CHAPTER IV

CHARACTERISTIC OF TRADITIONAL THAI SILK COCOON: COMPARISON BETWEEN NANG NOI, NANG LAI, DOK BUA AND LUANG PAIROTE SPECIES

4.1 ABSTRACT

This study aimed to investigate the characteristics of *Bombyx mori* Thai silk sericin and compare between four different species; Nang Noi, Nang Lai, Dok Bua and Luang Pairote. Silk sericin was extracted by heat under pressure and using freeze-drying technique in order to obtain dried silk sericin powder. Four species of silk sericin powder was characterized the solid contents by weighing method, the functional groups by Fourier Transform Infrared Spectroscope (FTIR), thermal stability by Thermogravimetric-Differential Thermal Analyzer (TG-DTA) and total amino acid compositions by High Performance Liquid Chromatography (HPLC). The FTIR spectra of four species showed the similar characteristic peaks of amino acid. Luang Pairote species provided the highest silk sericin content and Dok Bua gave the lowest silk sericin content. Thermal stability of Nang Noi, Nang Lai and Luang Pairote almost similarly but Dok Bua showed slightly lower decomposition temperature than others. The total amino acid compositions were different in each species. The majority of amino acids in silk sericin were serine, aspartic acid and glycine, respectively. Owing to the high content of amino acid with polar side groups, silk sericin exhibited the hydrophillicity that could be confirmed by initial weigh loss occurred from the absorbed moisture in TGA thermograms.

Keywords: Silk sericin; Thai silk; Silk protein; Sericin extraction

4.2 INTRODUCTION

Sericulture in Thailand has been established for more than 100^{th} years ago. Thai silk fabric and its products are famous, unique and valued products of Thailand. Thai silk farmers gain around two billion baht per year from sericulture and Thai silk exports amount around one billion baht annually (The government public relations department, 2004).

The most commercial silk originated from mulberry silk worm, *Bombyx mori*. Silk cocoon composed of two major proteins called fibroin and sericin formed in the form of fibroin-sericin composite. Fibroin is the fiber like protein surrounding by sericin, the globular glue like protein constituted around 20-30% of the cocoon (Kundu *et al.*, 2008). Silk sericin contained 18 amino acid and most of them have strongly polar side groups such as amino, hydroxyl and carboxyl groups. Silk sericin exhibited hydrophilic nature due to the high content of amino acid serine and aspartic acid, approximately around 33% and 16%, respectively (Zhang, 2002). In silk textile industry, sericin must be separated from fibroin by the process called "degumming" and mostly leaved in silk waste water resulting in the environmental contamination. In waste water contained silk sericin, the environmental pollution comes from the high oxygen demand for the degradation by microorganisms. The high concentration of BOD, COD and nitrogen make it difficult to treat (Vaithanomsat *et al.*,2008, Aramwit *et al.*, 2012). The recovery of silk sericin is the desirable way to solve this problem. Even though silk sericin is an invaluable by-product from silk industry, it also has the outstanding properties broadly ranges from cosmetics to biomedical purposes.

There are many methods to extract silk sericin from silk cocoon such as acid or alkali degumming but the most common way in traditional sericulture in Thailand is "hot water degumming". The advantage of this method is the obtained silk sericin has high purity and less contamination. In silk industry, they usually extract silk sericin by soap and detergent so as to reduce the fibroin damaging (Aramwit et al., 2012). To obtain high quality of silk sericin for the laboratory scale in further studies, extraction under pressure and heat is the most common method has been selected. In order to recovery silk sericin from the degumming water, several methods have been applied. Previous studied suggested that ethanol precipitation is the one of the effective method to recovery silk sericin because of the high yield of gained silk sericin (Wu *et al.*, 2007). Membrane filtration, freeze-drying and tray drying are the successfui methods to get silk sericin powder recommended from many studies.

Freeze-drying method is suitable to produced purified silk sericin powder in order to use this powder in the biomedical filed owing to the low protein denature (Vaithanomsat *et al.*, 2008).

