CHAPTER 3

RESULTS AND DISCUSSION

In this research, firstly, pigment extraction from silk waste was conducted to investigate the suitable extraction conditions. Next, sericin protein was extracted from the silk waste in alkali buffer and surfactant solutions, then precipitated with acetic acid. Finally, the degummed silk waste was dissolved by a mixed solution of $CaCl_2/CH_3CH_2OH/H_2O$. The sericin and fibroin proteins were purified by dialysis and then freeze dried to obtain powders. The quantities of pigments and also sericin and fibroin proteins were determined gravimetrically. The results in detail are described as the following.

3.1 Pigment Extraction and Determination of total pigments

Small piece of weighed silk cocoon from The Queen Sirikit Sericulture Centre, Nan Province was used to find the appropriate pigment extraction condition. The optimization was initially started by preliminary study of solvent selection. Then factors affecting the extraction of yellow Thai silk cocoon sample from Nan province were investigated and the quantity of total pigments (w_2) from two yellow Thai silk cocoon samples (*Nangnoi Si Sa Ket*) obtained from Nan and Chiang Mai provinces were also determined. The *Nangnoi Si Sa Ket* silk cocoon samples of *B. mori* silkworms obtained from Nan and Chiang Mai provinces in Thailand is shown in Fig.3.1.

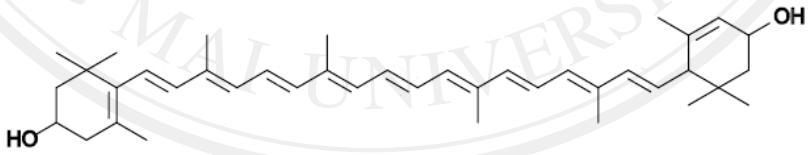
Fig. 3.1 *Nangnoi Si Sa Ket* silk cocoon samples of *B. mori* silkworms obtained from Nan and Chiang Mai provinces in Thailand.

3.1.1 Solvent selection

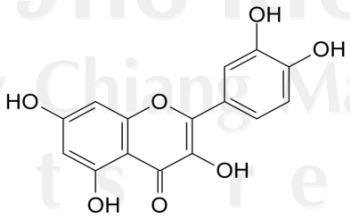
The solvents used for pigment extraction consist of hexane, toluene dichloromethane, ethanol, acetone, methanol and water of which their polarities are arranged in order of increasing polarity index. The yellow color of pigment extracted was observed and shown in Fig 3.2.

Fig. 3.2 The yellow color of pigment extracted by various solvents.

From Fig. 3.2, color intensity of the pigment extracted in the order from more to lower intense are ethanol > methanol > dichloromethane ≈ water > toluene > hexane ≈ acetone. Alcohol group provided the highest color intensity and the most efficient extraction when compared with other solvents. The effectiveness of solvent extraction depends on its ability to adequately dissolve pigment while leaving other materials unaffected. It appeared that the compound responsible for the unique yellow color mostly associated with carotenoids and flavonoids. The major carotenoid in the yellow color silk was reported to be lutein about 80 – 90% of total carotenoids as shown in Fig 3.3a whereas the β-carotene and α-carotene are minor components [56, 57] and phenolic compounds particularly flavonoid come from mulberry leaves as quercetin as shown in Fig 3.3b. Both of these pigments contain some hydroxyl groups (-OH), they can often be dissolved easily in alcohol which means that compounds extracted have similar polarity with the solvents. Thus, polar solvent of alcohols are suggested to be good enough for pigment extraction.



(a)



(b)

Fig. 3.3 Chemical structures of (a) lutein (carotenoid) and (b) quercetin (flavonoid).

3.1.2 Factors affecting pigment extraction

In general, there are some factors influencing the rate of extraction and quality of extracted compounds such as type of solvent, solvent concentration, particle size of the sample and also the temperature, pH and extraction time [115, 116]. In this study, the effect solvent type, solvent polarity, extraction time, acid–base condition and ionic strength of the extracting solvent were studied. The investigation was performed by measuring the absorption of the pigments extracted using UV–Vis spectrophotometry. The UV – Vis spectrum of pigment extracted from silk cocoon with 80% v/v EtOH scanned from 200 to 550 nm is shown in Fig 3.4.

