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APPENDICES

APPENDIX A

EXPERIMENTAL AND DATA ANALYSIS

A-1 Standard calibration curve of protein

Table A-1 Standard calibration curve data

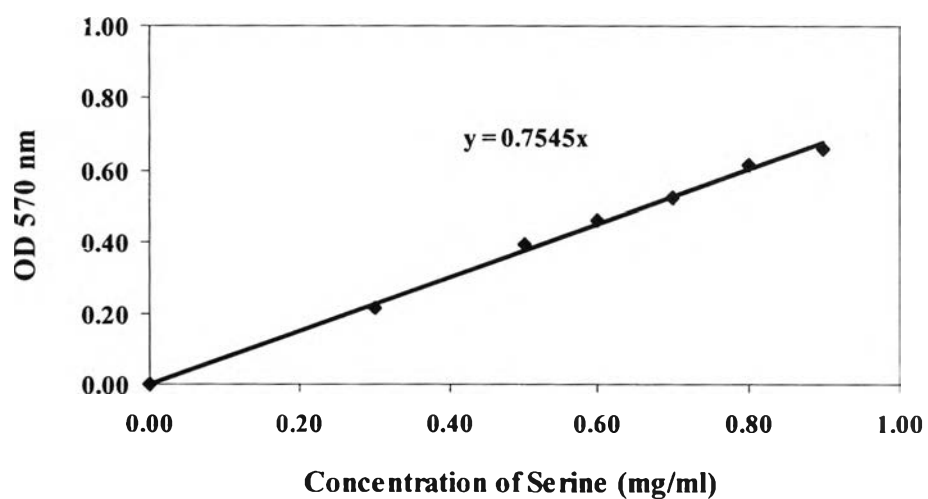| Concentration of BSA (mg/ml)	Absorbance at 750 nm.			
	Exp.1	Exp.2	Exp.3	Average
0.00	0.000	0.000	0.000	0.000
0.05	0.133	0.139	0.121	0.131
0.10	0.282	0.273	0.279	0.278
0.15	0.371	0.385	0.378	0.378
0.20	0.489	0.504	0.501	0.498
0.25	0.631	0.628	0.622	0.627
0.30	0.759	0.765	0.723	0.749
0.35	0.841	0.835	0.838	0.838
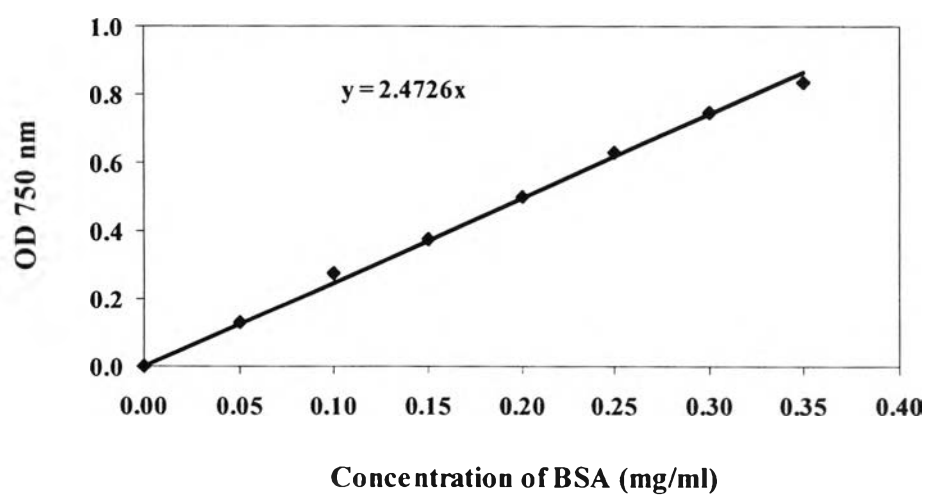
Figure A-1 Standard calibration curve of Protein

A-2 Standard calibration curve of amino acids

Table A-2 Standard calibration curve data

Concentration of Serine (mg/ml)	Absorbance at 570 nm.			
	Exp.1	Exp.2	Exp.3	Average
0.00	0.000	0.000	0.000	0.000
0.30	0.220	0.212	0.207	0.213
0.50	0.389	0.391	0.405	0.395
0.60	0.459	0.462	0.471	0.464
0.70	0.529	0.521	0.519	0.523
0.80	0.615	0.618	0.609	0.614
0.90	0.663	0.670	0.650	0.661

Figure A-2 Standard calibration curve of Serine

A-3 Standard calibration curve of amino acids

Table A-3 Standard calibration curve data

Concentration of Alanine (mg/ml)	Absorbance at 570 nm.			
	Exp.1	Exp.2	Exp.3	Average
0.00	0.000	0.000	0.000	0.000
0.20	0.123	0.133	0.129	0.128
0.40	0.259	0.261	0.264	0.261
0.50	0.328	0.321	0.330	0.326
0.60	0.412	0.410	0.420	0.414
0.70	0.502	0.500	0.502	0.501
0.80	0.602	0.603	0.605	0.602
0.90	0.688	0.690	0.658	0.679
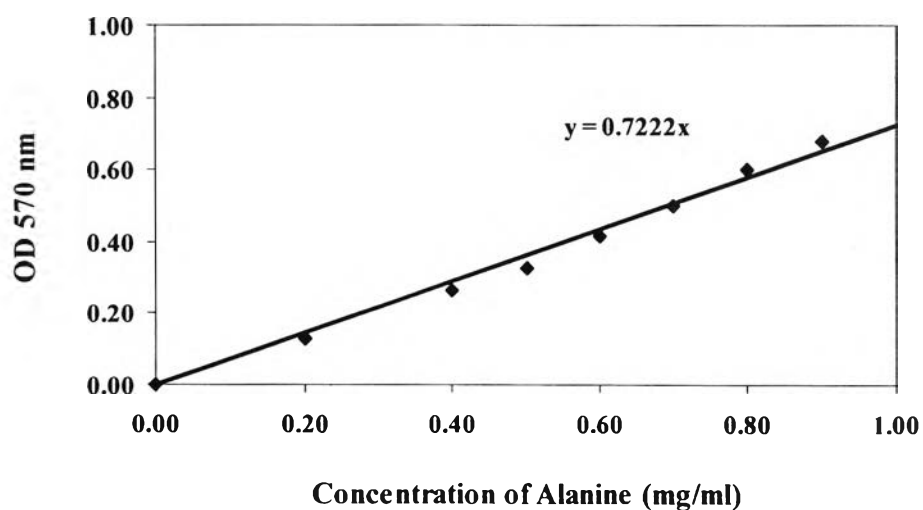
Figure A-3 Standard calibration curve of Alanine

APPENDIX B

EXPERIMENTAL DATA

B-1 Experimental data of sericin hydrolysis with subcritical water

B-1.1 Effect of temperature, reaction times, and silk to water ratio on weight of residue
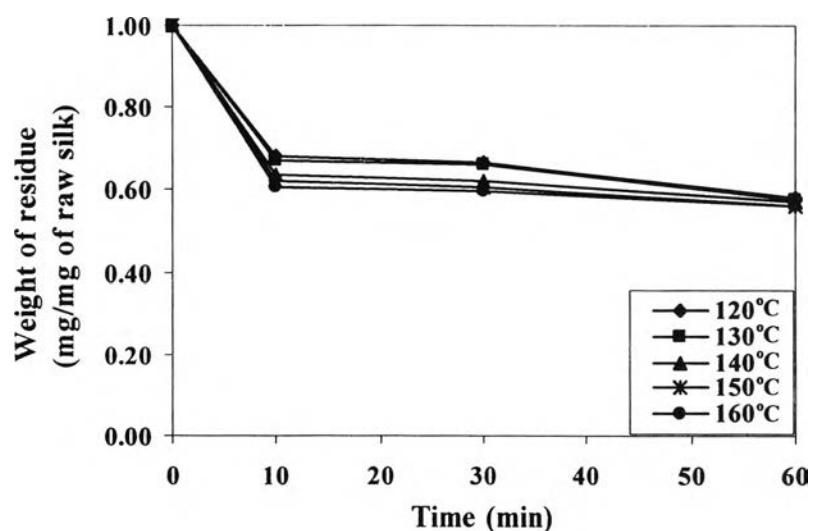
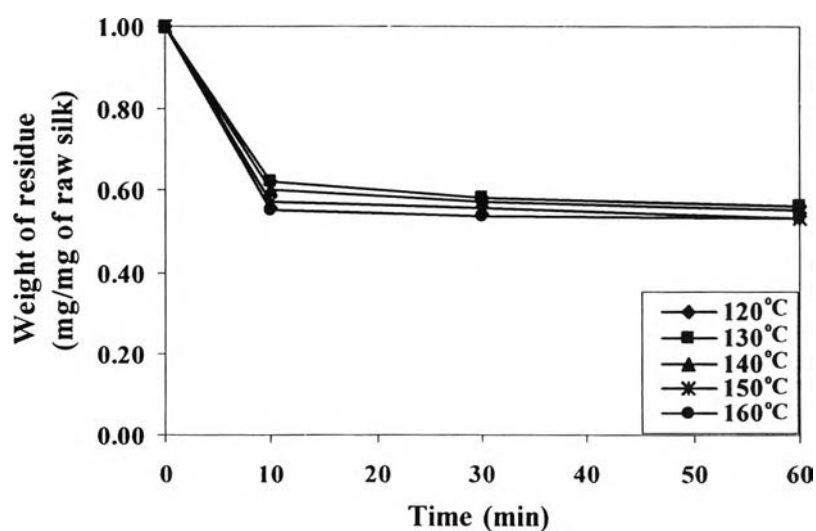**Figure B-1.1.1**: Effect of temperature and reaction time for hydrolysis for 1:20 silk to water ratio

Figure B-1.1.2: Effect of temperature and reaction time for hydrolysis for 1:50 silk to water ratio
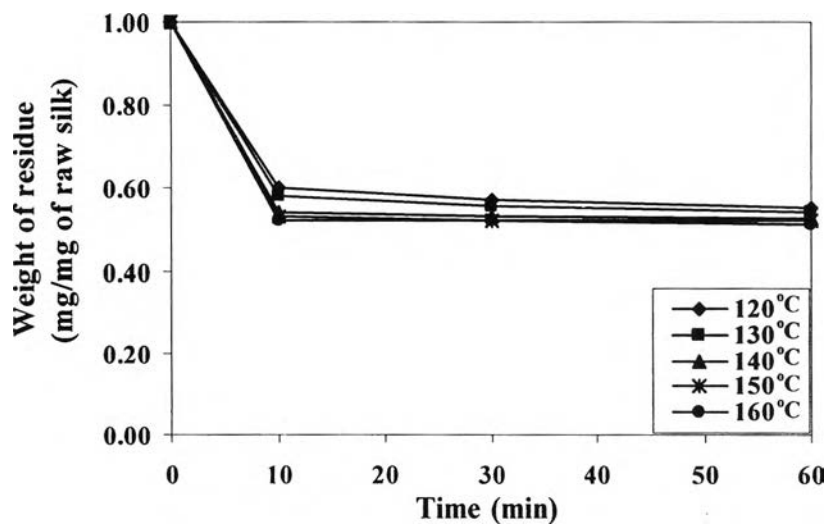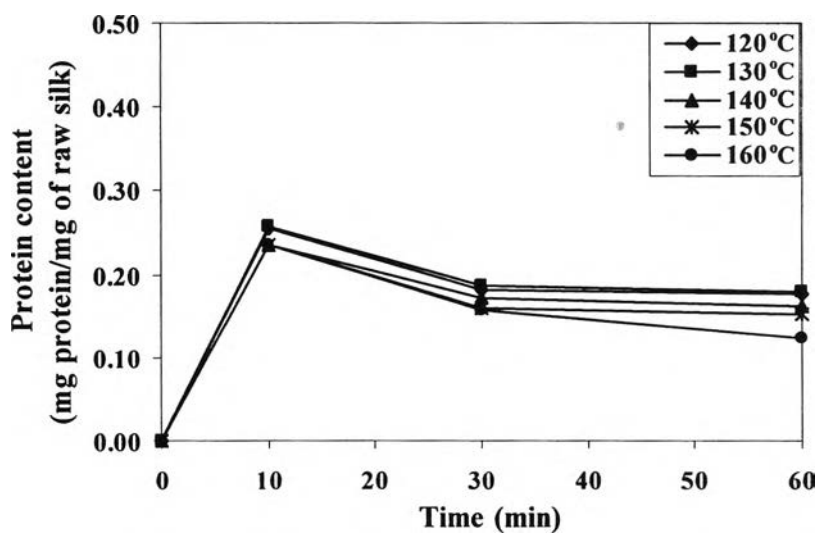
Figure B-1.1.3: Effect of temperature and reaction time for hydrolysis for 1:100 silk to water ratio