Silk has been attended in the medical and biological application for a period of time. Fibroin is well known for the excellent raw material used to create 2D or 3D scaffold for hard tissue due to high tensile strength. Silk sericin exhibited higher hydrophillicity hence silk sericin has been widely use in moisturizer, anti-wrinkle and anti-aging cosmetics, wound dressing and scaffold for soft tissue. Beside the ability to absorb moisture, promote cell growth, low immune response etc., silk sericin also has the unique biological activities such as anti-oxidant, anti-microbial, anti-tyrosinaes, UV protection etc. Sarovart *et al.* (2003), reported the anti-oxidant, anti-fungal and anti-bacterial activities of different species of Thai silk sericin; Nang Noi, Dok Bua and Jul. They found that different species showed dissimilar of biological activities. This result is an evident that different species of silk sericin has certain dissimilar characteristics which affected to the properties of silk sericin.

In this study, the characteristic of four different species of silk sericin from Thai silk; Nang Noi, Nang Lai, Dok Bua and Luang Pairote, were investigated. Silk sericin was extracted by using pressure and heat and freeze-dried to obtain silk sericin powder. Silk sericin powder from four species were determined the solid contents by weighing method, the functional groups by Fourier Transform Infrared Spectroscope (FTIR), thermal stability by Thermogravimetric-Differential Thermal Analyzer (TG-DTA) and total amino acid compositions by High Performance Liquid Chromatography (HPLC).

4.3 EXPERIMENTAL

4.3.1 Materials

Four different species of Thai silk cocoon (*Bombyx Mori*); Nang Noi, Nang Lai, Dok Bua and Luang Pairote were purchased from local silk sericulture in Thailand.

4.3.2 Extraction of silk sericin

20 g of silk cocoons were cut in to small pieces about 5×5 mm^2 and mixed with purified water. Silk cocoons were autoclaved under pressure of 0.8-0.9 atm at 120°C for 60 min. The waste cocoons (fibroin) were filtered out to obtain the silk sericin solution. This method was repeated 2 times in order to extract silk sericin from cocoons as much as possible.

4.3.3 Preparation of silk sericin powder

Silk sericin solution was frozen in the glass shells at -40°C for 12 hr. After freezing, the glass shells were put in to a freeze-dryer maintained at -110 °C for 48 hr. Then, freeze-dried silk sericin was grinded in to powder.

4.3.4 Characterizations

4.3.4.1 Solid Contents

The solid contents of silk sericin were determined by weighting method. After freeze-dried, silk sericin powder was weighted in order to obtain the dried weight of silk sericin. This method was repeated at less 3 times in each species. The percentage of solid content of silk sericin was calculated by

$$\%\, silk\ sericin = \frac{g\ of\ freeze\text{-}dried\ silk\ sericin\ powder}{g\ of\ dry\ silk\ cocoons} \times 100 \qquad \text{(eq. 4.1)}$$

4.3.4.2 Fourier Transform Infrared Spectroscope (FTIR)

The functional groups of freeze-dried silk sericin were analyzed by Thermo Nicolet Nexus 670 FTIR Spectrometer. About 1-2 mg of silk sericin powder was ground with KBr and was pelletized into the thin pellet with the thickness less than 0.5 mm. The spectra were recorded over the wavenumber range of 400-4,000 cm^{-1} with the resolution of 4 cm^{-1} and the number of scan at 64.

4.3.4.3 High Performance Liquid Chromatography (HPLC)

Amino acid composition of freeze-dried silk sericin was analyzed by Waters Alliance 2695 High Performance Liquid Chromatography using Hypersil Gold column C18 (4.6*150mm, 5um). The method was according to the in house method based on J. of AOAC, Vol.78 No.3, 1995 (CIF T001). Silk sericin was hydrolyzed in 6 N of HCl to obtain sericin hydrolysate. The internal standard was added in the hydrolysate and dilute with deionized water. The sample was derivatized by mix filtrate with AccQ-fluor derivatization buffer and AccQ-fluor reagent and heat at 55 °C for 10 minutes. Sodium acetate buffer pH 4.90 and 60 % acetonitrile were used as an eluents.