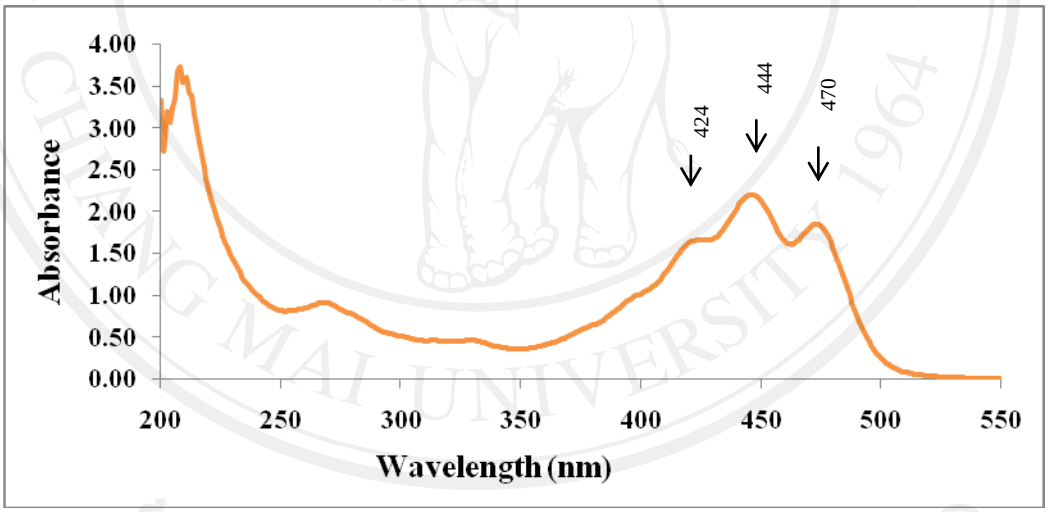


Fig 3.4 UV–Vis spectrum of pigment extracted from silk cocoon with 80%v/v EtOH (Numbers above the arrows indicate wavelengths of maximum absorbance).

From Fig 3.4, the broad peak of absorption bands was observed in the visible region of 350 – 500 nm with absorbance maxima at 424, 444 and 470 nm and small hump appears in the range of 250 – 350 nm. This can be confirmed that pigments extracted from silk cocoon include carotenoids and flavonoids by comparing

with the spectrum that has been reported as shown in Fig 3.5. The spectrum of this compound conforms to the absorption spectra of lutein–binding protein observed by Jouni & Wells [117] as shown in Fig 3.5a with characteristic band of three absorbance maxima in the visible region at 432, 460, and 492 nm. In addition, the UV–Vis of lutein dissolved in acetone – methanol reported by Rahman *et al*. [118] appears in the range 400 – 500 nm as shown in Fig. 3.4b which represent the characteristic three–headed absorption maxima in the blue part of the spectrum of carotenoid. However, the shifts of maxima to lower wavelengths are influenced by the polarity of the solvent. The lower the polarity of the solvent, the lower wavelength the shift occurs.

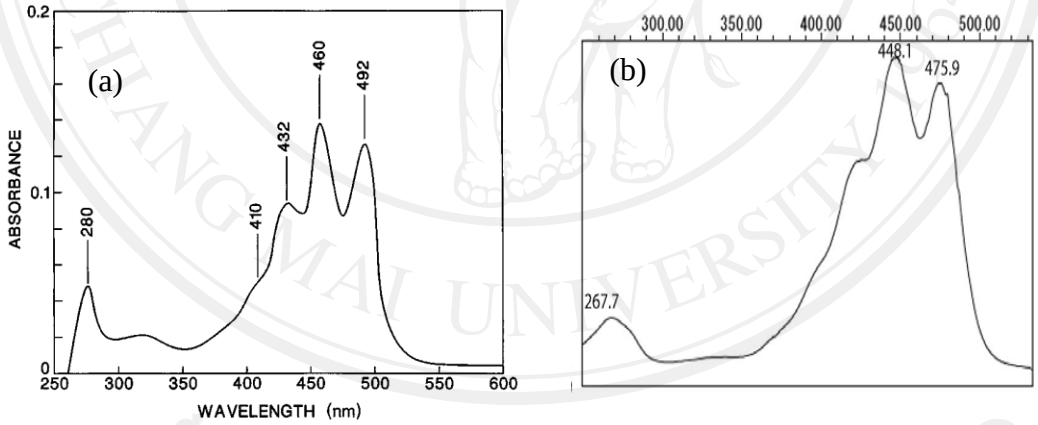


Fig. 3.5 (a) UV-Vis spectrum of the purified lutein- binding protein dissolved in 20 mM Tris-HCl, pH 7.0 solution, (b) UV-VIS spectrum of lutein dissolved in acetone/methanol [117, 118].

