CHAPTER IV

RESULTS AND DISCUSSION

4.1 Hydrolysis of silk fibre in subcritical water: Preliminary study

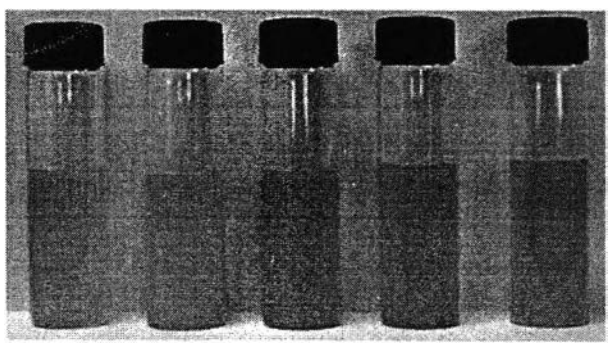
Hydrolysis of silk fibre was carried out in subcritical water at different temperatures and reaction times between 10 to 60 min. The reaction products consisted of two parts: solid residue and aqueous solution. In the preliminary study, general characteristics of these products were observed. The morphology of the solid residue was examined under a scanning electron microscope (SEM) and the molecular size distribution of the content in the soluble products was determined using sodium dodesyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

4.1.1 Soluble products

Figure 4.1 shows the photographs of the soluble reaction products obtained reaction of 30 min. The soluble product obtained was viscous at lower temperature (120 and 130 °C) and becomes less viscous at higher temperatures. In addition, at low temperatures of 120 and 130 °C, gelation of the soluble product was observed after allowing a period of cooling. The products shown in Figure 4 a were obtained in the temperature range between 120 and 160 °C. The yellow color was resulted from the pigment of the silk fiber extracted in to the solution. The weight of the remaining residue did not change significantly when the temperature increased from 120 to 160 °C, which indicates that the majority of fibroin fibre of the silk remained unhydrolyzed at these conditions. The the major component of these glue-like products in Figure 4 a was therefore resulted from sericin. They are consequently called *sericin solutions*.

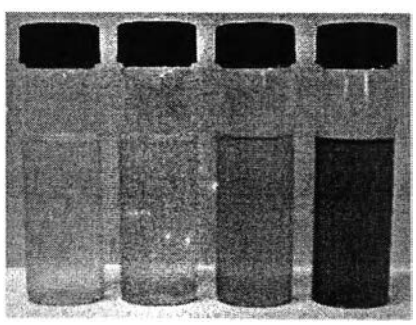
In order to hydrothermally decompose the fibroin, higher temperature was required. In the study of subcritical water hydrolysis of fibroin, a separate sets of experiments could be conducted using the sericin free fibroin fibre as the starting maerials. These samples were prepared from the raw silk waste by degumming in an

autoclave at 120 °C for 30 min, the condition found to be optimal for sericin removal. In a separate set of experiments, the fibroin hydrolysis study was carried out at various temperatures between 160-220 °C. The pictures of the resulting soluble products which we call *fibroin solution*, obtained after 30 min at various temperatures are shown in Figure 4.1 b. The color of the product obtained at 160 °C was lighter than the sericin solution. This was because most of the pigment had been removed along with sericin in the earlier step. At higher hydrolysis temperatures, the color of the solution as well as of the silk residue became dark brown due to the increase in the degree of carbonization. The morphology of the silk remained after hydrolysis at various temperatures was then observed under a scanning electron microscope and the soluble products were analyzed with sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE).



120 °C 130 °C 140 °C 150 °C 160 °C

(a)



160 °C 180 °C 200 °C 220 °C

(b)

Figure 4.1 Soluble products of silk fibre in subcritical water:

(a) Silk sericin solution, reaction time=30 min, 120-160 °C

(b) Silk fibroin solution, reaction time= 30 min, 160-220 °C.

4.1.2 Morphology of silk residue

Scanning electron microscopic images of raw silk and of the remaining silk residue after hydrolysis are shown in Figure 4.2. As seen in the figure, sericin, the glue-like protein that encases the silk fibroin fiber, could be removed completely after 30 min of hydrolysis at 120 °C. Although it is well known that silk sericin is soluble in boiling water, sericin removal with boiling water might give incomplete degumming. The SEM images demonstrated that at elevated temperature (120 °C), complete removal of sericin could be achieved in a short time period. At higher longer time period (Figure 4.2 c) or higher temperature of 160 °C (Figure 4.2 d), some amount of silk fibroin was also hydrolyzed, as can be seen from the resulting thinner fibre shown in the SEM images.

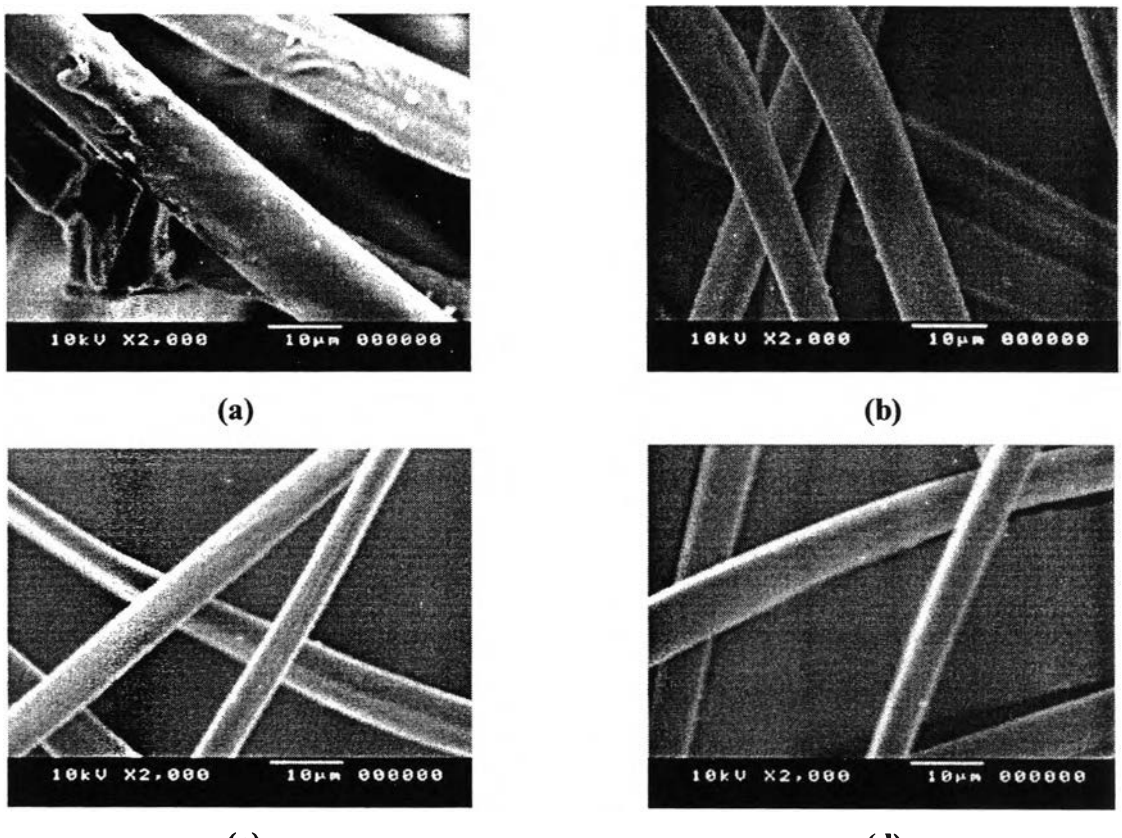
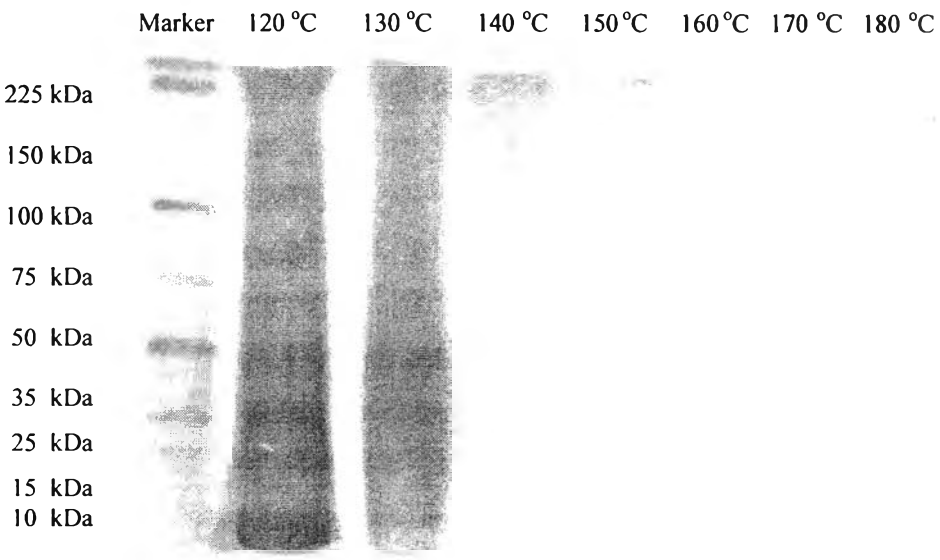


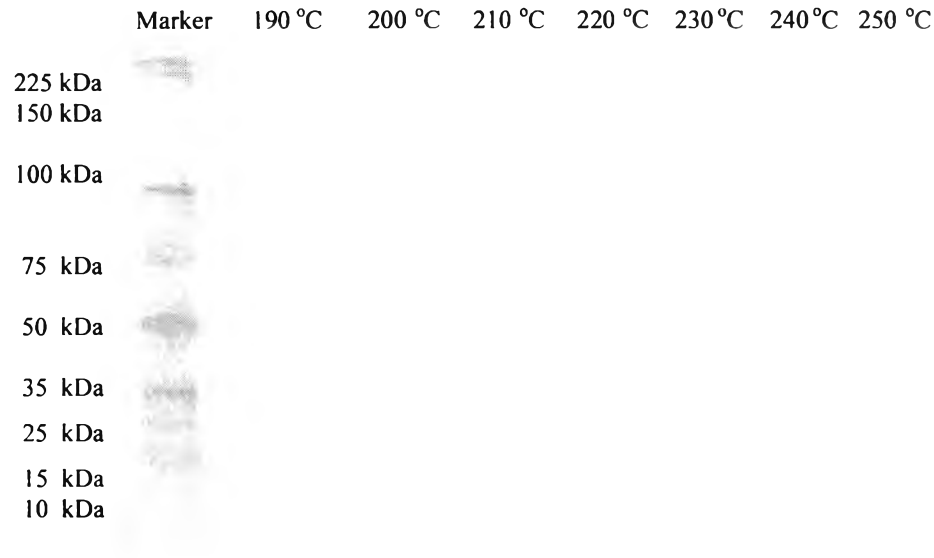
Figure 4.2 Scanning Electron Micrograph: (a) Raw silk (b) 1:50, 120 °C, 30 min (c) 1:50, 120 °C, 60 min (d) 1:50, 160 °C, 30 min

4.1. 3. Molecular size

The soluble fraction of the reaction product was analyzed by protein gel electrophoresis using 12 % polyacrylamide gradient gel prepared for the molecular range of proteins between 10-225 kDa. The hydrolyzed product obtained under different temperatures for the hydrolysis time of 30 min was analyzed and the results are shown in Figure 4.3. The product obtained at lower temperature of 120 and 130 °C contain complex mixture of multiple polypeptides ranging in size from approximately 10-225 kDa and mostly have the molecular size between 15-75 kDa. The soluble product obtained after 10 min of hydrolysis at the same temperatures was well retained on the gel and showed dark band in the higher molecular range of 100-225 kDa (Appendix B). This result seems to be consistent with literatures which describe sericin as complex mixture of polypeptides widely differing in molecular weights, ranging from about 10 to over 300 kDa (Zhang, 2003). At higher hydrolysis temperatures (>140 °C) the product was not retained on the gel, indicating that these hydrolysis products were peptides with lower molecular (<10 kDa) and even smaller amino acids.



