3.16	79.0	91.15	7.72*10 ⁻³	7.20*10 ⁻³

Table 4.10 Surface area and pore size diameter of untreated and pretreated of Napier grasses (Cont.)

Type of raw material	Surface area (m ² /g)		Pore diameter (Å)		Total pore volume (cc/g)	
	Untreated	Pretreated	Untreated	Pretreated	Untreated	Pretreated
Pakchong1 (Stem) Sa	3.03	3.66	73.13	66.9	5.55*10 ⁻³	6.12*10 ⁻³
Jakkapat (Leaf)	6.37	4.01	57.34	88.3	9.13*10 ⁻³	8.86*10 ⁻³
Jakkapat (Stem)	4.6	4.98	66.08	68.8	7.59*10 ⁻³	8.56*10 ⁻³

4.4.3 Thermal Analysis

Figure 4.19 shows the comparison of DTG curves of untreated and pretreated Napier grasses. At 220-315 °C, the peak of hemicellulose of untreated Pakchong 1 (Leaf) from Saraburi province disappeared, it was similar to Pakchong 1 (Leaf) from Kanchanaburi province. In contrast, Pakchong 1 (Stem) from Kanchanaburi province, Jakkapat (Leaf), and Jakkapat (Stem) displayed shoulder peak. After pretreatment, pretreated samples exhibited a small shoulder at 315 °C and a strong peak at 360 °C, which were attributed to cellulose.

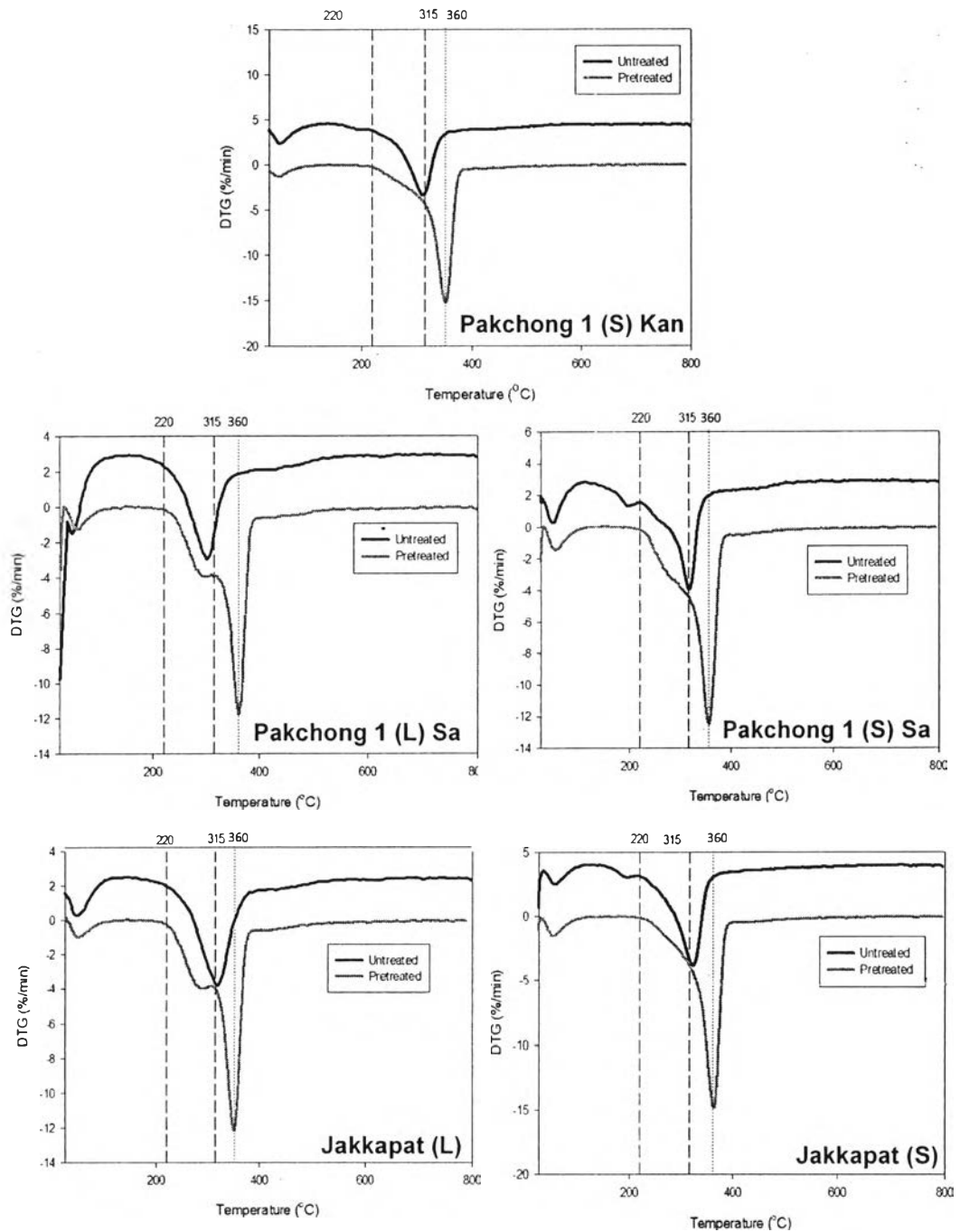


Figure 4.19 The DTG curves of untreated and pretreated samples of Pakchong 1 Napier grasses. Reaction conditions: at 147 °C, 76 min, 20 g/l biomass loading, and 1.287 volume% acetic acid.