For UV-Vis spectrum of quercetin, a main flavonoid compound mostly found in silk is shown in Fig 3.6 with two distinct absorption bands in the UV-Vis region. The first band peaking around 280 nm appears due to the presence of

aromatic ring(s). The second, longer wavelength absorption maximum is situated in the 300 – 360 nm range which could be particularly significant for visible radiation screening [119].

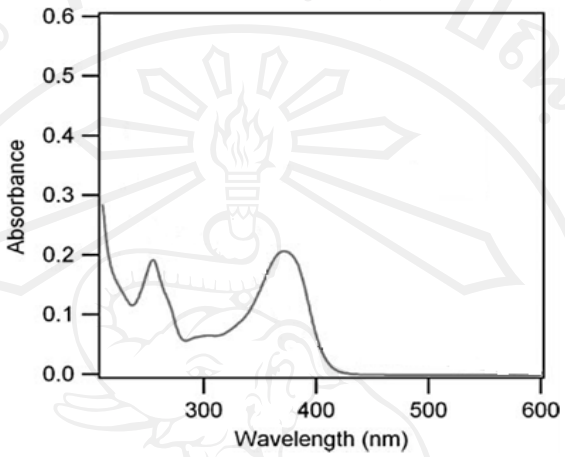


Fig. 3.6 UV–VIS spectrum of the quercetin [119].

From Fig 3.4, a small hump is also observed in the range of 250 – 350 nm which is expected to be a flavonoid compound. Absorption band due to the flavonoid in pigments extracted might be over shadowed by the broad peak of carotenoid.

3.1.2.1 Effect of methanol and ethanol concentrations

From the previous study in Fig. 3.2, it was observed that alcohol has a higher efficiency of extraction than other solvents and the concentration of each alcohol may affect the pigment extraction. By varying the MeOH and EtOH concentrations in the range of 60-100 %v/v for pigment extraction, the absorbance of each pigment extracted at various concentrations of MeOH and EtOH are shown in Fig 3.7 and 3.8, respectively by measuring at 424 nm 444 nm and 470 nm.

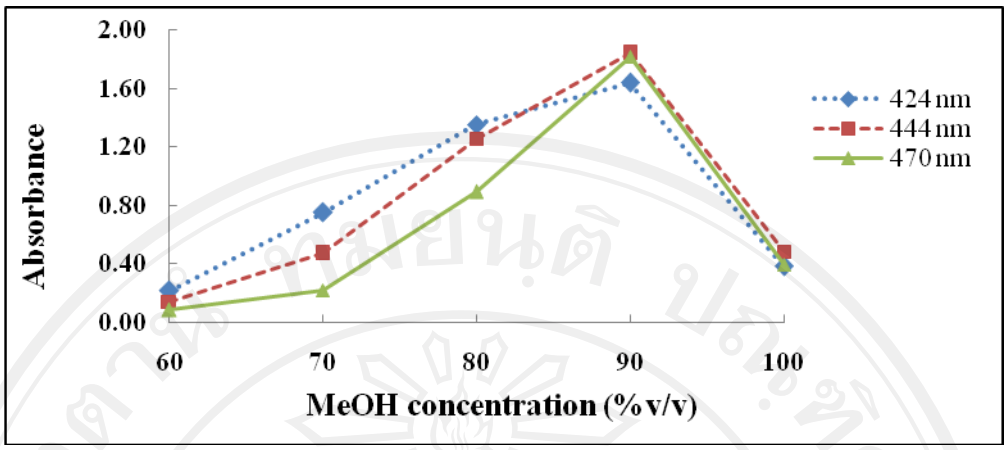


Fig. 3.7 Effect of MeOH concentrations on the extraction of pigments (no dilution).

