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APPENDICES

APPENDIX A

1. Reagent preparation

1.1 Reagent for protein assaying

- PBS (phosphate buffer saline)

A phosphate buffer saline tablets was dissolved in DI-water 200 mL (pH 7.4± 0.4)

- Standardize stock solution ovalbumin protein standard

The ovalbumin (100 mg) was dissolved with PBS solution (100 mL). The mixture was filtered through 0.45 μm filter with low protein binding. The standard solution was standardized by measurement the absorbance at 280 nm

- DOC solution

A sodium deoxycholate (0.15g) was dissolved in DI-water 100 mL.

- TCA solution

A trichloroacetic acid (72 g) was dissolved in DI-water 100 mL.

- PTA solution

A phosphotungstic acid (72 g) was dissolved in DI-water 100 mL.

- Color reagent

A sodium salt of bromophenol blue (0.1 g) was dissolved in DI-water 1 L.

- Reagent A

The reagent C 100 mL was mixed with reagent D (2 mL).

- Reagent B

The 2M Folin reagent (72 mL) was dissolved in DI-water 28 mL.

- Reagent C

A sodium carbonate (6 g) was dissolved in DI-water 100 mL.

- Reagent D

A cupper sulphate (1.5 g) and sodium citrate (3 g) were dissolved in DI-water 100 mL.

1.2 Reagent for chitinase activity assaying

- Color reagent

Potassium hexaferocyanate (K_3FeCN_6) (0.5 g) and Na_2CO_3 (45.34 g) were dissolved in DI-water 500 mL.

2. Preparation the calibration curve of *N*-acetyl-D-glucosamine for HPLC analysis

Calibration curve of GlcNAc was made by varying the concentration and measuring the peak area by HPLC.

Table A1 The concentration of standard solution of GlcNAc and peak area.

Standard No.	Conc. GlcNAc (mg/mL)	Conc. GlcNAc (mM)	Peak Area (mV*Sec)
1	0.0093	0.0420	14.983
2	0.0186	0.0841	27.667
3	0.0620	0.2803	98.046
4	0.1860	0.8408	308.345
5	0.1210	0.5470	195.536
6	0.2420	1.0940	387.937
7	0.4840	2.1880	749.393
8	1.2100	5.6055	1987.448

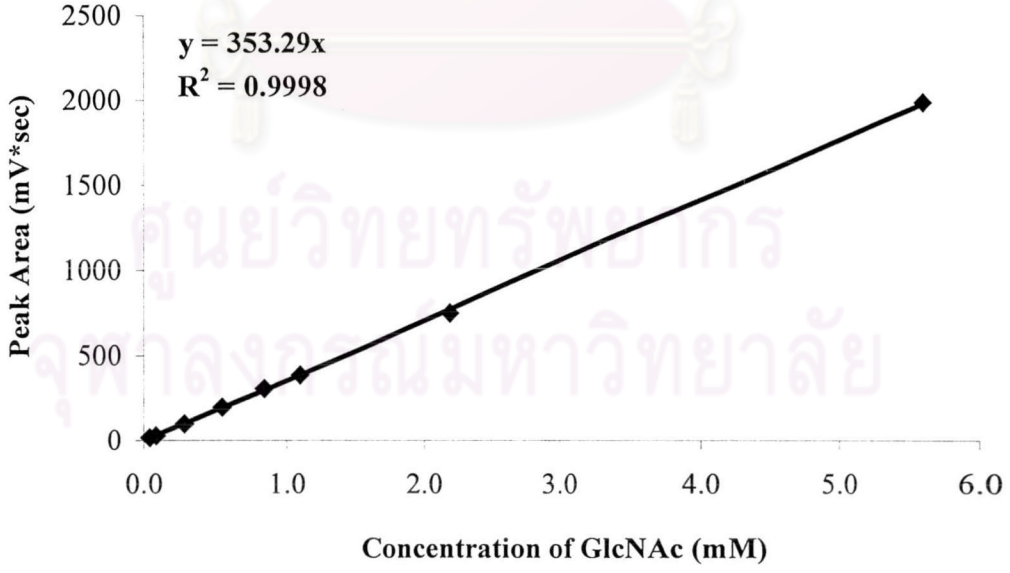


Figure A1 Correlation between concentration of standard *N*-acetyl-D-glucosamine and peak area by HPLC.

3. Preparation the calibration curve of *N,N'*-diacetylchitobiose for HPLC analysis

Calibration curve of $(GlcNAc)_2$ was made by varying the concentration and measuring the peak area by HPLC.

Table A2 The concentration of standard solution of $(GlcNAc)_2$ and peak area.

Standard No.	Conc. GlcNAc (mg/mL)	Conc. GlcNAc (mM)	Peak Area (mV*Sec)
1	0.05	0.1178	57.786
2	0.10	0.2356	111.022
3	0.20	0.4712	235.395
4	0.32	0.7540	380.000
5	0.60	1.4137	676.788
6	1.00	2.3562	1128.094
7	1.28	3.0159	1494.989
8	1.60	3.7699	1900.128

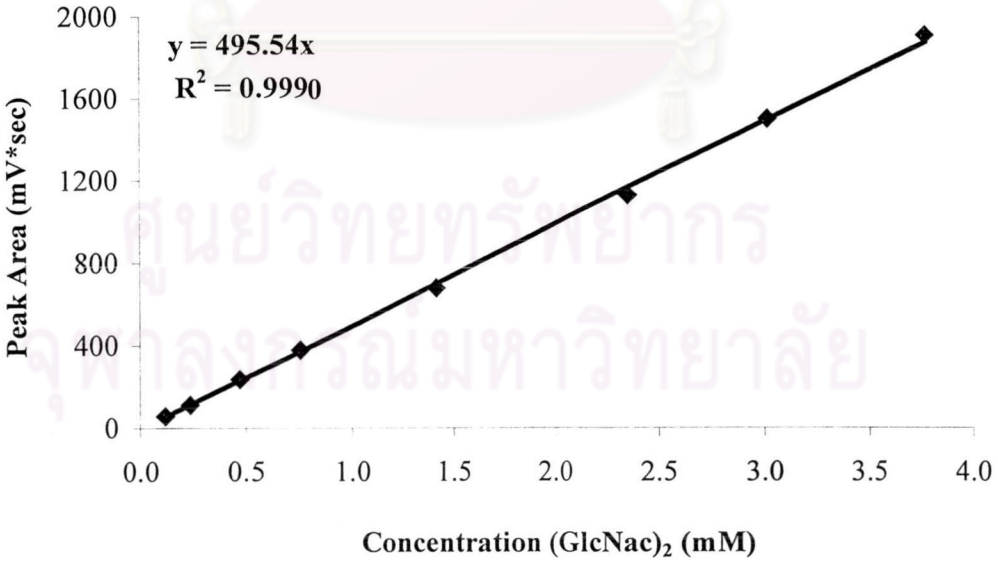


Figure A2 Correlation between concentration of standard *N,N'*-diacetylchitobiose and peak area by HPLC.

