CHAPTER IV

RESULTS AND DISCUSSION

The results and discussion were divided into four parts. The first part (section 4.1) involved the percentage of main components in durian and pineapple peel determined by a DTG/TGA. In this part, the determination of main components in durian and pineapple peel was reported. The second part (section 4.2) involved the determination of main components in durian and pineapple peel. The removable steps for sample preparation of TAPPI T203 test method and the comparison of the data with DTG/TGA was reported. The third part (section 4.3) involved the percentage of total reducing sugars obtained from dilute acid hydrolysis was presented. Finally, the fourth part (section 4.4) involved the determination of cellulosic ethanol in fermented broth was discussed.

4.1 DTG/TGA technique

The main components in durian and pineapple peel such as cellulose, hemicelluloses and lignin, were monitored in the range of 30-830°C by a DTG/TGA.

4.1.1 The percentage of main components in raw durian peel

The powder of raw durian peel was determined the main components such as hemicelluloses, cellulose and lignin. Their data was reported as DTG curve which reported types of degradation each of the main components while TGA curve which showed the percentage of weight loss (%), as expressed in Fig. 4.1 (a) and (b), respectively.

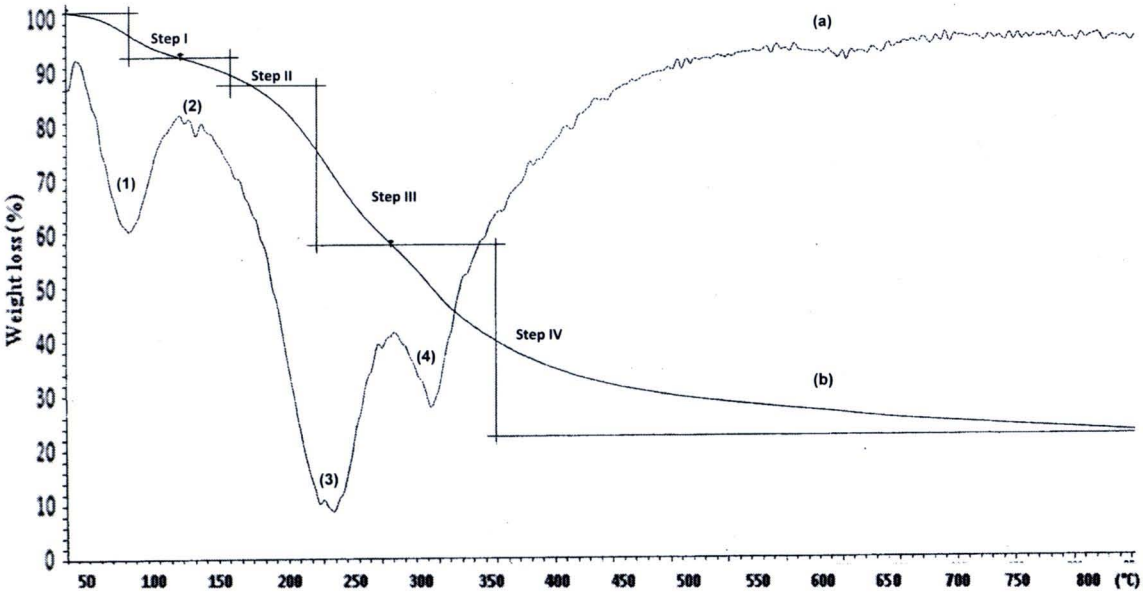

Figure 4.1 The DTG and TG curve of component in raw durian peel

The typical component in durian peel was reported by Haiping et al. (2006) that was the DTG curve as shown in Fig. 4.1 (a), the first event was moisture removal (1) up to temperature around 50°C followed by the second, third and fourth events around 125-300°C where the evolution of hemicelluloses (2) and cellulose (3) degradation, respectively. Degradation of lignin slowly took place in a wide temperature range and lasts to higher temperature (4). The TG curve showed maximum percentage of weight losses occurred in the range of 200 to 450°C. The first step could be attributed to hemicelluloses degradation of 1.25% whereas the second step was related to cellulose degradation of 33.03% and the final step was 35.16% of the lignin degradation, respectively, as shown in Fig. 4.1 (b). These results harmonized as report of Haiping et al. (2006).

4.1.2 The percentage of main components in raw pineapple peel

The main component in the powder of raw pineapple peel was determined. Their data was reported as DTG curve which showed types of degradation each of the main components while the TGA curve which expressed the percentage of weight loss (%) for comparison with durian peel, as reported in Fig. 4.2 (a) and (b), respectively.