(a)



(b)

Figure 4.3 The molecular weight range of sericin and fibroin solution determined by SDS-PAGE (a) 1:50, 30 min, 120-180 °C (b) 1:50, 30 min, 190-250 °C.

4.2 Noncatalytic hydrolyis of sericin

Hydrolysis of silk sericin at the temperatures range between 120 and 160 °C, and the reaction times between 10 and 60 min, was investigated. After each reaction, the weight of the remaining silk residue was measured and the soluble product was analyzed for the amount of protein and amino acids. In addition, the effect of silk to water ratio (1:20, 1:50, and 1:100) on conversion of silk into protein and amino acids were determined.

4.2.1 Weight of residue

The weight of the silk residue after the reaction was found to decrease with increasing hydrolysis temperature and reaction time. The results are shown in Figure 4.4 for the hydrolysis with the weight ratio of silk to water of 1:50. The other silk:water ratios showed similar trend (Appendix B). The highest amount of silk remained was found at the hydrolysis temperature of 120 °C (ratio 1:20) after 10 min to be 0.68 mg/mg

of raw silk, which means a 32 % conversion of the raw silk material into the soluble products. Sericin protein is known to constitute approximately 17–25%, of the raw silk fiber. This implied that even at the lowest temperature and time employed sericin should be almost completely removed. At higher temperature and extended extraction period (i.e. 160 °C, 60 min), only 0.51 mg/mg of raw silk was left, indicating that at this condition, silk fibroin had also been hydrolyzed.

The effect of silk to water ratio on the weight of the silk residue is shown in Figure 4.5 which demonstrated that when the weight ratio of silk was low (1:100), silk was converted into smaller protein and amino acid products more rapidly than that for the reaction with higher ratio of silk (1:50 and 1:20, respectively). The possible reason for this was that the sticky sericin protein could produce mass transfer resistance to the hydrolysis reaction. With the silk:water ratio of 1:100, this resistance can be minimized.

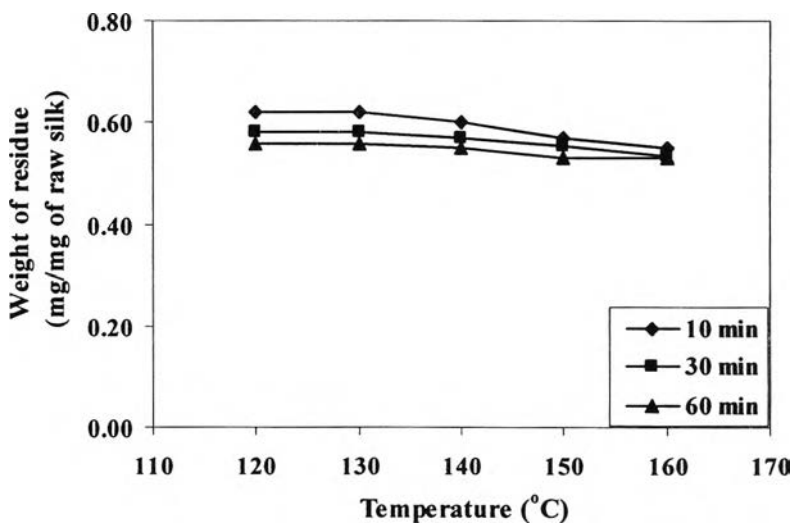
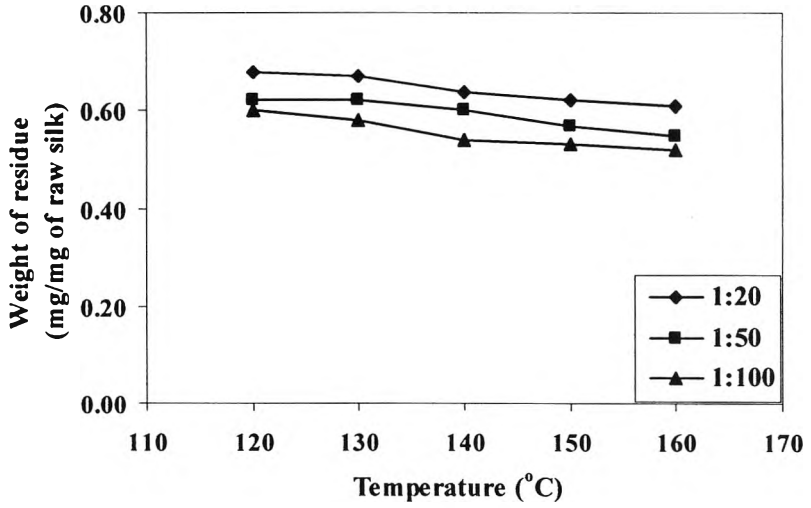
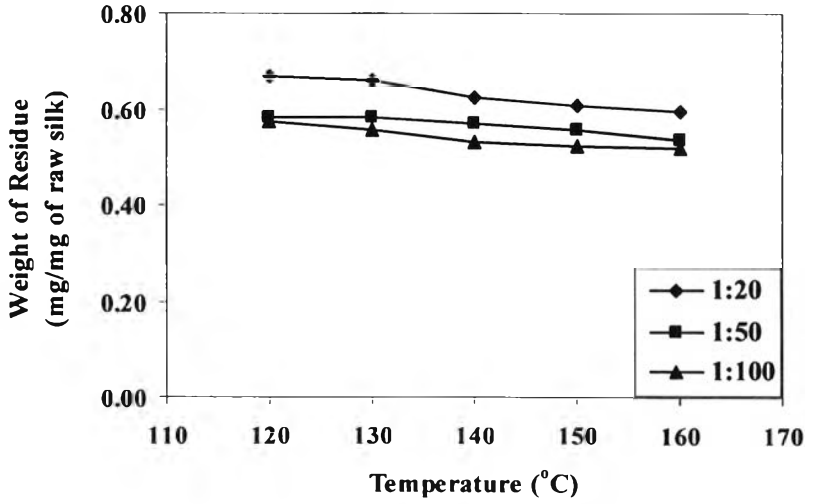


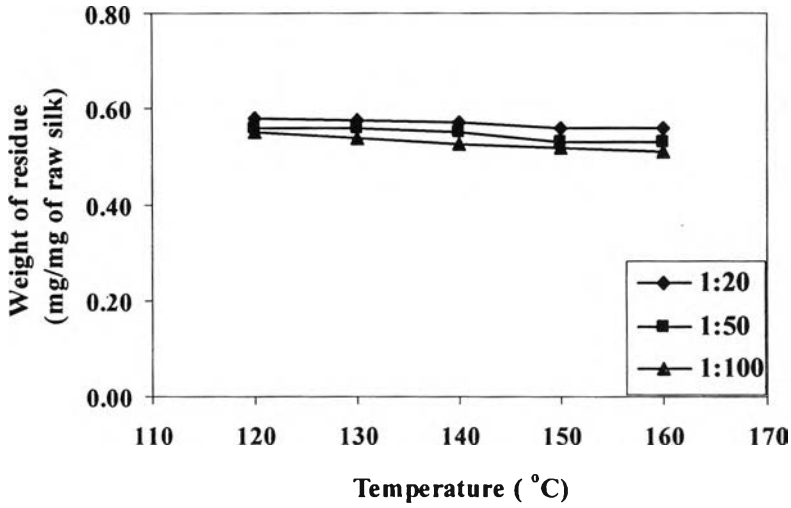
Figure 4.4 Weight of silk residue after subcritical water hydrolysis at different temperatures (1:50 silk:water ratio)


0.80
0.60
0.40
0.20
0.00
Weight of residue
(mg/mg of raw silk)
110
120
130
140
150
160
170
Temperature (°C)
1:20
1:50
1:100

(a)


0.80
0.60
0.40
0.20
0.00
Weight of Residue
(mg/mg of raw silk)
110
120
130
140
150
160
170
Temperature (°C)
1:20
1:50
1:100

(b)



(c)

Figure 4.5 Effect of silk to water ratio on weight of residue at different reaction time: (a) 10 min (b) 30 min (c) 60 min

4.2.2 Protein yield

Shown in Figure 4.6 and Figure 4.7 are the results for the yields of protein in the soluble products at different temperatures, times of extraction, and silk to water ratios. The highest yields (0.466 mg protein/mg of raw silk) were obtained at the lowest temperature (120 °C, 1:100, and 10 min, Figure 4.7 a) and the yield decreased slightly with increasing temperatures. The lowest protein yield was found to be 0.123 mg protein/mg of raw silk (160 °C, 1:20 and 60 min, Figure 4.7 c). The amount of protein in the sericin solution was not affected greatly by temperature and time of reaction for the ranges studied. This trend agreed with that observed for the weight of the silk residue which showed small effects of temperature and time of reaction. This result indicated that at these conditions, the rate of protein production was comparable to the rate of protein decomposition into smaller amino acids.

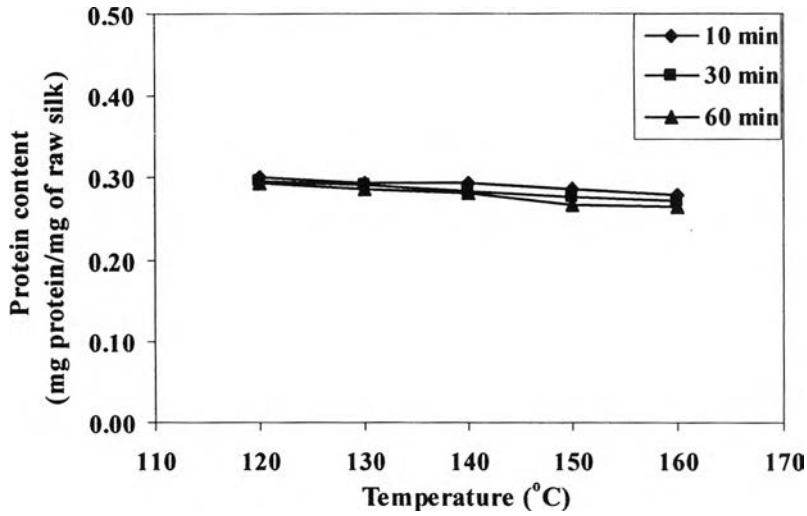
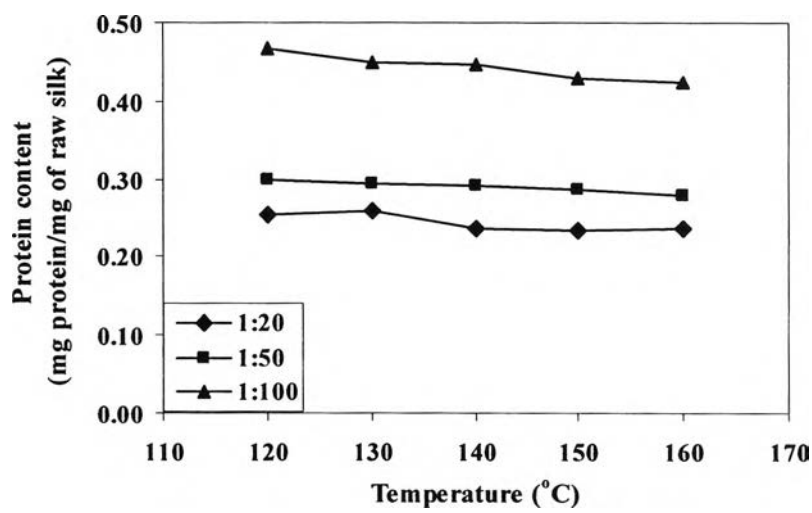
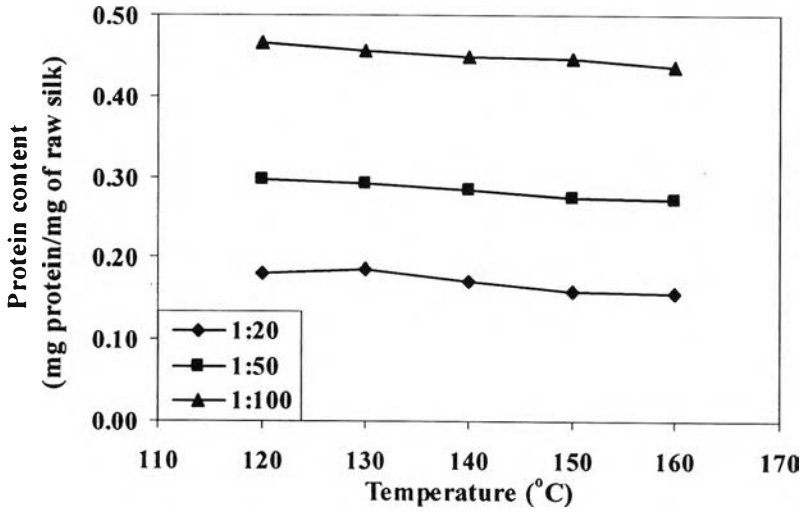