4. Preparation the calibration curve of *N,N',N''*-triacetylchitotriose for HPLC analysis

Calibration curve of $(GlcNAc)_3$ was made by varying the concentration and measuring the peak area by HPLC.

Table A3 The concentration of standard solution of $(GlcNAc)_3$ and peak area.

Standard No.	Conc. $(GlcNAc)_3$ (mg/mL)	Conc. $(GlcNAc)_3$ (mM)	Peak Area (mV*Sec)
1	0.050	0.080	64.77
2	0.200	0.319	281.76
3	0.400	0.637	557.62
4	0.600	0.956	827.87
5	0.800	1.275	1074.04
6	1.000	1.593	1376.31
7	1.200	1.912	1566.90
8	1.600	2.549	2081.22
9	2.000	3.187	2668.63

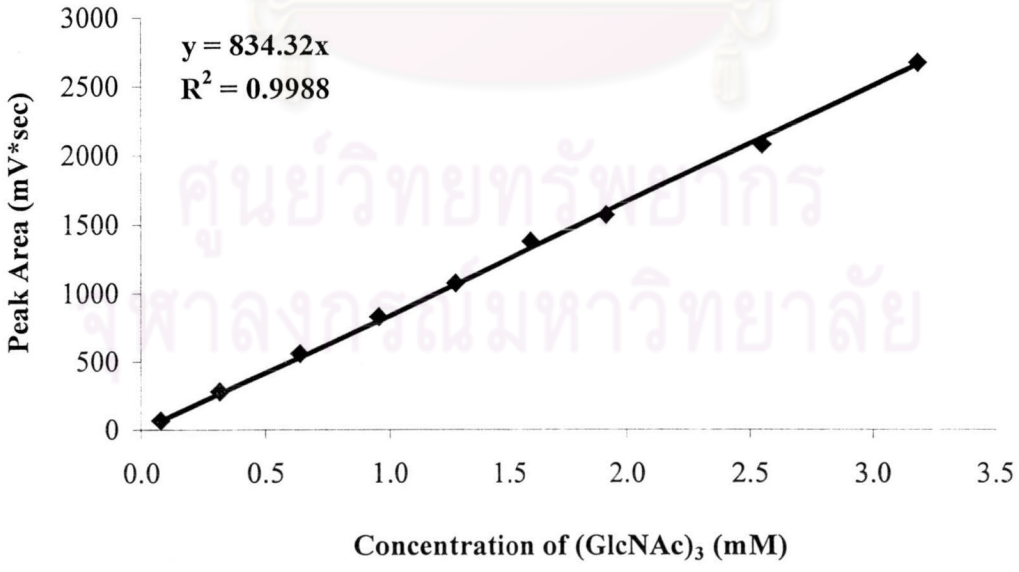


Figure A3 Correlation between concentration of standard *N,N',N''*-triacetylchitotriose and peak area by HPLC (Waters).

5. Prepartion calibration curve of N-acetyl-D-glucosamine for chitinolytic enzyme assay by colorimetric method.

Calibration curve for GlcNAc was made by determining the absorbance value at 420 nm of standard GlcNAc according to the method of Schales.

Table A4 The amount of standard solution of GlcNAc and ΔAbsorbance

Standard No.	Amount of GlcNAc (μmole)	ΔAbsorbance
1	0.8029	0.886
2	0.7025	0.761
3	0.6021	0.650
4	0.5018	0.541
5	0.5018	0.516
6	0.4014	0.411
7	0.3011	0.311
8	0.2007	0.187
9	0.1004	0.094

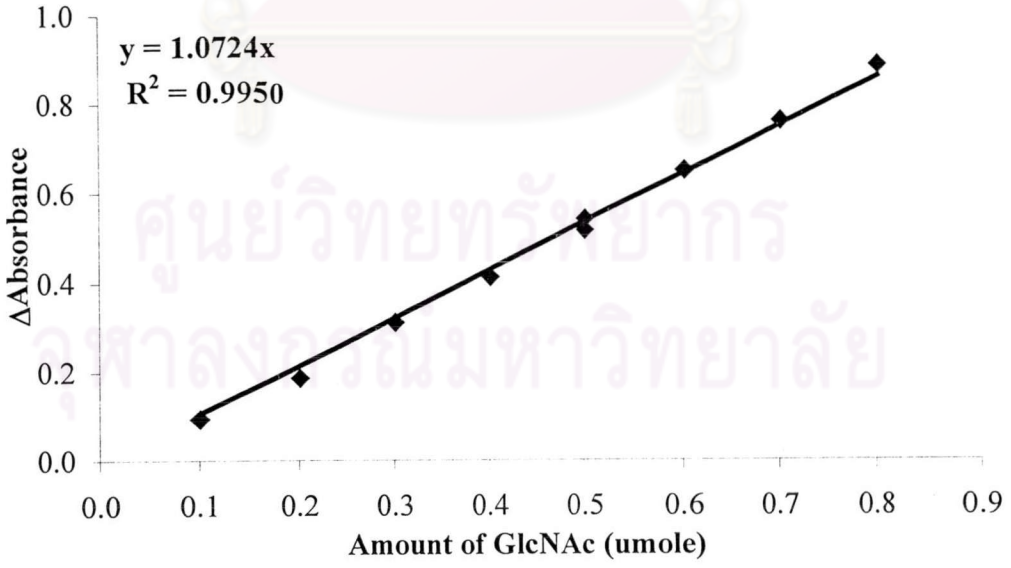


Figure A4 Correlation between amount of standard of *N*-acetyl-D-glucosamine and optical density (absorbance) at 420 nm.

6. Preparation calibration curve of protein concentration by Lawry's colorimetric method.

Calibration curve for ovalbumine protein was made by determining the absorbance value at 720 nm of standard ovalbumine according to the method of Lowry.

Table A5 The concentration of standard ovalbumine and absorbance

Standard No.	Concentration of Protein (mg/mL)	Absorbance
1	0.005	0.0498
2	0.01	0.1142
3	0.02	0.2016
4	0.04	0.4000
5	0.08	0.7232