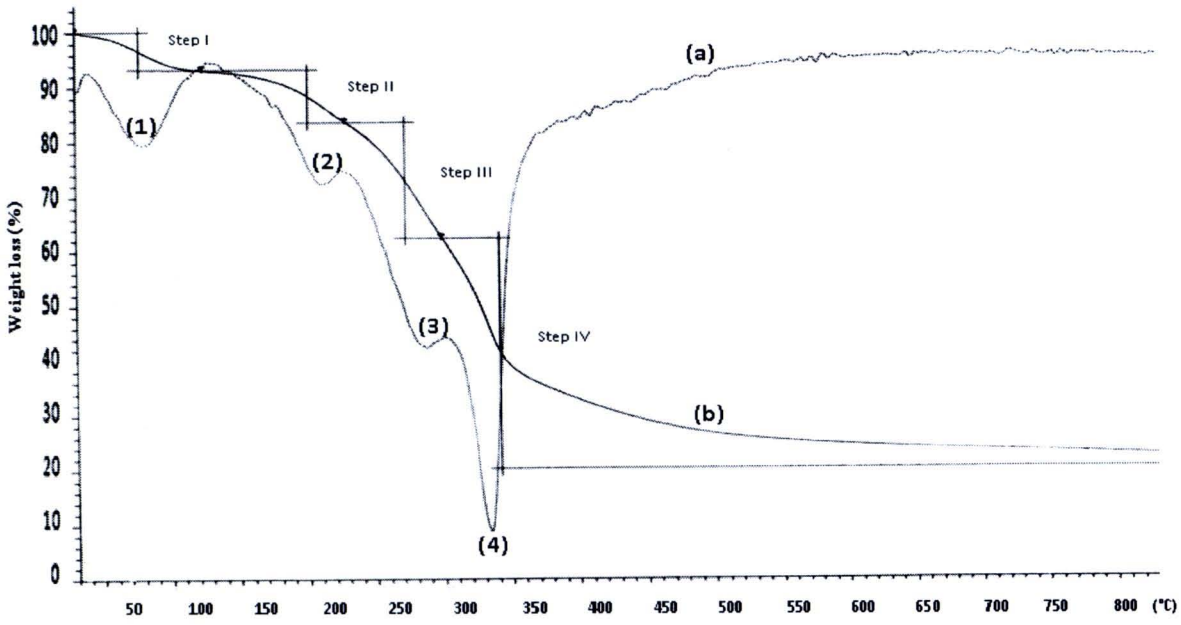

Figure 4.2 The DTG and TG curve of component in raw pineapple peel

Haiping et al. (2006) described the typical component, from the DTG curve showed in Fig. 4.2 (a), the first event was moisture removal (1) up to temperature around 150°C followed by the second, third and fourth events around 200-400°C where the evolution of hemicelluloses (2) and cellulose (3) degradation, respectively. Degradation of lignin slowly took place in a wide temperature range and last to higher temperature (4). The TG curve showed maximum percentage of weight losses occurred in the temperature range of 250°C to 450°C. The first step could be attributed to hemicelluloses degradation of 9.50% whereas the second step was related to cellulose degradation of 21.16% and the final step was 42.11% of the lignin degradation, respectively, shown in Fig. 4.2 (b). This analysis present the result could be accorded to Haiping et al. (2006).

4.1.3 Comparison of main components in raw durian and pineapple peel

The content of hemicelluloses, cellulose and lignin in raw durian peel was compared with raw pineapple peel which monitored by a DTG/TGA in the same of range temperature as expressed in Fig. 4.3.

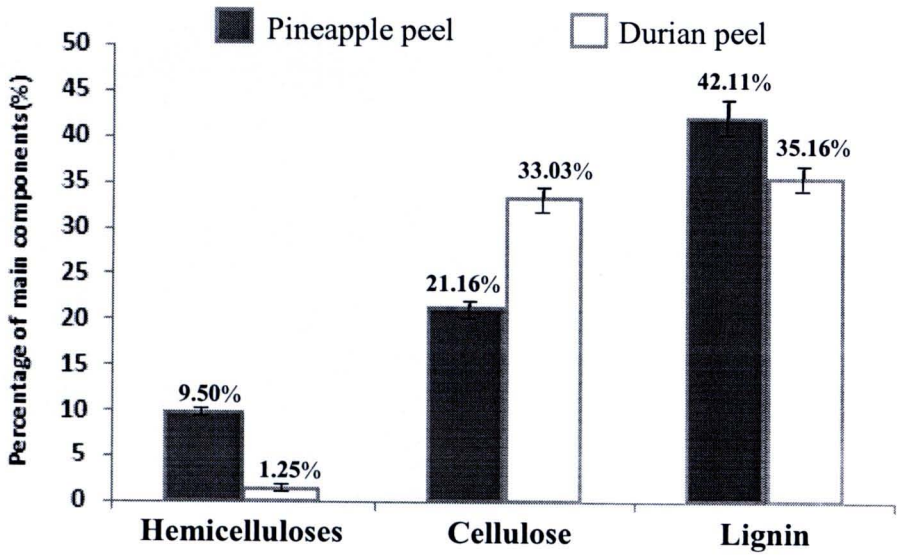

Figure 4.3 The bar charts of main components by DTG/TGA technique

The percentage of main components in durian peel. It composes the cellulose more than the pineapple peel was 33.03 and 21.16, respectively. Except, the percentage of hemicelluloses and lignin. It was found that the durian peel less than pineapple peel was 1.25, 9.50 of hemicelluloses and 35.16, 42.11 of lignin, respectively. The content of main components determined by a DTG/TGA was shown in Table A1.

4.2 TAPPI T203 test method

4.2.1 The percentage of main components via TAPPI T203 test method

TAPPI T203 test method (TAPPI, 1994-1995) could determine the contents of main components. It could investigate from the weight loss each of three samples done by classical gravimetric method. The raw durian and pineapple peel gave the percentage of main components of 1.21, 9.43 of hemicelluloses, 32.88, 20.44 of cellulose and 35.16, 41.21 of lignin, respectively which as shown in Fig. 4.4.

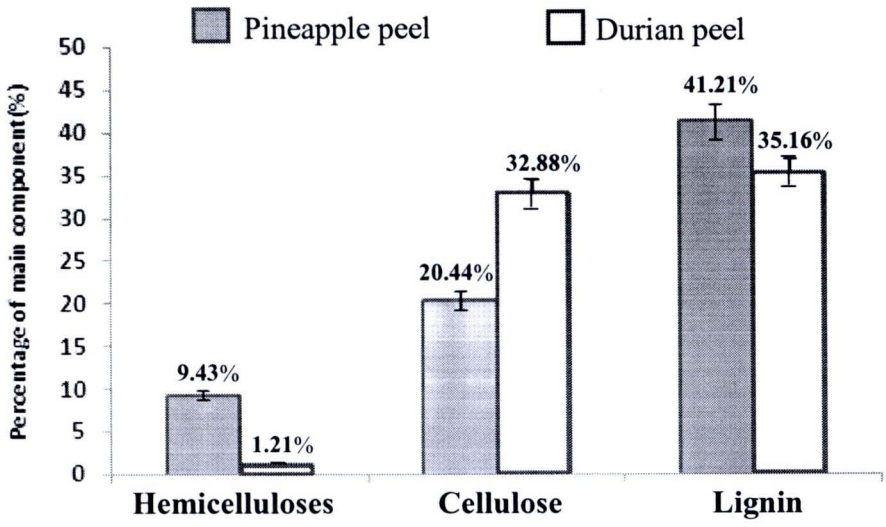

Figure 4.4 The bar charts of main component via TAPPI T203 test method

The percentage of main component in the durian peel. It composes of the cellulose more than the pineapple peel, was 32.88 and 20.44, respectively. Except, the percentage of hemicelluloses and lignin. It was found that the durian peel less than pineapple peel was 1.21, 9.43 and 35.16, 41.21, respectively. The content of main components determined via TAPPI T203 test method was shown in Table A2.

4.2.2 Comparison of main components in raw durian and pineapple peel between DTG/TGA techniques with TAPPI T203 test method

The obtained percentage of main components studied with TAPPI T203 test method could be accorded to DTG/TGA data, as presented in Fig. 4.5.

