



CHAPTER IV

RESULTS AND DISCUSSION

4.1 Raw Material Composition

The composition of *Napier grass* used in this study is presented in Table 4.1. The holocellulose fraction (or the total polysaccharide fraction) of 59.9% of the dry biomass consisted of cellulose being the major component at 36.3% and the remaining of 25.1% was belong to hemicellulose. Lignin levels were 16.55%. Water extractive compounds accounted for approximately 16% was identified as the non-structural disaccharide (NSD) sucrose. Further solvent extraction with ethanol resulted in 2.33% of materials, presumably composed of oils, pigments and waxes. The high polysaccharides content together with low lignin content in the raw material indicates that *Napier grass* is a promising lignocellulosic substrate for ethanol production.

Table 4.1 Chemical Composition of *Napier grass*

Composition	Raw Napier grass (% Dry matter)
Cellulose as glucan	36.3
Hemicellulose (xylan and arabinan)	25.1
Lignin	16.55
Water extractive	15.98
Ethanol extractive	2.33
Ash	2.99

4.2 Optimization of Microwave-Assisted Alkali Pretreatment

4.2.1 Effect of Time and Temperature

Napier grass was pretreated with either NaOH or NH₄OH under the following conditions: 0.5% volume of alkaline, 15:1 liquid to solid ratio, heating temperature in the range of 40°–160 °C, and heating time in the range of 5–60 min. Microwave-assisted NaOH system resulted in the highest amount of monomeric sugars, about 6.15 g/100 g biomass. The optimal temperature/time selected for the NaOH pretreatment were at 40 °C for 30 min to give the maximum glucose, xylose, and arabinose yields of 2.91, 2.88, and 0.34 g per 100 g biomass, respectively (Figure 4.1a).

While microwave-assisted NH₄OH system gave the highest monomeric sugar content of 6.19 g/100 g biomass. Those conditions selected for the NH₄OH pretreatment were at 40 °C for 30 min to give the maximum glucose, xylose, and arabinose yields of 2.88, 2.95, and 0.36 g per 100 g biomass, respectively (Figure 4.1b). Comparison of the results from using NaOH and NH₄OH is shown in Figure 4.3.

Alkali-based pretreatment efficiently delignifies lignocellulose by disrupting the ester bonds cross-linking lignin and xylan, leading to fractions enriched in both cellulose and hemicelluloses, as described by previous researchers that alkali pretreatments (sodium, calcium, potassium, and ammonium hydroxide) were very effective in lignin solubilization, exhibiting a lesser effect on glucan and xylan, as compared to acid pretreatments (Alvira *et al.*, 2010; Hendriks and Zeeman, 2009; Wyman *et al.*, 2005). Moreover, it was also found that alkali pretreatment processes generally utilized lower temperatures, pressures, and shorter residence times, comparing to other pretreatment technologies (McMillan *et al.*, 1994). Hu *et al.*, 2008 also showed that an increase of temperature resulted in not only a significant increase in lignin removal, but also a decrease in the total sugar yield, indicating the decomposition of sugars, as shown in Figures 4.1 and 4.2.

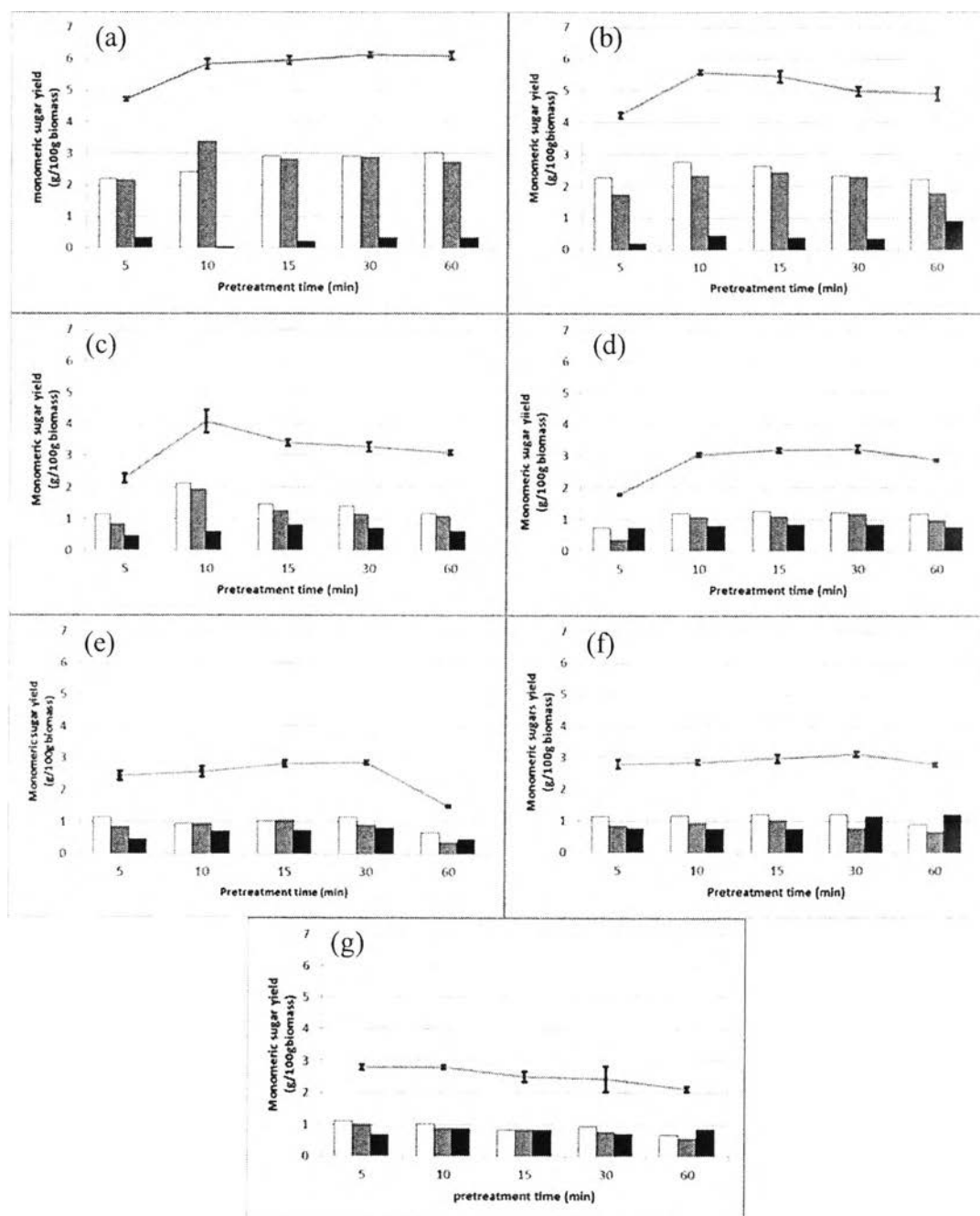


Figure 4.1 The glucose (□), xylose (▒), arabinose (■) components and total monomeric sugar yield (–x–) of *Napier grass* hydrolysate using NaOH 0.5 % (w/v) and different times and temperatures: (a) 40 °, (b) 60 °, (c) 80 °, (d) 100 °, (e) 120°, (f) 140 °, (g) 160 °C.