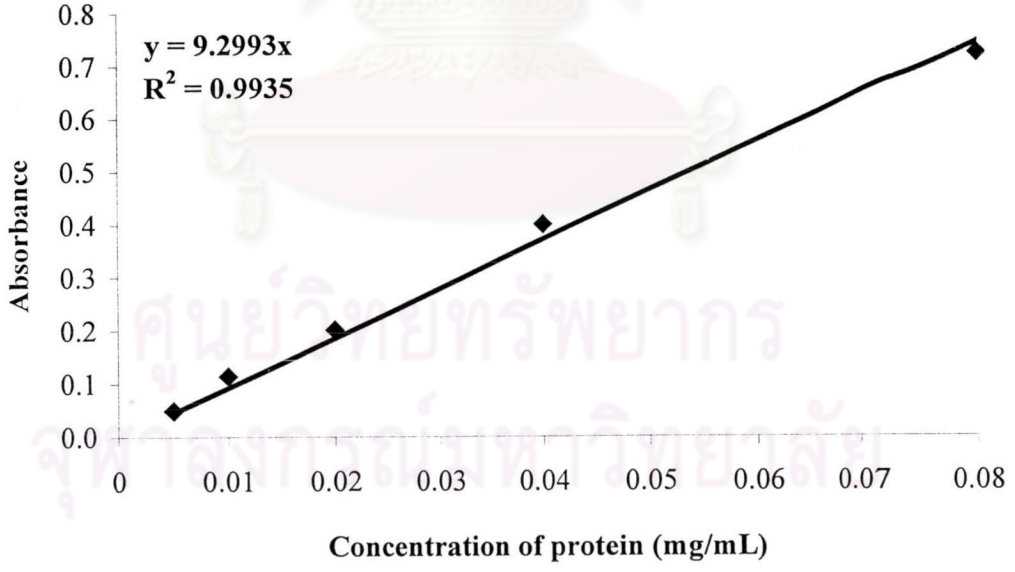
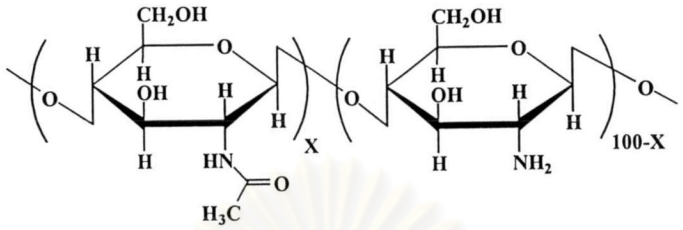


Figure A5 Relationship between standard protein (ovalbumine) concentration and optical density (absorbance) at 720 nm.

7. Standard curve of C/N ratio and %DA of chitin

The standard curve of C/N ratio and %DA was calculate from elemental analysis follow this equation.



$$\%C = \frac{(12.011)(8X + (6(100 - X)}{(203.197X) + 161.159(100 - X)}$$

$$\%N = \frac{(14.007)(X + (100 - X)}{(203.197X) + 161.159(100 - X)}$$

$$\therefore \textbf{The C/N ratio} = 0.0172X + 5.1452$$

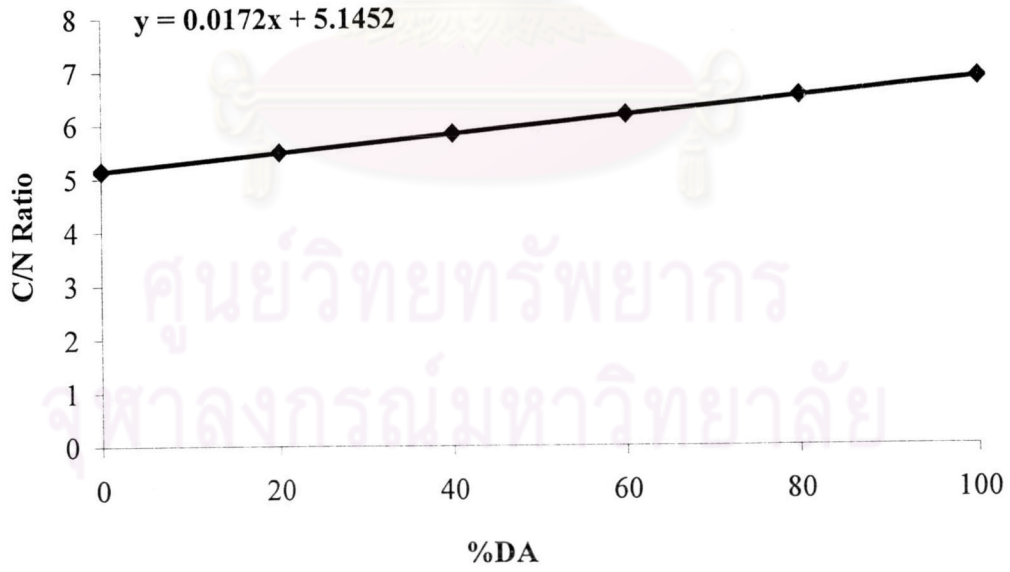
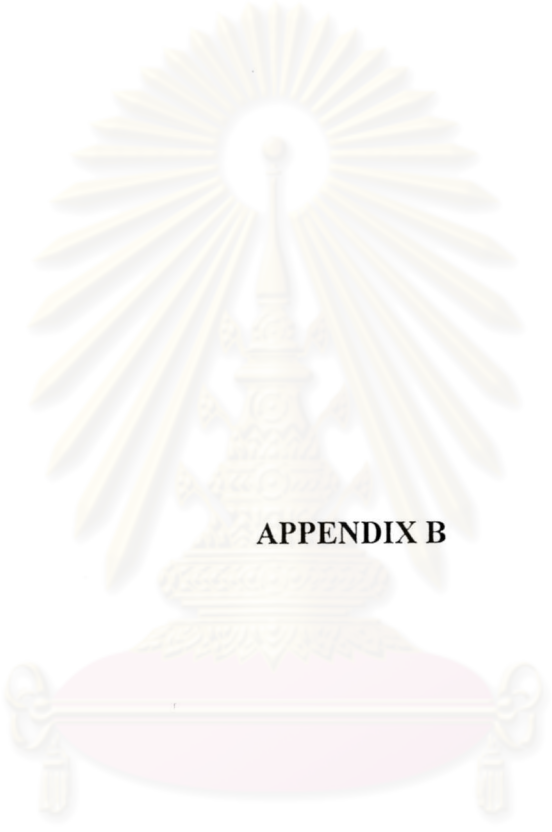


Figure A6 Relationship between %DA of chitin and C/N ratio by elemental analysis.



APPENDIX B

Table B1 The yield of GlcNAc, $(GlcNAc)_2$ and mole ratio of $(GlcNAc)_2$/GlcNAc by the effect of enzyme/chitin ratio on chitinolysis.

Enzyme/chitin ratio	Time (day)	[GlcNac] (mM)	[$(GlcNac)_2$] (mM)	Mole ratio $(GlcNAc)_2$/GlcNAc
	1	0.505	0.902	1.79
	2	0.827	1.833	2.22
0.11 mU/mg	4	1.487	3.066	2.06
	6	1.683	3.336	1.98
	8	1.831	3.633	1.98
	1	1.841	3.471	1.89
	2	2.481	4.795	1.93
0.54 mU/mg	4	2.862	5.689	1.99
	6	5.059	6.816	1.35
	8	5.448	7.943	1.46
	1	2.653	4.515	1.70
	2	3.827	7.618	1.99
1.07 mU/mg	4	4.909	8.093	1.65
	6	6.048	10.539	1.74
	8	7.704	11.781	1.53
	1	3.640	5.316	1.46
	2	4.410	7.715	1.75
1.61 mU/mg	4	5.300	9.151	1.73
	6	7.127	12.130	1.70
	8	8.593	13.479	1.57
	1	4.182	5.846	1.40
	2	5.094	8.731	1.71
2.15 mU/mg	4	7.096	11.254	1.59
	6	8.116	13.101	1.61
	8	9.733	14.575	1.50

The reactions were carried out in chitin concentration 20 mg/mL, acetate buffer solution pH 4.0 (0.05 M) and 37°C.