B-1.2 Effect of temperature, reaction times, and silk to water ratio on protein yield

Figure B-1.2.1: Effect of temperature and reaction time for hydrolysis for 1:20 silk to water ratio
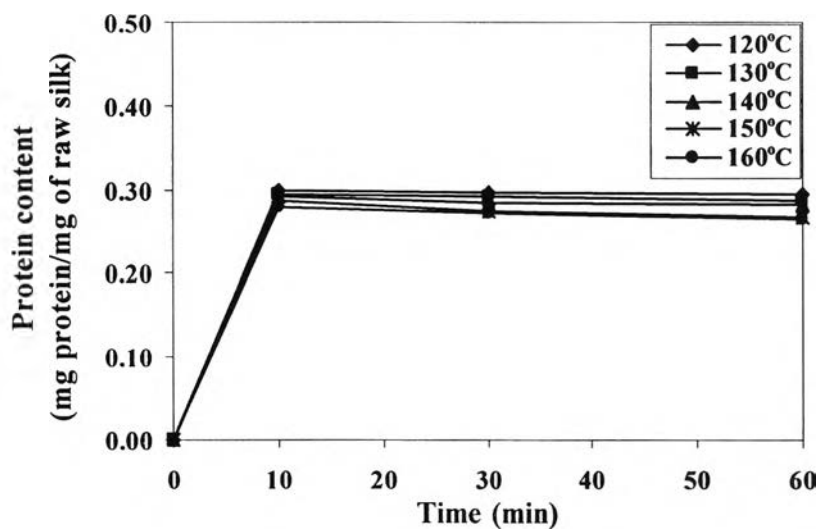
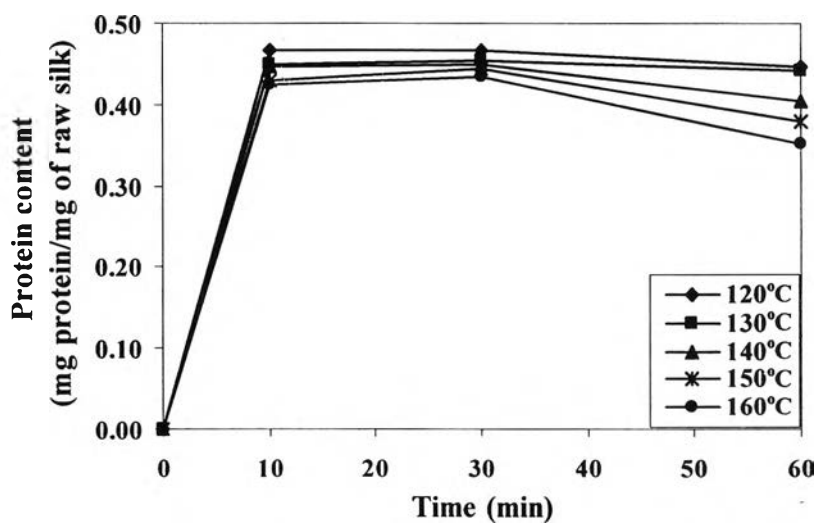**Figure B-1.2.2**: Effect of temperature and reaction time for hydrolysis for 1:50 silk to water ratio

Figure B-1.2.3: Effect of temperature and reaction time for hydrolysis for 1:100 silk to water ratio

B-1.3 Effect of temperature, reaction times, and silk to water ratio on amino acids yield.
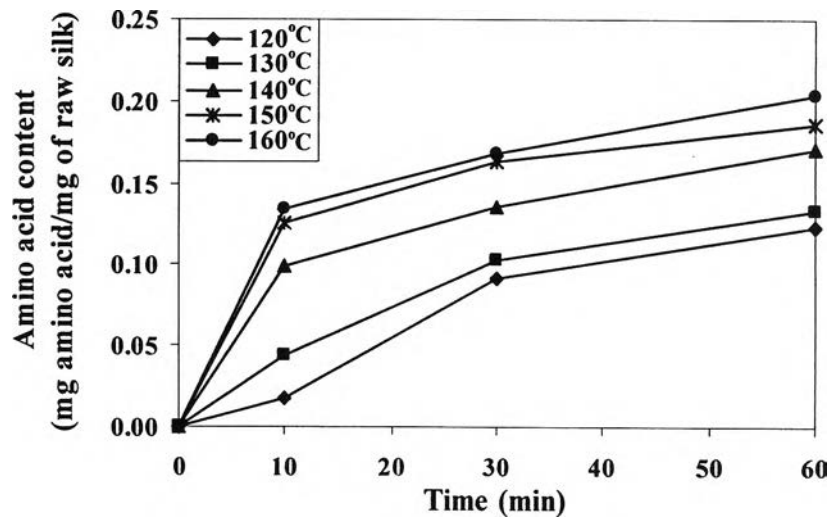

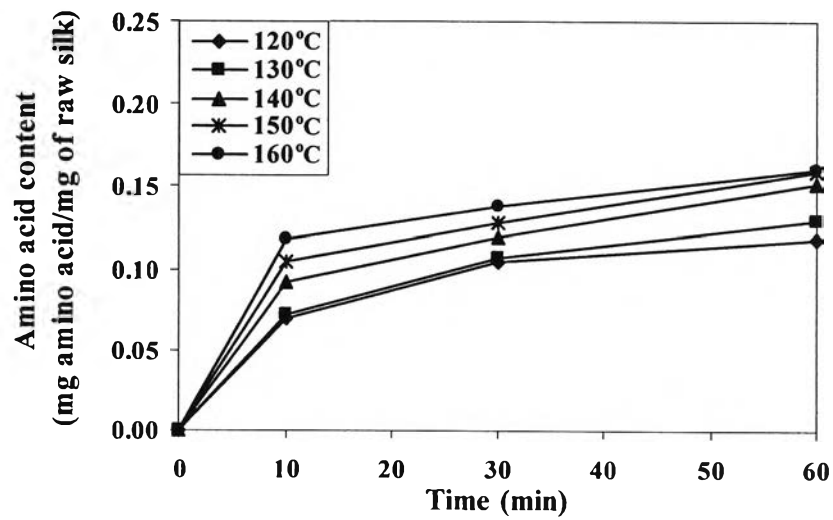**Figure B-1.3.1**: Effect of temperature and reaction time for hydrolysis for 1:20 silk to water ratio

Figure B-1.3.2: Effect of temperature and reaction time for hydrolysis for 1: 50 silk to water ratio
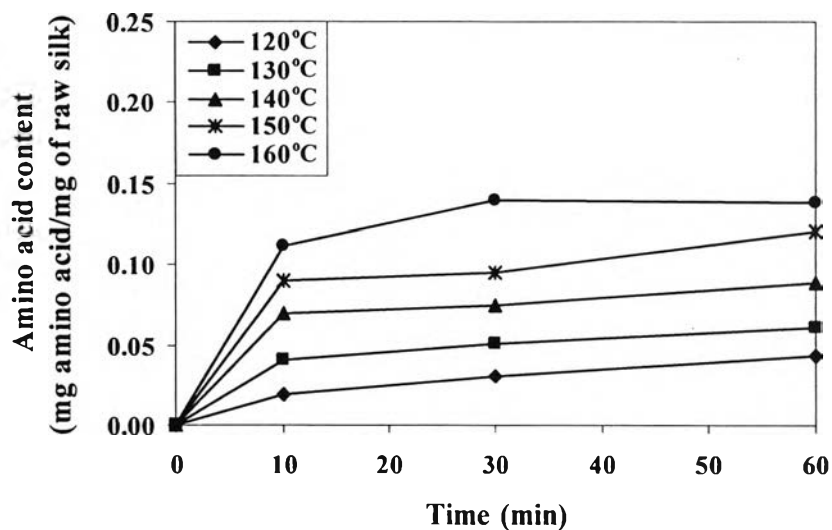
Figure B-1.3.3: Effect of temperature and reaction time for hydrolysis for 1:100 silk to water ratio

B-2 Experimental data of fibroin hydrolysis with subcritical water

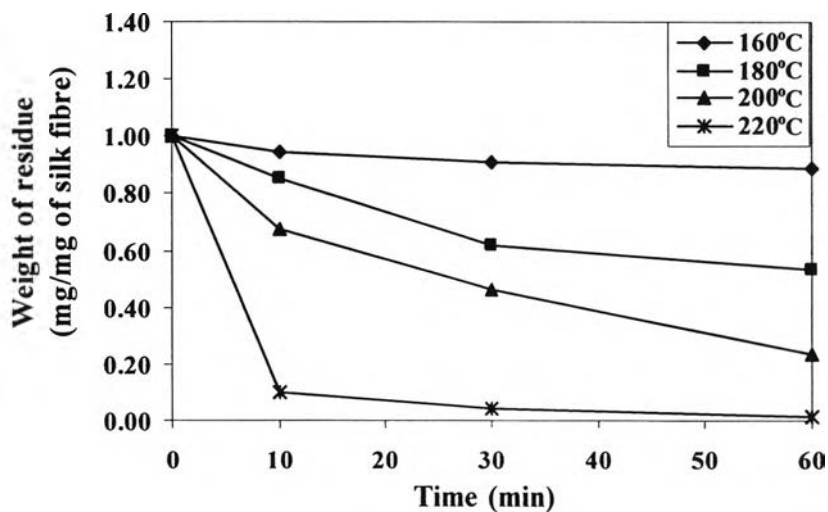### B-2.1 Effect of temperature, reaction times, and silk to water ratio on weight of residue

Figure B-2.1.1: Effect of temperature and reaction time for hydrolysis for 1:20 silk to water ratio
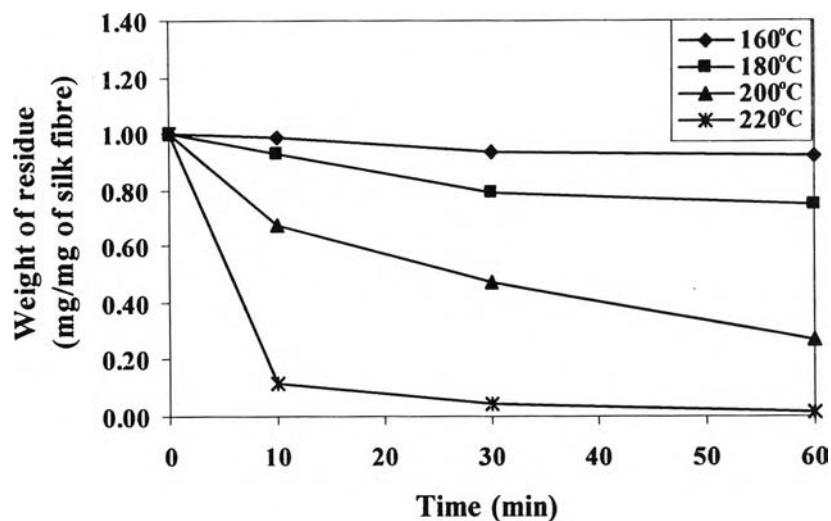
Figure B-2.1.2: Effect of temperature and reaction time for hydrolysis for 1:50 silk to water ratio

Figure B-2.1.3: Effect of temperature and reaction time for hydrolysis for 1:100 silk to water ratio

B-2.2 Effect of temperature, reaction times, and silk to water ratio on protein yield

Figure B-2.2.1: Effect of temperature and reaction time for hydrolysis for 1:20 silk to water ratio

Figure B-2.2.2: Effect of temperature and reaction time for hydrolysis for 1:50 silk to water ratio

Figure B-2.2.3: Effect of temperature and reaction time for hydrolysis for 1:100 silk to water ratio

B-2.3 Effect of temperature, reaction times, and silk to water ratio on amino acids yield.