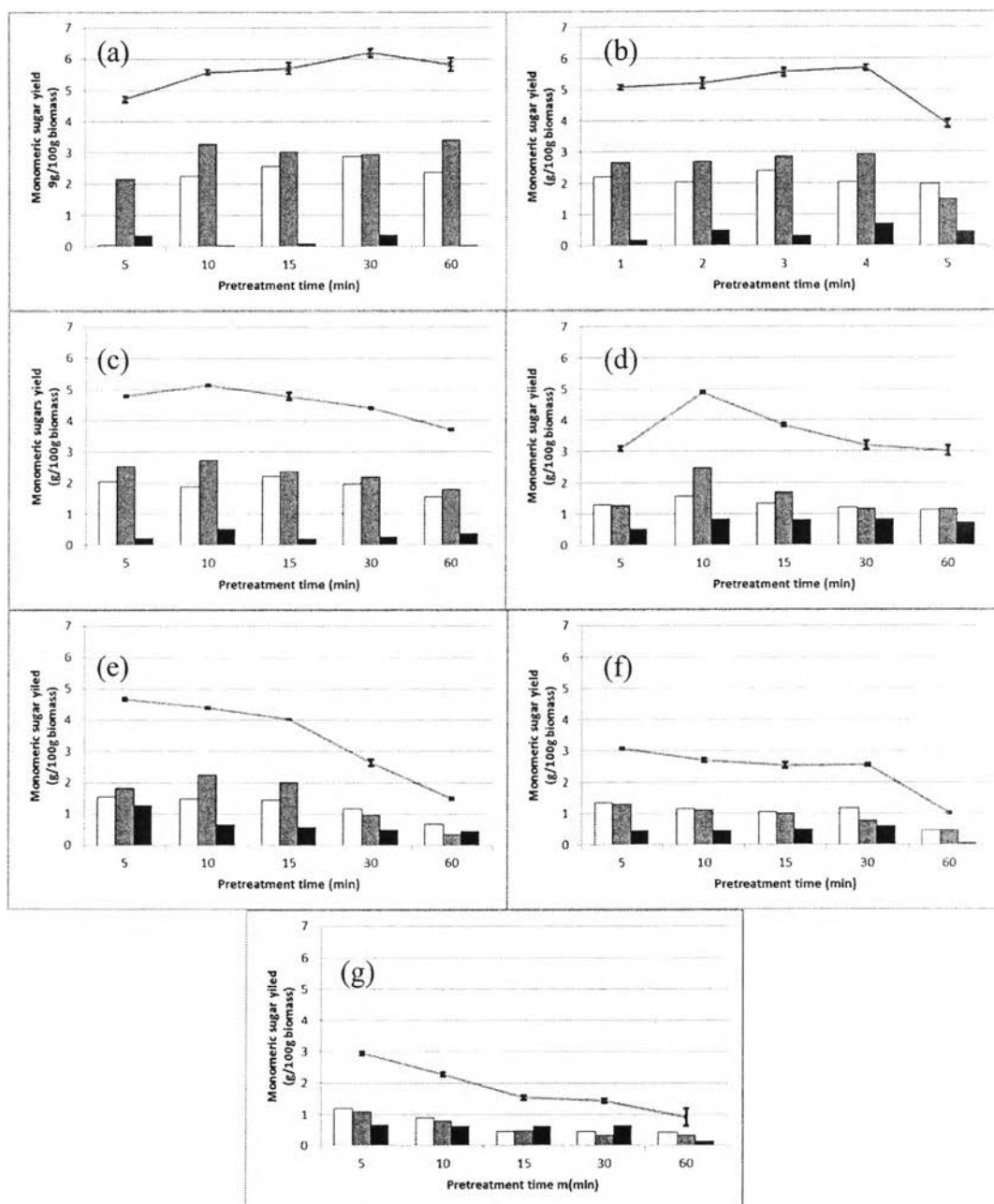


Figure 4.2 The glucose (\square), xylose (\blacksquare), arabinose (\blacksquare) components and total monomeric sugar yield ($-x-$) of *Napier grass* hydrolysate using NH_4OH 0.5 % (w/v) and different times and temperatures: (a) 40 °, (b) 60 °, (c) 80 °, (d) 100 °, (e) 120 °, (f) 140 °, (g) 160 °C.

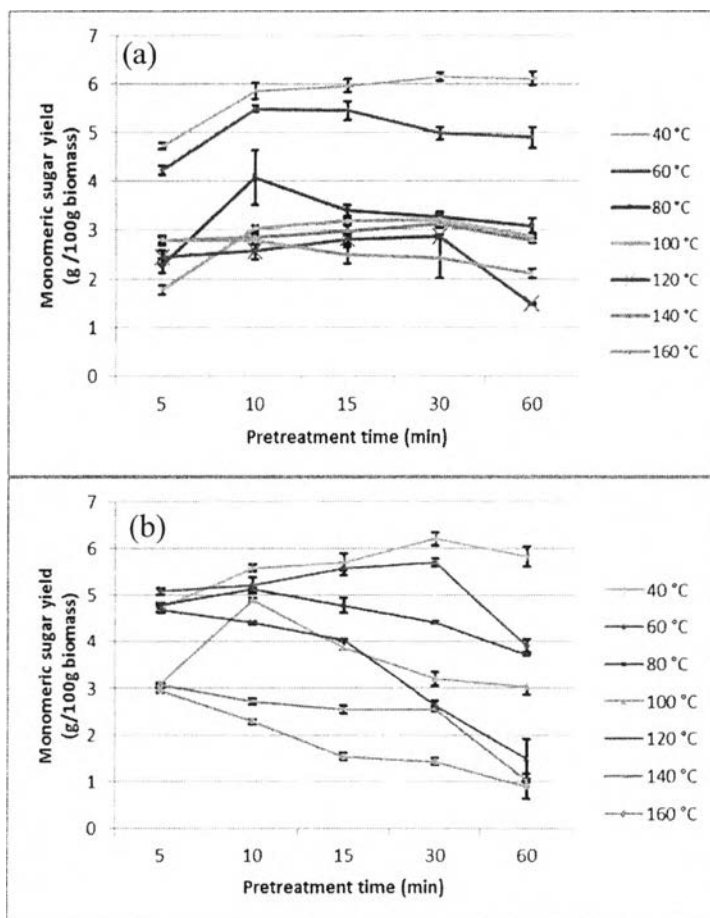


Figure 4.3 The comparison of the total yield of monomeric sugars at different temperatures and times using 0.5 % w/v of (a) NaOH and (b) NH₄OH.

4.2.2 Effect of Alkali Concentration

In the study, *Napier grass* was pretreated by microwave-assisted NaOH /NH₄OH using different alkaline concentrations in a range of 0.5 to 5% (w/v) at 15:1 LSR, 40°C for 30 min. For microwave-assisted NaOH, the maximum sugar yields from pretreating *Napier grass* were 6.15 g/100 g biomass (Figure 4.4a). The highest sugar yield came from the *Napier grass* samples pretreated with a 0.5% NaOH solution. As seen in Figure 4.4a, the higher concentrations of sodium hydroxide resulted in the more irreversible degradation of biomass, consistent to previous study by Kingsley *et al.*

(2010). In 2008, both Hu *et al.* and Wang *et al.* reported similar responses to the alkaline concentration; they recovered significantly less total sugars at elevated NaOH strengths.

For microwave-assisted NH_4OH , the pretreatment using smaller percentages of NH_4OH (0.5 to 1% w/v) was slightly different in quantity of sugar yields. The highest total monomeric sugar yield of 6.19 g/100 g biomass was achieved by using 0.5 % (w/v) NH_4OH , as shown in Figure 4.4b. Severe conditions of this pretreatment method caused a high degree of degradation of the released sugars, as indicated by the low yields of glucose, xylose and arabinose.

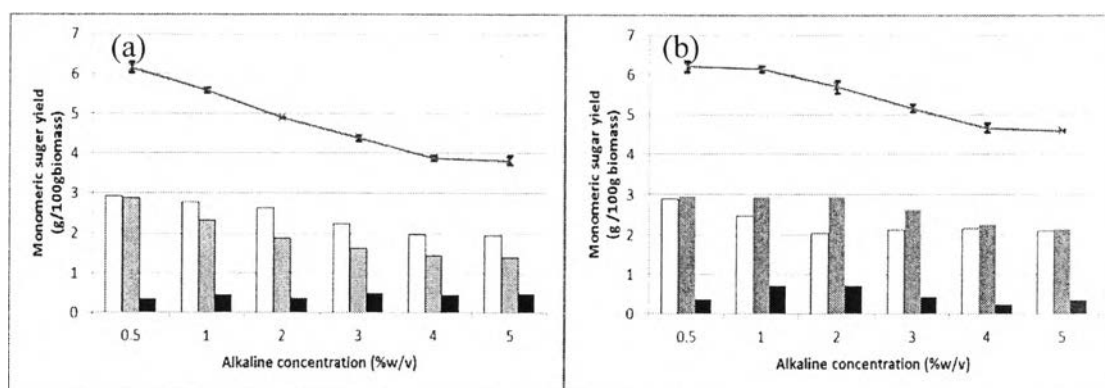


Figure 4.4 Effect of (a) NaOH and (b) NH_4OH concentrations on the release of monomeric sugars of pretreated *Napier grass* using 15:1 LSR at 40 °C for 30 min : glucose (□), xylose (▨), arabinose (■) and total monomeric sugar yield (–x–).