4.3.4.4 Thermogravimetric-Differential Thermal Analyzer (TG-DTA)

Thermal stability of freeze-dried silk sericin and silk sericin/PVA/clay aerogels were examined by Perkin-Elmer Pyris Daimond Thermogravimatric Analysis. The weight of sample was in the range of 5-7 mg and heated at the heating rate of 10 °C/min from 30-900 °C in nitrogen atmosphere under nitrogen flow at 30ml/min.

4.4 RESULTS AND DISCUSSION

4.4.1 Characteristic of four species of Thai silk

Table 4.1 The characteristic and background of four species of Thai silk

Species	Color of cocoons	Color of sericin	Country of origin	Details
Nang Noi[a]	Yellow	Yellow	Thai	Polyvoltine
Nang Lai[a]	Yellow	Yellow	Thai	Polyvoltine
Dok Bua[b]	Pale Yellow	Pale Yellow	Thai (Nang Noi) × Japan(Guang Nong No.3)	Polyvoltine × Bivoltine
Luang Pairote[b]	Yellow	Pale Yellow	Thai (Nang Lai) × Japan (J108)	Polyvoltine × Bivoltine

** Shape of cocoon [a)] Spindle shape and [b)] Oblong shape

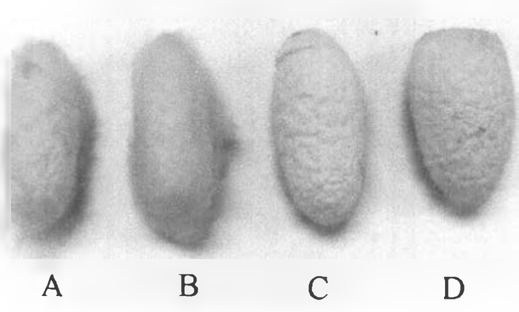

Figure 4.1 Four species of Thai silk cocoons : A. Nang Noi, B. Nang Lai, C. Dok Bua and D. Luang Pairote.

4.4.2 Chemical analysis of silk sericin

Figure 4.2 shows the FTIR spectra of freeze-dried silk sericin (*B. mori*) extracted from four species of Thai silk cocoon; Nang Noi, Nang Lai, Dok Bua and Luang Pairote. All species of Thai silk sericin presented almost similar absorption bands. The spectra showed the characteristic peak of amino acid, composed of N-H stretching band around 3288-3291 cm^{-1} (Sarovart *et al.*,2003), amide I band around 1653-1658 cm^{-1} associated with the C=O stretching, amide II band around 1529-1535 cm^{-1} associated with NH bending and CN stretching, amide III band around 1242-1246 cm^{-1} and amide IV band around 1072-1073 cm^{-1} (Srihanam *et al.*, 2009). Moreover, the board peak around 3600 cm^{-1} corresponded to OH stretching appeared due to the high content of amino acid serine, this characteristic peak appeared nearby the characteristic peak of N-H stretching hence overlapping of this two peaks was occurred.