Figure B-2.3.1: Effect of temperature and reaction time for hydrolysis for 1:20 silk to water ratio
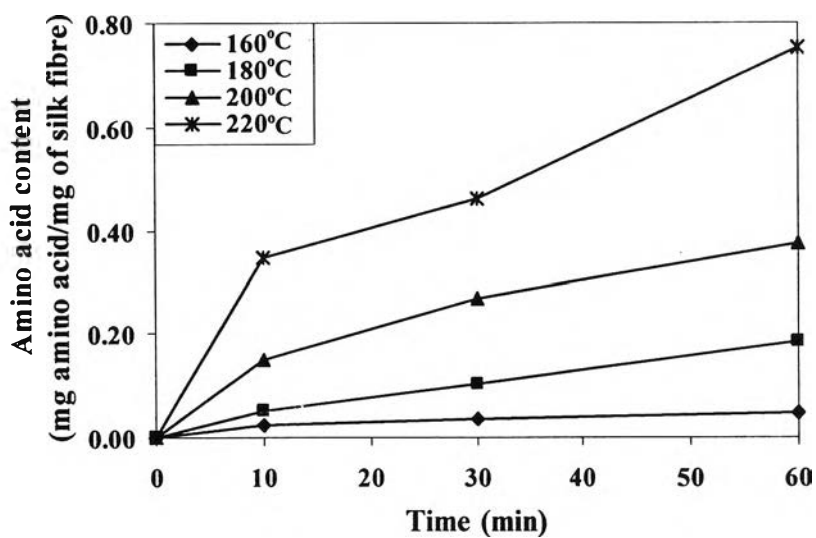
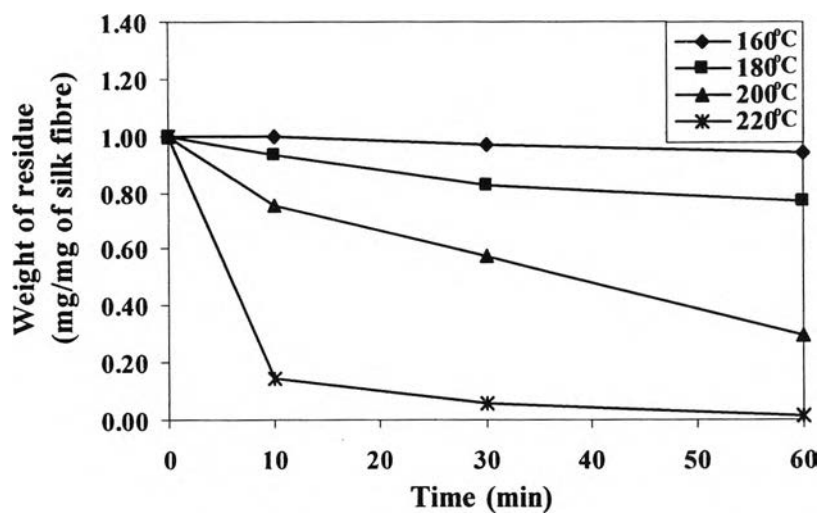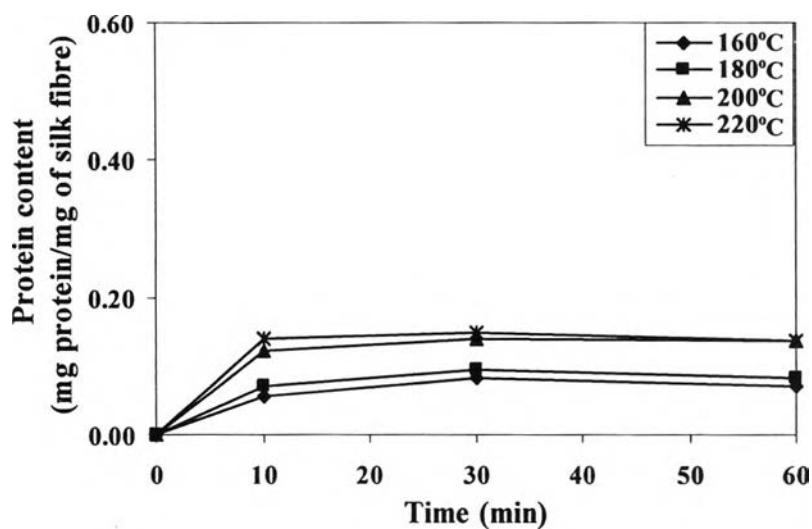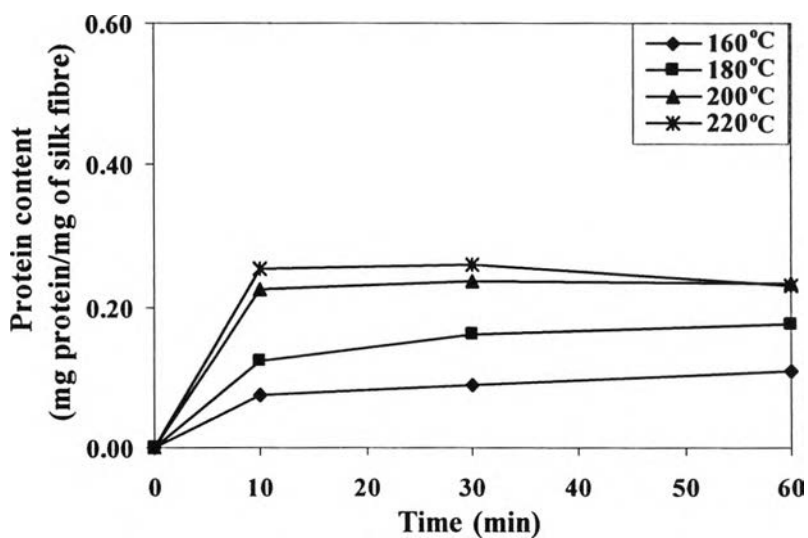**Figure B-2.3.2**: Effect of temperature and reaction time for hydrolysis for 1:50 silk to water ratio
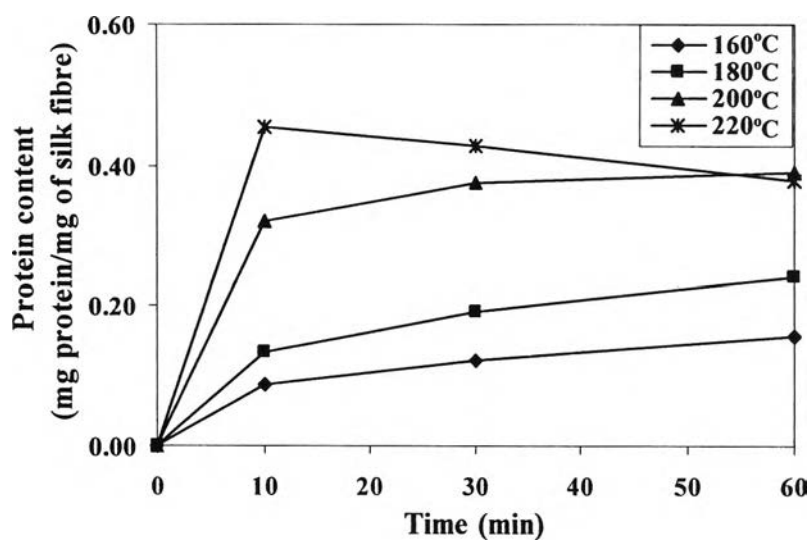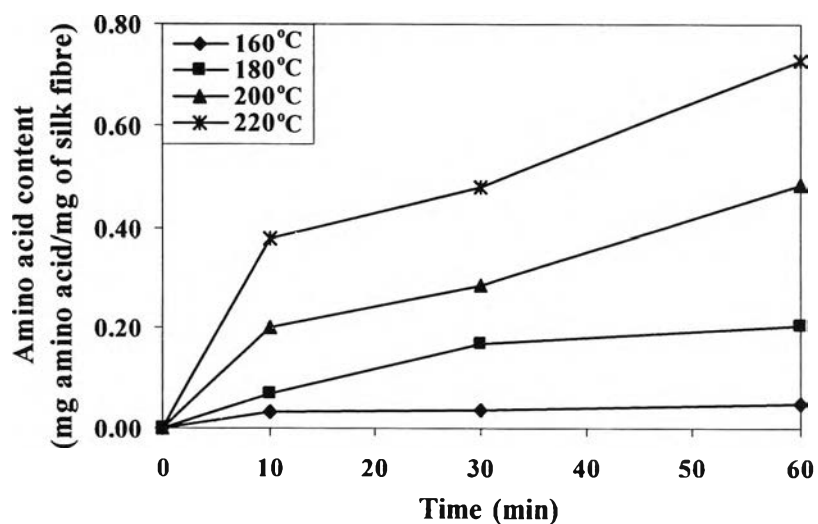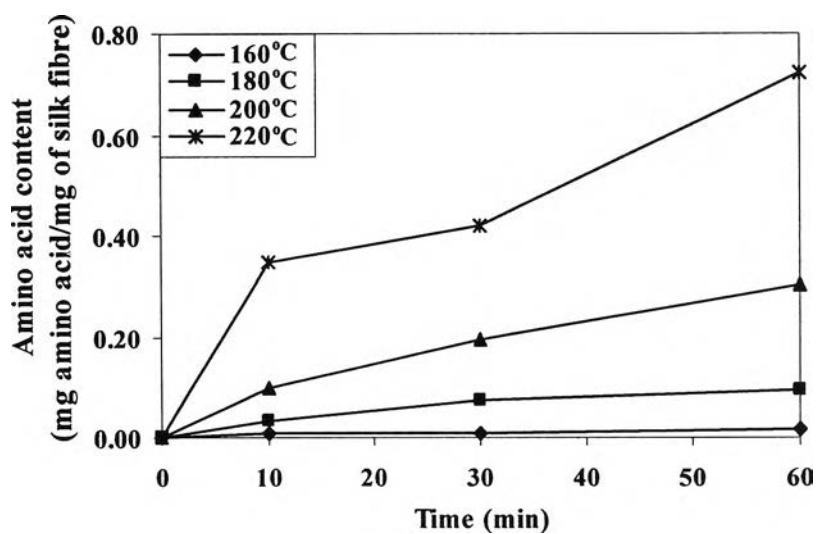

Figure B-2.3.3: Effect of temperature and reaction time for hydrolysis for 1:100 silk to water ratio

B-3 Experimental data of sericin hydrolysis with subcritical water

Table B-3.1 Weight of residue after subcritical water hydrolysis at 120°C -160°C, ratios 1:20, 1:50, and 1:100

Temperature (°C)	Reaction time (minutes)	Residual Weight * (mg/mg of raw silk)		
		Ratio 1:20	Ratio 1:50	Ratio 1:100
120	10	0.680	0.620	0.600
	30	0.669	0.582	0.571
	60	0.580	0.560	0.550
130	10	0.672	0.620	0.580
	30	0.659	0.580	0.556
	60	0.578	0.560	0.540
140	10	0.636	0.600	0.540
	30	0.623	0.570	0.533
	60	0.572	0.550	0.526
150	10	0.620	0.570	0.530
	30	0.605	0.555	0.523
	60	0.560	0.530	0.520
160	10	0.608	0.550	0.520
	30	0.595	0.535	0.520
	60	0.560	0.530	0.510

* Average Data

Table B-3.2 Protein yield of soluble product of subcritical water hydrolysis of sericin
at 120°C -160°C, ratios 1:20, 1:50, and 1:100

Temperature (°C)	Reaction time (minutes)	Protein Yield * (mg protein/mg of raw silk)		
		Ratio 1:20	Ratio 1:50	Ratio 1:100
120	10	0.2544	0.2998	0.4664
	30	0.1576	0.2968	0.4661
	60	0.1233	0.2942	0.4475
130	10	0.2582	0.2947	0.4501
	30	0.1602	0.2922	0.4560
	60	0.1520	0.2871	0.4429
140	10	0.2350	0.2927	0.4472
	30	0.1716	0.2836	0.4492
	60	0.1626	0.2826	0.4044
150	10	0.2346	0.2871	0.4303
	30	0.1860	0.2755	0.4449
	60	0.1792	0.2679	0.3802
160	10	0.2358	0.2786	0.4259
	30	0.1816	0.2715	0.4358
	60	0.1784	0.2639	0.3529

* Average Data

Table B-3.3 Amino acids yield of soluble product of subcritical water hydrolysis of sericin at 120°C -160°C, ratios 1:20, 1:50, and 1:100

Temperature (°C)	Reaction time (minutes)	Amino Acid Yields * (mg amino acid/mg of raw silk)		
		Ratio 1:20	Ratio 1:50	Ratio 1:100
120	10	0.0180	0.0700	0.0192
	30	0.0918	0.1049	0.0303
	60	0.1234	0.1183	0.0433
130	10	0.0440	0.0718	0.0408
	30	0.1036	0.1068	0.0510
	60	0.1329	0.1296	0.0611
140	10	0.0991	0.0928	0.0702
	30	0.1360	0.1196	0.0754
	60	0.1704	0.1518	0.0891
150	10	0.1258	0.1050	0.0897
	30	0.1636	0.1280	0.0954
	60	0.1857	0.1593	0.1210
160	10	0.1344	0.1178	0.1118
	30	0.1683	0.1378	0.1399
	60	0.2033	0.1604	0.1386

* Average Data

B-4   Experimental data of fibroin hydrolysis with subcritical water

Table B-4.1 Weight of residue after subcritical water hydrolysis at 120°C -160°C, ratios 1:20, 1:50, and 1:100

Temperature (°C)	Reaction time (minutes)	Residual Weight * (mg/mg of silk fibre)		
		Ratio 1:20	Ratio 1:50	Ratio 1:100
160	10	0.946	0.989	0.997
	30	0.908	0.938	0.966
	60	0.891	0.923	0.937
180	10	0.855	0.931	0.937
	30	0.615	0.792	0.826
	60	0.535	0.749	0.769
200	10	0.672	0.673	0.754
	30	0.461	0.474	0.573
	60	0.234	0.268	0.292
220	10	0.103	0.117	0.141
	30	0.042	0.042	0.054
	60	0.011	0.013	0.011