Table B2 The yield of GlcNAc, $(GlcNAc)_2$ and mole ratio of $(GlcNAc)_2$/GlcNAc by the effect of concentration of chitin on chitonolysis.

[chitin] (mg/mL)	Time (day)	[GlcNAc] (mM)	[$(GlcNAc)_2$] (mM)	Mole ratio $(GlcNAc)_2$/GlcNAc
	1	0.312	0.492	1.57
	2	0.540	1.076	1.99
5.0	4	0.8.65	1.779	1.84
	6	1.139	2.159	1.90
	8	1.420	2.768	1.95
	1	0.587	1.306	2.23
	2	0.922	1.675	1.82
10.0	4	1.283	2.683	2.09
	6	1.741	3.382	1.94
	8	1.958	3.673	1.88
	1	1.922	3.866	2.01
	2	2.832	5.377	1.90
20.0	4	4.017	7.865	1.96
	6	4.860	9.025	1.86
	8	5.641	10.435	1.85
	1	3.073	5.841	1.90
	2	4.223	7.634	1.81
30.0	4	5.513	10.487	1.90
	6	6.849	12.725	1.86
	8	7.937	14.545	1.83
	1	4.294	7.998	1.86
	2	5.378	9.661	1.80
40.0	4	8.913	16.408	1.84
	6	9.498	16.428	1.73
	8	10.883	18.836	1.73

Table B2 The yield of GlcNAc, $(GlcNAc)_2$ and mole ratio of $(GlcNAc)_2$/GlcNAc by the effect of concentration of chitin on chitinolysis (continued).

[chitin] (mg/mL)	Time (day)	[GlcNAc] (mM)	$[(GlcNAc)_2]$ (mM)	Mole ratio $(GlcNAc)_2$/GlcNAc
50.0	1	4.972	9.339	1.88
	2	6.287	10.691	1.70
	4	8.752	15.885	1.82
	6	10.775	18.645	1.73
	8	15.284	25.411	1.66
60.0	1	5.031	10.291	2.05
	2	7.844	15.375	1.96
	4	10.787	19.620	1.82
	6	13.703	23.557	1.72
	8	17.272	28.454	1.65
70.0	1	4.900	9.703	1.98
	2	7.070	13.839	1.96
	4	11.444	20.580	1.80
	6	17.711	30.943	1.75
	8	17.722	28.649	1.62

The reactions were carried out in chitin concentration 20 mg/mL, acetate buffer solution pH 4.0 (0.05 M) and 37°C.

Table B3 The yield of GlcNAc, $(GlcNAc)_2$ and mole ratio of $(GlcNAc)_2$/GlcNAc by the effect of pH of the reaction solution on chitinolysis.

Type of Buffer (pH)	Time (day)	[GlcNAc] (mM)	[$(GlcNAc)_2$] (mM)	Mole ratio $(GlcNAc)_2$/GlcNAc
McIlvan pH 2.0	1	7.037	13.077	1.86
	2	9.941	18.039	1.81
	4	14.916	25.552	1.71
	6	20.361	32.674	1.60
	8	22.484	34.655	1.54
McIlvan pH 2.5	1	7.698	14.732	1.91
	2	12.514	21.720	1.74
	4	16.903	28.092	1.66
	6	22.327	35.639	1.60
	8	22.486	34.513	1.53
McIlvan pH 3.0	1	7.133	14.052	1.97
	2	12.544	22.818	1.82
	4	16.529	28.118	1.70
	6	21.914	35.863	1.64
	8	22.168	34.725	1.57
McIlvan pH 3.5	1	6.420	11.984	1.87
	2	11.118	23.236	2.09
	4	16.331	28.479	1.74
	6	21.299	35.525	1.67
	8	22.494	35.812	1.59
McIlvan pH 4.0	1	5.047	10.367	2.05
	2	9.599	20.124	2.10
	4	13.180	24.080	1.83
	6	18.625	31.927	1.71
	8	19.566	32.916	1.68

Table B3 The yield of GlcNAc, $(GlcNAc)_2$ and mole ratio of $(GlcNAc)_2$/GlcNAc by the effect of pH of the reaction solution on chitinolysis (continued).

Type of Buffer (pH)	Time (day)	[GlcNAc] (mM)	[$(GlcNAc)_2$] (mM)	Mole ratio $(GlcNAc)_2$/GlcNAc
McIlvan pH 4.5	1	3.402	6.589	1.94
	2	5.549	12.208	2.20
	4	9.481	20.265	2.14
	6	11.530	22.051	1.91
	8	15.437	27.616	1.79
sodium acetate pH 4.0	1	8.842	17.059	1.93
	2	10.256	19.239	1.88
	4	15.498	27.041	1.74
	6	22.497	34.364	1.68
	8	22.972	37.602	1.64
sodium acetate pH 4.5	1	6.662	13.480	2.02
	2	7.644	17.266	2.26
	4	14.998	27.928	1.86
	6	17.200	30.833	1.79
	8	18.252	32.322	1.77
sodium acetate pH 5.0	1	2.659	5.887	2.21
	2	3.599	8.475	2.35
	4	5.617	13.251	2.36
	6	7.763	17.523	2.26
	8	11.249	23.564	2.09
sodium acetate pH 5.5	1	1.616	3.475	2.15
	2	1.972	4.670	2.37
	4	3.475	8.586	2.47
	6	4.112	9.844	2.39
	8	4.507	12.654	2.81

The reactions were carried out in chitin concentration 60 mg/mL, the enzyme/chitin ratio 0.22 mU/mg, and 37°C. All buffers concentration were 0.1M.

Table B4 The yield of GlcNAc, $(GlcNAc)_2$ and mole ratio of $(GlcNAc)_2$/GlcNAc by the effect of type of buffer on chitinolysis.

Type of buffer	Time (day)	[GlcNAc] (mM)	[$(GlcNAc)_2$] (mM)	Mole ratio ($GlcNAc_2$/GlcNAc
sodium acetate	2	9.860	17.762	1.80
	4	12.370	23.589	1.91
potassium acetate	2	9.451	19.077	2.02
	4	12.366	24.697	2.00
citrate phosphate	2	9.106	18.420	2.02
	4	15.728	26.320	1.67
sodium citrate	2	9.646	19.024	1.97
	4	13.667	24.912	1.82
potassium hydrogen phthalate	2	9.479	17.209	1.82
	4	15.682	24.767	1.60

The reactions were carried out in chitin concentration 60 mg/mL with the enzyme/chitin ratio 0.22 mU/mg at 37°C. All buffers concentration 0.1M which have pH 4.0.

Table B5 The yield of GlcNAc, $(GlcNAc)_2$ and mole ratio of $(GlcNAc)_2$/GlcNAc by the effect of temperature on chitinolysis.