4.2.3 Effect of Liquid-to-Solid Ratio (LSR)

In this case, *Napier grass* was pretreated using LSR of 15:1, 30:1 and 45:1 with 0.5 % (w/v) NaOH at 40°C for 30 min. The maximum sugar yields of 6.15 g/100 g biomass were obtained at 15:1 LSR of 0.5% NaOH, giving the highest glucose, xylose, and arabinose yields of 2.91, 2.88, and 0.34 g/100 g biomass, respectively (Figures 4.5 a).

And LSR were also studied with 0.5 % (w/v) NH_4OH at 40°C for 30 min. The result shown that 15:1 LSR of 0.5% (w/v) NH_4OH gave the maximum sugar yields

of 6.19 g/100 g biomass, containing glucose, xylose, and arabinose yields of 2.88, 2.95, and 0.36 g/100 g biomass, respectively (Figures 4.5 b).

When using lower than 15:1 LSR, non-homogeneous mixture was obtained. It was observed that an increase in LSR resulted in a decrease in the release of the monomeric sugars due to the more dilution of the mixture (Cara *et al.*, 2007).

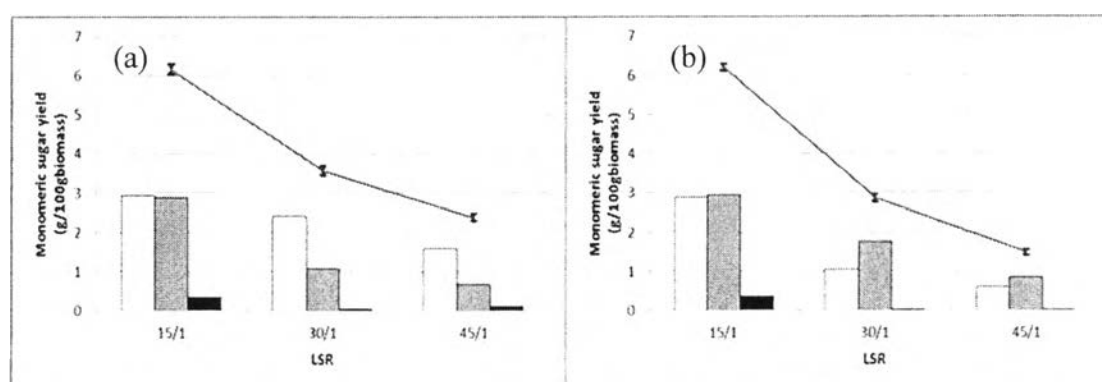


Figure 4.5 Effect of LSR on the release of the monomeric sugars using (a) NaOH and (b) NH₄OH (conditions: 0.5 % w/v of alkaline solution at 40 °C for 30 min) : glucose (□), xylose (▨), arabinose (■) and total monomeric sugar yield (–x–).

4.3 Optimization of Microwave-Assisted Acid Pretreatment

4.3.1 Effects of Time and Temperature

Acid pretreatment is considered as one of the most important techniques and expected to give high yields of sugars from lignocellulosics. H₂SO₄ is widely used for acid pretreatment among various types of acid because of its low cost and high efficiency. Boonmanumsin *et al.*, (2011) studied a release of monomeric sugar yield from *Miscanthus Sinensis*, and found that the total monomeric sugar produced from the H₃PO₄ pretreatment was much higher than the other acids, giving the highest total monomeric sugar yield of 62.28g/100 g biomass. Therefore, it was a great interest to study the efficiency of H₃PO₄ pretreatment in this work, comparing to H₂SO₄.

Napier grass was pretreated with H_2SO_4 and H_3PO_4 under the following conditions: 0.5% w/v volume of acid, 15:1 of liquid to solid ratio, heating temperature in the range of 60–160 °C, and heating time in the range of 5–60 min.

In this work, the optimal temperature and time selected for the H_2SO_4 pretreatment were at 160 °C for 15 min to give the maximum glucose, xylose, and arabinose yields of 5.93, 15.30, and 6.09 g per 100 g biomass, respectively (Figure 4.6f), and the optimal temperature and time selected for the H_3PO_4 pretreatment were at 140 °C for 30 min, giving the maximum glucose, xylose, and arabinose yields of 6.80, 13.63, and 4.00 g per 100 g biomass, respectively (Figure 4.7e). Comparison of the results from using H_2SO_4 and H_3PO_4 is shown in Figure 4.8.

The results illustrate the influence of the reaction temperature and time on the effectiveness of microwave-assisted $\text{H}_2\text{SO}_4/\text{H}_3\text{PO}_4$ pretreatment. The Figure 4.17 indicated that increases of the reaction temperature and time to a certain point gave higher monomeric sugar yields, but too high temperature resulted in further degradation of cellulose to furfural, 5-hydroxymethyl furfural (HMF), levulinic acid, and formic acid together with other substances (Gabhane *et al.*, 2011). H_2SO_4 and H_3PO_4 pretreatments showed that xylose was the main product. Since the acids attack the polysaccharides, especially hemicelluloses which are easier to hydrolyze than cellulose (Cardona *et al.*, 2009).