* Average Data

Table B-4.2 Protein yield of soluble product of subcritical water hydrolysis of sericin at 120°C -160°C, ratios 1:20, 1:50, and 1:100

| Temperature (°C)	Reaction time (minutes)	Protein Yield * (mg protein/mg of silk fibre)		
		Ratio 1:20	Ratio 1:50	Ratio 1:100
160	10	0.0554	0.0751	0.0869
	30	0.0831	0.0898	0.1220
	60	0.0726	0.1088	0.1572
180	10	0.0710	0.1253	0.1327
	30	0.0963	0.1610	0.1909
	60	0.0837	0.1755	0.2426
200	10	0.1225	0.2253	0.3211
	30	0.1401	0.2358	0.3769
	60	0.1382	0.2350	0.3898
220	10	0.1403	0.2540	0.4554
	30	0.1486	0.2584	0.4287
	60	0.1373	0.2321	0.3777

* Average Data

Table B-4.3 Amino acids yield of soluble product of subcritical water hydrolysis of sericin at 120°C -160°C, ratios 1:20, 1:50, and 1:100

| Temperature (°C)	Reaction time (minutes)	Amino Acid Yields * (mg amino acid/mg of silk fibre)		
		Ratio 1:20	Ratio 1:50	Ratio 1:100
160	10	0.0303	0.0218	0.0072
	30	0.0352	0.0358	0.0081
	60	0.0484	0.0485	0.0155
180	10	0.0692	0.0512	0.0332
	30	0.1706	0.1031	0.0738
	60	0.2054	0.1837	0.0934
200	10	0.1996	0.1478	0.0976
	30	0.2838	0.2690	0.1936
	60	0.4819	0.3752	0.3023
220	10	0.3795	0.3474	0.3490
	30	0.4782	0.4610	0.4190
	60	0.7274	0.7539	0.7222

* Average Data

B-5 The molecular weight range of subcritical water hydrolysis of sericin solution at 120°C -160°C determined by SDS-PAGE
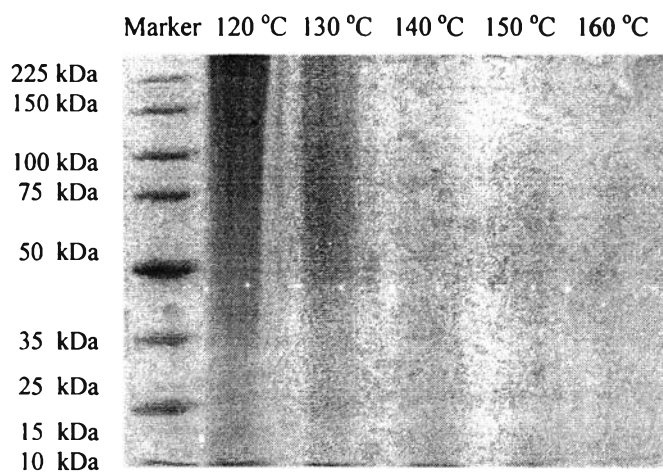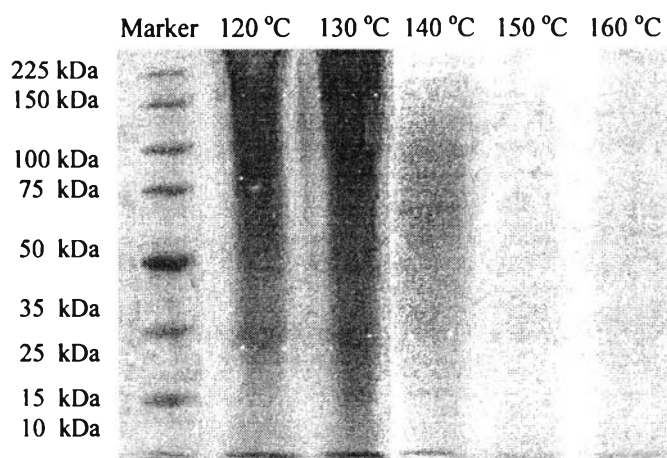

(a)
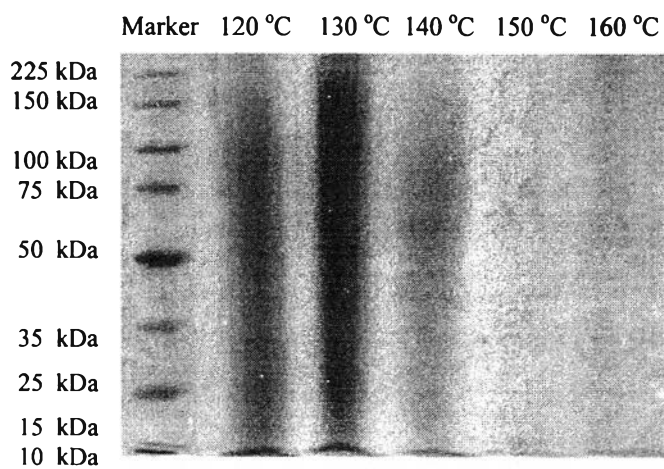
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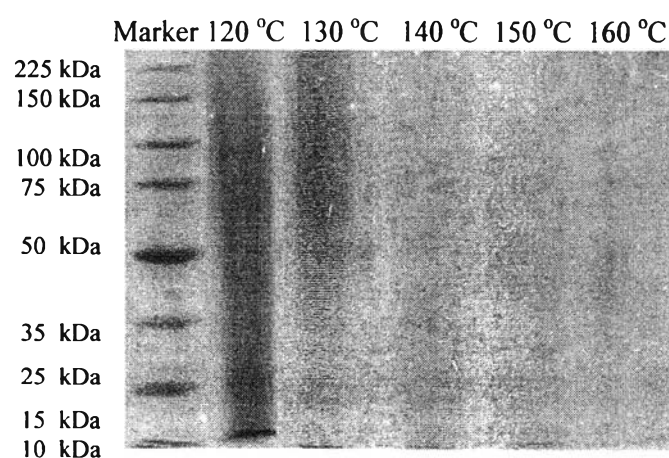
(c)

Figure B-5.1.1 The molecular weight range of sericin solution determined by SDS-PAGE (a) 1:20, 10 min, 120-160 °C (b) 1:20, 30 min, 120-160 °C (c) 1:20, 60 min, 120-160 °C

(a)

(b)

(c)

Figure B-5.1.2 The molecular weight range of sericin solution determined by SDS-
PAGE (a) 1:50, 10 min, 120-160 °C (b) 1:50, 30 min, 120-160 °C
(c) 1:50, 60 min, 120-160 °C
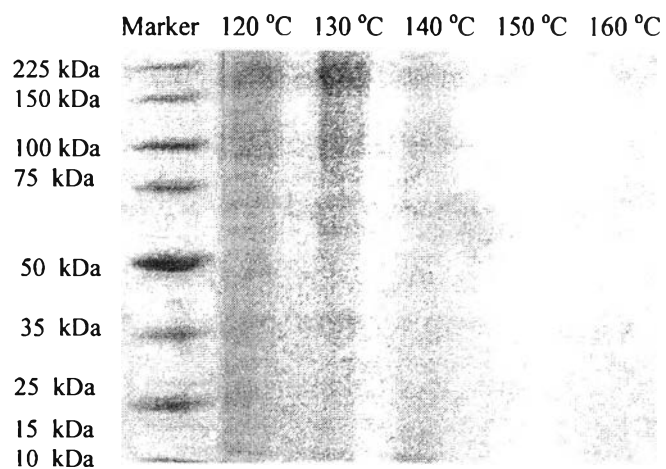
(a)

(b)

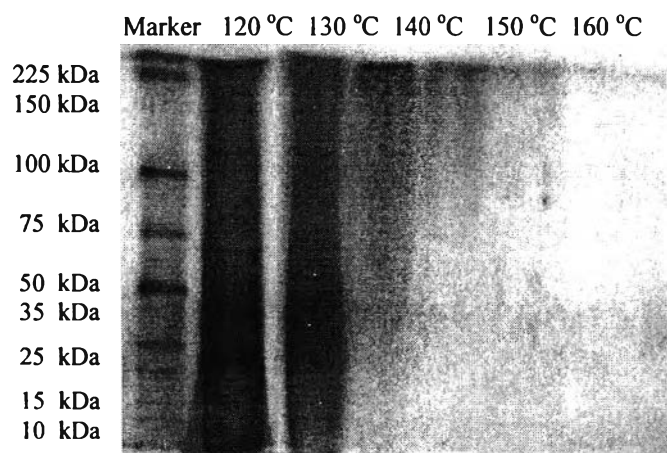
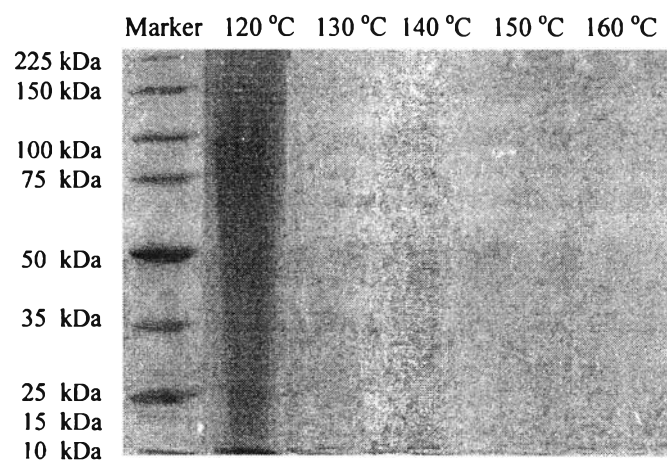**(c)**

Figure B-5.1.2 The molecular weight range of sericin solution determined by SDS-PAGE (a) 1:100, 10 min, 120-160 °C (b) 1:100, 30 min, 120-160 °C (c) 1:100, 60 min, 120-160 °C

B-4.1 The molecular weight range of subcritical water hydrolysis of fibroin solution at 160°C -220°C determined by SDS-PAGE

(a)

(b)

(c)

Figure B-4.4 The molecular weight range of fibroin solution determined by SDS-PAGE (a) 1:20, 10 min, 160-220 °C (b) 1:20, 30 min, 160-220 °C (c) 1:20, 60 min, 160-220 °C

Marker 160 °C 180 °C 200 °C 220 °C

225 kDa
150 kDa

100 kDa

75 kDa

50 kDa

35 kDa
25 kDa
15 kDa
10 kDa

(a)

Marker 160 °C 180 °C 200 °C 220 °C

225 kDa
150 kDa

100 kDa

75 kDa

50 kDa

35 kDa
25 kDa
15 kDa
10 kDa

(b)

Marker 160 °C 180 °C 200 °C 220 °C

225 kDa
150 kDa

100 kDa

75 kDa

50 kDa

35 kDa
25 kDa
15 kDa
10 kDa

(c)