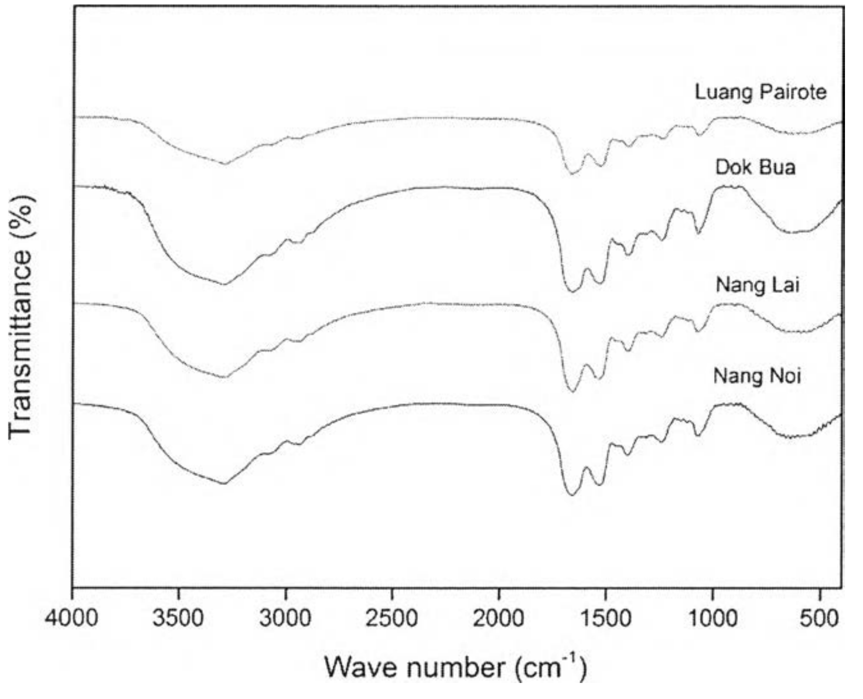

Figure 4.2 FTIR spectra of freeze-dried silk sericin extracted from four species of Thai silk cocoons.

4.4.2 <u>Solid contents of silk sericin</u>

Silk sericin is the glue-like glycoprotein constituted around 20-30% of silk cocoon (Kundu *et al.*, 2008). Table 4.2 shows the solid contents of silk sericin from four different species of Thai silk cocoon. The highest silk sericin content was found in Luang pairote species and the lowest silk sericin content was detected in

Dok Bua species. Because of high silk sericin content, Luang Pairote cocoons were dense and less fiber pilling observed on the surface. On the other hand, fiber pilling was observed in Nang Noi and Nang Lai cocoons due to the lower natural glue content in fibroin-sericin composite. Surprisingly, Dok Bua cocoons were dense and less fiber pilling but give the lowest solid content of silk sericin.

Table 4.2 Solid content of silk sericin from four species of Thai silk cocoon

Species of Thai silk	Sericin content per 20 g of silk cocoons (g)	Sericin content per 100g of silk cocoon (%)	Avg. weight of 1 cocoon	Sericin content per 1 cocoon (g)
Nang Noi	4.52±0.27	22.60	0.098	0.022
Nang Lai	4.40±0.10	22.00	0.094	0.021
Dok Bua	4.30±0.03	21.50	0.188	0.040
Luang Pairote	5.26±0.04	26.30	0.189	0.050

4.4.3 Total amino acid compositions of silk sericin

Silk sericin is hydrophilic protein contains 18 amino acids and most of which have strongly polar side groups such as hydroxyl, amino and carbonyl groups. The hydrophillicity of silk sericin comes from high amino acid serine content (Zhang *et al.*, 2002). Table 4.3 shows the total amino acid compositions of freeze-dried silk sericin from four species of Thai silk cocoons characterized by HPLC technique. The results showed the majority of amino acids in each species were serine, aspartic acid and glycine, respectively. In particulars, the amino acid serine, aspartic acid, and glycine constitute around 26 - 28 %, 17-18 % and 8 % of silk sericin, respectively. The total amino acid compositions were slightly different in each species. The HPLC chromatograms of four species of Thai silk cocoon are shown in Figure 4.3-4.6. Due to the high content of amino acid with strongly polar side groups made silk sericin can soluble in hot water. This property is necessary for the degumming process especially the extraction silk sericin by using high pressure and heat. For cell culture applications, the hydrophillicity of the silk sericin will be facilitated the cell attachment because it will be enhanced the penetration of aqueous cell suspension.

The amino acid composition showed in Table 4.3 was detected only 15 type of amino acid. The others 3 type were amino acid containing sulfur (methionine and cysteine) and aromatic ring (tryptophan) with had very small contents (Wu *et al.*, 2007).

Table 4.3 Total amino acid compositions of silk sericin from four different species of Thai silk cocoons

Amino acid	**g amino acid per 100 g of silk sericin**			
	Nang Noi	Nang Lai	Dok Bua	Luang Pairote
Aspartic acid	17.80	17.96	18.04	18.38
Serine	26.81	26.00	27.57	28.74
Glutamic acid	5.80	5.80	5.20	5.29
Glycine	8.53	8.50	8.80	8.60
Histidine	1.51	1.40	1.51	1.29
Arginine	4.32	4.17	4.56	4.46
Threonine	7.61	7.47	7.98	8.21
Alanine	3.51	3.55	3.64	3.42
Proline	0.74	0.76	0.73	0.60
Tyrosine	4.08	3.73	4.50	4.08
Valine	3.07	2.92	3.23	3.13
Lysine	3.60	3.66	3.24	3.25
Isoleucine	0.87	0.85	0.88	0.76
Leucine	1.30	1.28	1.31	1.16
Phenylalanine	0.56	0.53	0.53	0.45