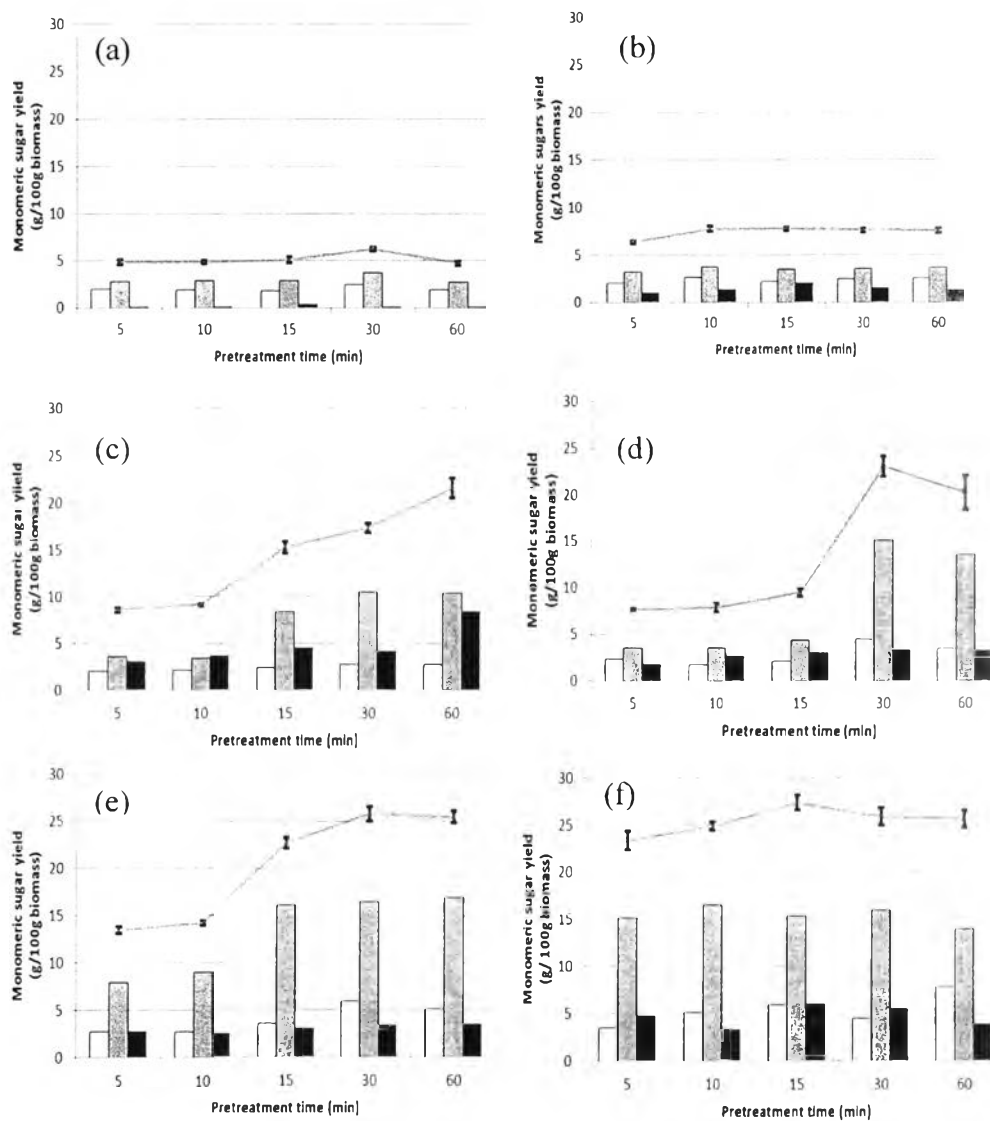


Figure 4.6 The glucose (□), xylose (▨), arabinose (■) components and total monomeric sugar yield (–x–) of *Napier grass* hydrolysate using H₂SO₄ 0.5 % w/v, different times and temperatures: (a) 60 °, (b) 80 °, (c) 100 °, (d) 120°, (e) 140 °, (f) 160 °C.

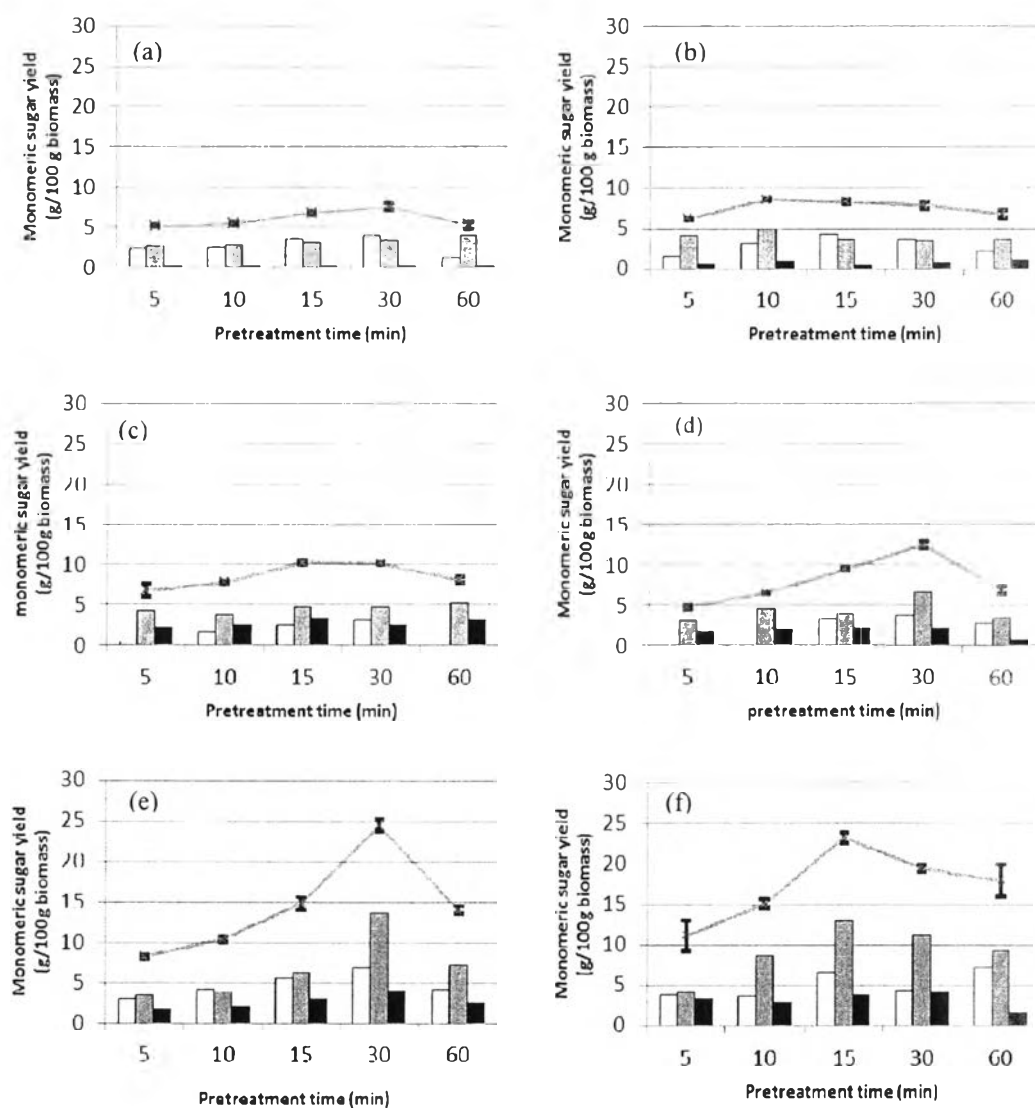


Figure 4.7 The glucose (□), xylose (▨), arabinose (■) components and total monomeric sugar yield (—x—) of *Napier grass* hydrolysate using H_3PO_4 0.5 % w/v, different times and temperatures: (a) 60 °, (b) 80 °, (c) 100 °, (d) 120 °, (e) 140 °, (f) 160 °C.

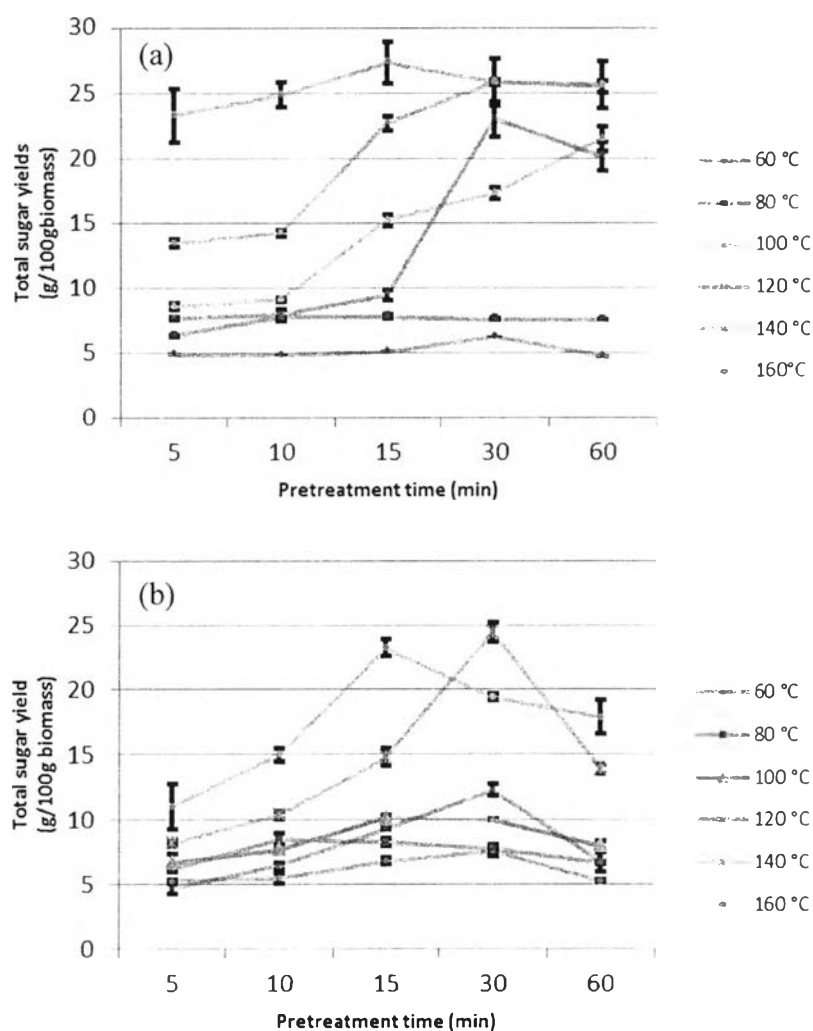


Figure 4.8 The comparison of the total yield of monomeric sugars at different temperatures using 0.5 % w/v of (a) H₂SO₄ and (b) H₃PO₄.