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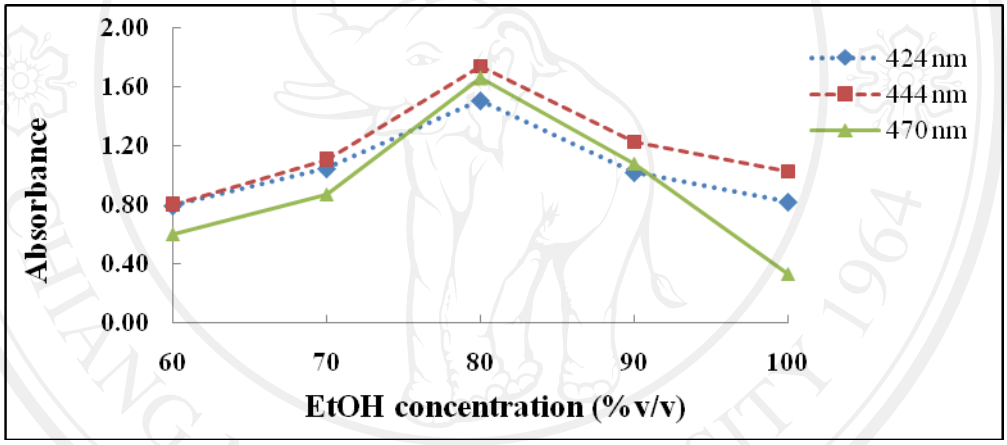


Fig. 3.8 Effect of EtOH concentrations on the extraction of pigments (Absorbance readings done after 2.5 folds of dilution).

From Fig. 3.7, when MeOH concentration increased from 60 to 90 %v/v, the absorbance of the pigment extracted increased significantly. For EtOH, its concentration effect can be seen in Fig. 3.8. It was found that the absorbance increased with the increase of EtOH concentration (60 to 80 %v/v). Referring to Fig. 3.4 and 3.5, they reveal that most of the pigment extracted is caratenoids and it was found that 80 % v/v EtOH was the most effective extracting solution observed from the maximum absorbance compared to other solvents. It has to be notified that all the

absorbance readings of EtOH extracts were done after 2.5 folds of dilution was made, thus the extraction with EtOH is more efficient than MeOH. Moreover, the absorbances of pigment extracted at various concentrations of MeOH appear to have wavelength shift as shown in Fig 3.9.

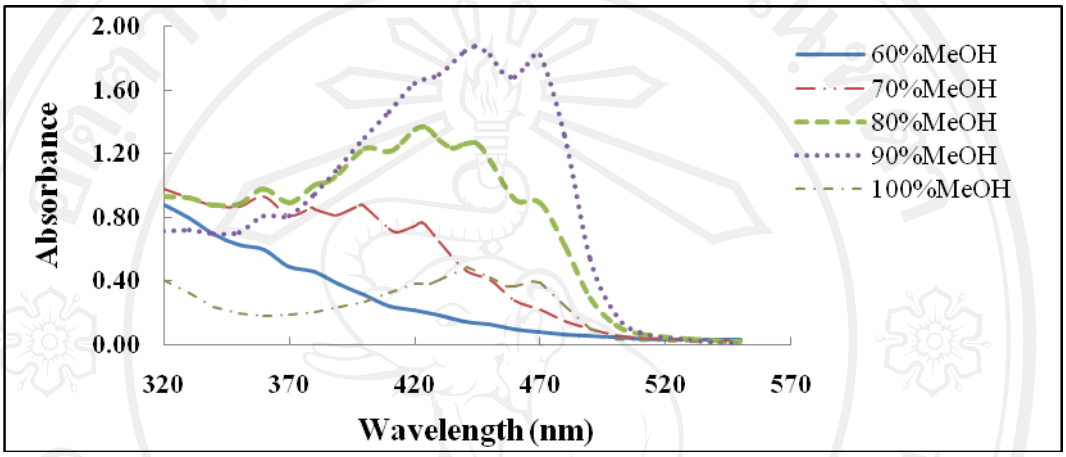


Fig. 3.9 Absorbances of the extracted pigments in various concentrations of MeOH solution at different wavelengths.