Figure 4.6 Protein yield after hydrolysis at different temperatures (1:50 silk:water ratio)

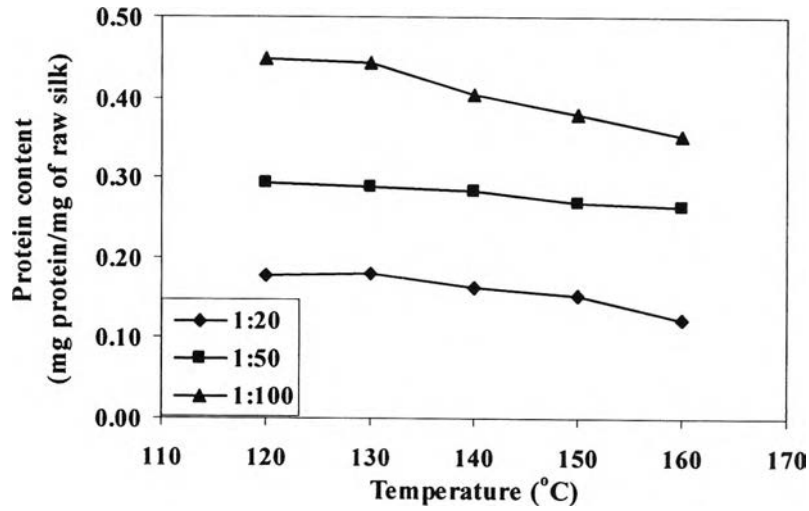
The effect of silk:water ratio on protein yield is shown in Figure 4.7. The higher amount of protein (0.466 mg protein/mg of raw silk) was produced when the silk:water ratio was low (1:100). This agreed with the result previously shown in Figure 4.4, in which the highest silk fiber conversion (weight loss) was observed for the silk:water of 1:100.



(a)



(b)



(c)

Figure 4.7 Effect of silk to water ratio on protein yield at different reaction time : (a) 10 min (b) 30 min (c) 60 min

4.2.3 Amino acids yield

In contrast to protein yield, amino acid yields increased with increasing temperatures and times of reaction as shown in Figure 4.8. This result suggested that, at these conditions, the rate of protein decomposition to amino acids was high, and that the production of amino acids was favored over the decomposition of amino acids to other products.

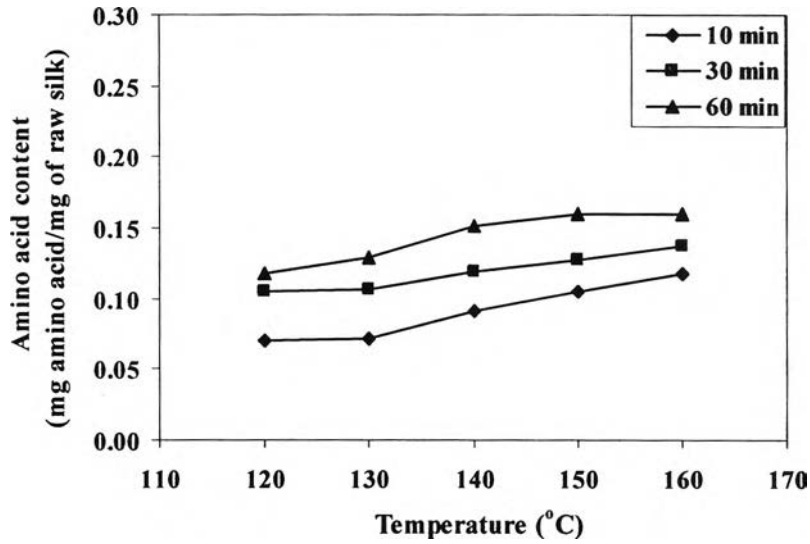
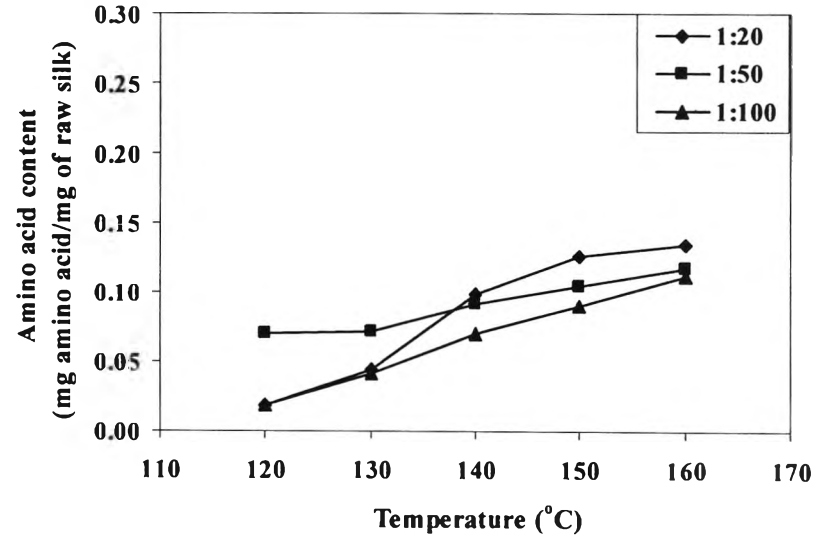
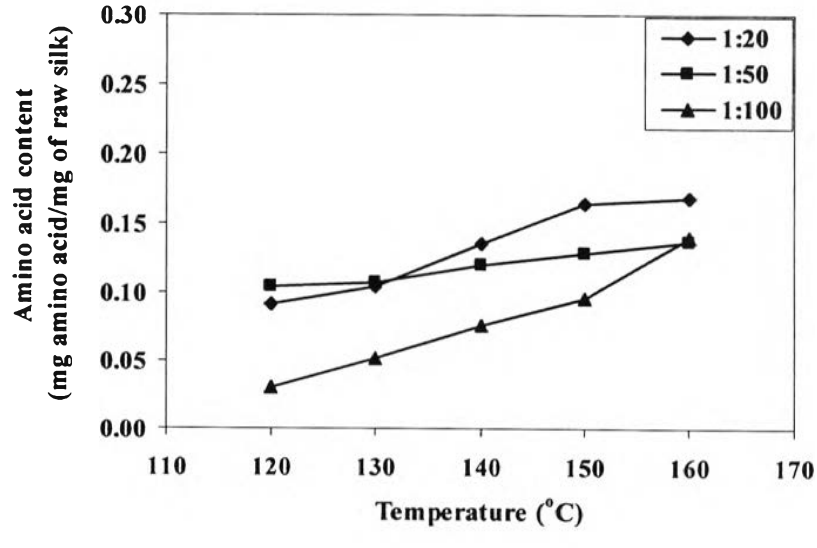


Figure 4.8 Amino acid yield after hydrolysis at different temperatures (1:50 silk:water ratio)

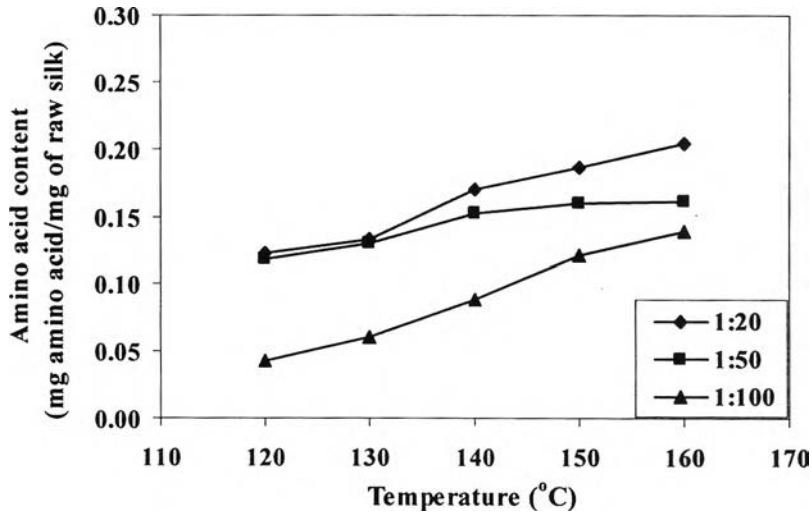
The effect of silk:water ratio on amino acid yield is shown in Figure 4.9, which indicated the higher production of amino acids was resulted in the case of the high silk:water ratio (ratio 1:20). A reasonable explanation for this is that the conversion of protein into amino acids is of homogenous nature. In such case, the high silk:water ratio means the higher protein (reactant) concentration, and thus higher amino acid yield would result. It should be noted that at lower temperature, silk:water ratio of 1:50 gave the highest yield of amino acids whereas at the higher temperatures, the silk:water ratio of 1:20 resulted in highest yield. As mentioned earlier, at low temperatures of 120 and 130 °C, viscous properties of silk sericin caused poor mixing of silk fiber and water, thus the silk:water ratio of 1:50 appeared to be optimal at this condition as it compromise the poor mixing and the concentration effect.


0.30
0.25
0.20
0.15
0.10
0.05
0.00
Amino acid content
(mg amino acid/mg of raw silk)
1:20
1:50
1:100
110
120
130
140
150
160
170
Temperature (°C)

(a)


0.30
0.25
0.20
0.15
0.10
0.05
0.00
Amino acid content
(mg amino acid/mg of raw silk)
1:20
1:50
1:100
110
120
130
140
150
160
170
Temperature (°C)

(b)



(c)

Figure 4.9 Effect of silk to water ratio on amino acid yield at different reaction time : (a) 10 min (b) 30 min (c) 60 min

4.3 Noncatalytic hydrolyis of fibroin

4.3.1 Weight of silk residue

Similar to sericin, the weight of the silk fibroin residue decreased with increasing hydrolysis temperature and time. Figure 4.10 shows the results for the hydrolysis with silk to water weight ratio of 1:50. At the temperature of 220 °C, most of the silk fibroin was converted after 10 min, and after 30 min and 60 min, the silk fibroin was converted completely.

