APPENDIX A

Appendix A-1 Reducing sugar determination by 3, 5-dinitrosalicylic acid (DNS) method (Miller, 1959)

1. Chemical reagent :

3, 5-dinitrosalicylic acid (Aldrich)	10.0 g
Na_2SO_3 (Ajax Finechem)	0.5 g
Na-K tratrate (APS Finechem)	182.0 g
NaOH (Merck)	10.0 g
Phenol (Merck)	2.0 g
Deionized water	1000 ml

NaOH 10 g are added into 700 ml of deionized water and mixed in order to add the 300 g Na-K tratrate. When the solution dissolved, 3, 5-dinitrosalicylic acid 10 g is then added and continuously stired. After that, the 0.5 g of Na_2SO_3 and 2.0 g of phenol are dissolved, respectively. Finally, the volume is adjusted to 1,000 ml by deionized water and kept away from light.

2. Reducing sugar determination procedure :

2.1 The samples 0.5 ml are mixed with 0.5 ml of DNS solution. The mixture is boiled for 10 min.

2.2 The sample is cooled down by immersing the sample tube into cold water immediately. Five ml of water is added. The mixture was mixed well, and measured at absorbance 540 nm.

2.3 Absorbance 540 nm is converted to glucose or galactorunic acid concentration with standard curve.

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Appendix A-2 Standard graph of reducing sugar

The relationship between reducingl sugar and absorbance at 540 nm is illustrated in Figure A-1, A-2.

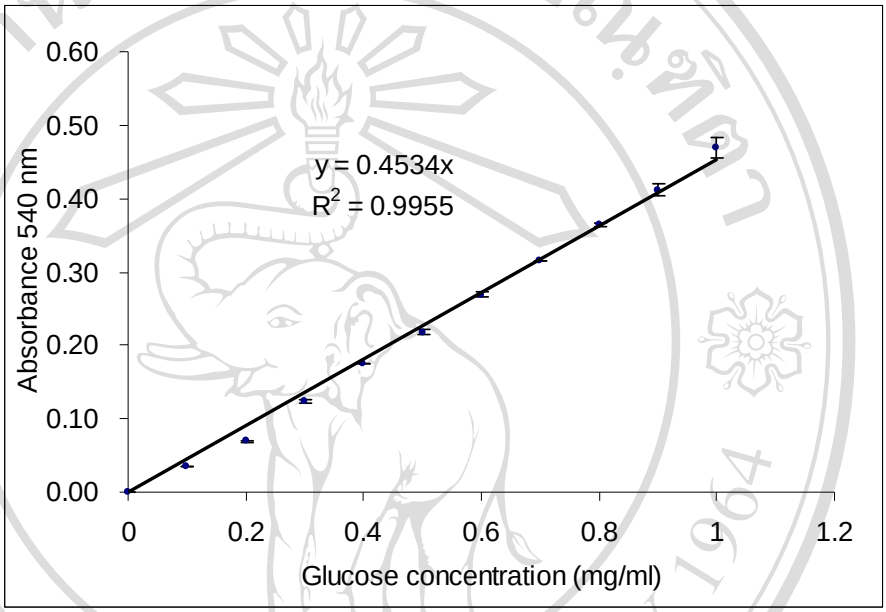

Figure A-1 Reducing sugar standard graph.

This, therefore, provides the equation below for the calculation of sugar concentration from the absorbance at 540 nm.

$$\text{Reducing sugar concentration (mg/ml)} = \frac{A540\,nm}{0.4534}$$

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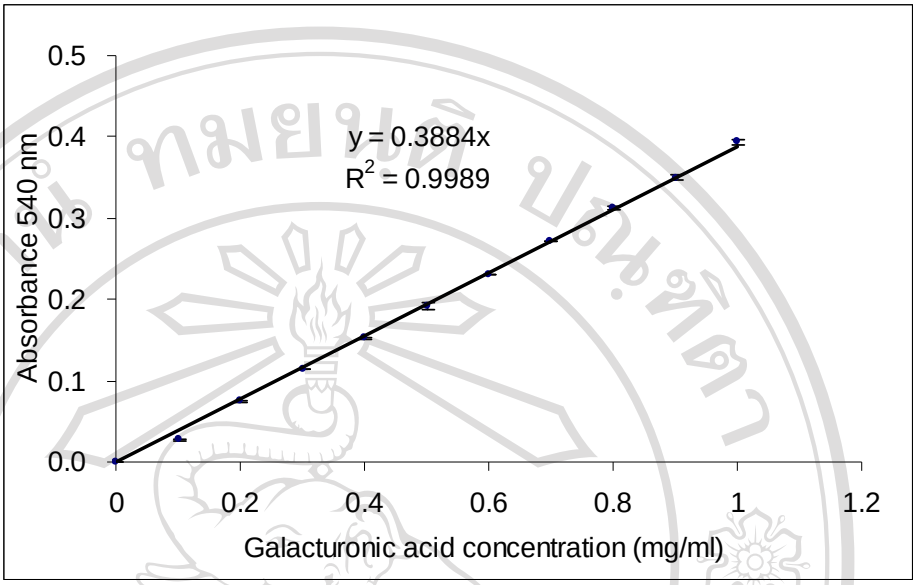

Figure A-2 Reducing sugar standard graph.