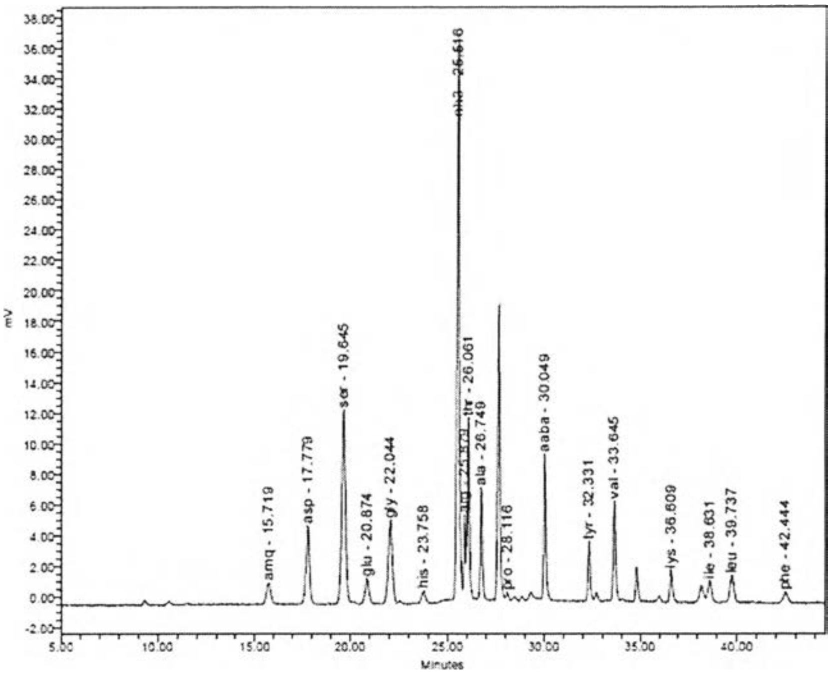

Figure 4.3 HPLC chromatogram of silk sericin from Nang Noi species.

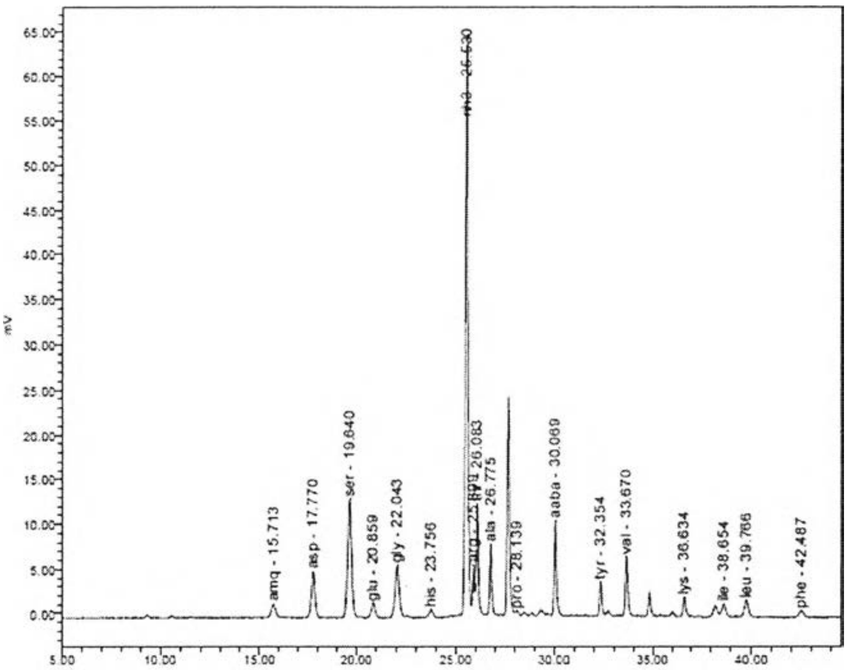

Figure 4.4 HPLC chromatogram of silk sericin from Nang Lai species.