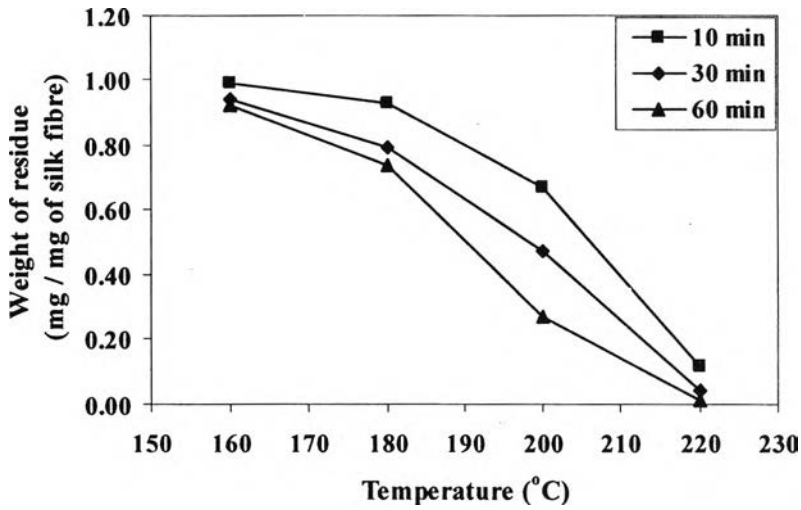
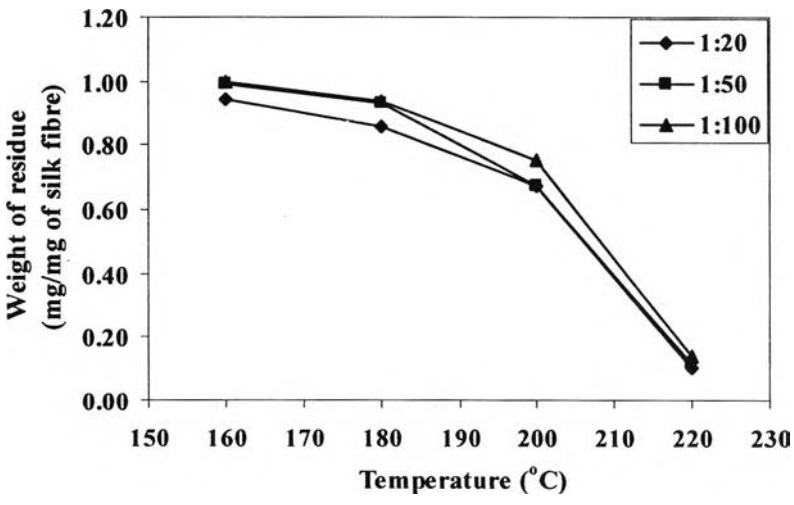
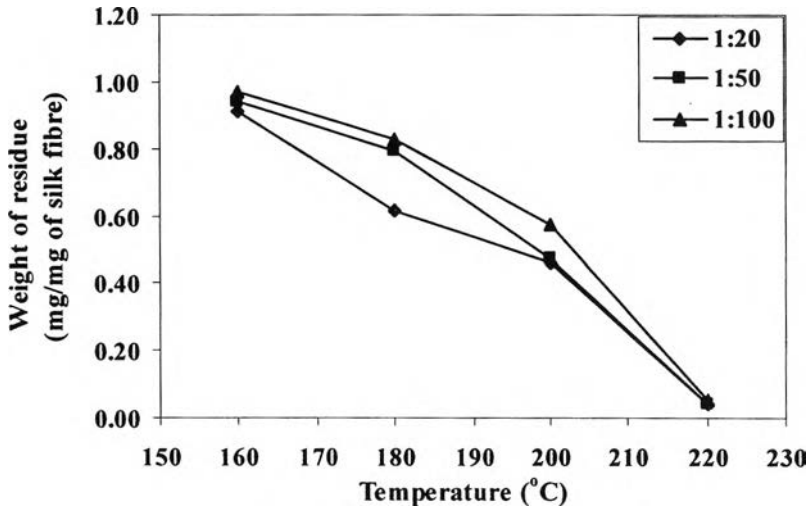


Figure 4.10 Weight of silk residue after hydrolysis at different temperatures (1:50 silk:water ratio)

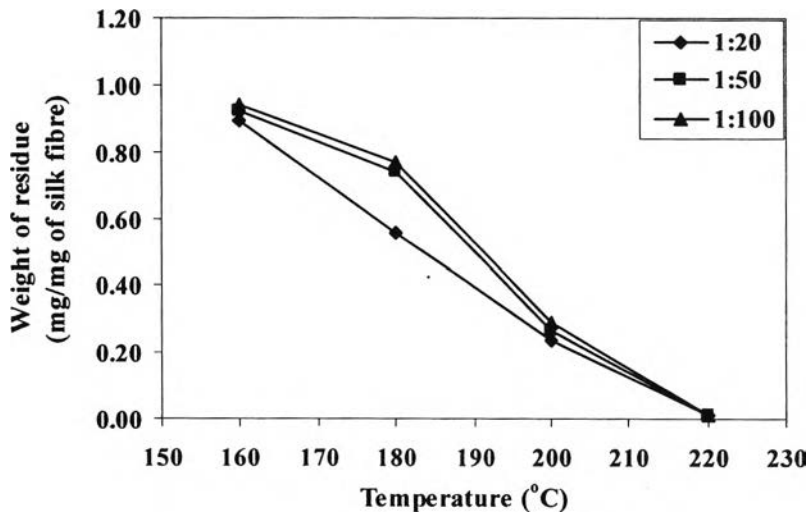
The effect of silk to water ratio on the weight of the silk residue is shown in Figure 4.11. Unlike sericin, the ratio of silk to water does not significantly affect silk conversion as seen by the comparable amount of residue left after the reaction. The reason for this observation is that silk fibroin solution was not viscous, mixing between water and silk fiber was not a limiting factor, particularly, the silk fibroin was presoaked in water for 5 min prior to the reaction.



(a)



(b)



(c)

Figure 4.11 Effect of silk to water ratio on weight of residue at different reaction times: (a) 10 min (b) 30 min (c) 60 min

4.3.2 Protein yield

The results for the yields of protein in the soluble products are shown in Figure 4.12 at different temperatures and times of extraction. The protein yield was generally found to increase with increasing temperature and time of hydrolysis. The maximum protein yield (0.455 mg protein/mg of silk fibre) was obtained at 220 °C (1:100, 10 min).

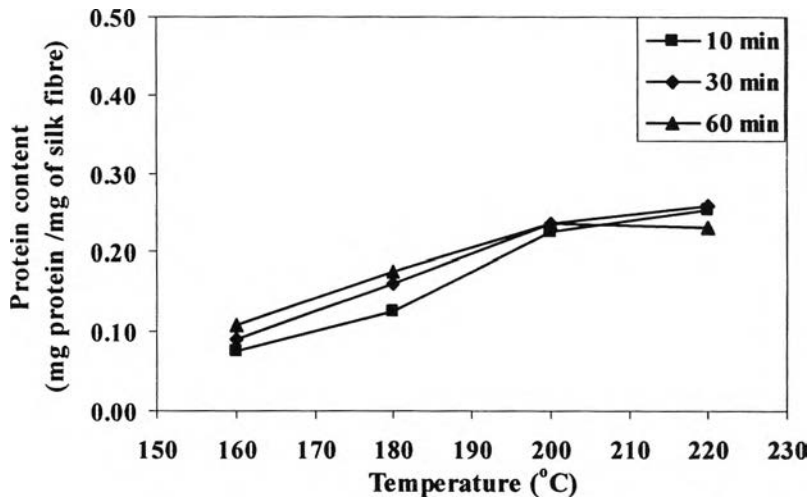
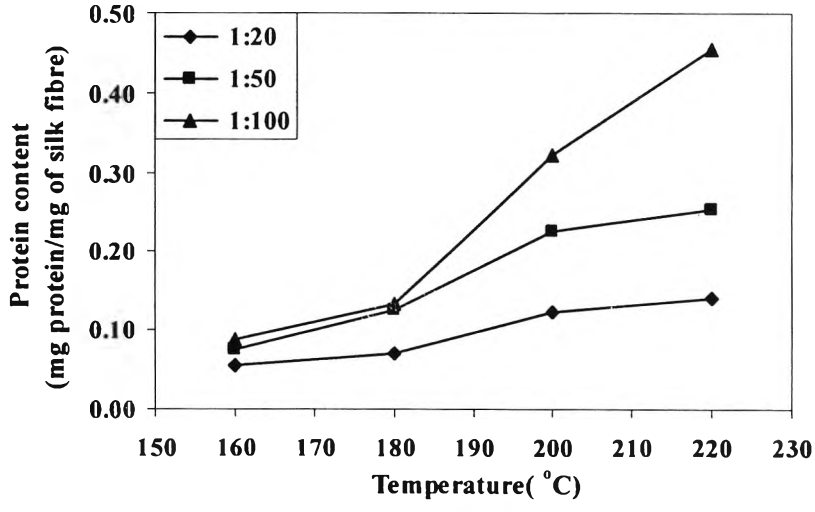
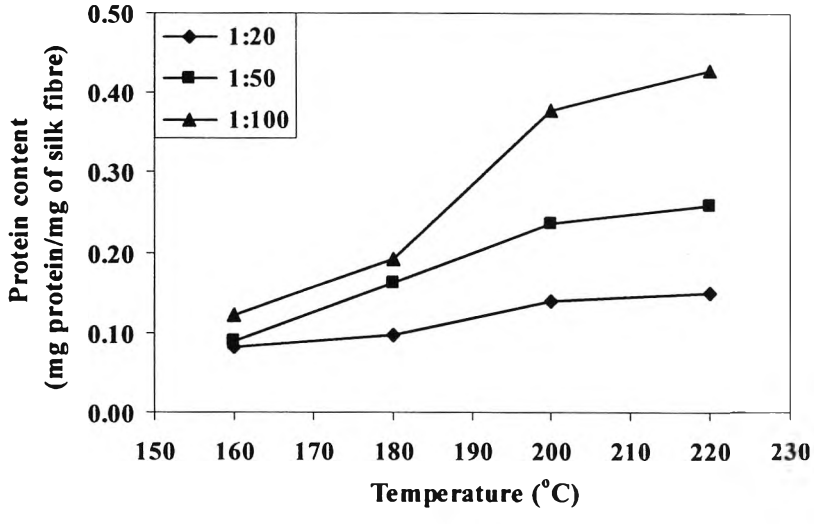


Figure 4.12 Protein yield after hydrolysis at different temperatures (1:50 silk:water ratio)

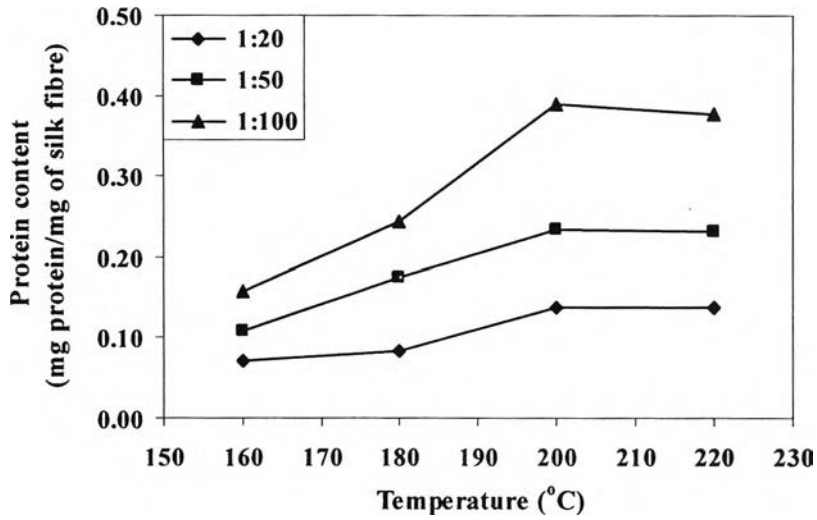
The effect of silk:water ratio on protein yield is shown in Figure 4.13 which showed that when silk:water ratio was lower (1:100), more protein was produced per gram of raw silk compared with at higher silk ratio, indicating that the high amount of water seems to favor the production of protein. Nevertheless the amount of water used causes increase in drying energy, thus if the product must be dried, the amount of water used in the reaction should be optimized concerning economic feasibility.