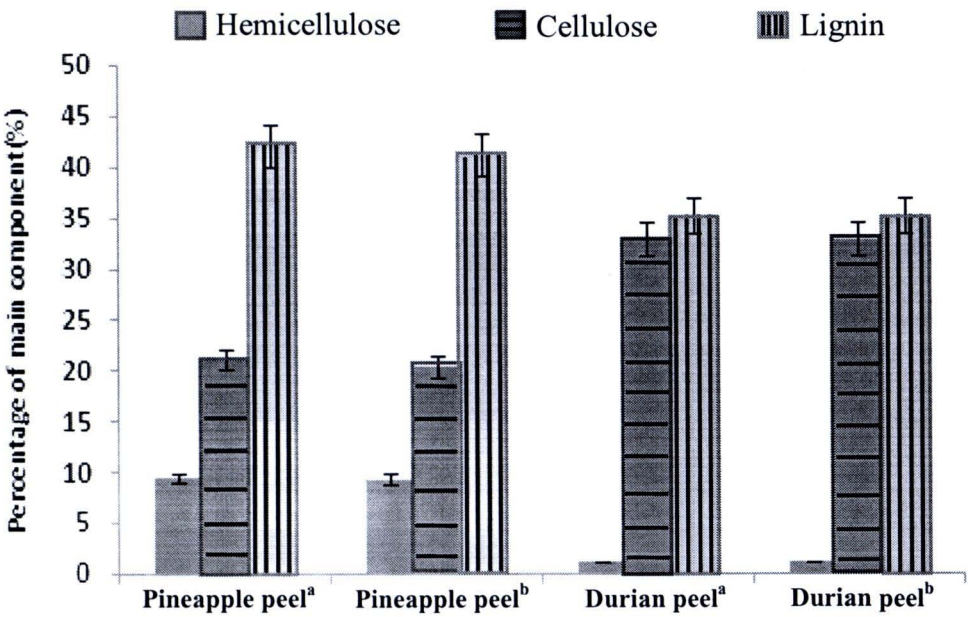

Figure 4.5 The bar charts show the comparison content of main component

The percentage of main components investigated by [b]TAPPI T203 test method could be accorded to [a]DTG/TGA technique. It was found that the obtained data from [b]TAPPI T203 test method was less than [a]DTG/TGA data because the filtration of the samples in the removable processes could be affected to weight of the samples lose.

4.2.3 Identification of main components in raw durian and pineapple peel by FTIR technique in each removable step

In each removable step of TAPPI T203 test method, the main components were removed. Their removable step was identified via FTIR technique.

4.2.3.1 Identification of main components in durian peel

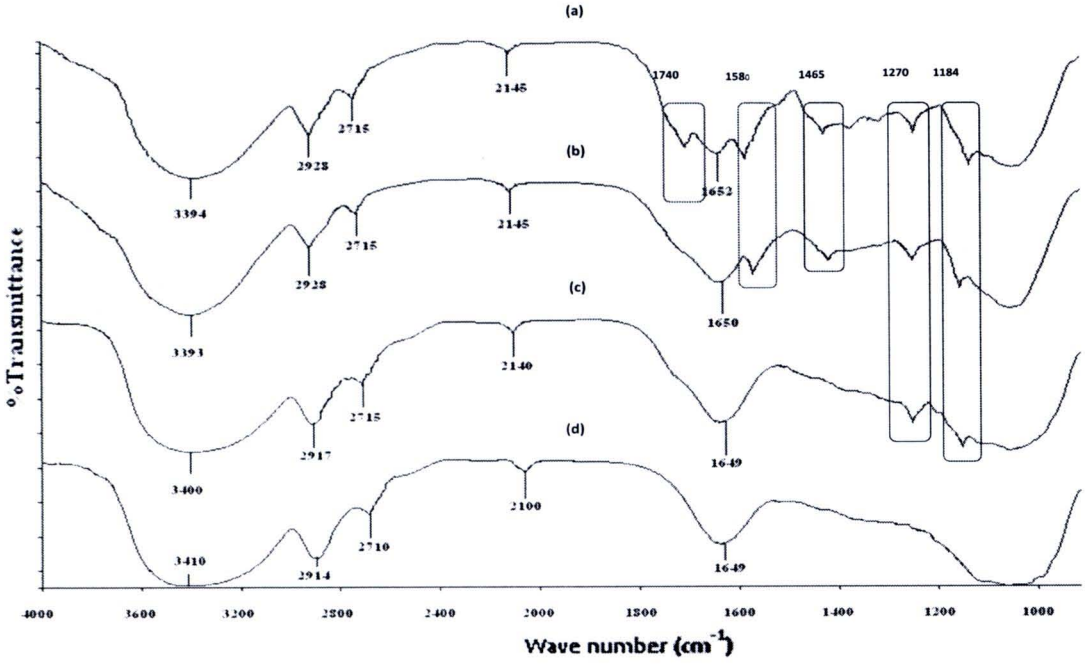

Figure 4.6 The IR spectra of the raw durian peel

The IR spectra of the raw durian peel for the mode assignment of O-H in reducing sugars appeared at 3600-3100 cm^{-1}, C=O ester in ester compounds occurred at 1740-1725 cm^{-1}, C=C aromatic in lignin occurred at 1580 cm^{-1} and 1465 cm^{-1}, C-O-C arabinose in hemicelluloses occurred at 1270 cm^{-1} and 1184 cm^{-1}, as shown in Fig. 4.6 (a). The mode assignments of functional groups harmonized to Crews et al. (1998). These confirmed main components present in the raw durian peel such as the reducing sugars in cellulose and hemicelluloses, the ester compounds and the lignin portion. From the remove of main components via TAPPI T203 test method showed the obtained sample 1 when ester compounds removed from raw durian peel, as expressed in Fig. 4.6 (b) showed no peak at 1740 cm^{-1}. The IR spectra of sample 2, as

shown in Fig. 4.6 (c) could disappear at 1580 cm^{-1} and 1465 cm^{-1} due to the absence of lignin. The peak of arabinose could disappear at 1270 cm^{-1} and 1184 cm^{-1} in the sample 3, as shown in Fig. 4.6 (d) due to the hemicelluloses removal. These results could be conformed as report of Pavia et al. (1996) and their data could be concluded in Table 4.1.