This, therefore, provides the equation below for the calculation of reducing sugar concentration from the absorbance at 540 nm.

$$\text{Reducing sugar concentration (mg/ml)} = \frac{A540\,nm}{0.3884}$$

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APPENDIX B

Appendix B-1 Total sugar determination by phenol sulfuric method (Dubois *et. al*., 1956).

1. Chemical reagent

Sulfuric acid (H_2SO_4)	95.5%
Phenol	5.0%

2. Total sugar determination procedure :

2.1 The samples 0.5 ml are mixed with 0.5 ml of phenol solution.

2.2 The mixture are mixed with conc. sulfuric 2.5 ml. The sample was mixed well, waited for 30 min and measured at absorbance 490 nm.

2.3 Absorbance 490 nm is converted to glucose concentration with standard curve.

Appendix B-2 Standard graph of total sugar

The relationship between total sugar and absorbance at 490 nm is illustrated in Figure B-1.

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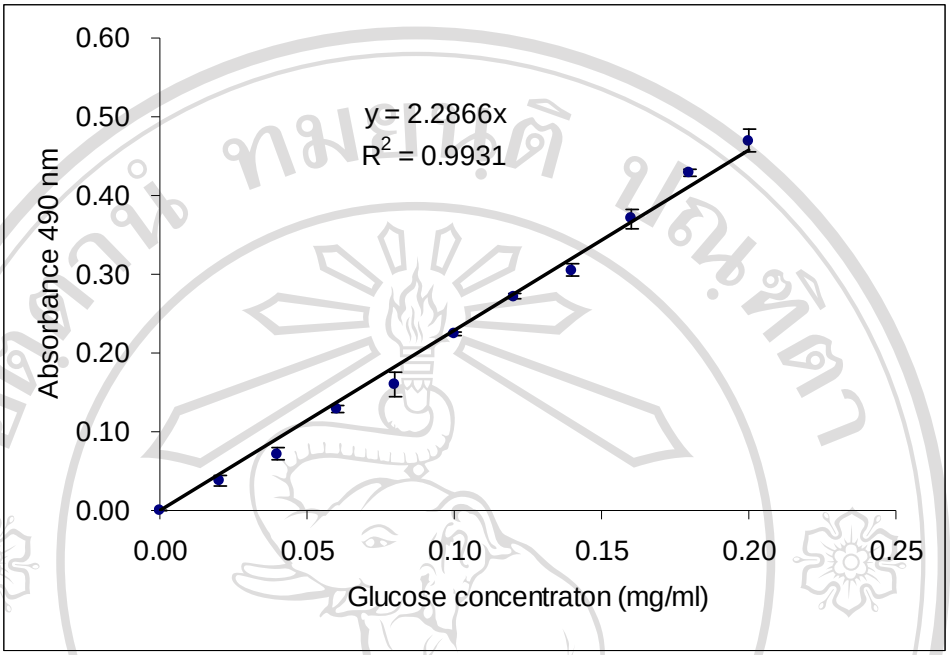

Figure B-1 Total sugar standard graph.

This, therefore, provides the equation below for the calculation of sugar concentration from the absorbance at 490 nm.

$$\text{Total sugar concentration (mg/ml)} = \frac{A490\,nm}{2.2866}$$

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APPENDIX C

Appendix C Total dissolve solids by (AOAC, 2000)

The samples were then centrifuged at 6000x*g* for 10 minute and the supernatant was collected for total dissolve solids. The samples 0.5 ml was added in evaporating dish and then drying at 70 °C for 24 h, followed by drying in a oven at 100°C for 1 h. The dish is then cooled in dessicator and weighed. The increase in weight of the dish represents the total dissolve solid.

$$\text{Total dissolve solids (mg/ml)} = \frac{\text{weight of dry sample}}{\text{weight of wet sample}}$$

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APPENDIX D

Appendix D-1 HPLC analysis (de Rodriguez *et al*., 2004)

1. Chemical reagent and equipments

   1.1 Column : Aminex HPX-87H column (BioRad, Herculas, USA)

   1.2 Detecter : Refractometer (Shimadzu RID-6A, Kyoto, Japan)

   1.3 Mobile phase : 5 mM H_2SO_4 in deionized water

   1.4 Flow rate : 0.75 mL/min

2. Total sugar determination procedure:

   2.1 Filter a sample via membrane filtration (0.2 µm pore size).

   2.2 Inject 20 µL sample to separate glucose, galacturonic acid, fructose, arabinose, maltose, xylose, fructose and sucrose.