Protein content
(mg protein/mg of silk fibre)
0.50
0.40
0.30
0.20
0.10
0.00
1:20
1:50
1:100
150
160
170
180
190
200
210
220
230
Temperature(°C)

(a)


Protein content
(mg protein/mg of silk fibre)
0.50
0.40
0.30
0.20
0.10
0.00
1:20
1:50
1:100
150
160
170
180
190
200
210
220
230
Temperature (°C)

(b)



(c)

Figure 4.13 Effect of silk to water ratio on protein yield at different reaction times: (a) 10 min (b) 30 min (c) 60 min

4.3.3 Amino acids yield

The results for the yield of amino acids in the soluble products are shown in Figure 4.14 at different temperatures and times. As expected, the amino acid yields increased with increasing temperatures and times of reaction. This means that the rate of decomposition of amino acids to smaller organic acids was smaller than its production rate at these conditions. The highest amino acid yield was found to be 0.754 mg amino acids/ mg of silk fibre (220 °C, 60 min).

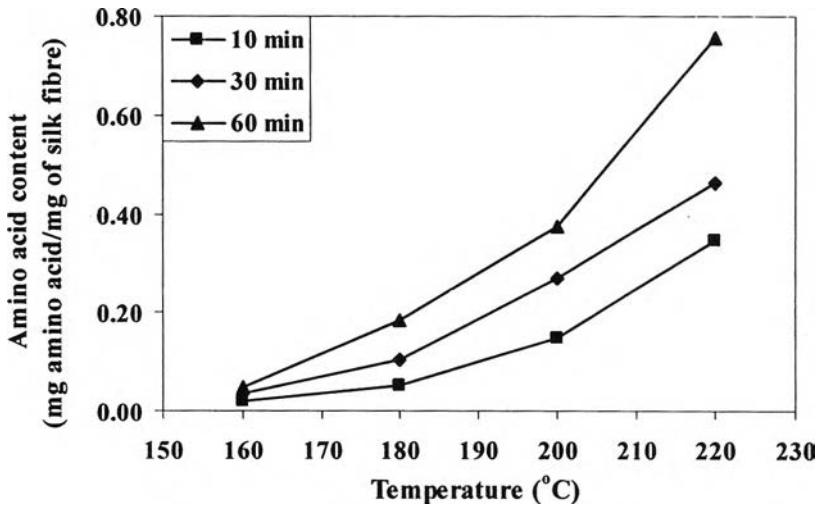
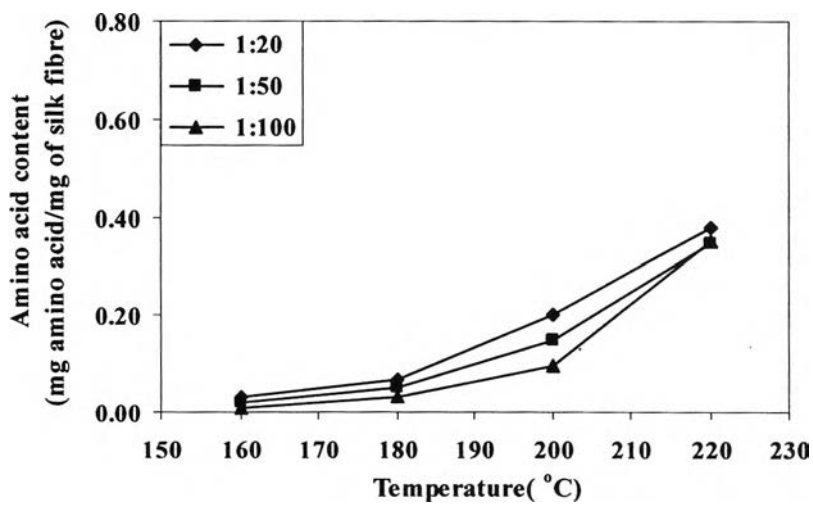
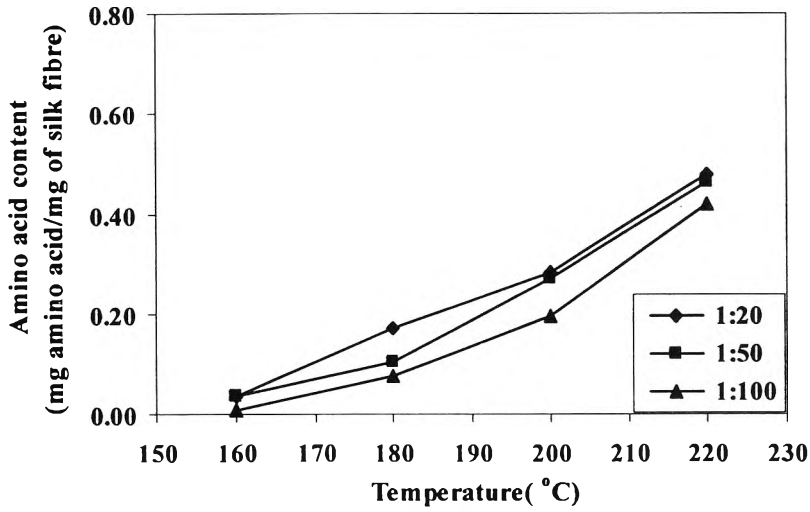


Figure 4.14 Amino acid yield after hydrolysis at different temperatures (1:50 silk:water ratio)

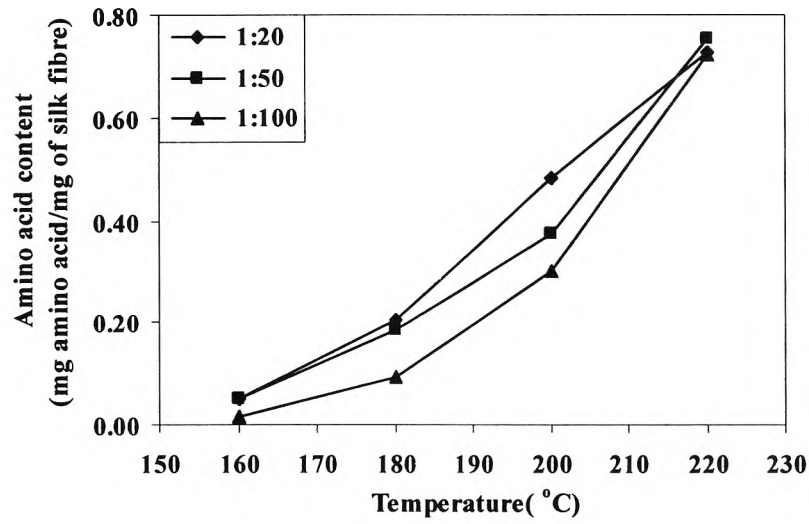
The effect of silk:water ratio on amino acid yield was shown in Figure 4.15. The results showed that more amino acids was produced per gram of raw silk when the silk to water ratio was higher (1:20). This is possibly because the conversion of protein to amino acids is influenced largely by homogeneous reaction. The system of lower silk to water ratio (1:100) might have resulted in more dilute reactant, thus the slower rate of amino acid production could achieved.



(a)



(b)



(c)

Figure 4.15 Effect of silk to water ratio on amino acid yield at different reaction times: (a) 10 min (b) 30 min (c) 60 min

4.4 Kinetic model for silk fibroin conversion in subcritical water

Kinetic model could be developed for the process of silk decomposition into protein and amino acids. Simplified reaction model could be depicted in Figure 4.16.

Silk sericin (s) → Sericin (aq)→ Protein solution (aq) → Amino acids (aq)
→ Degradation products (aq)

Silk fibroin (s) → Fibroin (aq)→Protein solution (aq) →Amino acids (aq)
→ Degradation products (aq)

Figure 4.16 Pathways of hydrolysis of silk sericin and fibroin to useful products

It is difficult to understand the kinetics of each of the reactions that take place, especially from the data we obtained with raw silk. The simplest kinetics model could however be proposed for the first step in which the silk fiber was converted in to the soluble product. However, based on the experimental data, sericin conversion occurred rapidly within 10 minute of reaction at low subcritical water conditions while conversion of fibroin occurred much more slowly. Due to the unavailability of data at the early times (<10 min) for sericin, it was not possible to develop a kinetic model for this reaction. Instead, we only deal with decomposition kinetics of fibroin. Two possible mechanisms of conversion of silk fibroin are depicted in Figure 4.17.

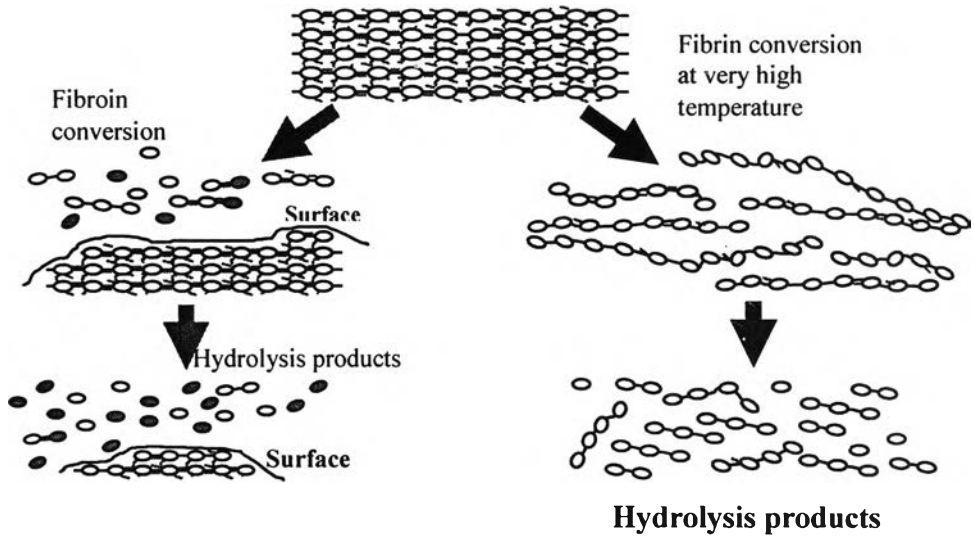


Figure 4.17 Reaction pathways of both a heterogeneous reaction and a homogeneous reaction

The first possible mechanism is a heterogeneous reaction, in which the reaction takes place at the surface of the silk fibroin sample. At very high temperature on the other hand, a homogeneous reaction becomes increasingly important. Since most of the conditions employed in this studied is considered quite moderate (subcritical region), heterogeneous reaction was used as a model. The surface reaction rate model is of the form:

$$\frac{dV(X)}{dt} = -k_s \cdot S(X) \tag{4.1}$$

where X is the conversion of silk fibroin determined from the change in weight of the silk fibre, k_s (cm s^{-1}) is the surface reaction rate constant, and S (cm^2) and V (cm^3) are the surface and the volume of the particle, respectively. From the micrograph of the silk fibre, it was seen that fibroin has a cylindrical shape. The following equation is resulted.

$$\frac{d\pi[r(X)]^2 l}{dt} = -k_s \cdot 2\pi r(X) l;$$

or

$$\frac{d[r(X)]^2}{dt} = -k_s \cdot 2r(X) \tag{4.2}$$

where r is the radius of the cylinder's cross sectional area, and l is the length of the cylinder.