Table 4.1 Mode assignment of raw durian peel

Peak assignment	Peak position (cm^{-1})			
	(a)	(b)	(c)	(d)
ν (O-H)	3394	3393	3400	3410
ν (C-H)	2928	2928	2917	2914
ν (C=O)	1652	1650	1649	1649
$\nu (C=O)_{ester}$	1740	-	-	-
ν (C=C)	1465, 1580	1465, 1580	-	-
ν (C-O-C)	1184, 1270	1184, 1270	1184, 1270	-

ν = stretching, - = no appear, durian peel (a), removal of fat and oil sample (b), removal of lignin sample (c) and cellulose sample (d)

4.2.3.2 Identification of main components in pineapple peel

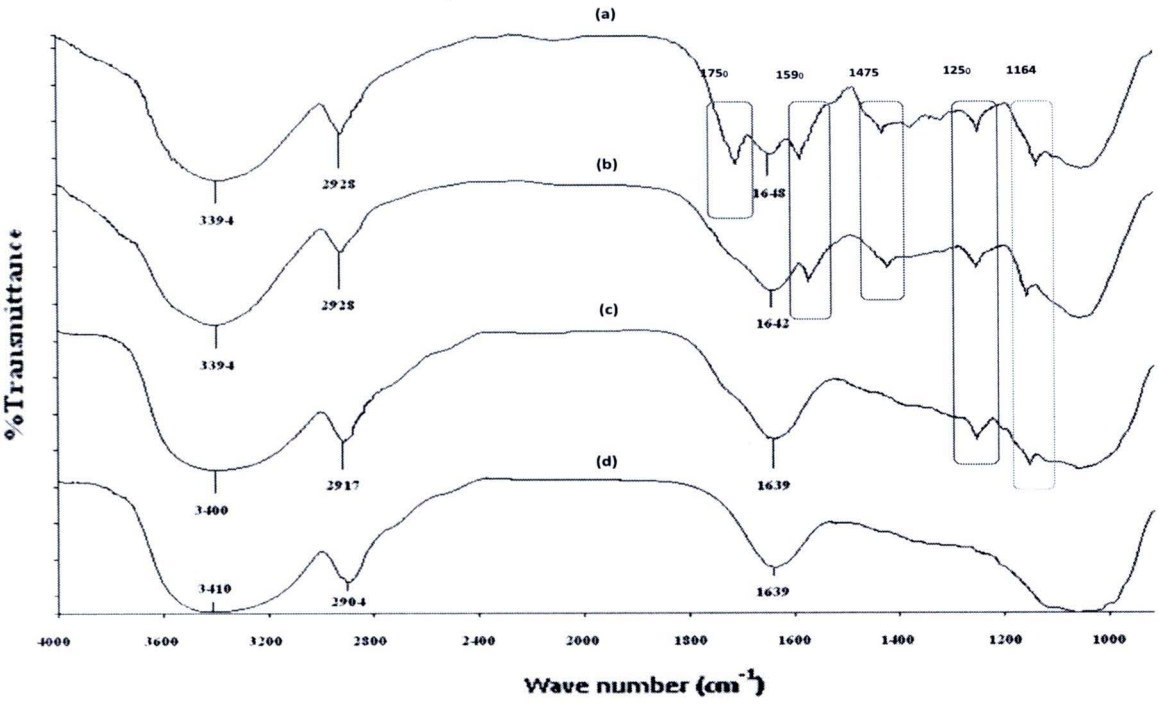

Figure 4.7 The IR spectra of raw pineapple peel

The IR spectra of the raw pineapple peel for the mode assignment of O-H in reducing sugars appeared at 3500-3200 cm^{-1}, C=O ester in ester compounds occurred at 1750-1735 cm^{-1}, C=C aromatic in lignin occurred at 1590 cm^{-1} and 1475 cm^{-1}, C-O-C arabinose in hemicelluloses occurred at 1250 cm^{-1} and 1164 cm^{-1}, as shown in Fig. 4.7 (a). The mode assignments of functional groups harmonized to Crews et al. (1998). These confirmed main components present in the raw pineapple peel such as the reducing sugars in cellulose and hemicelluloses, the ester compounds and the lignin portion. From the remove of main components with TAPPI T203 test method showed the obtained sample 1 when ester compounds removed from raw pineapple peel, as shown in Fig. 4.7 (b) showed no peak at 1750 cm^{-1}. The IR spectra of sample 2, as expressed in Fig. 4.7 (c) could disappear at 1590 cm^{-1} and 1475 cm^{-1} due to the absence of lignin. The peak of arabinose could disappear at 1250 cm^{-1} and 1164 cm^{-1} in the sample 3, as shown in Fig. 4.7 (d) due to the hemicelluloses removal. These results could be conformed to the results reported by Pavia et al. (1996) and their data could be concluded in Table 4.2.

Table 4.2 Mode assignment of raw pineapple peel

Peak assignment	Peak position (cm^{-1})			
	(a)	(b)	(c)	(d)
ν (O-H)	3394	3394	3400	3410
ν (C-H)	2928	2928	2917	2904
ν (C=O)	1648	1642	1639	1639
$\nu (C=O)_{ester}$	1750	-	-	-
ν (C=C)	1475, 1590	1475, 1590	-	-
ν (C-O-C)	1164, 1250	1164, 1250	1164, 1250	-

ν = stretching, - = no appear, pineapple peel (a), removal of fat and oil sample (b), removal of lignin sample (c) and cellulose sample (d)