4.3.2 Effect of Acid Concentration

Various H₂SO₄ acid concentrations (0.5–5% w/v) at 160°C for 15 min and various H₃PO₄ acid concentrations (0.5–5 % w/v) at 140 °C for 30 min were investigated. From Figure 4.9a showing the results obtained from the microwave-assisted H₂SO₄, it was found that releasing monomeric sugars were increased when increasing acid concentration from 0.5 to 1.0% w/v. Among monomeric sugars released,

glucose amount did not seem to be affected, but when H_2SO_4 concentration was increased above 1.0% w/v, the glucose yield decreased owing to too high concentration of acid causing the decomposition of sugars, as also observed by Cardona *et al.* (2010). The highest total monomeric sugar yield of 30.93 g/100 g biomass, containing glucose, xylose, and arabinose yields of 6.44, 20.20, and 4.28 g/ 100 g biomass, respectively, obtained from this work was to use 1%w/v H_2SO_4 . As discussed earlier, hemicelluloses, mainly composing of xylan, are easier to break down in acid pretreatment than cellulose, resulting in much higher yield of xylose than glucose (Wang *et al.*, 2010, Liao *et al.*, 2006).

The microwave-assisted H_3PO_4 also showed the same trend as the microwave-assisted H_2SO_4 , see Figure 4.9b. The highest total monomeric sugar yield of 24.99 g/100 g biomass, containing glucose, xylose, and arabinose yields of 6.46, 15.18, and 3.44 g/ 100 g biomass, respectively, was obtained from using 1% w/v H_3PO_4 .

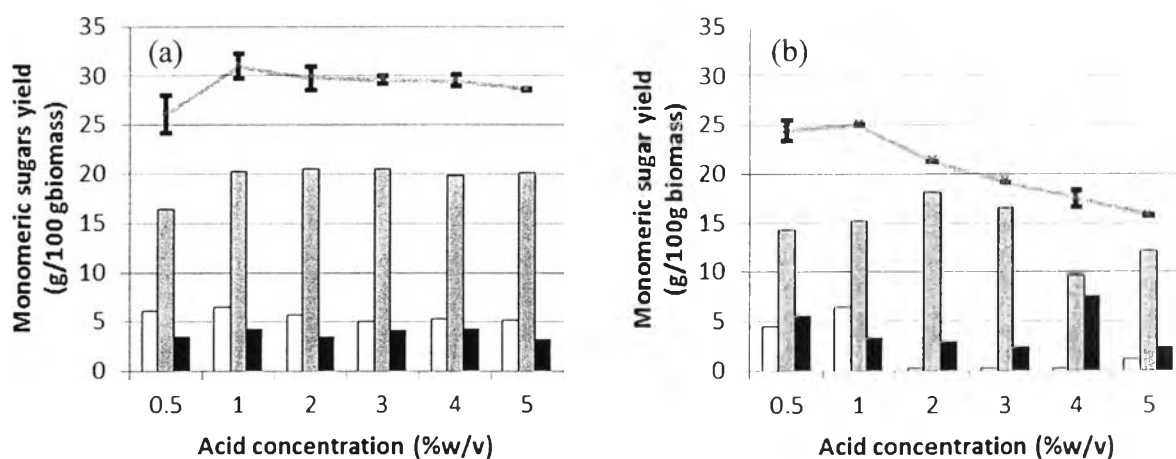


Figure 4.9 Effect of (a) H_2SO_4 and (b) H_3PO_4 concentration on the release of monomeric sugars of pretreated *Napier grass* using 15:1 LSR at 160 °C for 15 min and 140 °C for 15 min, respectively: glucose (□), xylose (▨), arabinose (■) and total monomeric sugar yield (—x—).

4.3.3 Effect of Liquid-to-Solid Ratio (LSR)

To investigate the effect of LSR on the release of monomeric sugar, *Napier grass* was evaluated using LSR of 15:1, 30:1, and 45:1 at 1.0 % (w/v) H_2SO_4 , 160 °C for 15 min and at 1.0 % (w/v) H_3PO_4 , 140 °C for 30 min. The pretreatment was not performed at lower 15:1 LSR because of non-homogenous mixture obtained.

The 15:1 LSR of 1% (w/v) H_2SO_4 gave the maximum sugar yields of 30.93g/100 g biomass, containing glucose, xylose, and arabinose yields of 6.44, 20.20, and 3.28g/100 g biomass, respectively (Figure 4.10 a).

The H_3PO_4 pretreatment also showed the same trend as the H_2SO_4 process, see Figure 4.10b. The maximum sugar yields of 24.99 g/100 g biomass were obtained at 15:1 LSR of 1.0 % (w/v) H_3PO_4 , giving the highest glucose, xylose, and arabinose yields of 6.46, 15.18, and 3.44 g/100 g biomass, respectively.

The LSR results of H_2SO_4 and H_3PO_4 revealed that an increase in LSR resulted in a decrease in the release of the monomeric sugars, similar to those obtained from the NaOH and NH_4OH pretreatments. The increase in LSR makes the mixture more dilute, thus less catalyst concentration, resulting in less efficiency in producing the monomeric sugar

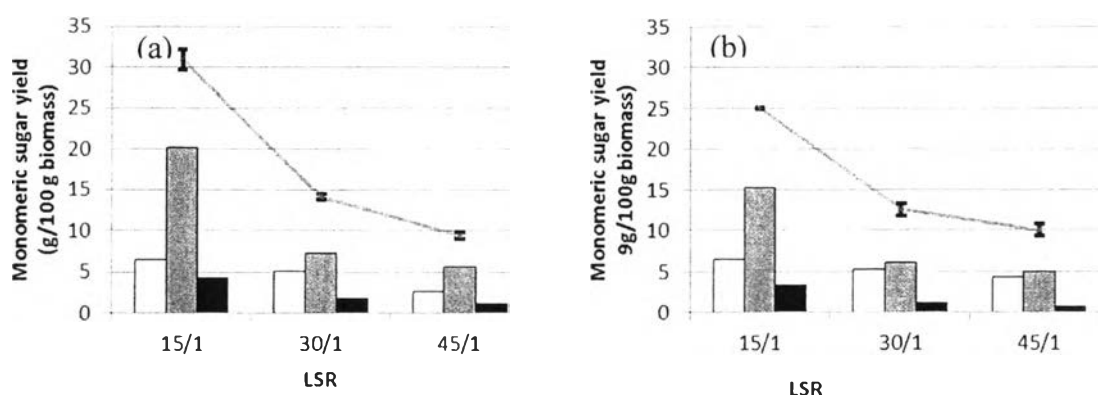


Figure 4.10 Effect of LSR on the release of the monomeric sugars of (a) H_2SO_4 (conditions: 1 % H_2SO_4 w/v at 160°C for 15 min) and (b) H_3PO_4 (conditions: 1 % H_3PO_4 w/v at 140°C for 30 min) : glucose (□), xylose (▨), arabinose (■) and total monomeric sugar yield (–x–).