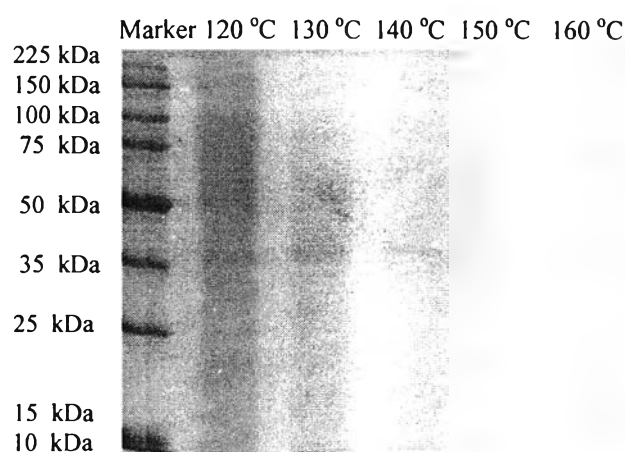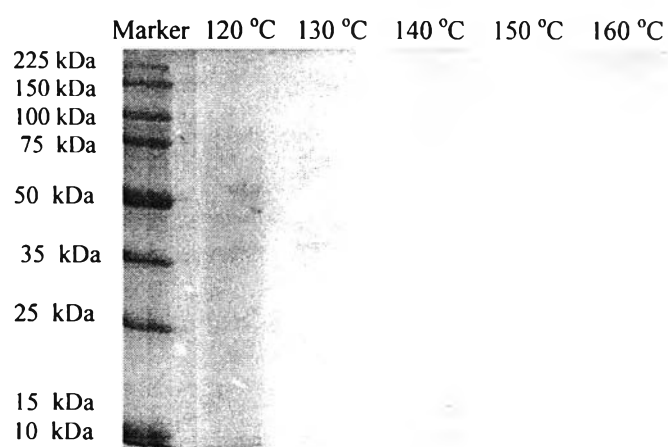**Figure B-4.** The molecular weight range of fibroin solution determined by SDS-
PAGE (a) 1:50, 10 min, 160-220 °C, (b) 1:50, 30 min, 160-220 °C
(c) 1:50, 60 min, 160-220 °C
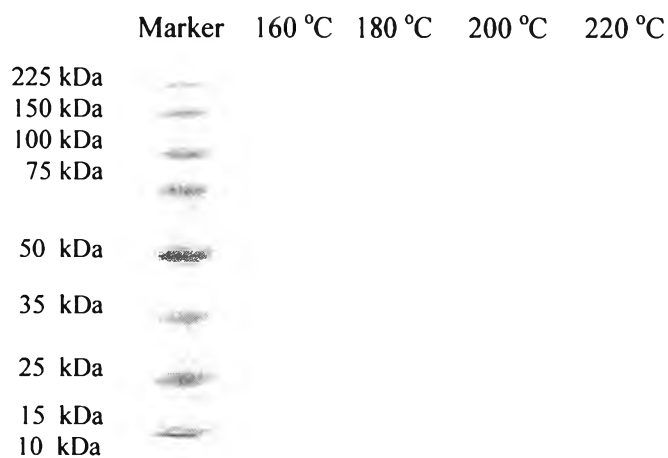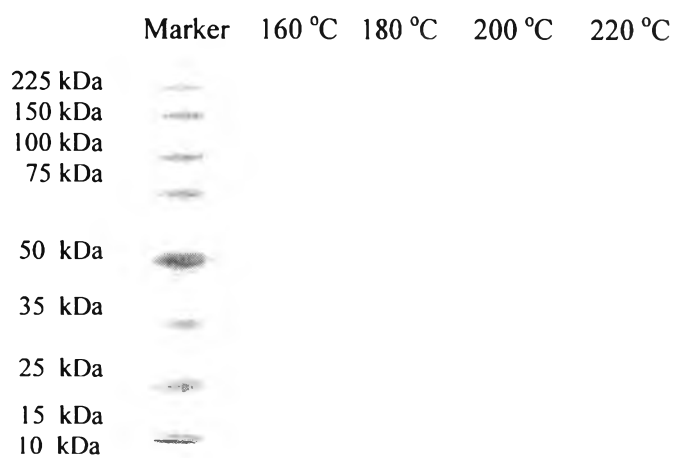
Marker 160 °C 180 °C 200 °C 220 °C

225 kDa
150 kDa
100 kDa
75 kDa

50 kDa

35 kDa

25 kDa

15 kDa
10 kDa

(a)

Marker 160 °C 180 °C 200 °C 220 °C

225 kDa
150 kDa
100 kDa
75 kDa

50 kDa

35 kDa

25 kDa

15 kDa
10 kDa

(b)

Marker 160 °C 180 °C 200 °C 220 °C

225 kDa
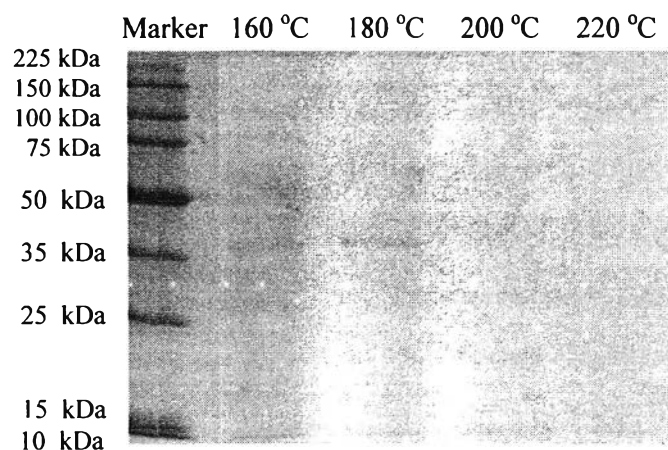150 kDa
100 kDa
75 kDa
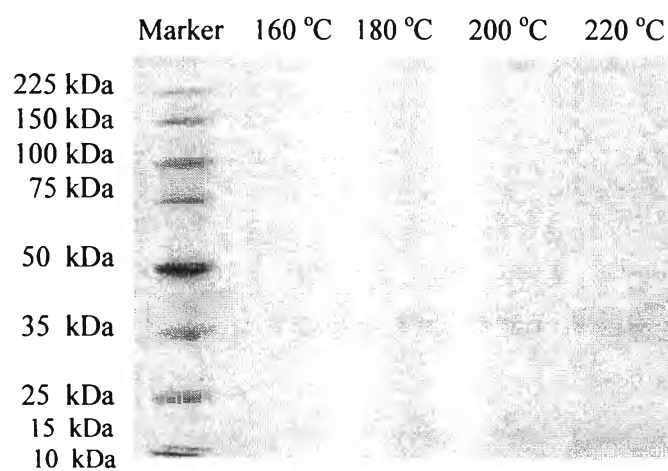
50 kDa

35 kDa

25 kDa

15 kDa
10 kDa

(c)

Figure B-4. The molecular weight range of fibroin solution determined by SDS-PAGE (a) 1:100, 10 min, 160-220 °C, (b) 1:100, 30 min, 160-220 °C, (c) 1:100, 60 min, 160-220 °C
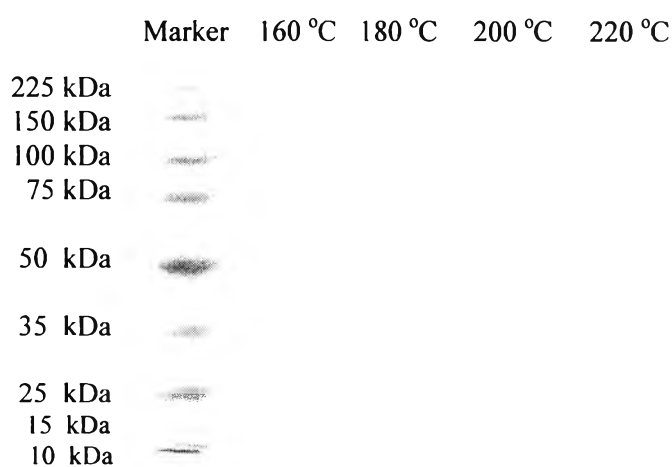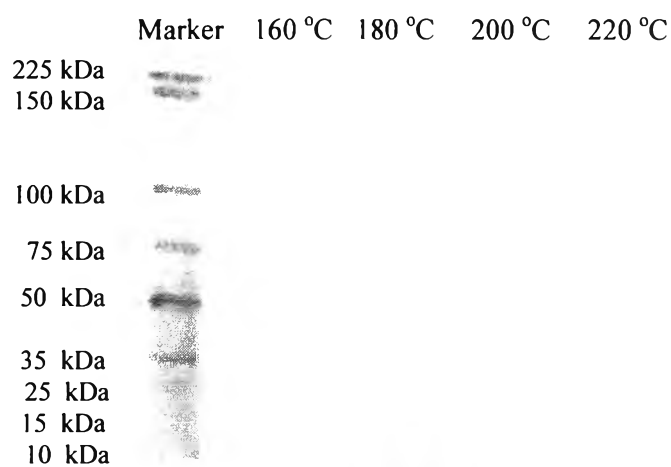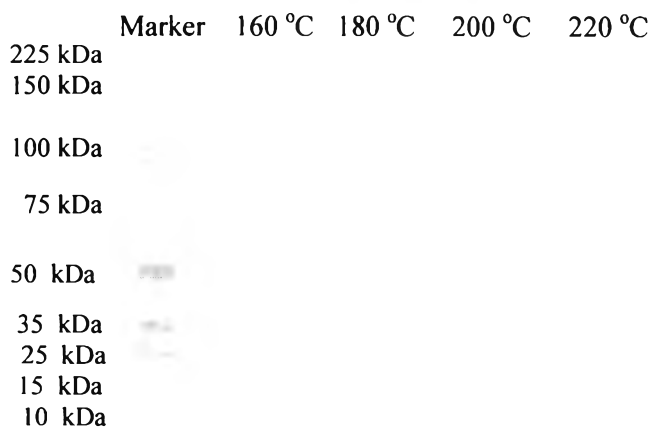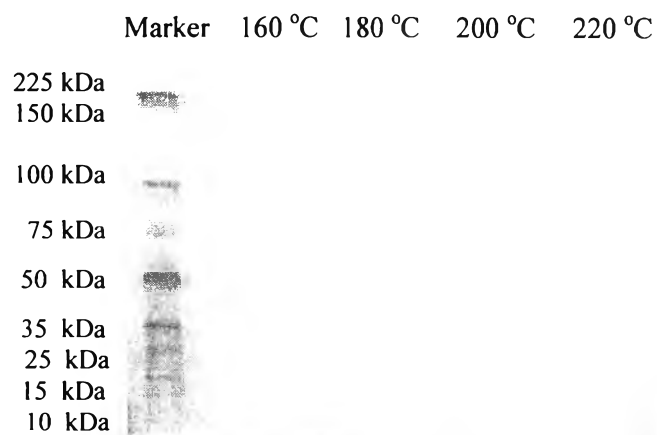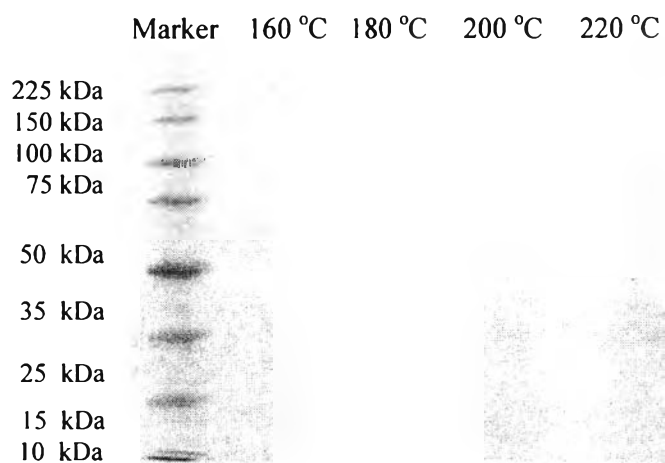
APPENDIX C

PROCEDURE FOR HPLC ANALYISIS OF AMINO ACIDS AND PRELIMINARY DATA

C-1. Procedure for high performance liquid chromatography

The analysis of animo acids by HPLC was performed with a Prevail C-18 (5 μm particle, 4.6x250 mm ID.), with a evaporative light scattering detector (ELSD, Altech, USA). This technique requires no derivatization of the sample. For the analysis, the temperature was adjusted to 50°C and nitrogen gas flow rate to 2.8 L/min. The sample injection volume was 50 μl. A standard calibration curve was constructed from a plot of peak areas versus concentrations for a mixture of standard amino acids solutions.

The eluent systems consisted of two components:

Eluent A was a 5 mM Heptafluorobutyric acid (pH 1.0) with 0.7 % Trifluoroacetic acid (TFA).

Eluent B was 100 % Acetonitrile.

The conditions for the gradient program are shown in Table C-1.

Table C-1 Chromatographic gradient conditions for HPLC analysis.

Time (min)	% Eluent A	% Eluent B
0	100	0
6	100	0
8	85	15
25	65	35

The standard free amino acids (Glycine, Serine, Glutamic aicd, Aspartic acid, Asparagene, Threonine, Alanine, Leusine, Tryocine) was prepared in 0.01 M HCl. The concentrations of 22.727-250.000 µg/ml were used to obtain the calibration curve.

C-2 Experimental Data

C-2.1 Chromatrogram of mixture of standard amino acids solution.

Retention times (min)
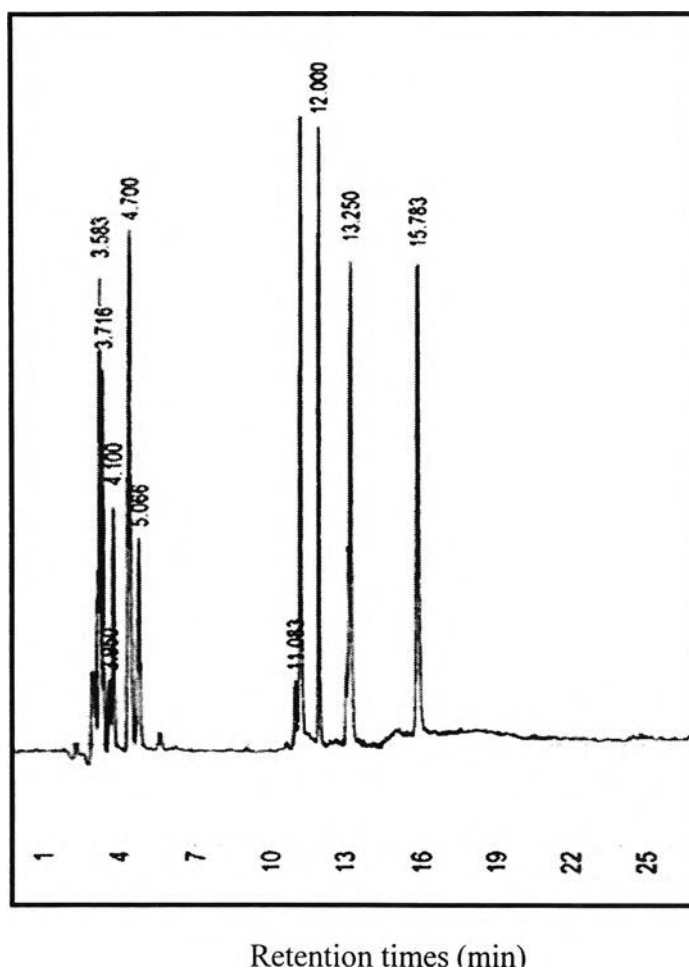
Figure C-2 Chromatrogram of mixture of standard amino acids solution.