   2.3 Quantify the concentration of each chromatographic peak using a standards graph (Appendix C-3).

standards mixed at 10000 ppm graph

Appendix D-2 Standard graph of HPLC analyses

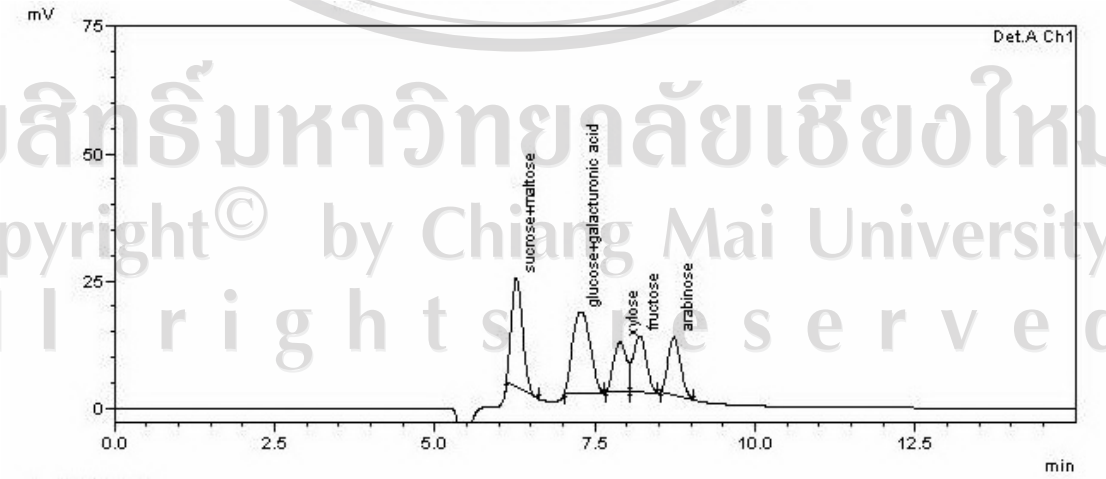

Figure D-1 Chromatogram of sucrose, maltose, glucose, galacturonic acid, xylose fructose and arabinose analysis.

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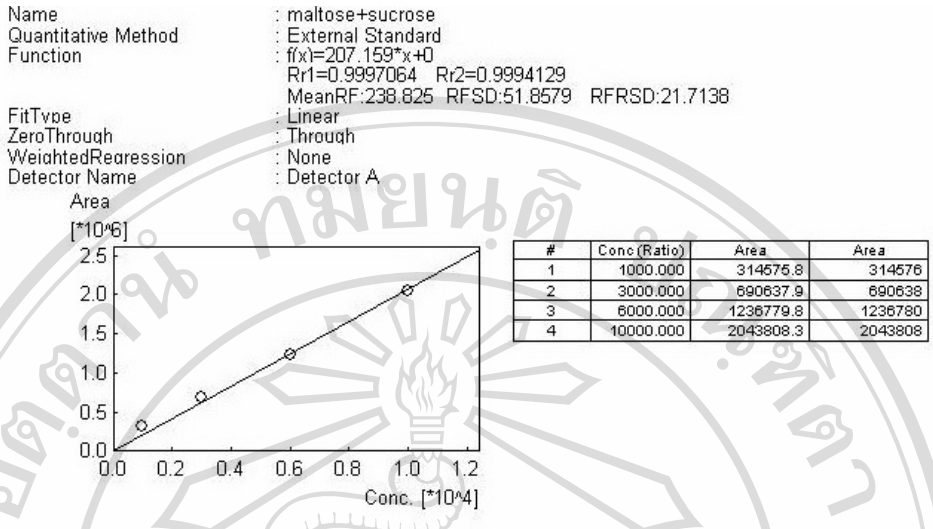
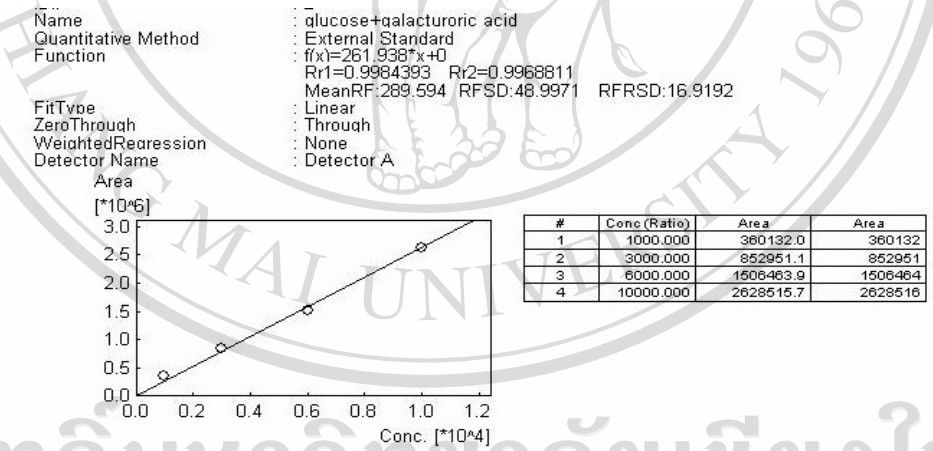