4.4 Two-stage Pretreatment (Dilute Sodium Hydroxide/Dilute Ammonium Hydroxide Followed by Dilute Sulfuric Acid Pretreatment)

From above study, both microwave-assisted NaOH/NH₄OH pretreatments provided similar amounts of the releasing monomeric sugar, which are 6.15 and 6.19 g/100 g biomass, respectively. Therefore, they both were interesting for further study by bringing both solid residues after the base pretreatment to wash with distillation water until pH became 7 before pretreating with the optimal conditions of the H₂SO₄ pretreatment (1.0 % (w/v) H₂SO₄ using 15:1 LSR at 160 °C for 15 min. As can be seen in Figure 4.11, the total sugar yield of one-step and two-stage pretreated *Napier grass* is compared. Both glucose and xylose yields for the two-stage treated samples were higher, as compared to that pretreated with the one-step method. Since hemicellulose and lignin were (partially) removed from the surface of cellulose with alkaline from the first pretreatment, more binding sites were available to cellulose and crystallinity of cellulose was decreased due to swelling, which may contribute to higher digestibility of cellulose obtained in the two-stage pretreated samples (Wang *et al.*, 2010).

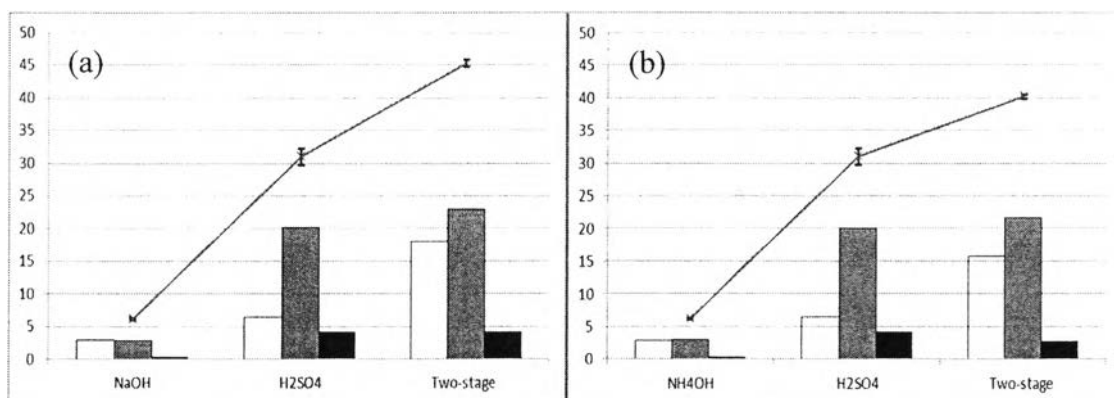


Figure 4.11 The effect of pretreatment processes on the release of the monomeric sugar yields via (a) microwave-assisted NaOH followed by H₂SO₄ and (b) microwave-assisted NH₄OH followed by H₂SO₄: glucose (□), xylose (▨), arabinose (■) and total monomeric sugar yield (—x—).

The microwave-assisted NH_4OH pretreatment followed by the H_2SO_4 pretreatment resulted in the total monomeric sugar yield of 40.16 g/100 g biomass, containing glucose, xylose, and arabinose yields of 15.74, 21.61 and 2.81 g/100 g biomass, respectively. However, the highest total monomeric sugar yield of 45.28 g/100 g biomass, composing of 18.01 g glucose, 23.02 g xylose and 4.24 g arabinose/100 g biomass, was achieved when using the microwave-assisted NaOH pretreatment followed by the H_2SO_4 pretreatment.

4.5 Effect of Pretreatment on Chemical Composition

The percentages of basic constituents, including hemicellulose, cellulose, lignin, and ash, contained in the raw *Napier grass* and the pretreated materials are analyzed and listed in Table 4.2. These data are to provide an insight into the variation of the constituents affected by microwave-assisted chemical.

Table 4.2 Chemical composition of the solid residues from each treatment

Method	%Recovered (mg)	%Glucan (mg)	%Xylan (mg)	%Arabinan (mg)	%Lignin (mg)	%Ash (mg)
Untreated	100 (300)	36.3 (108.9)	21.1 (63.3)	2.5 (7.7)	16.5 (49.5)	3 (9)
Microwave/ NH_4OH	71.2 (213.4)	48.8 (103.8)	23.9 (51)	2.2 (4.7)	12.4 (27.6)	1 (2.2)
Microwave/ NaOH	69.7 (207.3)	49.3 (102)	25.8 (53.5)	2.9 (5.9)	11 (22.9)	0.9 (1.9)
Microwave/ H_3PO_4	59.8 (166.8)	55.6 (92.8)	5.7 (9.5)	0 (0)	19 (31.7)	1.9 (3.2)
Microwave/ H_2SO_4	54.2 (162.6)	57.1 (92.9)	2.9 (4.8)	0 (0)	18.72 (30.2)	1.7 (2.7)
Microwave/ NH_4OH / H_2SO_4	50.7 (151.2)	48 (72.6)	0.6 (1)	0 (0)	10.2 (18.1)	0.92 (1.4)

Method	%Recovered (mg)	%Glucan (mg)	%Xylan (mg)	%Arabinan (mg)	%Lignin (mg)	%Ash (mg)
Microwave/NaOH /H ₂ SO ₄	48.6 (145.8)	44.5 (64.5)	0.3 (0.5)	0 (0)	8.3 (12.1)	0.7 (1.0)

For microwave assisted alkaline pretreatment have significantly reduced lignin content when compared to the untreated after pretreatment, a 33% delignification was observed in NaOH treated *Napier grass*, as compared to only 25% in NH₄OH treated samples. This is typical for the alkaline pretreatment, which generally removes more lignin and less hemicellulose than the dilute acid pretreatment, producing recovered material with lower levels of residual lignin and higher levels of residual xylan (Chenlinet *al.*, 2010). The alkali pretreatment is the most effective with low lignin content biomass like agricultural residues, but becomes less effective as lignin content of the biomass increases.

The microwave assisted acid produced cellulose enriched solids, comparing to the raw *Napier grass*. The glucan fraction was found to be the major component present in the pretreated solid residue (57.1 and 55.6% for H₂SO₄ and H₃PO₄, respectively), while the raw *Napier grass* contains only 36.3%. The increase observed in the glucan content was in agreement with the reduction of the xylan content determined in the *Napier grass*. As expected, most of the lignin was not removed from the *Napier grass* by dilute acid pretreatment, as shown in Table 4.2 (Qi *et al.*, 2010).

For the microwave assisted alkaline followed acid pretreatment, the xylose content of the solid residue after these pretreatments was found to be low and ranged from 0.6 to 0.3% of the microwave assisted NH₄OH followed H₂SO₄ pretreatment and the microwave assisted NaOH followed H₂SO₄ pretreatment, respectively, implying that there was a nearly complete hydrolysis of xylan during the pretreatment. The glucose content in the treated sample was observed to be decreased when comparing with the microwave-assisted acid pretreatment due to a high release of the glucose content into prehydrolysate during the two-stage pretreatment, see Figure 4.11. Moreover, the

highest lignin removal was obtained from the two-stage pretreatment. The amount of lignin removed was ranged from 63.4 to 75.7% of the initial lignin in the untreated *Napier grass*.

4.6 FT-IR Analysis

The untreated samples generally show peak at 1734 cm^{-1} due to strong carbonyl groups in branched hemicelluloses (Choudhary *et al.*, 2012). This peak is missing from the spectra of the acid, the alkaline, and the two-stage pretreatments, indicating the removal of hemicellulose from the sample. This shows that the pretreatment is responsible for the hemicellulose removal. It is, therefore, in agreement with the results of the composition analysis after pretreatment in Table 4.2.

The peak at $1,253\text{ cm}^{-1}$ is belonging to aromatic C–O stretching out of lignin (Xu *et al.*, 2006). This band is also missing from the spectra of the alkaline and the two-stage pretreated samples (Figure 4.12), indicating removal of the lignin. The impact of the dilute acid pretreatment on lignin contained in *Napier grass* is explored in Figure 4.12B. From the spectrum, the $1,253\text{ cm}^{-1}$ peak was also observed. This result reflects that the structure of lignin is hardly affected by the dilute acid pretreatment.