Temperature (°C)	Time (day)	[GlcNAc] (mM)	[$(GlcNAc)_2$] (mM)	Mole ratio $(GlcNAc)_2$/GlcNAc
30	1	6.381	11.541	1.81
	2	9.771	16.923	1.73
	4	12.046	20.083	1.67
	6	12.855	23.133	1.80
	8	16.446	28.162	1.71
37	1	6.964	13.392	1.92
	2	11.692	20.854	1.78
	4	18.260	30.076	1.65
	6	22.121	35.927	1.62
	8	25.070	40.453	1.61
45	1	12.188	24.055	1.97
	2	17.147	31.438	1.83
	4	24.321	40.737	1.67
	6	28.355	44.713	1.58
	8	35.660	51.058	1.43
55	1	13.736	21.575	1.57
	2	16.903	30.258	1.79
	4	20.953	36.948	1.76
	6	25.877	41.265	1.59
	8	31.037	42.974	1.38

The reactions were carried out in chitin concentration 60 mg/mL, the enzyme/chitin ratio 0.22 mU/mg, acetate buffer solution pH 4.0 (0.1 M).

Table B6 The yield of GlcNAc, $(GlcNAc)_2$ and mole ratio of $(GlcNAc)_2$/GlcNAc by the effect of concentration of buffer on chitinolysis.

[acetate buffer] (M)	Time (day)	[GlcNAc] (mM)	[$(GlcNAc)_2$] (mM)	Mole ratio $(GlcNAc)_2$/GlcNAc
0.0	1	1.156	4.023	3.48
	2	2.682	8.476	3.16
	4	4.466	13.935	3.12
	6	6.281	18.315	2.92
	8	7.742	23.455	3.03
0.05	1	3.841	9.902	2.58
	2	7.131	18.532	2.60
	4	8.420	19.793	2.35
	6	18.092	34.755	1.92
	8	20.965	39.841	1.90
0.1	1	5.762	13.064	2.27
	2	13.707	26.769	1.95
	4	17.975	33.950	1.89
	6	28.770	44.505	1.55
	8	34.350	51.514	1.50
0.2	1	8.914	18.223	2.04
	2	17.229	30.774	1.79
	4	19.457	34.165	1.76
	6	27.338	40.030	1.46
	8	29.860	44.763	1.50
0.4	1	9.451	19.077	2.02
	2	19.052	33.346	1.75
	4	20.774	35.239	1.70
	6	28.007	40.941	1.46
	8	30.679	47.288	1.54

The reactions were carried out in chitin concentration 60 mg/mL, the enzyme/chitin ratio 0.22 mU/mg, acetate buffer solution pH 4.0, and 45 °C.

Table B7 The yield of GlcNAc, $(GlcNAc)_2$ and mole ratio of $(GlcNAc)_2$/GlcNAc by substrate dependence on chitinolysis.

Substrate	Time (day)	[GlcNAc] (mM)	[$(GlcNAc)_2$] (mM)	Mole ratio $(GlcNAc)_2$/GlcNAc
fibrous chitin[a] (100 × 50 μm)	2	5.762	13.064	2.2
	4	13.707	26.769	2.0
	6	17.975	33.950	1.9
	8	28.770	44.505	1.6
fibrous chitin[a] (50 × 25 μm)	2	19.598	39.587	2.0
	4	24.486	44.494	1.8
	6	30.457	52.380	1.7
	8	42.257	56.605	1.4
powder chitin[a] (3.0 μm)	2	21.876	37.427	1.7
	4	22.363	41.151	1.8
	6	27.376	45.957	1.7
	8	40.615	54.698	1.4
fibrous chitin[b] (100 × 50 μm)	2	9.403	15.846	1.7
	4	11.674	18.968	1.6
	6	14.243	22.657	1.6
	8	15.890	24.982	1.6
colloidal[b] chitin	2	5.655	7.895	1.5
	4	7.146	11.036	1.5
	6	11.646	18.914	1.6
	8	11.905	19.493	1.6

The reactions were carried out with the enzyme/chitin ratio 0.22 mU/mg, acetate buffer solution pH 4.0 (0.1 M) at 45 °C.

[a]concentration of chitin = 60 mg/mL

[b]concentration of chitin = 20 mg/mL

Table B8 The yield of GlcNAc, $(GlcNAc)_2$ and mole ratio of $(GlcNAc)_2$/GlcNAc by Hydrolysis with increasing enzyme/chitin ratio on chitinolysis.

Enzyme/chitin ratio	Time (day)	[GlcNAc] (mM)	[$(GlcNAc)_2$] (mM)	Mole ratio $(GlcNAc)_2$/GlcNAc
0.22 mU/mg	2	8.737	16.312	1.9
	4	19.603	32.730	1.7
	6	28.636	43.431	1.5
	8	29.462	50.526	1.7
0.44 mU/mg	2	15.374	25.109	1.6
	4	30.340	44.466	1.5
	6	43.589	57.692	1.3
	8	37.827	58.674	1.5
0.66 mU/mg	2	22.106	34.094	1.5
	4	31.801	44.120	1.4
	6	40.390	53.899	1.3
	8	34.625	54.213	1.5
0.88 mU/mg	2	23.166	35.390	1.5
	4	30.515	46.894	1.5
	6	33.946	50.360	1.4
	8	35.531	53.447	1.5

The reactions were carried out in chitin concentration 60 mg/mL, acetate buffer solution pH 4.0 (0.1 M), and 45 °C.

Table B9 The yield of GlcNAc, $(GlcNAc)_2$ and mole ratio of $(GlcNAc)_2$/GlcNAc by product inhibition by GlcNAc on the chitinolysis.

Initial concentration of GlcNAc (mM)	Time (day)	[GlcNAc] (mM)	$[(GlcNAc)_2]$ (mM)
0.00	2	20.749	33.516
	6	32.358	44.999
9.31	2	20.077	34.299
	6	31.489	44.439
18.17	2	16.552	31.457
	6	29.331	43.489
28.21	2	14.084	30.921
	6	34.483	47.424
36.16	2	18.265	30.689
	6	34.521	46.153
47.19	2	19.961	42.007
	6	31.032	45.225

The reaction was carried out with fibrous β-Chitin concentration 60 mg/mL, serum *Hb* 64.8 mU., acetate buffer pH 4.0 (0.1 M), temperature 45 °C.

Table B10 The yield of GlcNAc, $(GlcNAc)_2$ and mole ratio of $(GlcNAc)_2$/GlcNAc by using dialysis tubing **System A1**.

Time (day)	[GlcNAc] (mM)	$[(GlcNAc)_2]$ (mM)	Mole ratio $(GlcNAc)_2$/GlcNAc
1	0.294	1.021	2.5
3	0.614	1.483	2.4
5	0.704	1.760	2.5
5	0.704	1.754	2.5
7	0.889	2.281	2.6
10	0.742	2.243	3.0
11[a]	0.830	2.251	2.7
12	0.226	0.575	2.5
13	0.447	1.028	2.3
17	0.627	1.434	2.3
18	0.645	1.560	2.4
21	0.792	1.865	2.4

The reaction was carried out with fibrous β-Chitin 3.6 g, serum *Hb* 0.75 U, acetate buffer pH 4.0 (0.1 M), temperature 45 °C, [a]The outer solution (500 mL) was refreshed and another portion of the serum Hb (0.75 U) was also added inside the bags.