The conversion of silk fibroin could be evaluated from the change of the silk weight or volume before (W_0 or V_0) and after hydrolysis time t (W and V), described by the following equation.

$$X = 1 - \frac{W(X)}{W(0)} = 1 - \frac{V(X)}{V(0)} = 1 - \frac{\pi[r(X)]^2 l}{\pi r_0^2 l} = 1 - \frac{[r(X)]^2}{r_0^2} \qquad (4.3)$$

Differentiating the above equation, we get

$$\frac{dX}{dt} = -\frac{1}{r_0^2}\frac{d[r(X)]^2}{dt} \qquad (4.4)$$

Also rearrangement of Equation 4.3 gives:

$$r(X) = r_0^2 (1 - X)^{1/2} \qquad (4.5)$$

After substituting equation (4.4) and (4.5) into (4.2), we get

$$\frac{dX}{dt} = \frac{2k_s}{r_0}(1 - X)^{1/2} \qquad (4.6)$$

By integrating this equation,the following equation was obtained:

$$k = \frac{k_s}{r_0} = \frac{1 - (1 - X)^{1/2}}{t} \qquad (4.7)$$

where k (min^{-1}) is the overall conversion rate constant of silk fibroin.

The $1-(1-X)^{1/2}$ values at all the reaction conditions were plotted against time. Figure 4.18 shows the typical results at 160, 180, 200, and 220 °C (silk:water ratio of 1:50). The values were proportional to t for all temperatures except at 220 °C. At 220 °C, silk fibroin was rapidly and completely decomposed into the solution after 10 min, which suggested that the reaction could be of homogeneous nature. At such temperature, the hydrogen linkages in fibroin is broken, the fibroin might disperse into high temperature water and form a homogeneous fibroin water reaction atmosphere. At each of the other reaction conditions, the k value can be determined from the slope of the straight line and the results are shown in Table 4.1.

Table 4.1 shows the reaction rate constant for conversion reaction of fibroin at difference ratio hydrolysis

Ratio of silk to water	Overall rate constant k (min^{-1})		
	160 °C	180 °C	200 °C
Ratio 1:20	0.0011	0.0051	0.0092
Ratio 1:50	0.0007	0.0025	0.0087
Ratio 1:100	0.0005	0.0023	0.0079

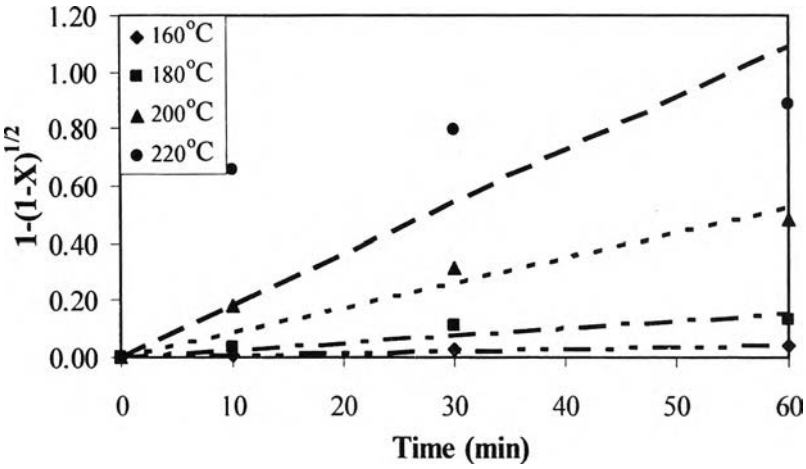


Figure 4.18 Relationship between the $1-(1-X)^{1/2}$ and the reaction time of hydrolysis (t) on the reaction of crystalline silk fibroin in subcritical water at ratio 1:50

Table 4.1 summarize the rate constants determined from the experimental data. For the kinetics of fibroin decomposition, the dependence of the rate constant on temperature could be explain by an Arrhenius Equation. Figure 4.19 shows the Arrhenuis plot of the of the experimental data obtained in the range 160-200 °C for 1:50 silk:water ratio. Based on the quantification of the kinetic data, the activation energy, E_A, was found to be 105.26 kJ/mol, and the frequency factors were 1.179×10^8, 5.946×10^9, and 79.022×10^9 min^{-1} for 1:20, 1:50, and 1:100 silk to water ratios, respectively.

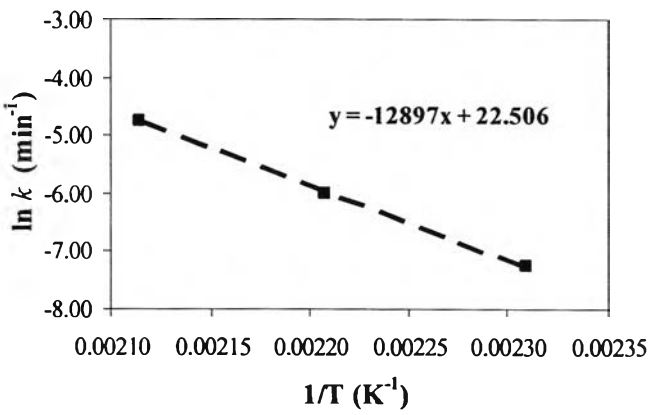


Figure 4.19 Arrhenius pot of the rate constant of conversion of silk fibroin (k) in subcritical water.

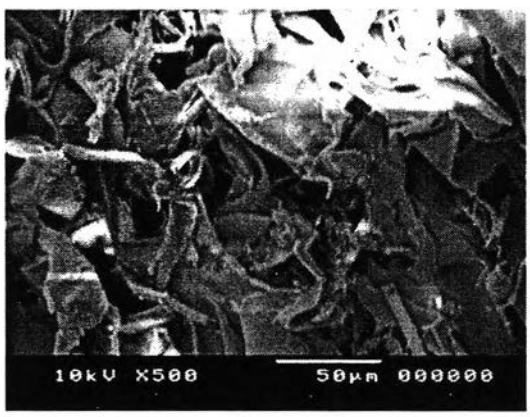
4.5 Characterization of powder of hydrolysis products

It is commonly known that particle size, morphology, conformation, and crystal structures could greatly depend on how the sample was prepared. For example, the drying process such as spray-drying or freeze-drying affect the particle size and shape and the rate at which particle cools can affect the crystalinity of the particles. In this study, it is not the main objective to determine such effects on the characteristics of the silk sericin and silk fibroin particles. However, the particles prepared from sericin and fibroin solutions obtained from selected conditions were characterized. The method employed for particle formation is freeze-drying at -40 °C for 24 h, after which the dried sample was mechanically ground into small particles. The particle size and size distribution was measured by a particle analyzer. The morphology of samples silk fibres and silk sericin and fibroin particles was examined under a scanning electron microscope at an acceleration voltage of 10 kV. The molecular structures of the sericin and fibroin powder were examined using a FT-IR spectrophotometer and an X-ray diffractometer. It should be noted that not all the powder prepared at all conditions could be characterized. Sericin resulted from hydrolysis reaction at 120 °C and 130 °C formed aggregate as they absorb water and was not possible to be ground into smaller particles. Thus, only more brittle sericin specimen (such as sericin prepared at 160 °C) could be analyzed under SEM, FT-IR, and XRD. In case of fibroin, the particles are quite brittle and were easier to be ground into small particle after freeze drying process. The particles prepared from the product obtained at hydrolysis condition of 200 °C, 30 min, in which protein and amino acid yield was the highest was selected for the analysis.

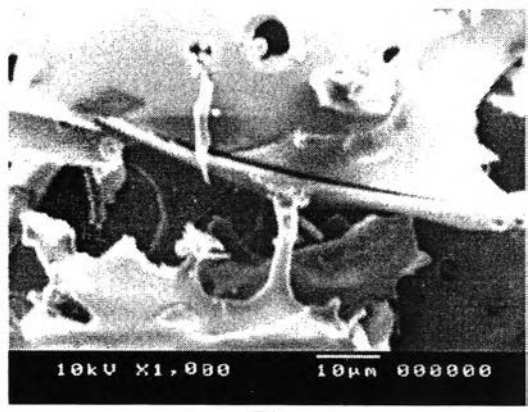
4.5.1 Particle size and morphology

The scanning electron micrograph freeze dried sample of sericin and fibroin and that of the ground samples are shown in Figure 4.20 (a-d). The freeze dried sericin sample in Figure 4.20 a (160 °C, 30 min) was found to be more condensed and compact and less brittle when compared to that of fibroin sample in Figure 4.20 b (200 °C, 30 min) which was more brittle and loosely packed. As mentioned earlier, sericin sample obtained

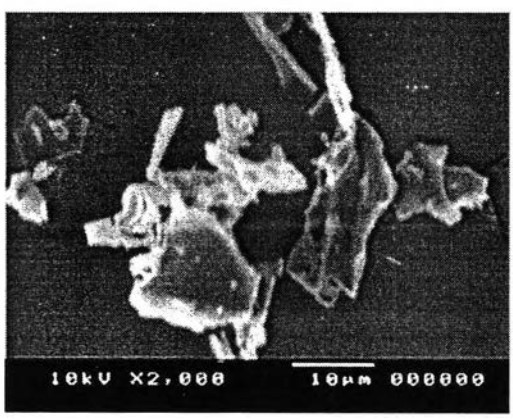
from hydrolysis at 120 and 130°C was viscous, and after drying, the sample was rather sticky and could not easily be disintegrated into small particles. At higher temperatures (140 °C and 160 °C), the sample brittleness increased and could be more easily flaked apart. The morphology of the disintegrated flakes of sericin was similar to those of fibroin sample. The particle analysis suggested that sericin and fibroin particles prepared in this study had an average size of 95.77 and 110.97 μm. The size is largely depends on the degree of mechanical force applied during breaking up the flakes. If desired, smaller particles could be prepared by mechanically grinding the particle further. Moreover, the method of drying could influence the particle size and shape as shown in Figure 4.20 (e and f) that sericin and fibroin particles prepared by spray drying has a spherical shape and smaller sizes (Sheng et al., 2001).