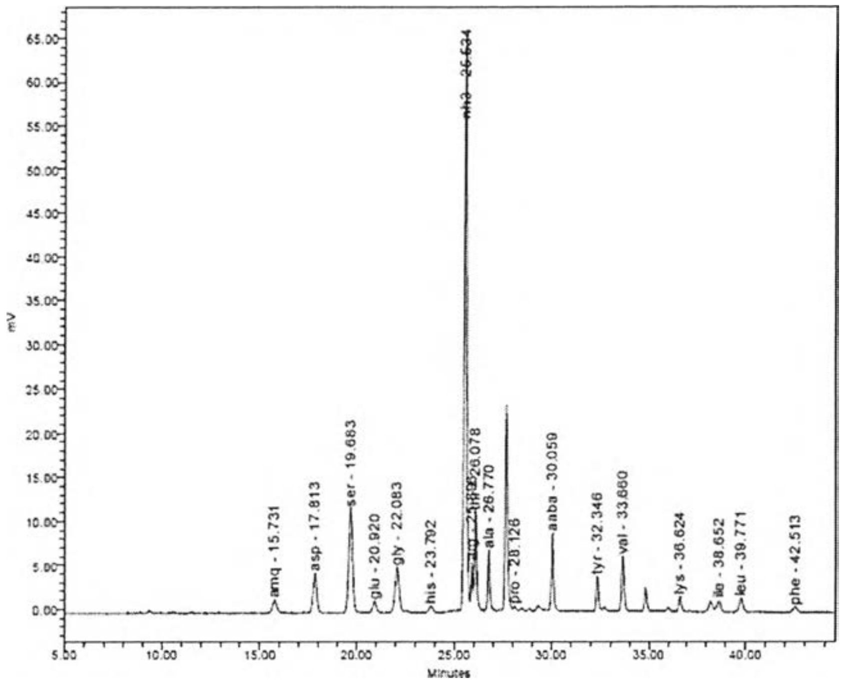

Figure 4.5 HPLC chromatogram of silk sericin from Dok Bua species.

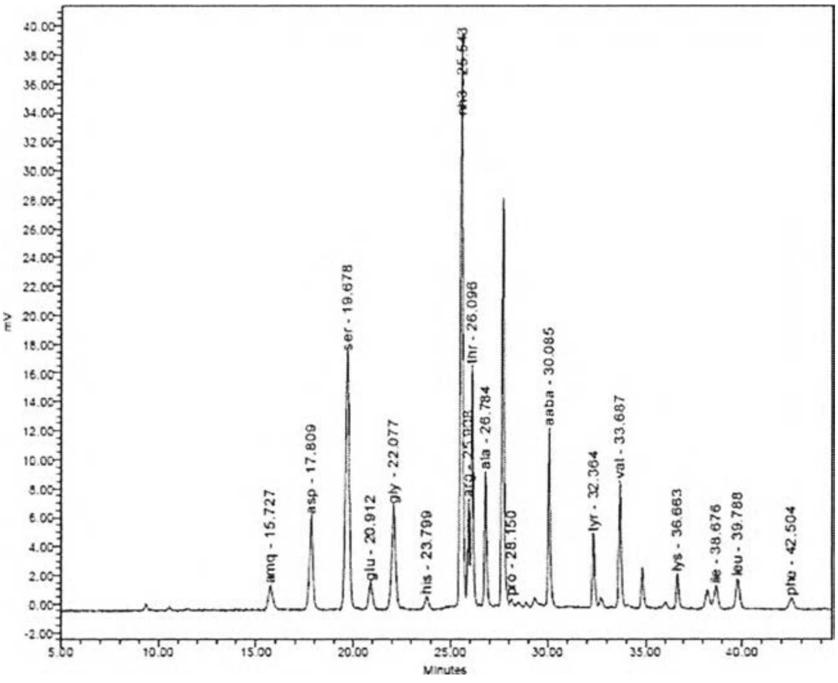

Figure 4.6 HPLC chromatogram of silk sericin from Luang Pairote species.