Table B11 The yield of GlcNAc, $(GlcNAc)_2$ and mole ratio of $(GlcNAc)_2$/GlcNAc by using dialysis tubing **System A2**.

Time (day)	[GlcNAc] (mM)	$[(GlcNAc)_2]$ (mM)	Mole ratio $(GlcNAc)_2$/GlcNAc
1	0.285	0.736	2.6
3	0.452	1.167	2.6
5	0.490	1.352	2.8
7	0.694	2.044	2.9
9	0.777	2.307	3.0
11[a]	0.827	2.428	2.9
12	0.339	1.012	3.0

Table B11 The yield of GlcNAc, $(GlcNAc)_2$ and mole ratio of $(GlcNAc)_2$/GlcNAc by using dialysis tubing **System A2** (continued).

Time (day)	[GlcNAc] (mM)	$[(GlcNAc)_2]$ (mM)	Mole ratio $(GlcNAc)_2$/GlcNAc
14	0.377	1.080	2.9
16	0.508	1.404	2.8
18	0.528	1.587	3.0
20	0.549	1.603	2.9
21	0.568	1.676	2.9

The reaction was carried out with swollen chitin 3.6 g, serum *Hb* 1.59 U., acetate buffer pH 4.0 (0.1 M), temperature 45 °C, [a]The outer solution (500 mL) was refreshed.

Table B12 The yield of GlcNAc, $(GlcNAc)_2$ and mole ratio of $(GlcNAc)_2$/GlcNAc by using dialysis tubing **System B**.

Time (day)	[GlcNAc] (mM)	$[(GlcNAc)_2]$ (mM)	Mole ratio $(GlcNAc)_2$/GlcNAc
1	1.449	3.116	2.1
2	1.995	4.277	2.1
4	2.034	4.455	2.2
6	2.318	5.126	2.2
7	2.699	5.945	2.2
9	2.627	5.707	2.2
11[a]	2.875	6.255	2.2
12	1.232	2.697	2.2
14	1.363	2.896	2.1
17	1.484	3.120	2.1
18	1.811	3.674	2.0
21	2.043	4.251	2.1

The reaction was carried out with fibrous β-Chitin 7.2 g, serum *Hb* 3.2 U., acetate buffer pH 4.0 (0.1 M), temperature 45 °C, [a]The outer solution (500 mL) was refreshed.

Table B13 The yield of GlcNAc, $(GlcNAc)_2$ and mole ratio of $(GlcNAc)_2$/GlcNAc by using dialysis tubing **System C**.

Time (day)	[GlcNAc] (mM)	$[(GlcNAc)_2]$ (mM)	Mole ratio $(GlcNAc)_2$/GlcNAc
1	3.562	4.980	1.4
2	4.987	7.455	1.5
4	6.441	10.015	1.6
7	7.429	11.786	1.6
9	7.933	12.588	1.6
11[a]	8.338	13.342	1.6
12	3.335	5.226	1.6
14	3.761	6.036	1.6
18	4.423	7.336	1.7
20	4.516	7.530	1.7
21	5.071	8.494	1.7

The reaction was carried out with fibrous β-Chitin 14.4 g, serum *Hb* 6.4 U., acetate buffer pH 4.0 (0.1 M), temperature 45 °C, [a]The outer solution (500 mL) was refreshed.

Table B14 The yield of GlcNAc, $(GlcNAc)_2$ and total % yield by production the *N*-acetyl-D-glucosamine in one step.

Mixing ratio[a] Pectinase/Serum Hb	Time (day)	[GlcNAc] (mM)	$[(GlcNAc)_2]$ (mM)	Total %Yield
0.03	2	32.079	42.400	42
	4	37.549	45.326	45
	6	41.289	48.356	50
	8	44.935	50.315	47
0.16	2	41.050	33.985	39
	4	57.220	35.514	46
	6	70.833	29.593	47
	8	75.456	30.269	49

Table B14 The yield of GlcNAc, $(GlcNAc)_2$ and total % yield by production the *N*-acetyl-D-glucosamine in one step (continued).

Mixing ratio[a] Pectinase/Serum Hb	Time (day)	[GlcNAc] (mM)	[$(GlcNAc)_2$] (mM)	Total %Yield
0.33	2	52.311	25.675	37
	4	73.519	26.449	46
	6	116.457	6.658	47
	8	125.362	3.314	50
1.65	2	98.193	4.002	39
	4	109.659	5.917	44
	6	117.475	1.737	45
	8	130.525	1.882	49
3.29	2	96.447	4.638	39
	4	115.390	3.129	45
	6	129.235	2.565	49
	8	134.052	2.385	50

The reaction was carried out with β-Chitin concentration 60 mg/mL , serum *Hb* 64.8 mU, acetate buffer pH 4.0 (0.1 M), temperature 45 °C. [a]Unit of chitinase of serum *Hb* = 1.24 U/mL, Unit of chitobiase of pectinase *An* = 2.56 U/mg, Mixing ratio = Unit by pectinase *An*/Unit by serum *Hb*

Table B15 Relative viscosity of the hydrolysate as a function of the hydrolysis time.

Time for hydrolysis (hrs)	Falling time (s)		
	21 %DA	16 %DA	13 %DA
0	186.26	150.13	136.32
3	120.46	113.84	123.26
6	112.04	107.50	115.21
24	93.51	96.48	99.02
48	90.23	92.66	95.51
72	89.13	91.21	93.86
96	89.01	90.91	93.55

The chitosans (150 mg) were hydrolyzed by serum *Hb* (3.8 mU) in acetate buffer pH 4.0 (0.1M) at 45 °C. The viscosity was measured by Ubbelohde viscometer.