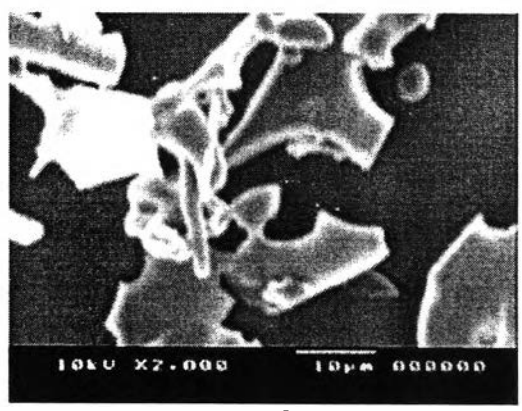
(a)



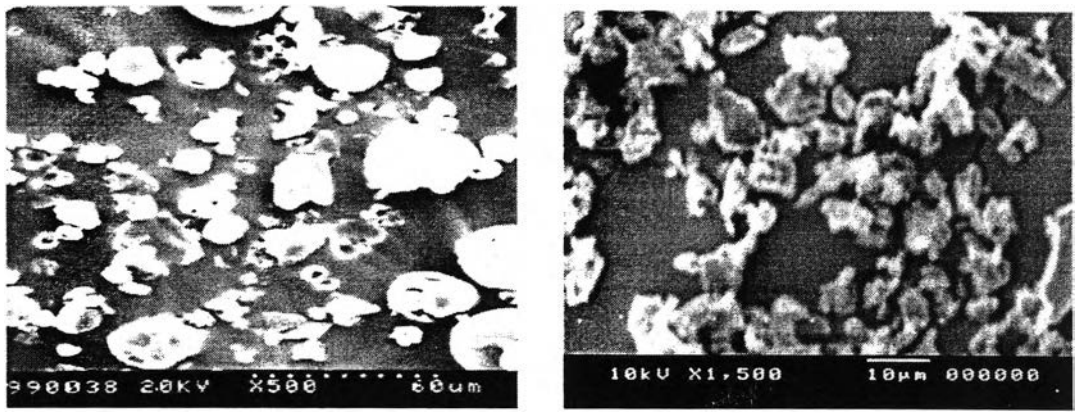
(b)



(c)



(d)

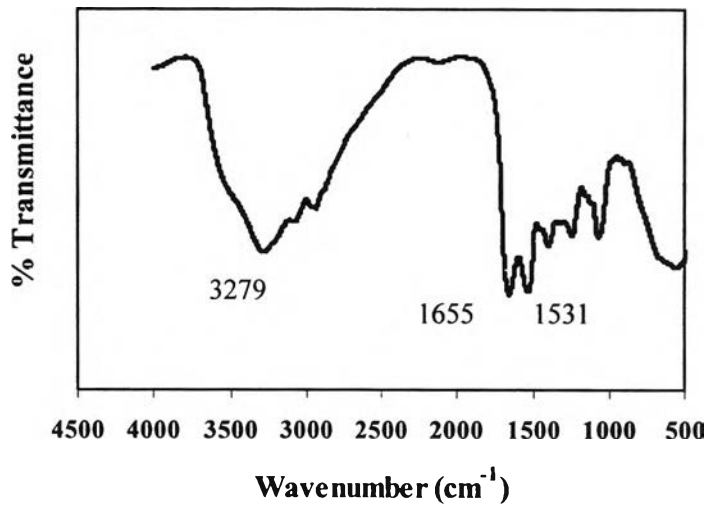


(e) (f)

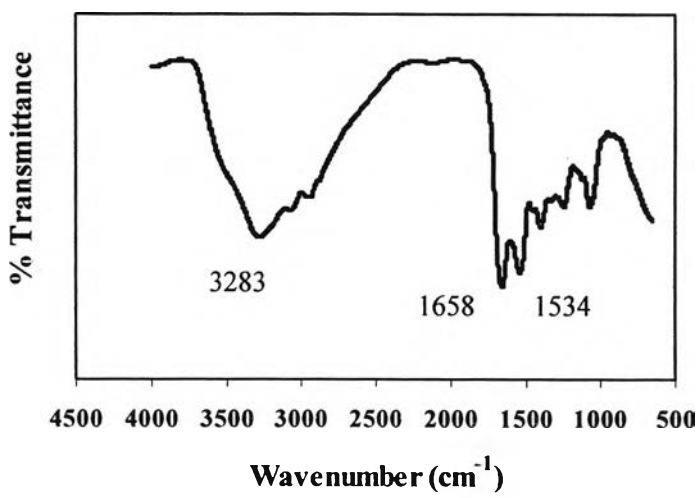
Figure 4.20 SEM of sericin and fibroin powder: (a) freeze-dried sericin powder from solution prepared at at 1:50, 160 °C and 30 min (b) freezed-dried fibroin powder from solution prepared at 1:50, 200 °C and 30 min (c) ground sericin powder from solution prepared at 1:50, 160 °C and 30 min (d) ground fibroin powder from solution prepared at 1:50, 200 °C and 30 min (e) Spray-dried sericin powder (Sheng et al.,2001) (f) spray-dried fibroin powder.

4.5.2 Fourier Transform Infrared Spectroscopy (FT-IR)

In this work, to determine the molecular conformation of silk powder, infrared spectra of silk fibers and particles were obtained by means of FTIR spectrometer (PerkinElmer, Spectrum One), in the spectral region of 4000-400 cm^{-1}. Figure 4.21 shows FTIR spectra of silk sericin obtained from autoclave (sercin powder I, a) and that was prepared from hydrolysis solution obtained at 150 °C and 30 min (sericin powder II, b). For the sericin powder I, the characteristic peaks were at 1531, 1655, and 3279 cm^{-1}. These peaks were those for amide II, amide I, and N-H stretching bands and represented the β-sheet and α-helix/random coil conformation, respectively (Hang et al., 2005 and Tomoaki et al., 2003). As seen from the figure, sericin powder II showed similar peaks patterns and whose peaks appeared at 1534, 1658 and 3283 cm^{-1}, indicating the prepared powder has β-sheet conformation and α-helix/random coil conformation similar to that obtained with the autoclaved product (Donna et al., 2000, Wang et al., 2005a and 2005b, and Hino et al., 2003).

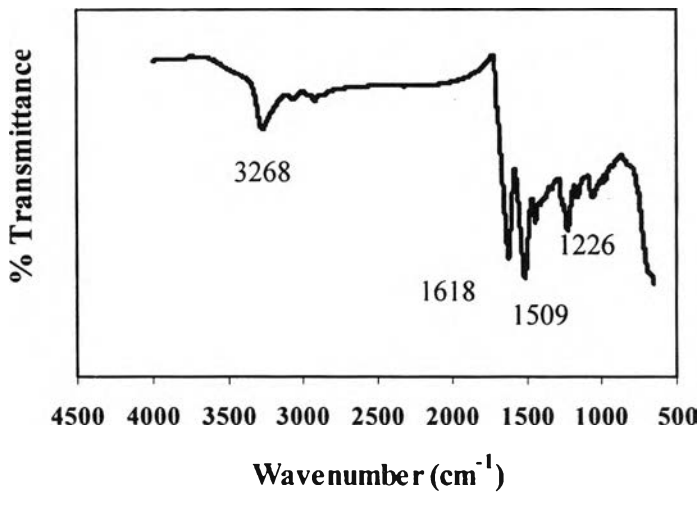


(a)

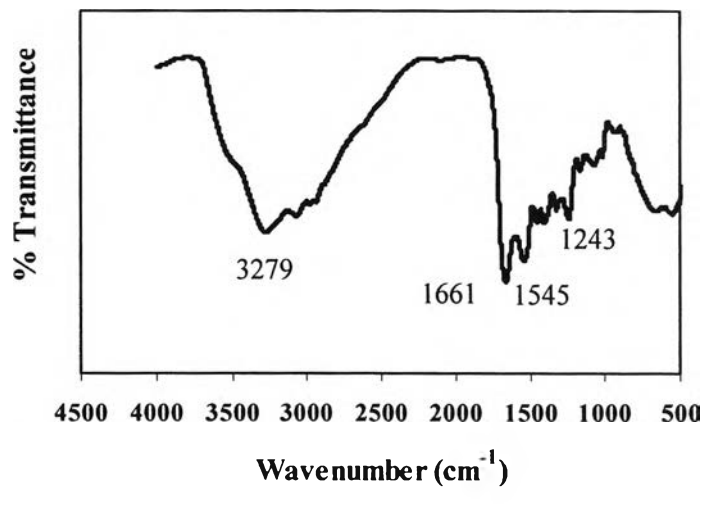


(b)

Figure 4.21 (a) Sericin powder was prepared by autoclave at at 1:50 120 °C 30 min (b) Sericin powder was prepared at 1:50 150 °C 30 min by subcritical water hydrolysis



(a)



(b)

Figure 4.22 (a) Fibroin fibre after degumming in autoclave (120 °C and 30 min) (b) Fibroin powder from solution prepared by subcritical water hydrolysis at 200 °C, 30 min, 1:50.

Figure 4.22 shows FTIR spectra of the starting fibroin fibre obtained after degumming at 120 °C for 30 min in an autoclave (a) and fibroin particles prepared from freeze-drying the solution obtained from at 200 °C and 30 min (b). For the former, the characteristic peaks appeared at 1226, 1509, 1618, and 3289 cm^{-1}. These peaks were

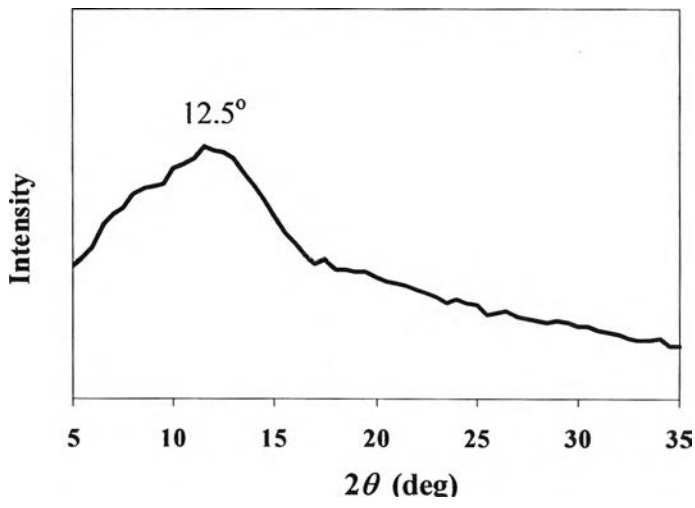
those for amide III, amide II, amide I, and N-H stretching bands and represent the β-sheet conformation (Wang et al., 2005a and 2005b, and Hino et al., 2003). The chracteristic peaks shifted to 1243, 1545, 1661 and 3279 cm^{-1} for the particles prepared from the hydrolyzed product, which indicated α-helix/random coil conformation (Donna et al., 2000, Wang et al., 2005a and 2005b, and Hino et al., 2003). This irregular α-helix/random coil conformation was a result of the cleavage of hydrogen bonds in the β-sheet structure of the orginial fibre during the hydrolysis reaction at high temperature (higher K_w). In addition, the results indicated that the method and condition of freeze-drying employed in this study did not allow recrystallization of fibroin to take place.

4.5.3 X-ray diffraction (XRD)

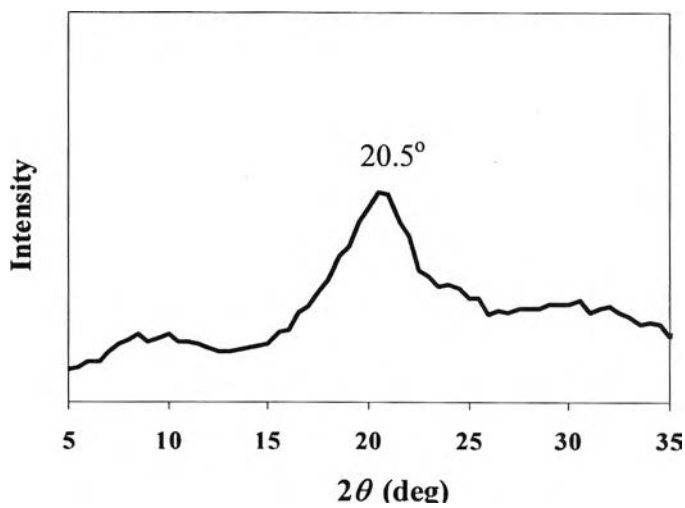
It is well known that silk sericin takes amorphous form while fibroin have both ordered (crystalline) and disordered (amorphous) regions due to the presence of long and flexible molecular chains which hinder further crystallization (Hino et al., 2003). Through X-ray diffraction measurement, some information about crystalline structure of silk fibroin and its conformations of α-helix and *β*-sheets can be obtained from its crystal structure transition. The principal X-ray diffraction peaks of silk I (α-helix) are 7.25×10^{-10} m (2θ = 12.2° with Cu Kα), 4.50×10^{-10} m (2θ = 19.7° with Cu Kα) and 3.60×10^{-10} m (2θ = 28.2° with Cu Kα), while those of silk II (*β*-sheets) are 9.7×10^{-10} m (2θ = 9.1° with Cu Kα), 4.69×10^{-10} m (2θ = 18.9° with Cu Kα), 4.3×10^{-10} m (2θ = 20.7° with CuKα) and 3.67×10^{-10} m (2θ = 24.3° with Cu Kα) (Wang et al., 2005a and 2005b).