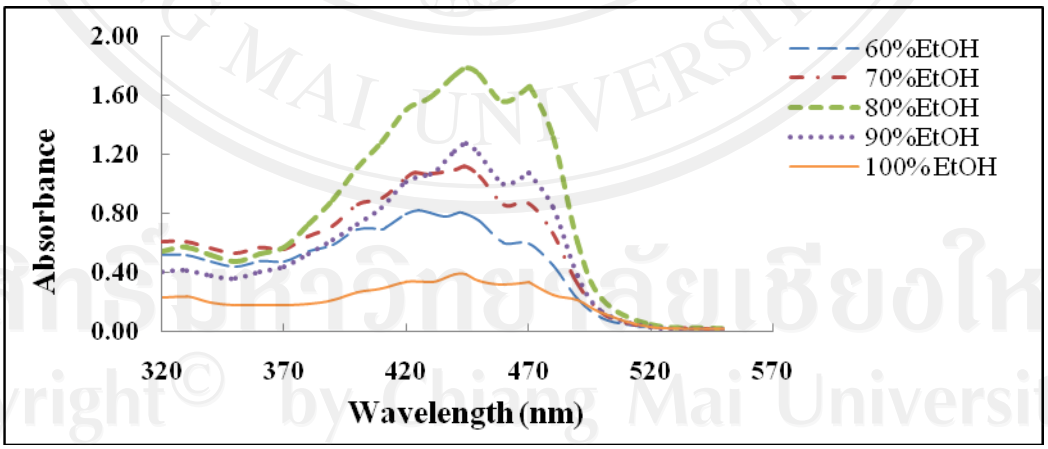


Fig. 3.10 Absorbances of the extracted pigments in various concentrations of EtOH solution at different wavelengths (Absorbance readings done after 2.5 folds of dilution).

It can be seen also that the shape of the spectrum changes with the increase of MeOH concentration from 60 to 100 %v/v, the absorption maxima for the isolated pigment suffered a bathochromic shift when the polarity of solvent decreased. But for EtOH, the shape of the spectrum is slightly changed with the change of EtOH concentration.

Moreover, from the result of changing MeOH and EtOH concentrations, higher concentrations of MeOH and EtOH (60 – 90 %v/v) evidently show more efficiency for extracting polyphenolic compounds than pure water and absolute MeOH or EtOH [120]. This is because the concentration of alcohol used exhibited different effect in changing the fluid polarity and thus had diverse effect on the solubility enhancement of the pigments [121]. The optimal extraction yield may be fulfilled when the polarity of the fluid and its pigments are matched.

3.1.2.2 Effect of temperature and extraction time

Since 80% EtOH was chosen as a solvent for extraction of pigments, the extraction was performed at various conditions of temperatures (50, 60, 70 and 80˚C) and extraction times (15, 30, 60,180 and 360 min) in a reflux extraction system. The absorbance of pigments extracted was measured at 444 nm to reveal the effect of temperature and extraction time as shown in Fig. 3.11.

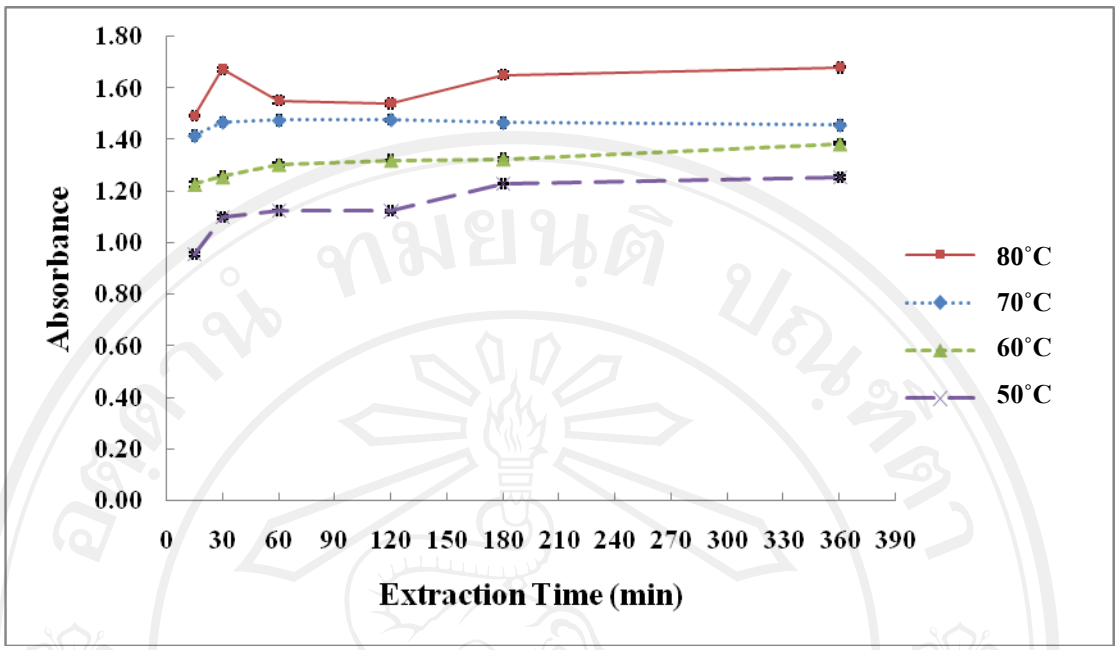


Fig. 3.11 The effect of temperature and extraction time on pigment extraction.