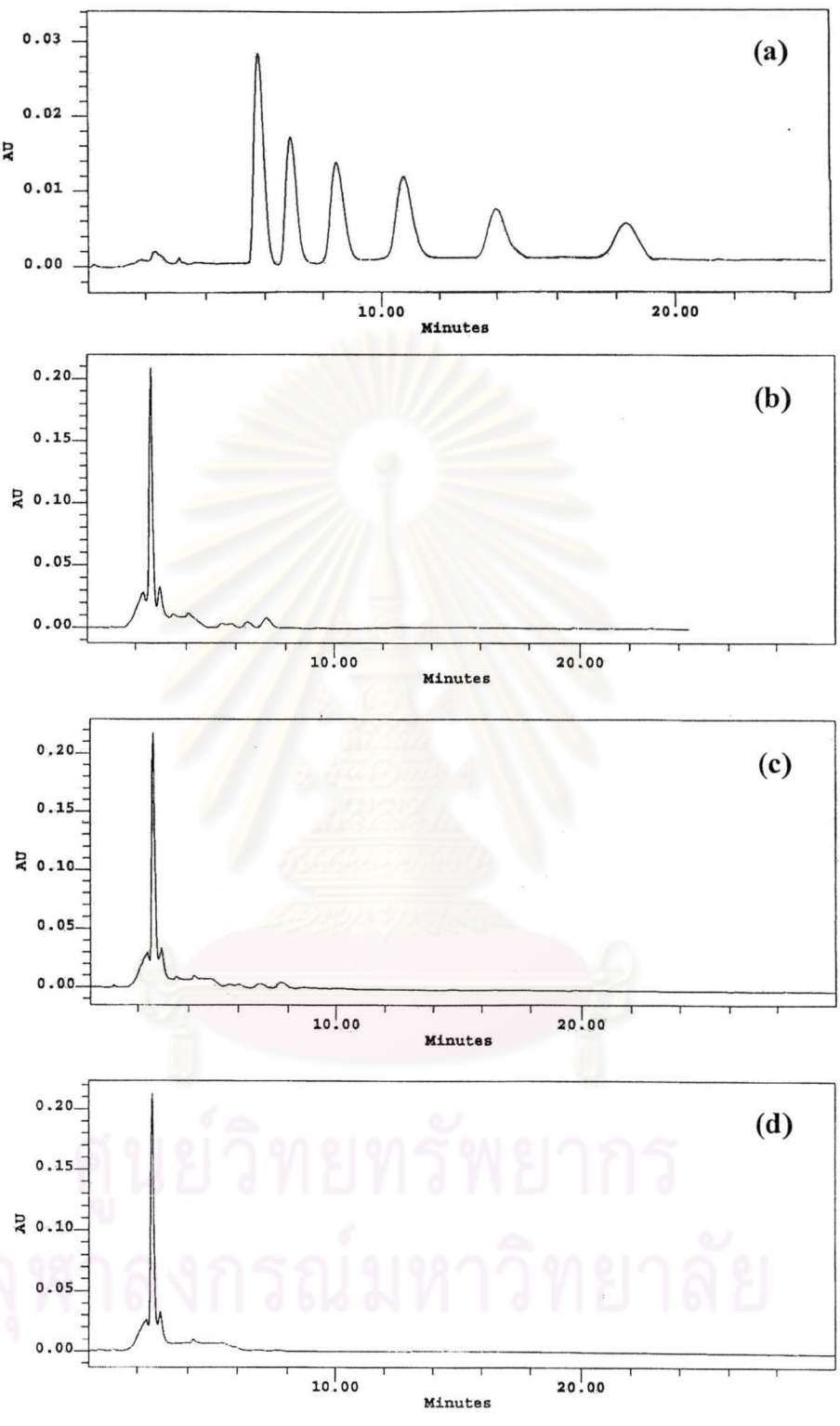
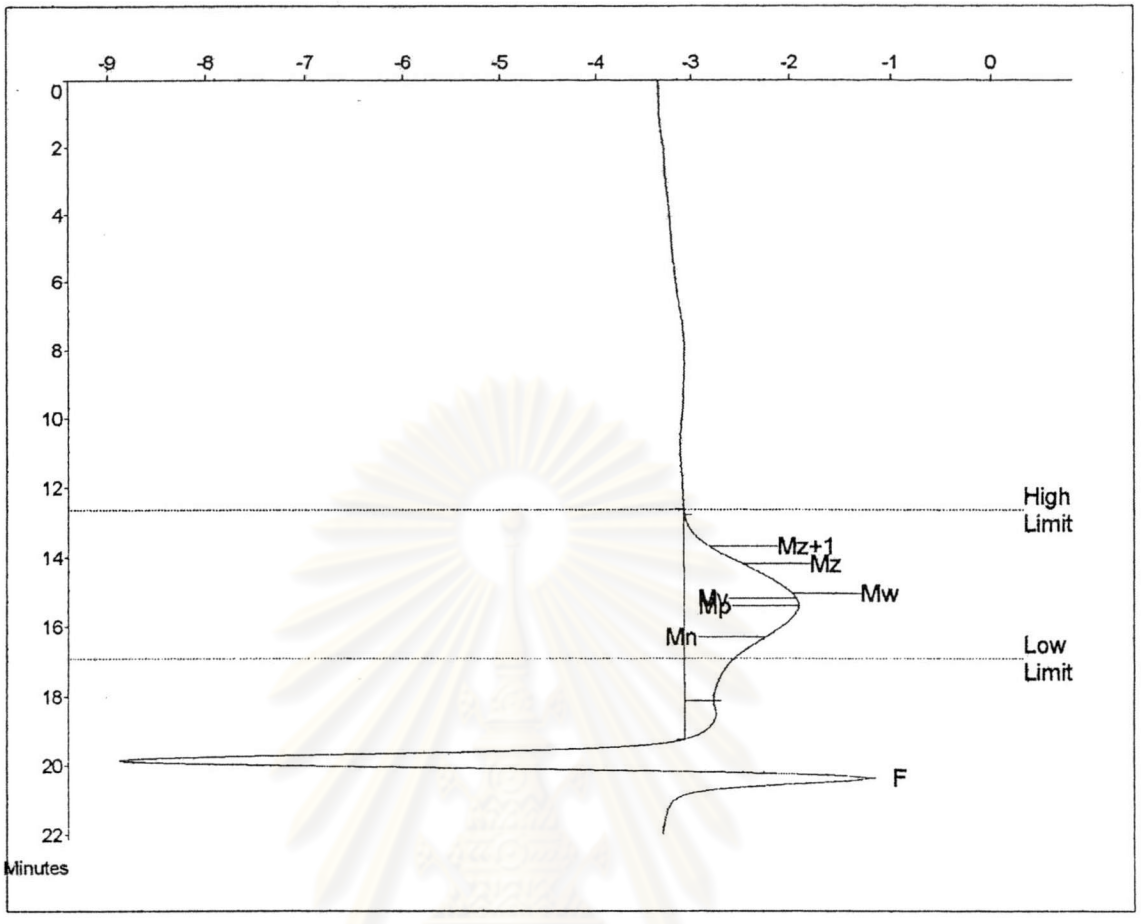