4.4.4 Thermogravimetric-differential thermal analyzer

Figure 4.7 shows the TGA thermograms of silk sericin from four different species of Thai silk cocoons. The initial weight loss at below 100 °C was due to water evaporation (~5-10 % weight loss) because of the hydrophillicity of silk sericin. The weight loss was mainly occurred around 250 °C and the peak degradation temperature of silk sericin was around 300 °C. Silk sericin Dok Bua had lower decomposition temperature than other species. However, the silk sericin did not completely decompose at 900 °C (Srihanam *et al.*, 2009).

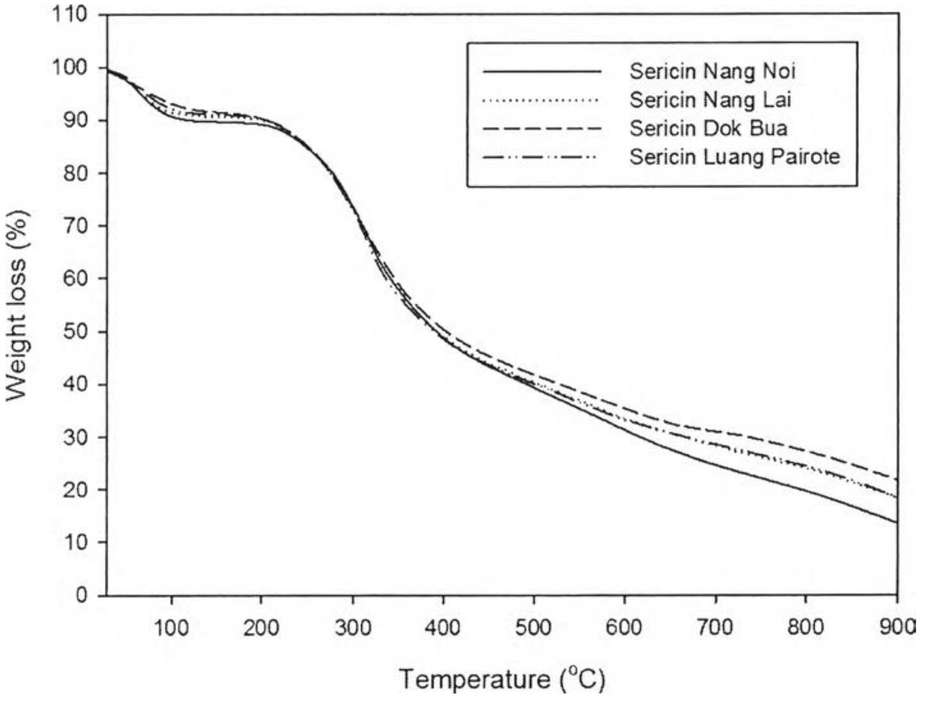

Figure 4.7 TGA thermograms of silk-sericin from four different species of Thai silk cocoons.

Table 4.4 Thermal stability of Thai silk sericin from four different species

Species of silk sericin	T_d onset (°C)	Peak decomposition temperature (°C)	Char residual (%)	Water absorbed (%)
Nang Noi	258.5	314.2	21.7	10.1
Nang Lai	257.9	315.0	25.7	8.8
Dok Bua	244.0	307.9	29.3	7.6
Luang Pairote	255.6	312.8	24.7	8.3

4.5 CONCLUSIONS

Silk sericin powder from four different species of Thai silk cocoon: Nang Noi. Nang Lai, Dok Bua and Luang Pairote, was prepared by freeze-drying technique. The slightly dissimilar characteristics in each species were exposed. The highest silk sericin content was found in Luang Pairote and the lowest silk sericin content was found in Dok Bua. Dok Bua showed the slightly lower in thermal behavior compared with other species. The total amino acid compositions suggested the hydrophillicity of silk sericin came from the high content of amino acid serine. In conclusion, the different in the origin of silk is not significantly affected on the characteristic like chemical analysis, thermal stability. The dissimilar characteristic was detected in total amino acid compositions and the solid content of silk sericin.

4.6 ACKNOWLEDGEMENTS

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