Table C-2.1 Retention times of different amino acids

Amino Acids	Retention times (minutes)
Glycine	3.555
Serine/Asparagine	3.766
Aspartic Acid	4.183
Alanine/Threonine	4.716
Glutamic Acid	5.100
Arginine	12.950
Valine	13.516
Tyrosine	14.783
Leucine	16.500

Table C-2.2 Standard calibration curve data of mixture of amino acids solutions

Concentration of Standard Mixture (µg/ml)	Peak Area								
	Glycine	Serine / Asparagine	Aspartic Acid	Alanine / Threonine	Glutamic Acid	Arginine	Valine	Tyrosine	Leucine
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
22.727	147.760	114.642	202.141	370.606	132.641	884.597	306.947	430.532	224.347
83.333	693.142	474.563	567.684	838.314	553.166	1906.435	860.605	1355.469	1175.143
250.000	2402.741	1674.331	1726.023	3000.361	1858.862	5032.380	3633.412	4350.532	4217.124

C-3 Experimental data of sericin solution by hydrolysis with subcritical water.

C-3.1 Amino acids profile in sericin solution by hydrolysis with subcritical water.

Table C-3.1 Amino acid yields of sericin solution obtained after 30 min hydrolysis at different ratios at 150 and 160 °C.

Amino Acids (mg/g of raw silk)	Temperature (°C)					
	150 °C			160 °C		
	Ratio 1:20	Ratio 1:50	Ratio 1:100	Ratio 1:20	Ratio 1:50	Ratio 1:100
Glycine	12.625	11.835	10.987	17.728	14.164	8.644
Serine/Asparagine	-	-	-	-	-	-
Alanine/Threonine	0.567	-	-	3.118	-	-
Valine	22.723	30.002	15.760	-	22.179	2.721
Leucine	-	0.353	-	0.419	0.5276	-
Aspartic Acid	12.136	9.050	11.364	10.249	11.738	12.402
Glutamic Acid	-	-	-	-	-	-
Arginine	28.084	22.287	12.956	33.134	24.998	59.843
Tyrosine	0.766	0.830	1.368	4.042	0.593	0.945

Table C-3.2 Amino acids profile of sericin solution obtained after different times of hydrolysis at 160 °C, with 1:50 silk:water ratio.

Amino Acids	Reaction times (minutes)		
(mg/g of raw silk)	10 min	30 min	60 min
Glycine	10.982	14.164	0.017
Serine/Asparagine	-	-	0.038
Alanine/Threonine	-	-	0.917
Valine	26.705	22.179	18.392
Leucine	-	0.5276	0.529
Aspartic Acid	10.377	11.738	160.590
Glutamic Acid	-	-	-
Arginine	28.847	24.998	40.594
Tyrosine	1.077	0.593	3.136

C-4 Experimental data of fibroin solution by hydrolysis with subcritical water.

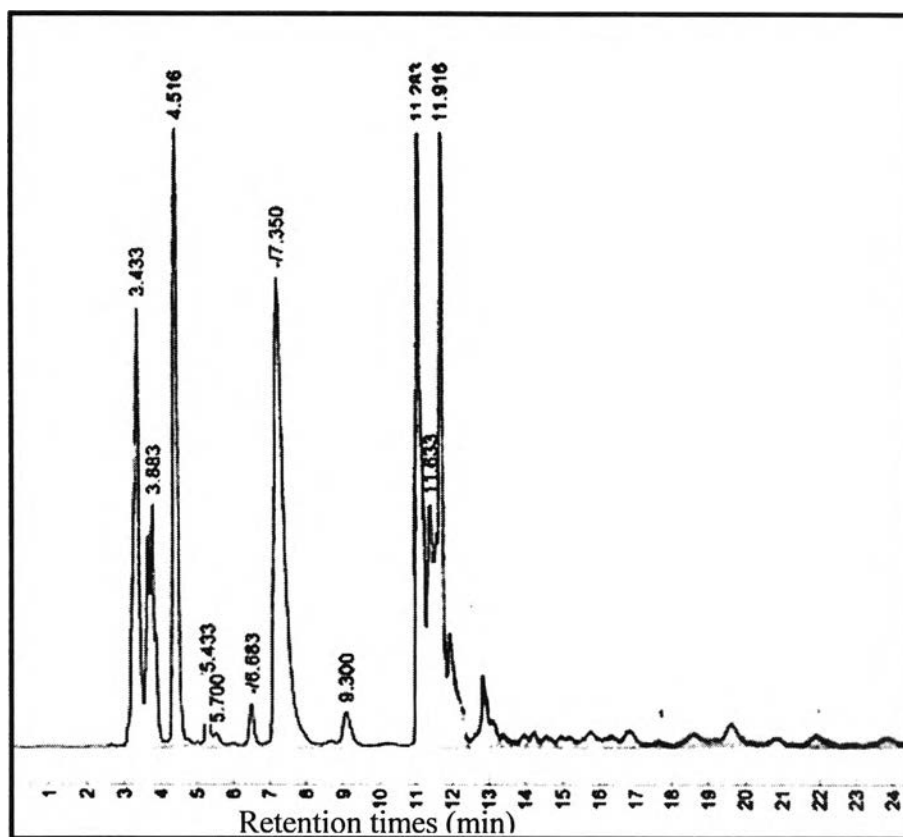## C-4.1 Amino acids profile in fibroin solution by hydrolysis with subcritical water.

Figure C-4 Chromatrogram of Fibroin solution at 1:20, 220 °C, 30 min.

Table C-4.1 Amino acids profile of fibroin solution obtained after 30 min of hydrolysis at different ratios and temperatures.

Amino Acids (mg/g of silk fibre)	Temperature (°C)											
	160 °C			180 °C			200 °C			220 °C		
	1:20	1:50	1:100	1:20	1:50	1:100	1:20	1:50	1:100	1:20	1:50	1:100
Glycine	1.141	-	-	1.367	1.178	-	62.821	18.407	8.125	63.181	22.808	35.547
Serine/Asparagine	-	-	-	2.418	-	-	-	54.052	7.871	-	61.033	46.772
Alanine/Threonine	2.960	-	-	0.975	-	-	63.255	19.106	-	62.829	27.760	52.640
Valine	-	-	-	-	-	-	38.006	-	38.435	8.748	57.767	45.564
Leucine	-	-	-	0.448	0.443	0.377	3.449	7.955	1.447	3.786	5.598	2.911
Aspartic Acid	-	3.698	-	7.310	8.587	10.896	63.244	24.942	20.994	64.291	23.354	33.133
Glutamic Acid	-	-	-	-	-	-	9.482	3.415	4.106	-	-	-
Arginine	6.996	8.931	19.759	8.459	13.918	21.798	37.293	48.419	34.222	36.989	59.586	57.241
Tyrosine	-	-	0.182	0.656	0.410	0.078	4.299	6.588	2.359	4.393	5.979	5.944

Table C-4.2 Amino acids profile of fibroin solution obtained after different times of hydrolysis at different temperature with 1:50 silk to water ratio.

Amino Acids (mg/g of silk fibre)	Temperature (°C)					
	200 °C			220 °C		
	10 min	30 min	60 min	10 min	30 min	60 min
Glycine	38.504	18.407	538.039	149.026	22.808	24.504
Serine/Asparagine	-	54.052	45.466	24.111	61.033	38.475
Alanine/Threonine	-	19.106	58.210	9.250	27.760	89.923
Valine	-	-	-	53.381	57.767	51.994
Leucine	0.517	7.955	2.148	1.886	5.598	2.890
Aspartic Acid	11.127	24.942	28.926	11.335	23.354	-
Glutamic Acid	-	3.415	4.554	-	-	12.699
Arginine	17.348	48.419	51.164	22.171	59.586	29.502
Tyrosine	-	6.58	4.389	1.818	5.979	9.948

APPENDIX D

HYDROTHERMAL DECOMPOSITION OF YEAST POWDER

D-1 Subcritical water hydrolysis

In this study, baker's yeast was used as a model for spent brewer's yeast for the hydrothermal decomposition study due their close resemblance in physical compositions (Table D-1).

Table D-1. Approximate gross composition of dried yeast cells.

Compositions	Chemical composition (% as dry matter)	
	Brewer's yeast (*Saccharomyces uvarum*) [Thornton, 1992]	Baker's yeast (*Saccharomyces cerevisiae*) [Kemal et al., 2001]
Protein	48	42-46
Carbohydrates	36	30-37
Minerals	N/A	7-8
Nucleic Acids	N/A	6-8
Lipids	1	4-7
Moisture	7	2-5
Ash	8	N/A

Baker's yeast (Oriental Yeast Co., Ltd., Japan) was suspended in distilled water to about 10% solids content. Then, 5 ml of this yeast suspension was charged into a 5 ml stainless steel (SUS-316) closed batch reactor (AKICO Co., Japan). The reactor was then heated with an electric heater to the desired temperature (100-250 °C). The schematic diagram of the apparatus is shown in Figure D-1.
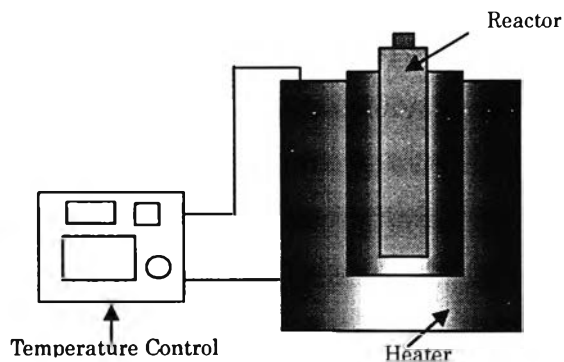
Figure D-1. Schematic diagram of subcritical water hydrolysis apparatus

The pressure in the reactor was estimated based on saturated steam to be between 101.35 kPa and 3.97 MPa for the temperature range studied. After a desire reaction time (5-30 minutes), the reactor was immediately cooled to room temperature by immersion in a cool water bath. The liquid and solid contents in the reactor were collected and the remaining solid in the reactor was washed out with water. The residual yeast was separated from the soluble product with a filter paper (Whatman no. 1) and weighed after drying in a vaccum oven at 50 °C. The soluble portion was assayed for the amount of protein, amino acids, and total organic carbon (TOC).

D-2 Autolysis

Yeast suspension was adjusted to obtain the pH of 5.15 using 0.5 N hydrochloric acid solution. Then autolysis was allowed to take place in a stirred vessel (Julabo HC-2/8, Labortechnik GMBH, Germany) at 50 °C. After autolysis for 19 h, the autolysate was heated to 85 °C for 20 min to terminate the enzyme activity. After autolysis, the weight of the yeast residue was measured and TOC and the protein and amino acid contents of the soluble product were measured.

D-3 Analytical Methods

The protein content of the soluble portion was assayed using Lowry, 1951 method [Lowry et al., 1951] using bovine serum albumin (BSA) as a standard. Amino acids content was analyzed by Ninhydrin assays [Moore et al., 1957] using L-Glutamic acid as a standard. Briefly, Ninhydrin reagent, containing 1 ml of 1%w/w ninhydrinsolution, 2.4 ml of 55 % v/v glycerol solution, 0.2 ml of 0.5 M citrate buffer

and 100 mg/ml manganese chloride, is added to 0.2 ml of the sample solution. The mixture was then shaken and boiled for 12 minutes, after which it was cooled in a water bath. Spectroscopic absorbance of the sample mixture was then measured at 570 nm in triplicates.

The TOC of the soluble reaction products was measured with a TOC analyzer (Shimadzu TOC-5050A), into which 7 μl of aqueous phase was injected. TOC was calculated by subtracting inorganic carbon (IC) from total carbon (TC). The IC values were less than 10% of the TC values for all samples examined.

The TOC yield of the aqueous phase was defined by the following equation:

$$TOCyield = \frac{TOC \times V}{w}$$

where TOC, V, and w were TOC of the aqueous phase [mg/l], volume of the aqueous phase [l], baker's yeast weight [mg], respectively.