#	Conc (Ratio)	Area	Area
1	1000.000	314575.8	314576
2	3000.000	690637.9	690638
3	6000.000	1236779.8	1236780
4	10000.000	2043808.3	2043808

Figure D-2 Maltose and sucrose standard graph.



#	Conc (Ratio)	Area	Area
1	1000.000	360132.0	360132
2	3000.000	852951.1	852951
3	6000.000	1506463.9	1506464
4	10000.000	2628515.7	2628516

Figure D-3 Glucose and galacturonic acid standard graph.

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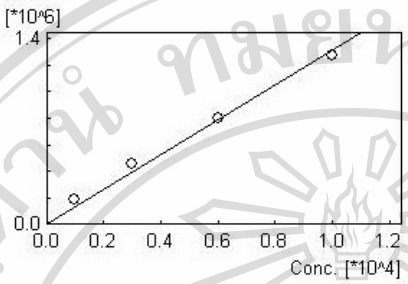
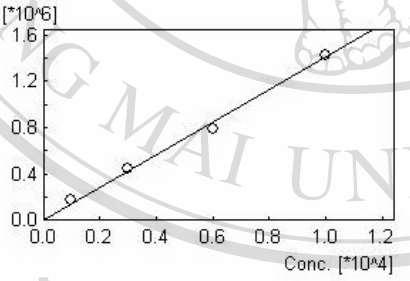
Name : xylose
Quantitative Method : External Standard
Function : f(x)=130.738*x+0
Rr1=0.9996927 Rr2=0.9993854
MeanRF:149.493 RFSD:26.3146 RFRSD:17.6025
FitType : Linear
ZeroThrough : Through
WeightedRegression : None
Detector Name : Detector A



#	Conc (Ratio)	Area	Area
1	1000.000	186118.0	186118
2	3000.000	451746.8	451747
3	6000.000	804464.4	804464
4	10000.000	1271962.2	1271962

Figure D-4 Xylose standard graph.

Name : fructose
Quantitative Method : External Standard
Function : f(x)=140.256*x+0
Rr1=0.9971143 Rr2=0.9942368
MeanRF:150.264 RFSD:20.6057 RFRSD:13.713
FitType : Linear
ZeroThrough : Through
WeightedRegression : None
Detector Name : Detector A



#	Conc (Ratio)	Area	Area
1	1000.000	179201.0	179201
2	3000.000	445233.6	445234
3	6000.000	785805.9	785806
4	10000.000	1424757.9	1424758

Figure D-5 fructose standard graph.

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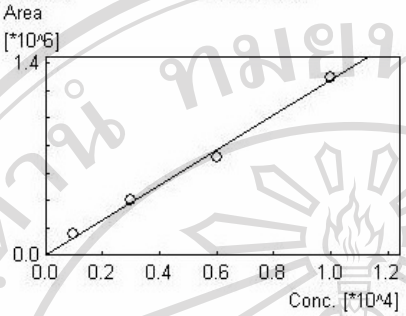
Name : arabinose
Quantitative Method : External Standard
Function : f(x)=127.417*x+0
Rr1=0.9977921 Rr2=0.9956890
MeanRF:134.935 RFSD:14.8546 RFRSD:11.0087
FitType : Linear
ZeroThrough : Through
WeightedRegression : None
Detector Name : Detector A



#	Conc.(Ratio)	Area	Area
1	1000.000	155020.7	155021
2	3000.000	406993.4	406993
3	6000.000	719870.0	719870
4	10000.000	1290773.1	1290773

Figure D-6 Arabinose standard graph.

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