As mentioned in Chapter I, cellulose is a homopolysaccharide composing of β -D-glucopyranose units linked together by (1-4)-glycosidic bonds (Balat *et al.*, 2008). Figure 4.12 depicts that a sharp band at 895 cm^{-1} in the spectrum of cellulose is attributed to β -glycosidic linkages between the sugar units. When the raw *Napier grass* was examined, the band of 895 cm^{-1} was not notable, probably due to the coverage of cellulose by hemicellulose and lignin. However, when the biomass was pretreated with single-stage and two-stage pretreatments, the band of 895 cm^{-1} was clearly exhibited. Moreover, the other polysaccharides peaks (1108 , 1164 , 1260 , 1325 , and 1378 cm^{-1}) became sharper, as compared with the untreated *Napier grass*, corresponding well to the increase in polysaccharides content after the pretreatment (Wang *et al.*, 2010).

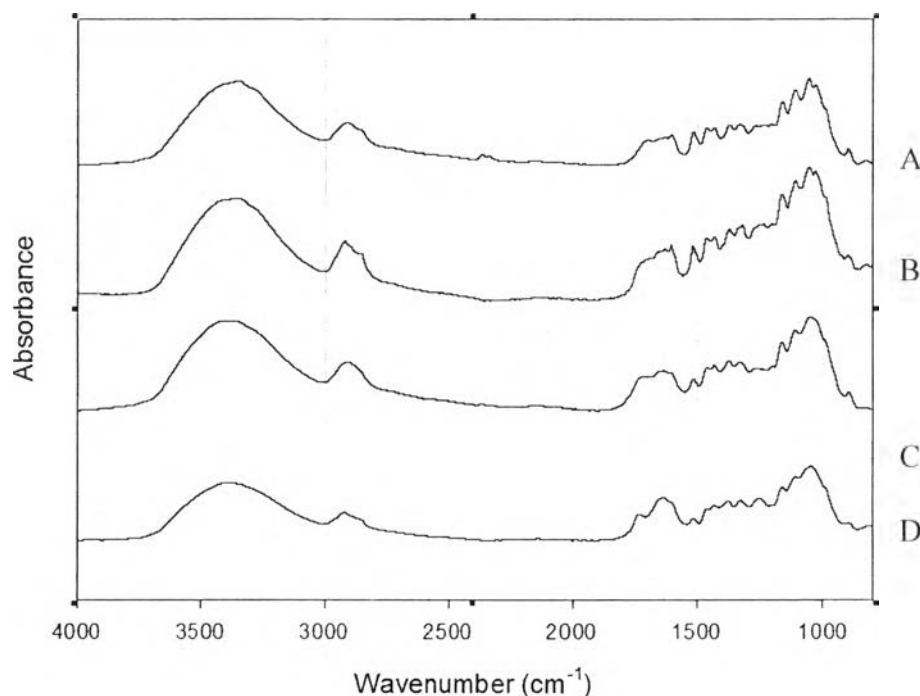


Figure 4.12 FTIR spectra of (A) raw *Napier grass*, (B) microwave-assisted alkali, (C) microwave-assisted acid, and (D) Two-stage pretreated *Napier grass*.

4.7 Solid Loss (%) and pH

It was initially observed that the pretreatment of *Napier grass* with alkaline resulted in dark colored hydrolysate, while the acid reagent gave a yellow hydrolysate, and that the color intensity generally increased with pretreatment severity. An increase in the severity of the pretreatment by increasing the pretreatment temperature or time resulted in significantly higher solid losses. Each pretreatment variable in this study contributed to the loss of solids. It was found that the pretreatment temperature affected a greater loss of solid than the pretreatment time. Others have reported similar reduction in solids during the pretreatment (Chen *et al.*, 2004). The solid losses were ranged from 14–16% under mild conditions (5 min; 60 °C) of each reagent pretreatment to the

highest losses of 73, 55, 62, and 47% for the pretreatments at 160 °C for 60 min of H₂SO₄, H₃PO₄, NaOH, and NH₄OH, respectively (Figure 4.13).

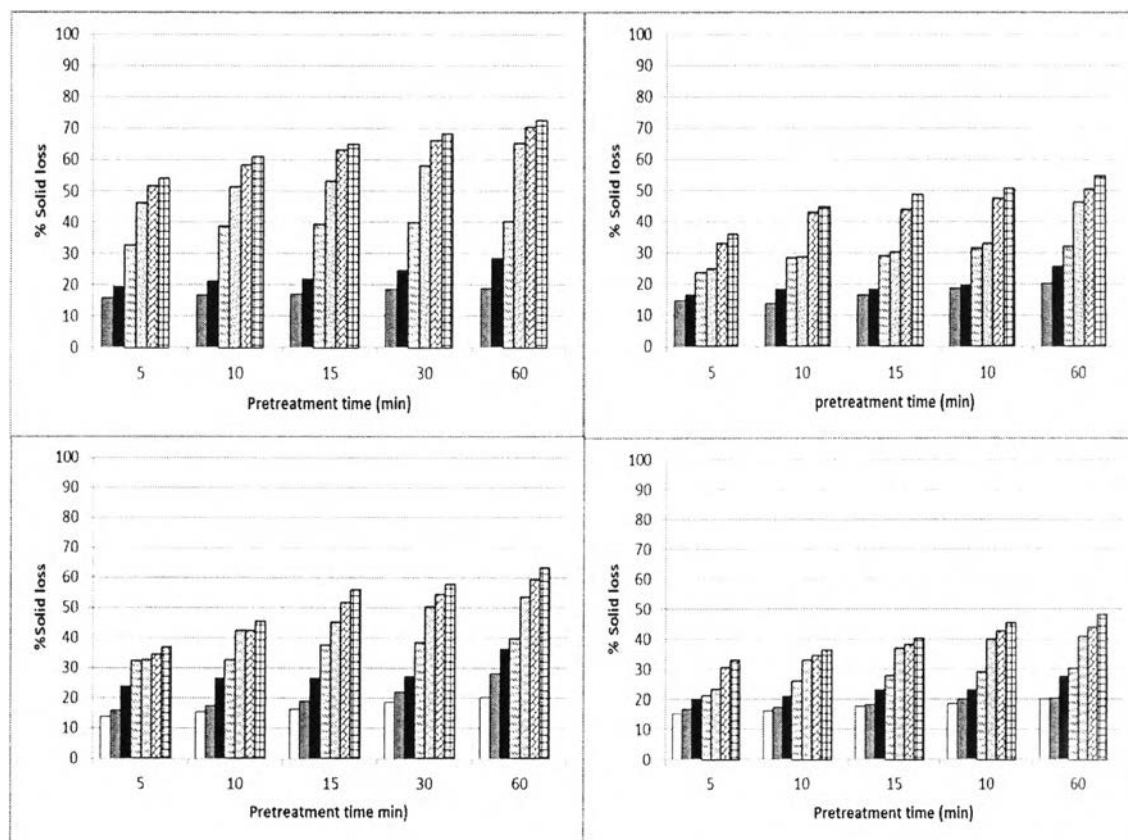


Figure 4.13 % Solid loss of *Napier grass* after the pretreatment using 0.5 % (w/v) of (a) H₂SO₄, (b) H₃PO₄, (c) NaOH, and (d) NH₄OH for various heating times and temperatures : 40°C (□), 60°C (▣), 80°C (■), 100°C (▤), 120°C (▥), 140°C (▦) and 160°C (▧).