D-4 Results and discussion

D-4.1 Amount of residual yeast and TOC of soluble product

The reaction products consisted of two parts: solid yeast residue and aqueous solution. Figure D-4.1.1 shows the photographs of the soluble reaction products at 125, 200, and 250 ^{0}C after the reaction time of 10 min. The color of the soluble product became darker as more organic content are dissolved. Also, cells started to burn at high temperature.
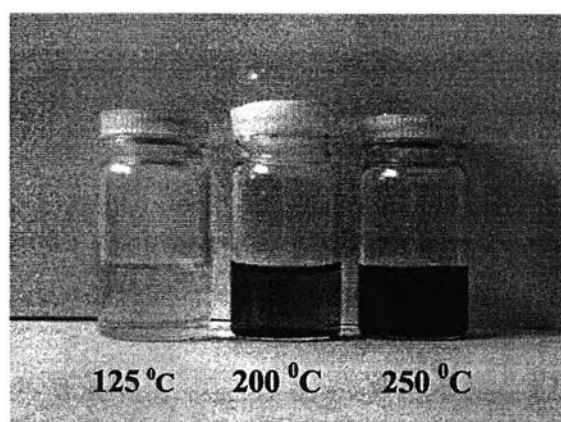
FigureD-4.1.1 Soluble reaction product obtained at 125, 200, and 250 °C after 10 min

The weight of residual yeast decreased with an increase in temperature because at high temperature, hydrolysis proceeded to the larger extent than at low temperature (Figure D-4.1.2). When the reaction temperature was increased to 250 °C (3.97 MPa), the residual weight of solid was about 0.22 mg/mg of dry yeast. This is a 78 % reduction of the total solid waste, which is significantly higher than that obtained after 19 h of autolysis in which only 32% was hydrolyzed. The conversion of yeast cells into soluble products was supported by the TOC of the soluble products which was generally found to increase as the temperature increases (Figure D-4.1.3).
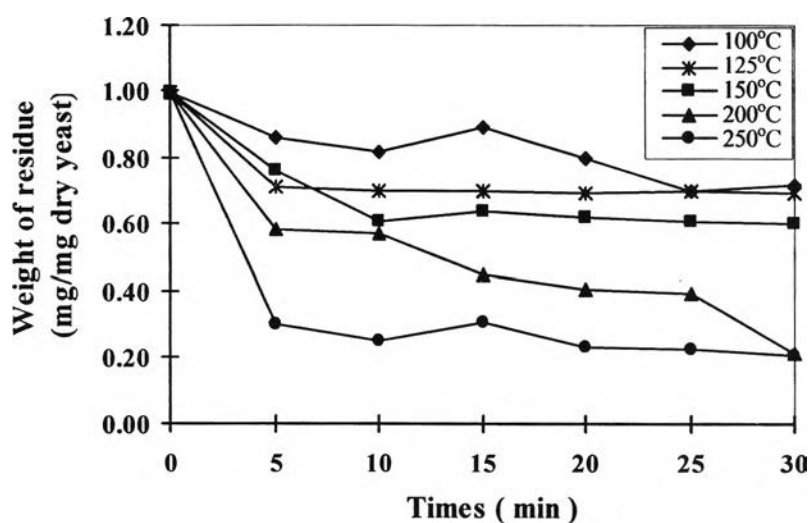

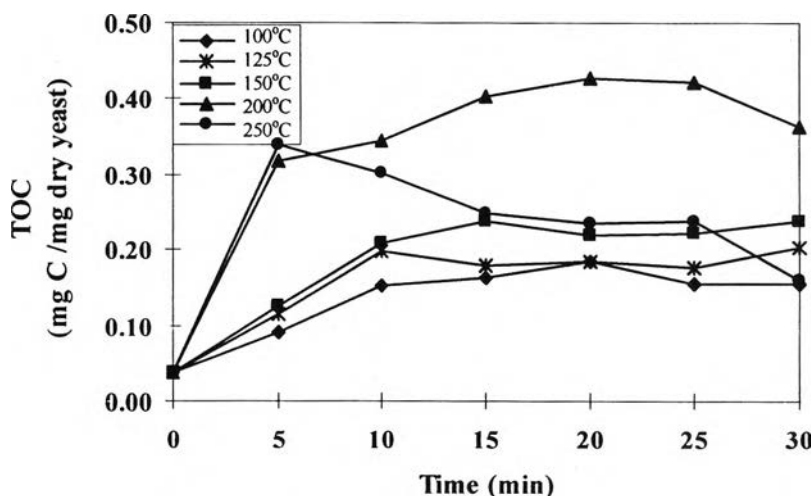**Figure D-4.1.2.** Effect of reaction time and reaction temperature on residual weight of dry yeast

Figure D-4.1.3 Effect of reaction time and reaction temperature on TOC of supernatant

From Figure D-4.1.3, it can be seen that TOC yields increased and became stable after 10 min under temperature conditions lower than 200 °C. TOC yields were

found to increase from 0.04 mg C /mg of dry yeast at the start of the reaction and reached the highest yield of about 0.42 mg C/mg of dry yeast after 20 min at the reaction temperature of 200 °C (1.55 MPa), indicating that about 42 % of the organic carbon in dry yeast cells was recovered into soluble solution. The TOC yield of the soluble product after a 19 h autolysis process was found to be only about 22.8 %, indicating that the lower amount of organic carbon in dry yeast cells was recovered. This was about 50% lower than the TOC yield obtained by hydrolysis with subcritical water (at 200 °C, 20 min). However, at the temperature of 250 °C, the TOC value decreased at higher reaction times and were lower than those at 200 °C. This result suggested that formation of gaseous product might have occurred at these conditions and that some part of water-soluble may be converted to water-insoluble through carbonization.

D-4.2 Soluble proten product

The effect of the temperature and time of subcritical water on the hydrolysis yield was determined. As shown in Figure D-4.2, the yield of protein almost reached plateaus by 5 min for all reaction temperatures. The amount of protein produced increased with an increase in temperature and was the highest for the reaction that took place at 250 °C (3.97 MPa) for 20 min, which was 0.16 mg/mg of dry yeast. The product was found to decrease at higher temperatures and after the reaction was extended beyond 20 min. When compared with the protein yield of baker's yeast obtained by autolysis process (0.095 mg/mg of dry yeast after 19 hours), the protein yield obtained by hydrolysis in subcritical water was higher at the above condition. This demonstrates that hydrothermal decomposition yields valuable protein products in favorable amount in a short time.
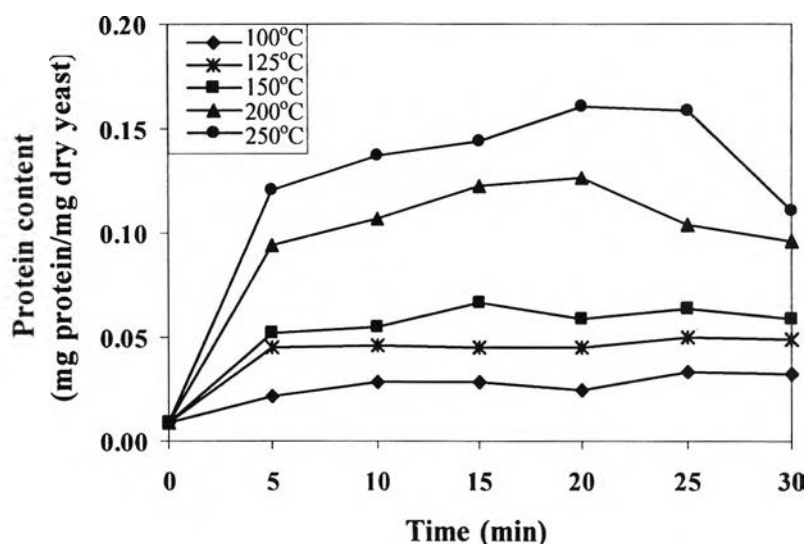
Figure D-4.2. Effect of reaction time and reaction temperature on protein production.
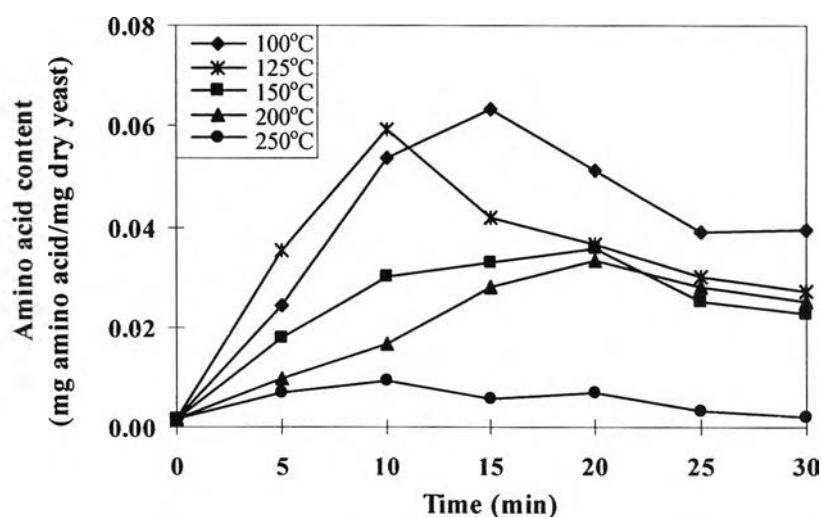
D-4.3 Total amino acids product

Figure D-4.3. Effect of reaction time and reaction temperature on amino acids production.

The results in Figure D-4.3 revealed that the amount of total amino acids produced decreased with an increase in temperature. The highest yield was obtained at 100 °C (101.35 kPa) for 15 min reaction time, and was found to be 0.063 mg/mg of dry yeast. Compared with the total amino acids yield obtained after 19 h of autolysis (about 0.45 mg/mg of dry yeast), the amino acids yields obtained by hydrolysis of baker's yeast in subcritical water was much inferior, despite the higher TOC values.

This strongly suggests that amino acids decomposed at high reaction temperature and longer reaction times into low-molecular-weight carboxylic acids and gaseous products [Kang et al., 2004; Quitain et al., 2002].

D-5 Conclusions

This study demonstrates the feasibility of using subcritical water to potentially hydrolyze spent brewer's yeast cells, organic waste from brewing industries into more valuable proteins and amino acids. The amount of protein produced increases with an increase in temperature, while that of amino acids decreases with increasing temperatures. The highest yield of proteins and amino acids were 0.1606 and 0.0634 mg/mg of dry yeast, respectively. The amount of total organic carbon was found to increase with increasing temperatures. However, the value was decreased at the temperature as high as 250 ^{0}C.

APPENDIX E

LIST OF PUBLICATION

Wiwat Lamoolphak, Motonobu Goto, Mitsuru Sasaki, Manop Suphantharika, Chirakarn Muangnapoh, Artiwan Shotipruk, "Subcritical Water Hydrolysis of Yeast Cells for the Production of Proteins and Amino Acids", Proceedings of Thai Institute of Chemical Engineering and Applied Chemical Conference 15th, Chonburi, Thailand, 27-28 October, 2005, Ref. No.BE05.

Subcritical Water Hydrolysis of Yeast Cells for the Production of Proteins and Amino Acids

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ABSTRACT

Spent brewer's yeast is an organic waste, which can be used for the production of yeast extract. This study was to examine a new alternative method for hydrolysis of spent yeast cells with subcritical water while producing proteins and amino acids. Hydrolysis was carried out in subcritical water in a closed batch reactor at various temperatures between 100 and 250 ^{0}C. The amount of proteins and amino acids produced were analyzed by Lowry's and Ninhydrin assays, respectively. The results of this study demonstrated that the amount of yeast residue decreased with increasing hydrolysis temperature while the total organic carbon (TOC) of the products measured with a TOC analyzer was higher at higher temperatures. The amount of protein produced increased with an increase in temperature, while that of amino acids decreased with increasing temperature. These results demonstrated the feasibility of using subcritical water to potentially hydrolyze and convert spent yeast into more valuable products.

Keyword: Subcritical Water; Proteins; Amino Acids; Hydrolysis; Baker's Yeast

1. INTRODUCTION

Spent brewer's yeast, by-product from the brewing industries, is being produced in large amount annually from main beer manufacturers due to an increase in volumes of beer production. It is generally sold primarily as inexpensive animal feed after inactivation by heat, and much of this by-product is considered industrial waste. However, because of the high level of proteins, vitamin B complex, and minerals remaining, the production of a higher value product such as yeast extract from spent brewer's yeast has become recognized [1].