In this study, XRD was conducted at ambient temperature on a diffractometer with CuKα radiation of wavelength 1.542 Å. The acceleration voltage was 30 Kv and 20 mA scaning rate was 0.5°/min and range was 2θ = 5 ~ 35° was used. The sericin and fibroin powder were studied and the XRD are shown in Figure 4.23. The XRD of sericin prepared from autoclaved sample showed a broad peak at 1:100, 120 °C and 30 min, indicate an α-helix form (Silk I). On the other hand, XRD of fibroin showed a pronounced peak at 2θ = 20.5°, which represented *β*-sheet conformation of Silk II. This

pattern was guite similar to the result of Hino et al. (2003). The coexistence of the peak means fibroin consists of crystalline portion.



(a)



(b)

Figure 4.23 XRD of (a) sericin powder prepared from autoclave sample (b) fibroin powder

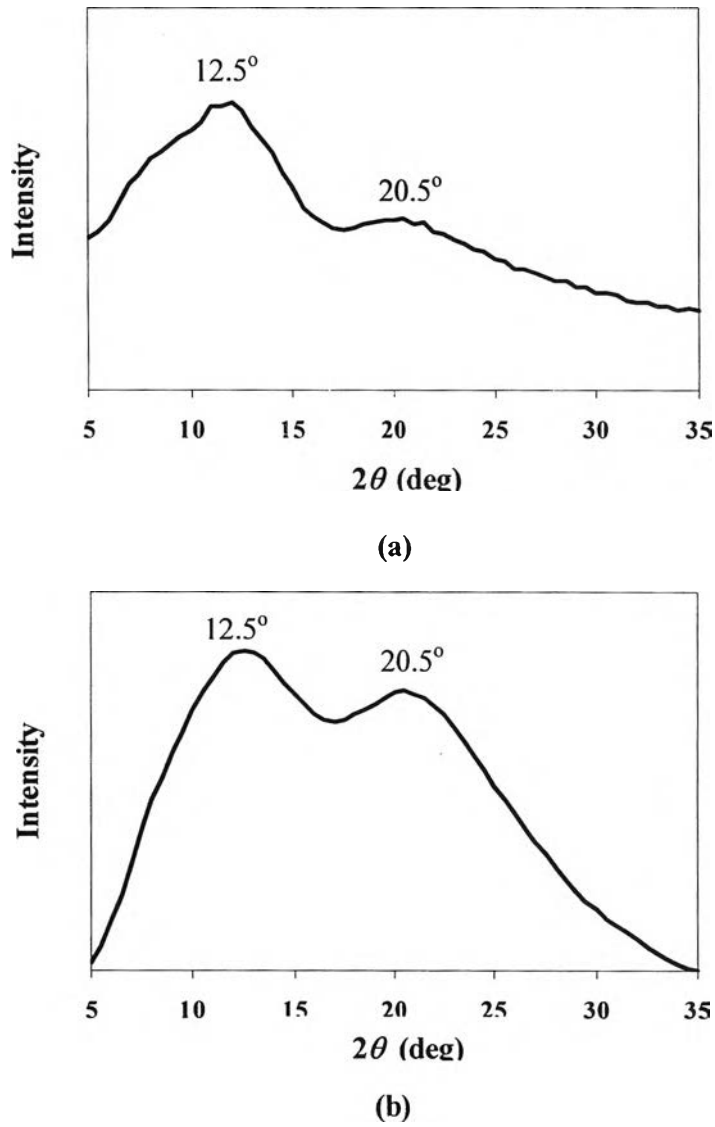


Figure 4.24 (a) XRD of sericin powder (1:50, 150 °C, and 30 min (b) XRD peak for fibroin powder (1:50, 200 °C and 30 min)

Figure 4.24 showed the XRD of sericin and fibroin particles obtained from the product of hydroysis. Broad peaks are observed at 12.5 and 20.5°, appear at the same location with the maximum peak of silk sericin and fibroin in Figure 4.23. For sericin, the peak intensitiy at 2θ = 20.5°, was low, while the intensity of the same peak was higher for the fibroin particles. In the case of sericin particles, the existence of two peaks instead of a single peak at 12.5° (as in the autoclaved sample) was due to the fact that the sericin

solution prepared in subcritical water at as high temperature as 160 °C actually contained the mixture of both sericin and fibroin. It should be noted however that these peaks are very broad, which means that the powders are highly amorphous. This could be a result of rapid cooling by quenching the reaction in cool water or rapid drying at the low freeze drying tempature of -40 °C. The latter explanation is supported by the study of Li et al. (2001) who reported that lyophilized silk fibroin was mainly amorphous if the freezing temperature was below −20 °C, while silk I was prepared if the temperature was above −20 °C. Based on this line of reasoning, it seems that the crystal structure of the particle formed could be greatly controlled by the method of particle preparation. Other report existed which indicated that the spray-drying process which involved rapid evaporation of water also caused the conformational change of fibroin from crystalline to amorphous structure (Ueno et al., 1998). Further studies are needed to determine the effect of cooling method and cooling rate on the conformation and the structure of these silk particles prepared from the aqueous solution.

4.5.4 Differential Scanning Calorimetry (DSC)

The thermal behaviors of samples were examined using a differential scanning calorimeter (NETZSCH DSC 204 F1, Germany), with the heating rate of 10 °C/min from room temperature to 350 °C.

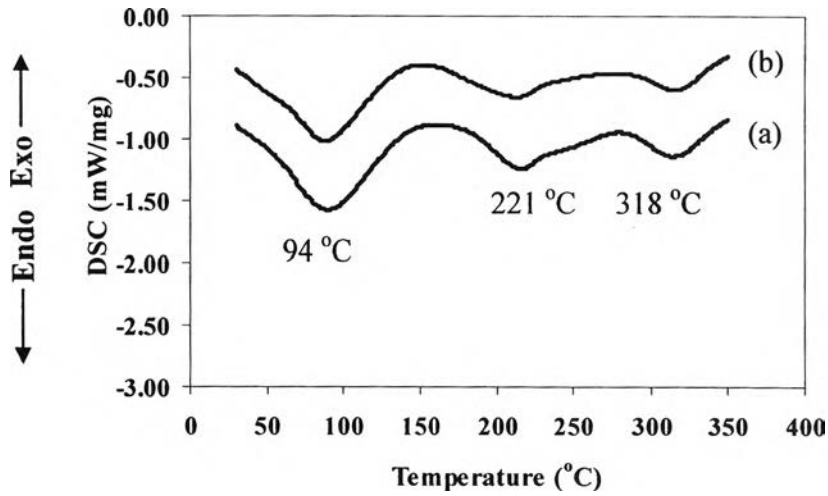


Figure 4.25 Thermogram of sericin powder obtained (a) at 120 °C, 30 min in an autoclave (b) at 150 °C 30 min in an SW reactor.

As shown in Figure 4.25 a, sericin particles obtained at 120 °C 30 min in an autoclave shows a water evaporation peak at around 94 °C and two endothermic peaks at around 221 and 318 °C. This suggested that the degradation temperatures, Td, of sericin powder began to occur at 221 °C. The powder prepared in subcritical water hydrolysis shows similar differential scanning calorimetric patterns (Figure 4.25 b), which implies that the conditions used for subcritical water hydrolysis of sericin did not induce significant molecular conformational changes.

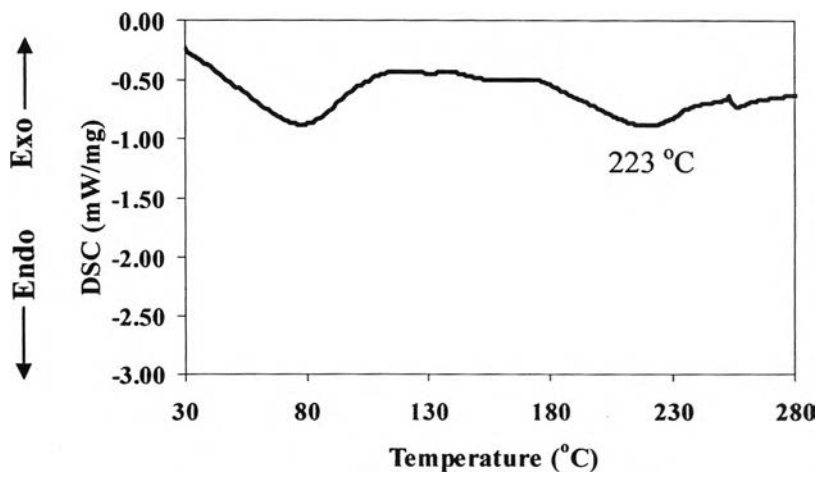


Figure 4.26 Thermogram of fibroin powder obtained at 200 °C, 30 min in an SW reactor

Figure 4.26 show the thermogram of the fibroin particles obtained by subcritical water hydrolysis at 200 °C, 30 min. The water evaporation peak appeared at around 100 °C and an endothermic peak appeared at 223 °C, at which thermal degradation began to occur (Td=223 °C). Compared with the Td of 317.27 °C reported in literature for fibroin fibre (Sheng et al., 2001), the fibroin powder prepared in this study degraded more readily. The result agreed more closely with the degradation temperature of the regenerated silk fibroin of 285 °C reported by Nam et al. (2001), for regenerated fibroin was prepared by dissolving in ternary solvent system of $CaCl_2/H_2O$/EtOH solution, freezing, and lyophilization.

The lower T_D obtained for the fibroin powder prepared in this study was due to the change in from β-sheet to α-helix/random coil conformation resulted by high temperature process.

4.6 Possible application of silk sericin and fibroin products

Based on the results in this study, it could be observed that by varying reaction temperature as well as particle preparation, hydrolysis products of different molecular sizes, composition, morphology, and structure could be obtained. These products have specific characteristics and are suitable for different applications. Based on molecular weight, generally, high molecular weight silk peptides (greater than 20 kDa) are mostly used as medical biomaterials, degradable biomaterial, compound polymers, functional biomembranes, hydrogels, and functional fibers and fabrics. Reviews of application of materials modified with sericin and sericin-fibroin and other sericin composites are given by Zhang (2002). The small sericin peptides are reported to be water soluble and have excellent moisture absorption and release as well as important biological activities such as antioxidation, tyrosinase activity inhibition, and pharmacological functions such as anticoagulation, anti-cancer activities, cryoprotection, and digestion promotion. Lower molecular weight sericin peptides (less than 20 kDa) are used in cosmetics including skincare and hair care products, health products, and medications.

An increasingly important application of silk fibroin is that related to tissue engineering. Silk fibroin offers versatility in matrix scaffold design for a number of tissue engineering needs in which mechanical performance and biological interactions are major factors for success, including bone, ligaments, tendons, blood vessels, and cartilage. Silk fibroin can be solubilized in appropriate solvent and has been successfully processed into foams, films, fibers and meshes. Thus, it is of interest to further examine the possibility of applying the fibroin solution prepared in subcritical water for this application.

When the hydrolysis reaction was carried out at high temperature or for a long period of time, smaller amino acids are produced. These smaller amino acid products also have wide uses and applications pharmaceuticals, food products, animal nutrition, and cosmetic industries. As medicine, they can be used for the treatment of various diseases such as renal, gastrointestinal, endocrinal, and dermal among others. In the food industry, amino acids are utilized as taste enhancer and animal feeds [Kang, 2004]. Moreover, amino acids undergo further decomposition in subcritical water to other organic compounds such as organic acids. Among the decomposition products are amino acids including formic, lactic acid, acetic acid and propionic acid, which are important starting materials for various chemical industries.