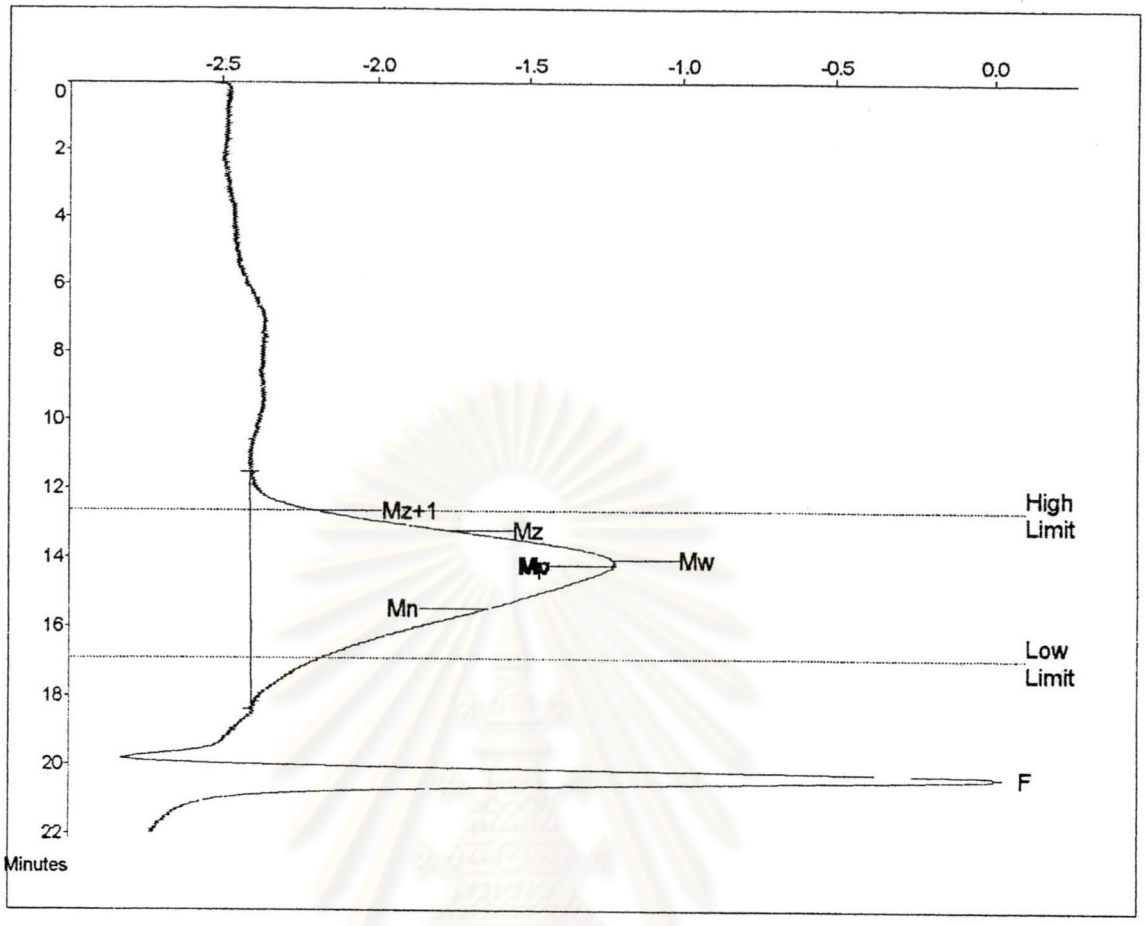
Figure B1 The chromatogram by HPLC analysis of hydrolysis chitosan (a) standard GlcNAc-$(GlcNAc)_6$, (b) 21%DA (Koyo, Japan), (c) 16%DA (Seafreash, Thailand), and (d) 13%DA (Ta-ming, Thailand).



Molecular Weight Averages

Mp	=	34,733	Mz	=	13
Mn	=	12,664	Mz+1	=	238,925
Mw	=	52,970	Mv	=	44,287
Polydispersity	=	4.182	Peak Area	=	39,934

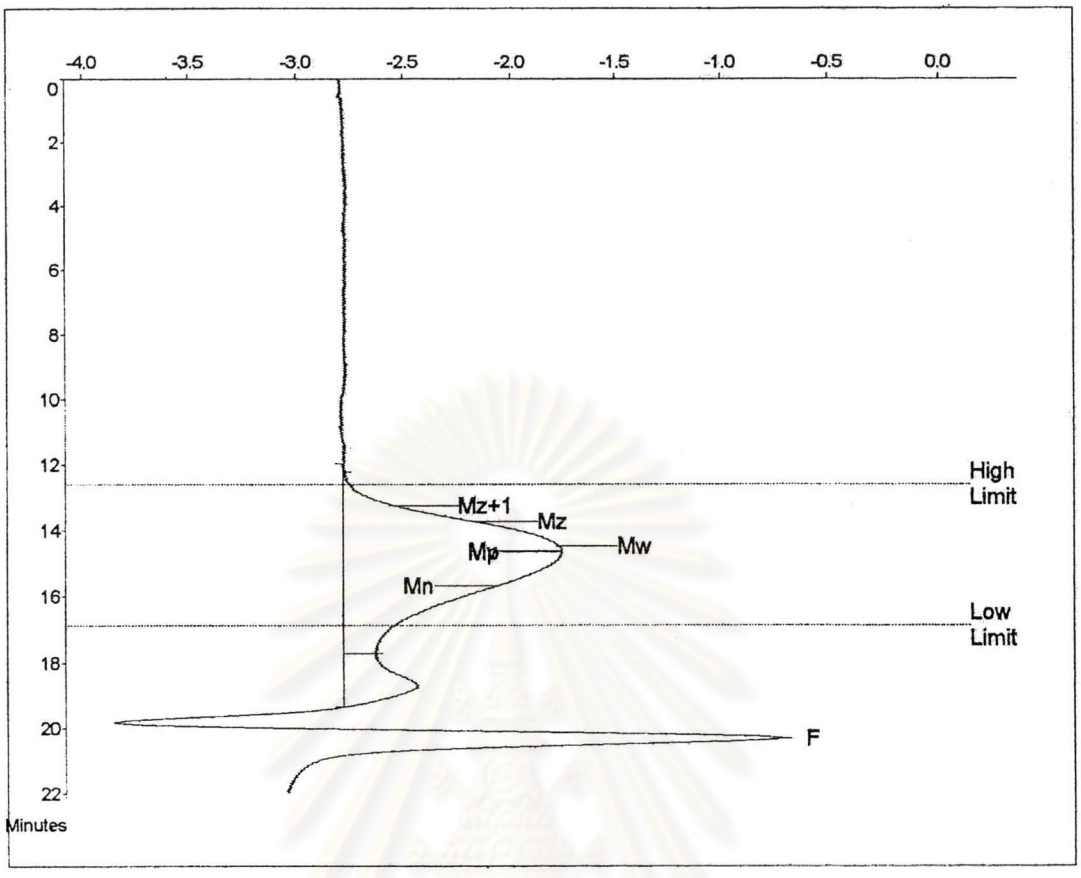
Figure B2 GPC Elution profile of products from hydrolysis chitosan 13 %DA from Ta-ming (Thailand) by serum *Hb*.



Molecular Weight Averages

Mp	=	131,613	Mz	=	414,379
Mn	=	32,342	Mz+1	=	781,756
Mw	=	158,429	Mv	=	132,452
Polydispersity	=	4.899	Peak Area	=	41,395

Figure B3 GPC Elution profile of products from hydrolysis chitosan 16 %DA from Seafresh (Thailand) by serum *Hb*.



Molecular Weight Averages

Mp	=	77,183	Mz	=	222,617
Mn	=	24,743	Mz+1	=	384,442
Mw	=	94,532	Mv	=	80,730
Polydispersity	=	3.821	Peak Area	=	33,473

Figure B4 GPC Elution profile of products from hydrolysis chitosan 21 %DA from Koyo (Japan) by serum *Hb*.

VITAE

Mr. Akamol Klaikherd was born on September 11th, 1977 in Samutsakhon, Thailand. He received a Bachelor Degree of Science, majoring in Chemistry from Chulalongkorn University, in 1999. Since 2000, he has been a graduate student studying Organic Chemistry as his major course at Chulalongkorn University. During his studies towards the Master's Degree, he was awarded a teaching assistant scholarship by the Faculty of Science during 2001-2002 and was supported by a research grant for his Master degree's thesis from the Graduate School, Chulalongkorn University.

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