4.2.4 The photograph of durian and pineapple peel in each removable step of TAPPI T203 test method

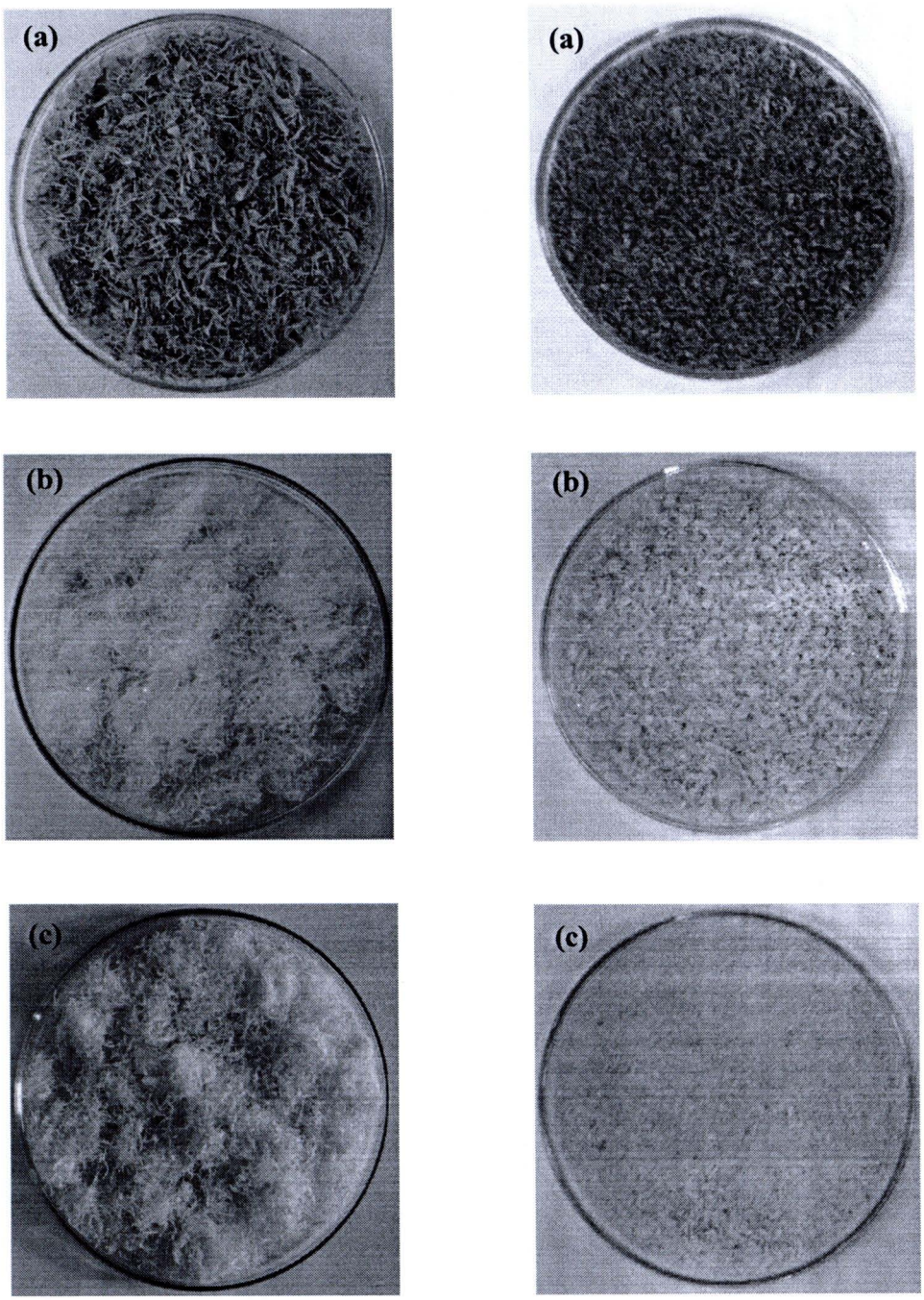

Figure 4.8 The photograph of durian and pineapple peel sample

The photograph of both durian and pineapple peel could be described as follows in step of the fat and oil removal (1), both durian and pineapple peel had the color of peels like the raw peel sample but they had fewer odours, as shown in Fig. 4.8 (a). The lignin removal (2), both durian and pineapple peel had the less odour. The durian peel had the white pearl but pineapple peel had the light yellow because the lignin in pineapple peel was more than in durian peel, as shown in Fig. 4.8 (b). The hemicelluloses removal (3), both durian and pineapple peel had the lightening white, less odour and homogenized sample, as shown in Fig. 4.8 (c).

4.3 The dilute acid hydrolysis

4.3.1 The optimum conditions for dilute acid hydrolysis

The working conditions for dilute acid hydrolysis were modified from the experiment as described by Xu et al. (2003). The three samples were hydrolyzed with 0.2 M sulfuric acid using electrical autoclaving at 120°C, 15 psi for 30 min, as shown in Table 4.3. These optimum conditions were applied for dilute acid hydrolysis on a hotplate.

Table 4.3 The optimum conditions for dilute acid hydrolysis

Parameters	
Sample weight (g)	10
Temperature for hydrolysis (°C)	120
Time for hydrolysis (min)	30
Sulphuric acid volume (mL)	100
Sulphuric acid concentration (M)	0.2
Pressure for hydrolysis (psi)	15

4.3.2 The analytical performance characteristics of glucose

The analytical performance characteristic of the standard glucose by a UV-Vis spectrophotometer was evaluated using optimum conditions; the calibration plot, LOD and LOQ, precision and recovery were investigated. The standard glucose was prepared by DNS method in concentration range of 1.0-10.0 mg L^{-1}, as shown in Fig. 4.9.

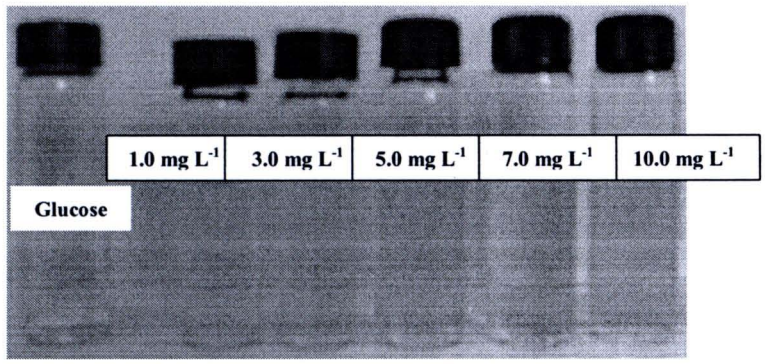

Figure 4.9 The photograph of standard glucose

4.3.2.1 Calibration plot

The calibration curve was studied between 1.0 and 20.0 mg L^{-1} and the linearity was maintained until 10.0 mg L^{-1} with a correlation coefficient (r^2) greater than 0.990 as shown in Fig. C1.

4.3.2.2 LOD and LOQ

The LOD and LOQ were usually determined by decreasing glucose concentration following by the DNS method until the signal of a UV-VIS spectrophotometer disappears. The lowest concentration that still gives an acceptable recovery is defined as method detection limit. The LOD and LOQ of a UV-VIS spectrophotometer for DNS method were 0.010 mg L^{-1} and 0.015 mg L^{-1}.

4.3.2.3 Precision

The precision of the DNS method is expressed in terms of relative standard deviation (RSD), estimated from 10 replicated and calculated at concentration of 1.0 and 5.0 mg L^{-1} glucose were 4.41% and 5.72%, respectively.