The production of yeast extract generally involves autolysis in which hydrolysis occurs as a result by activation of the intracellular enzymes within yeast cell. The process has some disadvantages such as difficulty in solid-liquid separation due to high content of residue in autolysate, risk of deterioration due to microbial contamination, and long process time, thus may not be practical for industrial applications. Alternatively, plasmolysis, the process in which inorganic salts or non-polar organic solvents are added to yeast suspension during autolysis process, is utilized [2-3]. However this process resulted in yeast extract with high salt or carcinogenic content [4]. Acid hydrolysis is the most efficient method of solubilizing yeast cells, and gives high production yield but the acid must be washed with large amount of water, causing a great deal of environmental problem. Enzyme hydrolysis, carried out by cell wall lysis enzyme, proteolytic enzymes, or the combination of these enzymes [1] produces product low in salt content, however the high cost of enzymes makes the method less favorable.

In this study, the feasibility of an alternative yeast cell hydrolysis method, namely hydrolysis in subcritical water, is investigated. Water at sub and supercritical conditions has been used for oxidation of municipal sludge [5] and harmful substances such as sodium sulfate [6]. Subcritical water, which produces milder reaction conditions than supercritical water, has also been applied for hydrolysis of fish meat [7] and silk fibroin [8] to produce organic acids and amino acids. These studies demonstrated that the process could be devised to recover a large amount of a variety of useful substances from organic waste in a short time. It is therefore the aim of this study to examine the effect of subcritical water temperature and hydrolysis time on the amount of proteins and amino acids produced. Due to the physiological

similarity of spent brewer's yeast and baker's yeast, baker's yeast was used in this investigation due to the ease of storage and handling.

2. EXPERIMENTAL METHODS

2.1. Subcritical water hydrolysis

Baker's yeast (Oriental Yeast Co., Ltd., Japan) was suspended in distilled water to about 10% solids content. Hydrolysis of the yeast suspension was carried out in subcritical water in a closed 5 cm^3 stainless steel pressure reactor, the schematic diagram is shown in Figure1. at various temperatures and times for hydrolysis. The pressure in the reactor was estimated from a steam table. After reaction, the reactor was immediately cooled to room temperature by placing it into a water bath. The yeast residual was separated from the soluble product with a filter paper (Whatman no. 1) and weighed after drying in a vaccum oven at 50 °C. The soluble portion was assayed for the amount of proteins, amino acids, and total organic carbon (TOC)
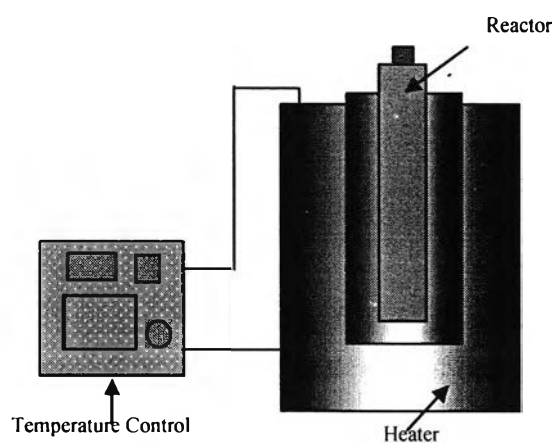

Figure 1. Schematic diagram of subcritical water hydrolysis apparatus

2.2. Analytical Methods

2.2.1 Analysis of protein and amino acids

The protein content of the yeast cells and yeast extract was assayed using Lowry method [9] using bovine serum albumin as a standard. Amino acids produced were analyzed by Ninhydrin assays [10] using L-Glutamic acid as a standard

2.2.2 TOC measurement

The TOC reaction products was measured with a TOC analyzer (Shimadzu TOC-5050A), into which 7 µl of aqueous phase was injected. TOC was calculated by subtracting inorganic carbon (IC) from total carbon (TC).

3. RESULTS AND DISCUSSSION

3.1. Effect of subcritical water hydrolysis temperature and time on soluble product and weight residual yeast

Figure 2. shows the reaction products at 125, 200, and 250^0C after the reaction time of 10 min. The color of the soluble product became darker as the reaction temperature increased because the yeast cells started to burn at high temperatures.
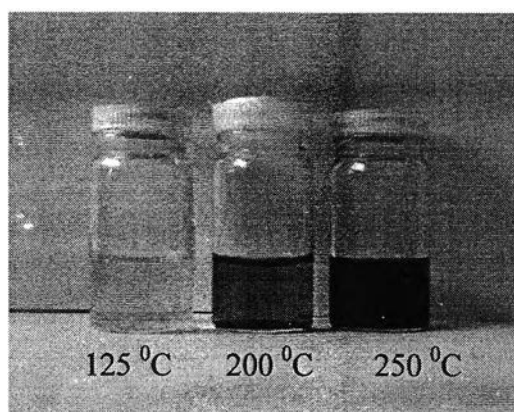

Figure 2. Supernatant of reaction product 125, 200, and 250 ^0C at 10 min

The weight of residual yeast decreases with an increase in temperature because at high temperatures hydrolysis proceeds to the larger extent than at low temperature (Figure 3). The conversion of yeast cells into soluble products was supported by the TOC of the soluble products which was generally found to increase as the temperature increases (Figure 4). At the temperature as high as 250 ^0C however, TOC was lower than that of the product obtained at hydrolysis temperature of 200 ^0C although the residual weight was lower than that of 200 ^0C. This indicated that there was some solid loss while attempting to recover it from the pressure vessel after the reaction.
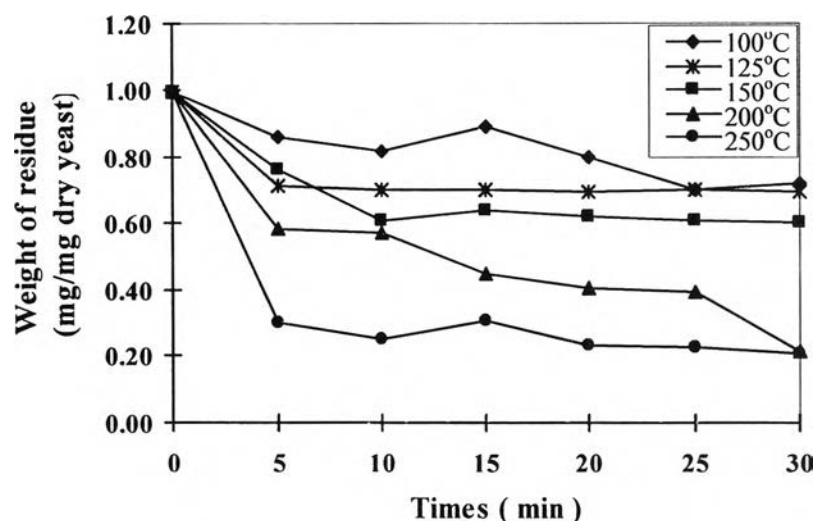
Figure 3. Effect of reaction times and temperatures on residual weight of dry yeast.
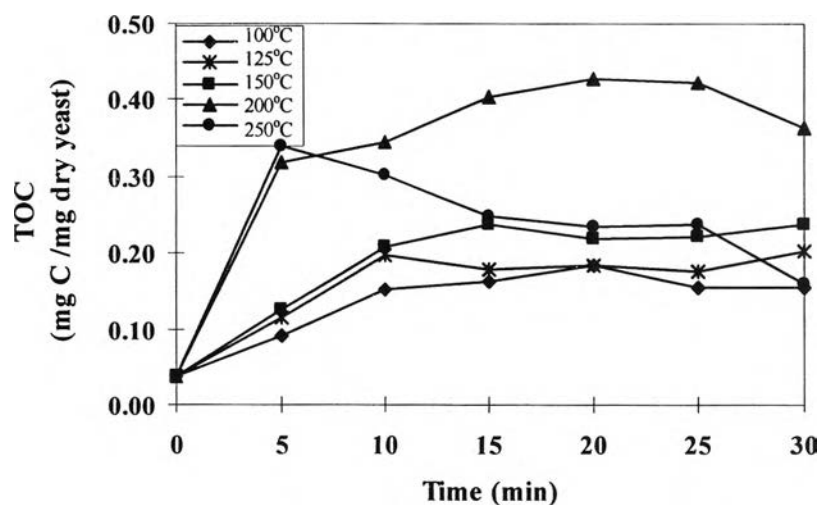

Figure 4. Effect of reaction times and temperatures on TOC of supernatant.

3.2. Effect of subcritical water hydrolysis temperature and time on protein production

The effect of the temperature and time of subcritical water on the hydrolysis yield was determined. As shown in Figure 5, the amount of protein produced increases with an increase in temperature and was the highest for the reaction that took place at 250 ^{0}C for 20 min (0.1606 mg/mg of dry yeast) The product was found to decrease at higher temperatures and after the reaction was extended beyond 20 min indicating product decomposition at these conditions.
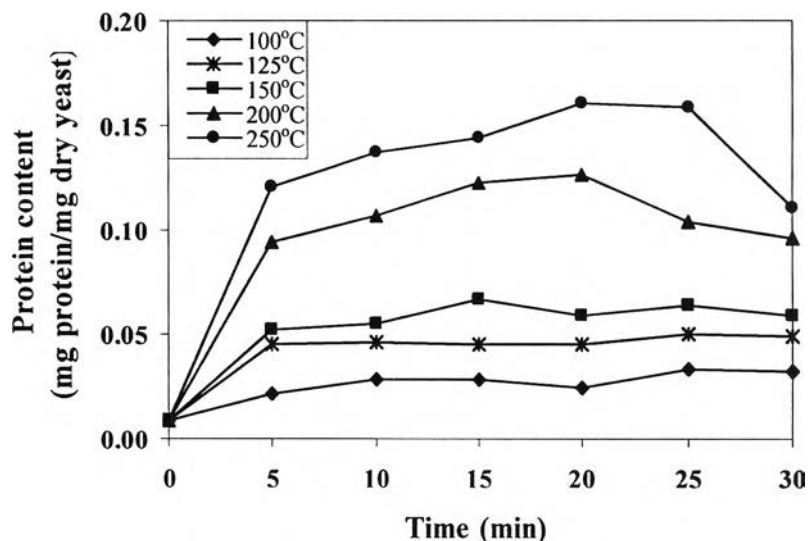
Figure 5. Effect of reaction times and temperatures on protein production .

3.3. Effect of subcritical water hydrolysis temperature and time on total Amino Acids production
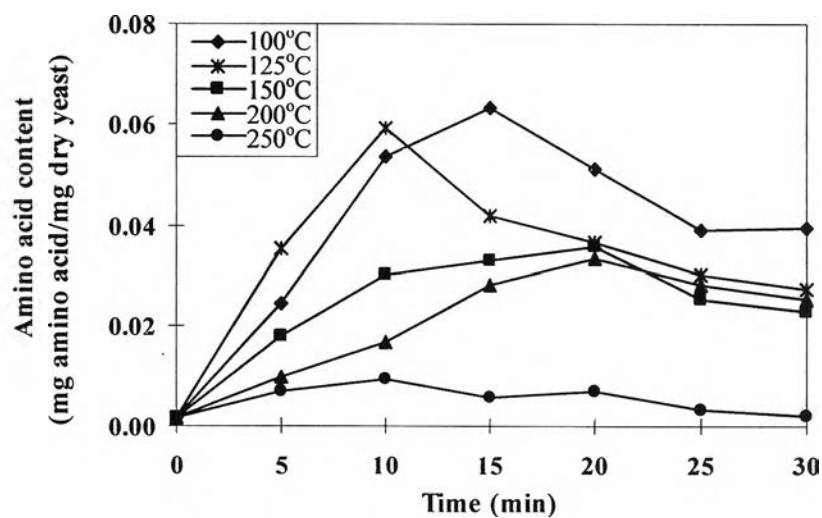

Figure 6. Effect of reaction times and temperatures on amino acids production.

The results in Figure 6 reveal that the amount of total amino acids produced was found to decrease with an increase in temperature and was the highest yield at 100 ^{0}C for 15 min reaction time (0.0634 mg/mg of dry yeast). This strongly suggests that product decomposition occurs at high reaction temperatures.

3.4. Total Organic Carbon (TOC)

The effect of the reaction temperatures and times on the TOC yield is shown in Figure 4. The amount of total organic carbon was found to increase with increasing temperatures and reached stable after 10 min. However, the value was decreased at the temperature as high as 250 ^{0}C.

The increase of TOC with the reaction temperature is well explained by the decrease of the solids of residual weight of dry yeast with the reaction temperature shown in Figure 3.

4. CONCLUSIONS

This study demonstrates the feasibility of using subcritical water to potentially hydrolyze spent brewer's yeast cells, organic waste from brewing industries into more valuable proteins and amino acids. The amount of protein produced increases with an increase in temperature, while that of amino acids decreases with increasing temperatures. The highest yield of proteins and amino acids were 0.1606 and 0.0634 mg/mg of dry yeast, respectively. The amount of total organic carbon was found to increase with increasing temperatures. However, the value was decreased at the temperature as high as 250 ^{0}C.

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