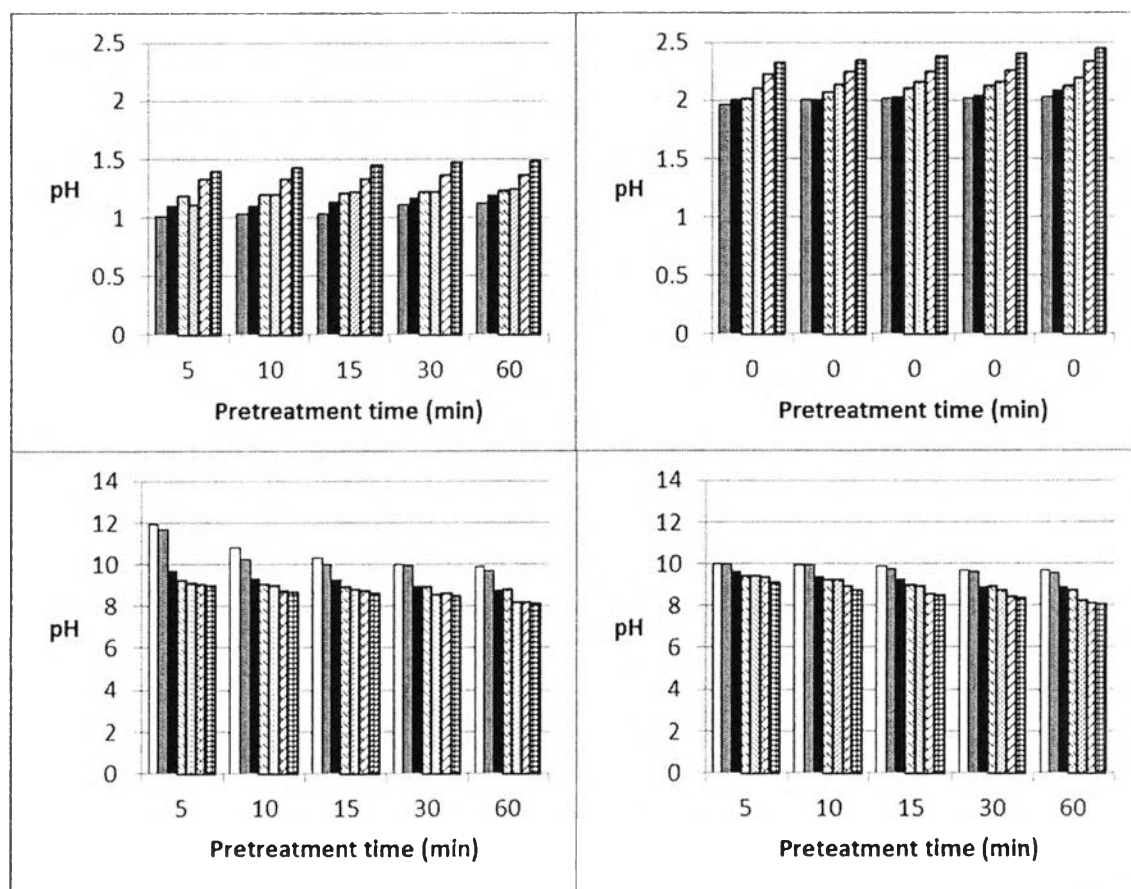


Figure 4.14 The pH of *Napier grass* hydrolysate using 0.5 % (w/v) of (a) H_2SO_4 , (b) H_3PO_4 , (c) NaOH , and (d) NH_4OH for 5, 10, 15, 30, and 60 min at different temperatures : 40°C (\square), 60°C (\boxtimes), 80°C (\blacksquare), 100°C (\boxplus), 120°C (\boxdot), 140°C (\boxtimes) and 40°C (\boxtimes).

The pHs of the acid and the alkaline hydrolysates were performed in this work. Changing in pH of a hydrolysate was affected by the pretreatment temperature or time. It was found that the pH of the acid hydrolysate was increased after increasing the severity of the pretreatment. The pH of the H_2SO_4 hydrolysate was increased from 1.01 to 1.48 (Figure 4.14a) and from 1.97 to 2.43 for the H_3PO_4 hydrolysate (Figure 4.14b). When

acidic media were used, the proton acts as a catalyst to protonate the oxygen atom linking between the cellulose and the hemicellulose chains of biomass, as shown in Figure 4.15. Thus, the hydrolysis of the cellulose and the hemicellulose chains was easily taken place (Krassig *et al.*, 2002, Harmsen *et al.*, 2010). On the other hand, the pH of an alkaline hydrolysate was decreased after increasing the severity of the pretreatment due to the hydroxy ion used to cleavage the ether bond of lignin in the alkaline solution for delignification, as shown in Figures 4.16 and 4.17 (Gierer *et al.*, 1985, Lin *et al.*, 2002, Harmsen *et al.*, 2010). The pH of the NaOH hydrolysate was decreased from 11.95 to 8.11 (Figure 4.14c) and from 9.99 to 8.03 for the NH₄OH hydrolysate (Figure 4.14d).

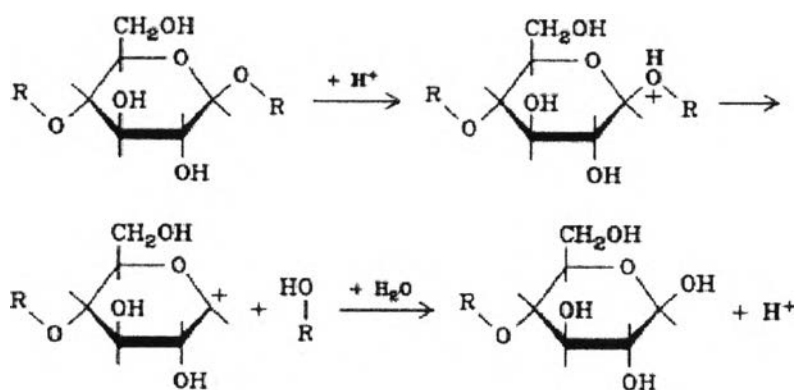


Figure 4.15 Hydrolysis of cellulose in acidic media (Krassig *et al.*, 2002).

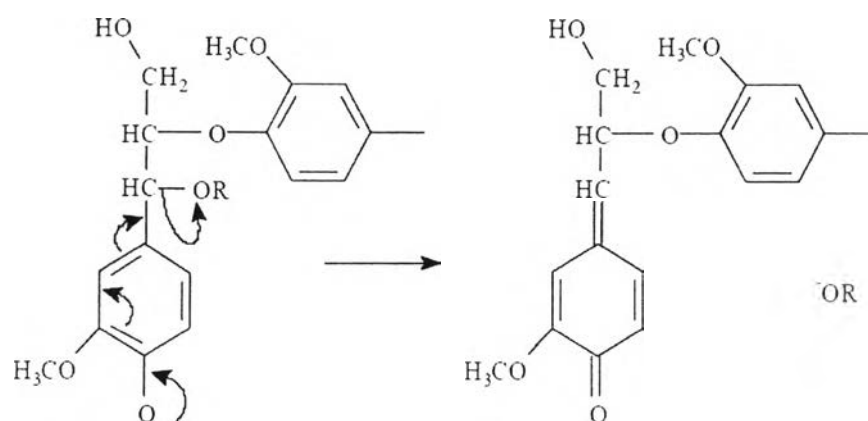


Figure 4.16 Alkaline cleavage of α -aryl ether bonds (Giereret *al.*, 1985).

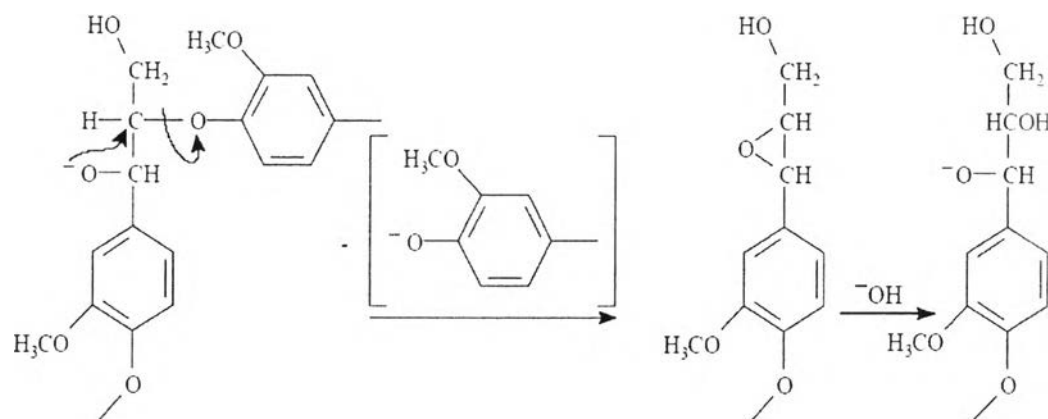


Figure 4.17 Alkaline cleavage of β -aryl ether bonds (Giereret *al.*, 1985).