4.3.2.4 Recovery

The recovery was studied by spiking the 0.03, 0.06 and 0.09 mg L^{-1} glucose into each durian peel and pineapple peel hydrolyzed solution and was analyzed by a UV-VIS spectrophotometer. The percentage recoveries of glucose for all types of hydrolyzed solution were found in the range from 98.55 to 106.7% as, summarized in Table 4.4.

Table 4.4 The percent recoveries of glucose in spiked sample

Hydrolyzed solution	Compound	Concentration (mg L^{-1})		Recovery (%)
		Added	Found ±S.D.	
Durian peel	Glucose	0	0.009 ± 0.012	-
		0.03	0.032 ± 0.016	106.7
		0.06	0.060 ± 0.037	100.3
		0.09	0.082 ± 0.050	91.07
Pineapple peel	Glucose	0	0.008 ± 0.045	-
		0.03	0.029 ± 0.031	96.56
		0.06	0.059 ± 0.033	98.55
		0.09	0.082 ± 0.043	91.12

4.3.3 Real sample analysis

The DNS method was used for the determination of the total reducing sugars in the hydrolyzed solution of durian and pineapple peel which hydrolyzed by an electrical autoclave and a hot plate under the optimum condition by a UV-VIS spectrophotometer, as shown in Fig. 4.10. The percent recovery of spiked glucose in sample 1, sample 2 and sample 3 was shown in Table 4.5. The content of total reducing sugars in samples, as shown in Table A3 and A4, respectively.

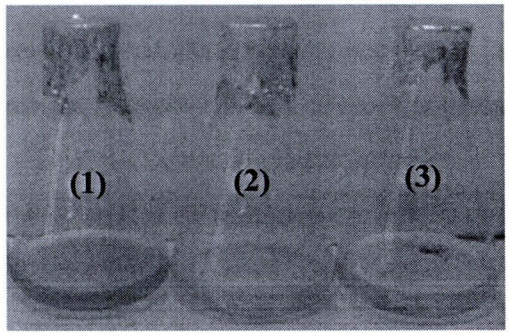

Figure 4.10 The hydrolyzed solution of each sample

Table 4.5 The recovery of spiked glucose in each sample

Hydrolyzed solution	Type of samples	Recovery (%); n=3
Durian peel	[a]Sample 1	106.7 ± 0.85
	[b]Sample 2	99.89 ± 0.77
	[c]Sample 3	91.21 ± 0.73
Pineapple peel	[a]Sample 1	96.61 ± 0.96
	[b]Sample 2	98.61 ± 1.07
	[c]Sample 3	91.23 ± 0.93

Sample 1 = lignin + hemicelluloses + cellulose, Sample 2 = hemicelluloses + cellulose and Sample 3 = cellulose, spiked with [a]0.03, [b]0.06 and [c]0.09 mg L^{-1}

4.3.3.1 The percentage of total reducing sugars via an electrical autoclave

An electrical autoclave was used for dilute acid hydrolysis of three samples of durian and pineapple peels. It could complete hydrolysis and good for production of total reducing sugars. The content of total reducing sugars was shown in Fig. 4.11.

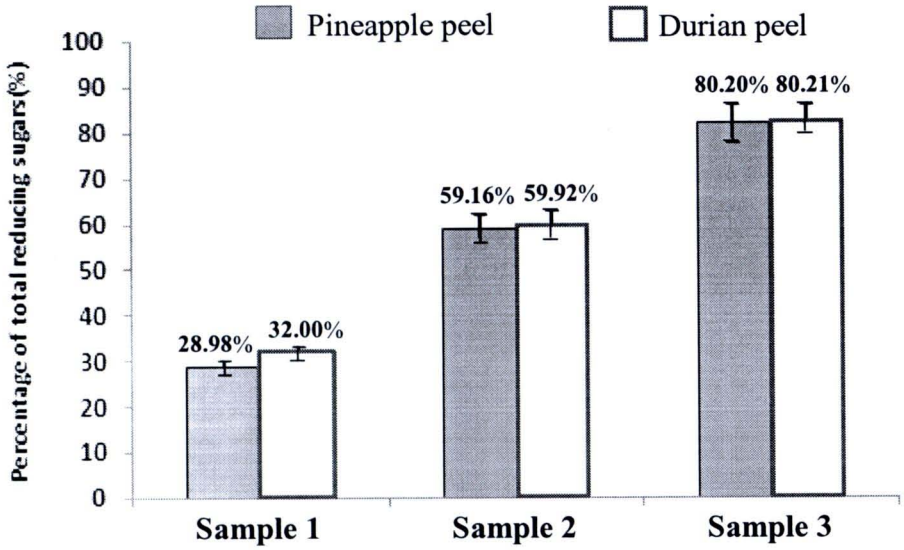

Figure 4.11 The percentage of the total reducing sugars by an autoclave

The hydrolyzed solution of sample 1 gave percentage of total reducing sugars were 32.00 and 28.98, respectively. Its total reducing sugars that was quite low because the effect on high amount and complicated structure of lignin, including long and rowdy branches of hemicelluloses. The sample 1 of pineapple peel could give the total reducing sugar less than durian peel because the pineapple peel had the highest content of the lignin. The hydrolyzed solution of sample 2 and sample 3 of durian and pineapple peels which hydrolyzed in an electrical autoclave gave the percentage of total reducing sugars were 59.9, 59.16 and 80.2, 80.20, respectively. The sample 3 of durian and pineapple peels could give the nearly highest content of total reducing sugars which was all the glucose. Because it had the highest amount of cellulose and normally, the structure of the cellulose was linear chain polymers then could be hydrolyzed easier than the others (Updegraff, 1969). The hydrolyzed

solution of sample 2 also had glucose and other reducing sugars from the hydrolyzed hemicelluloses. In fact, the structure of hemicelluloses was long and rowdy branches which could be a blocker in diluted sulfuric acid hydrolysis (Ebringerova et al., 2005).

4.3.3.2 The percentage of total reducing sugars via a hot plate

A hot plate could use for produce the total reducing sugars of three samples of durian and pineapple peel. It could not control the pressure of the process but it saves and could produce the total reducing sugars. The content of total reducing sugars was shown in Fig. 4.12.

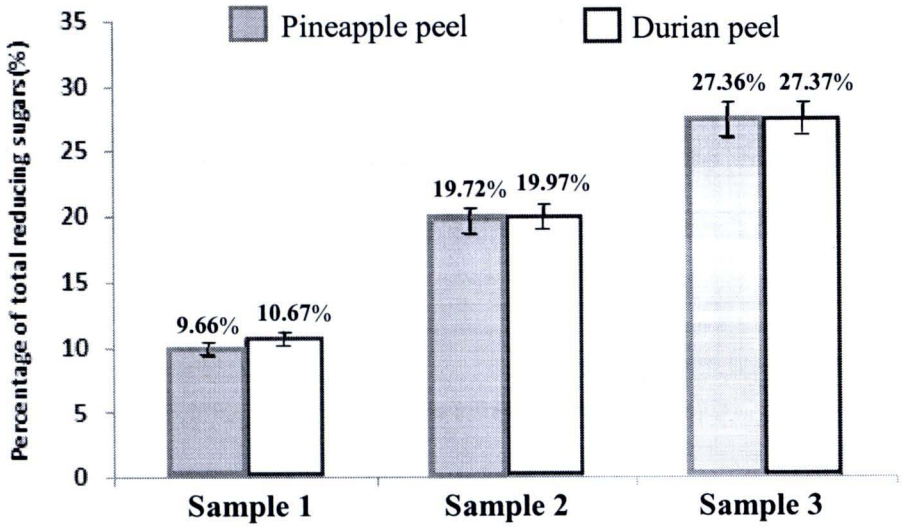

Figure 4.12 The percentage of the total reducing sugars on a hot plate

The sample 1 gave the lowest percentage of the total reducing sugars were 10.67 and 9.66, respectively. The result obtained in this study is agreed with previous study reported by Updegraff and Ebringerova. The hydrolyzed solution from durian and pineapple peel gave the nearly percentage of total reducing sugars. The sample 3 was 27.37 and 27.36, respectively. The sample 2 could give 19.97 and 19.72, respectively.

4.3.3.3 Comparison of the total reducing sugars between electrical autoclaving with hot plate

The percentage of the total reducing sugars obtained from was compared between an electrical autoclave with a hot plate. The performance of dilute acid hydrolysis with two methods was investigated, as shown in Fig. 4.13.

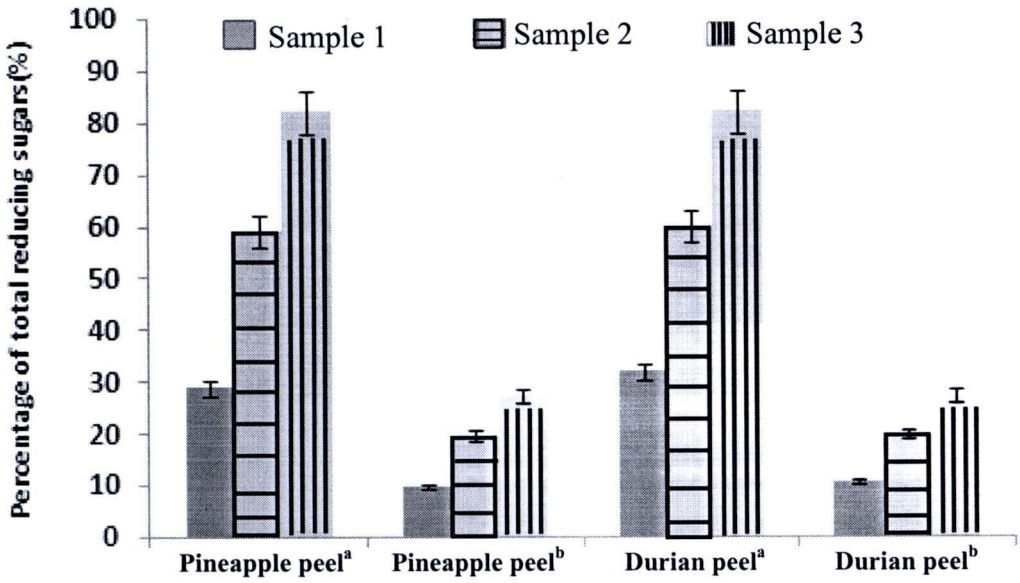

Figure 4.13 The comparison of total reducing sugars

The dilute acid hydrolysis by an [a]electrical autoclave could give the total reducing sugars higher than a [b]hot plate. In the fact, the [a]electrical autoclave could still and complete heat for all the time and it had the optimum pressure which the [b]hot plate could not. Therefore an [a]electrical autoclave was chosen for diluted acid hydrolysis for total reducing sugars production.

4.4 The contents of cellulosic ethanol

4.4.1 The optimum condition for GC-FID

The details showed as following; injector was operated at 250°C. The flame ionization detector was kept at 200°C. Nitrogen gas was used as carrier gas at flow rate of 30 mL/min. The temperature was programmed at 120°C for 1.4 min, from 120°C to 240°C at 30°C/min, then hold 5 min at 240°C. The internal standard used was 2-propanol. They were followed from Caylak and Vardar, as presented in Table 4.6.

Table 4.6 The optimum temperature program of GC-FID

Parameters	
Initial temperature (°C)	120
Initial hold time (min)	1.4
Heat rate (°C/min)	30
Final temperature (°C)	240
Final hold time (min)	5
Flow rate of carrier gas (mL/min)	30.0
Injection temperature (°C)	250
FID temperature (°C)	200
Flame of hydrogen gas : air ratio	0.6 : 0.5

4.4.2 The retention time of standard ethanol and 2-propanol

The retention time (t_R) of ethanol was 1.485 min and the retention time (t_R) of 2-propanol was 1.521 min.

4.4.3 The analytical performance characteristics of ethanol

The analytical performance characteristic of the standard ethanol by a GC-FID was evaluated using optimum conditions; the calibration plot, LOD and LOQ, precision and recovery were investigated, as shown in Fig. 4.14.

4.4.3.1 Calibration plot

The calibration curve was studied between 1.0 and 15.0 M and the linearity was maintained until 9.0 M with a correlation coefficient (r^2) greater than 0.990 as shown in Fig. C2.

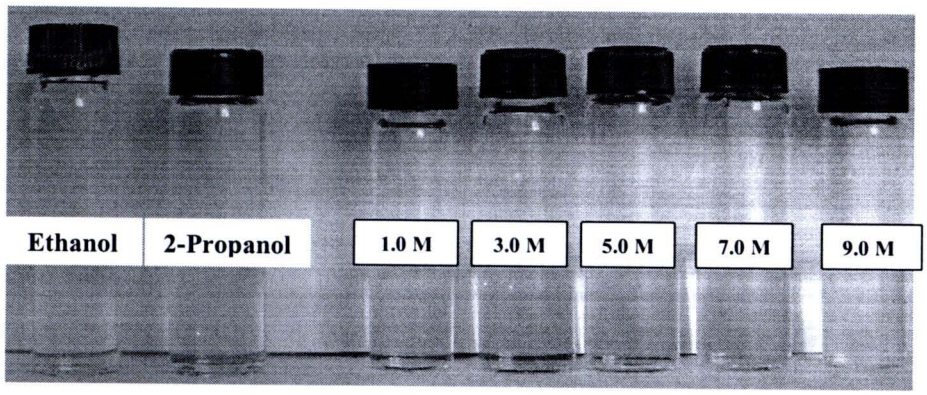

Figure 4.14 The photograph of standard ethanol analyzed by a GC-FID

4.4.3.2 LOD and LOQ

The LOD and LOQ were usually determined by decreasing glucose concentration following by the GC system until the signal of a GC-FID disappears. The lowest concentration that still gives an acceptable recovery was defined as method detection limit. The LOD and LOQ were 0.7 and 1.0 M, respectively.

4.4.3.3 Precision

The precision of the GC system was expressed in terms of relative standard deviation (RSD), estimated from 10 replicated and calculated at concentration of 1.0 and 5.0 M glucose were 5.32% and 7.72%, respectively.

4.4.3.4 Recovery

The recovery was studied by spiking the 1.0, 5.0 and 9.0 M ethanol into durian peel and pineapple peels fermented broth and was analyzed by a GC-FID. The percentage recoveries of ethanol for all types of fermented broth from durian and pineapple peel were in the range of 67.01 to 100.00 % as summarized in Table 4.7.

Table 4.7 The percent recoveries of ethanol in spiked sample

Fermented broth	Compound	Concentration (M)		Recovery (%)
		Added	Found ±S.D.	
Durian peel	Ethanol	0	0.90 ± 0.032	-
		1.0	1.30 ± 0.018	86.82
		5.0	4.90 ± 0.017	80.11
		9.0	6.63 ± 0.030	73.69
Pineapple peel	Ethanol	0	0.50 ± 0.045	-
		1.0	1.00 ± 0.031	100.00
		5.0	3.35 ± 0.024	67.01
		9.0	6.52 ± 0.013	72.52

4.4.4 Real sample analysis

The optimum condition of GC system was used for the determination of the cellulosic ethanol in the fermented broth of hydrolyzed solution of durian and pineapple peel which hydrolyzed in electrical autoclaving, as shown in Fig. 4.15. The percent recovery of spiked ethanol in sample 1, sample 2 and sample 3, as shown in Table 4.8. The content of cellulosic ethanol was shown in Fig. 4.16. The content of cellulosic ethanol in samples, as shown in Table A5.

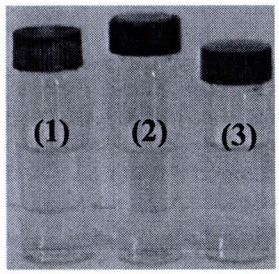

Figure 4.15 The photograph of obtained cellulosic ethanol

Table 4.8 The recovery of spiked ethanol in each sample

Fermented broth	Type of samples	Recovery (%); n=3
Durian peel	[a]Sample 1	87.27 ± 0.35
	[b]Sample 2	97.84 ± 0.12
	[c]Sample 3	89.76 ± 0.45
Pineapple peel	[a]Sample 1	99.61 ± 0.16
	[b]Sample 2	68.21 ± 0.07
	[c]Sample 3	75.23 ± 0.33

Sample 1 = lignin + hemicelluloses + cellulose, Sample 2 = hemicelluloses + cellulose and Sample 3 = cellulose, spiked with [a]1.0, [b]5.0 and [c]9.0 M.

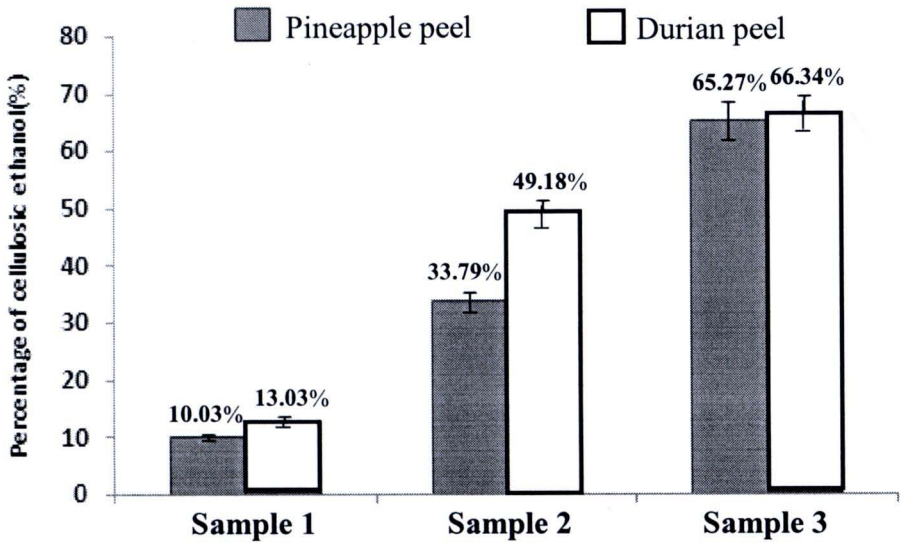

Figure 4.16 The percentage of obtained cellulosic ethanol

The fermented broth of sample 1 gave percentage of cellulosic ethanol were 13.03 and 10.03 only. It was the lowest percentage of bioethanol due to the interfering affectation from high content of the lignin. The fermented broth of sample 3 from durian and pineapple peels gave the highest and nearly the percentage of cellulosic ethanol were 66.34 and 65.27 because it had all glucose which could be bioethanol by *S. cerevisiae* better than the other and no interfering affectation from other components (Lui and Shen, 2008). The cellulosic ethanol in fermented broth of sample 2 from durian peel higher than pineapple peel was 49.18 and 33.79, respectively. Because durian peel contains cellulose more than pineapple peel and the other reducing sugars from hydrolyzed hemicelluloses could not be converted to bioethanol by *S. cerevisiae* (Marek et al., 2007). A regular increased in production of bioethanol was observed until 18 hrs of batch fermentation and declined thereafter. The *S. cerevisiae* showed as a promising strain for utilization of total sugar presented in hydrolyzed lignocellulose which is affected on the higher bioethanol production with a greater yield.