Fig 3.11 shows that the absorbance of pigment extracted increases gradually with the increase of extraction time (15 – 360 min) and mostly becomes stable after 30 min. This indicates that the pigments can physically interact effectively as the temperature of extraction increases because of its kinetic effect solvent efficiently. Likewise, the extraction can proceed more effectively as the temperature of extraction increases because of its kinetic effect. Hence, the extraction done at 80 ˚C for 30 min is considered optimum and this condition was used in the subsequent experiments.

3.1.2.3 Effect of solvent polarity

Pigment in silk cocoon was extracted using solvents with various polarities solvents. The absorbance of the extracts was measured at 424, 444 and 470 nm. Since 80 %v/v EtOH was selected as the extractant, its polarity was further varied in order to evaluate the efficiency of extraction. It was therefore modified with either hexane or acetone or acetic acid of which their polarity ranking is such that acetic acid > acetone > hexane. Result of modifying solvent polarity by adding each concentrations modifier at two different (5 and 10%) in 80% EtOH solution is shown in Fig 3.12. It was found that, in all cases of having additional solvent in the system, the extraction efficiency decreases as evidently illustrated through the lowering of absorbance values at each wavelength of absorption.

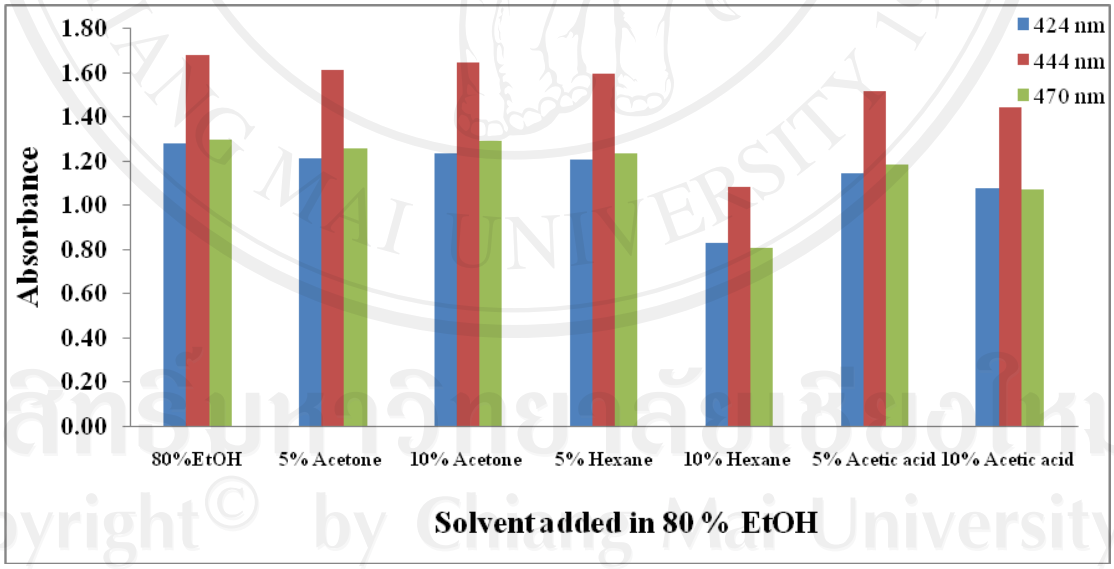


Fig. 3.12 Absorbances of the pigment extracted by various polarities solvents (Absorbance readings done after 2.5 folds of dilution).

A general principle in solvent extraction is “like dissolves like”, which means that solvents only extracts those pigments which have similar polarity with the solvents [122]. However, the present for adding hexane or acetone or acetic acid to solution which have affects on the extraction of pigment from the decrease of the absorbance, It is not necessary to add these solvents into solution. Thus, 80% EtOH was chosen as a solvent for subsequent extraction of pigment and effect of acidity/alkalinity and salt in solvent were determined.

3.1.2.4 Effect of acidity/alkalinity

The extraction condition was modified further by the addition of either acetic acid (CH_3COOH) or sodium hydroxide (NaOH) at different concentrations. The extraction result using acetic acid at different concentrations is shown in Fig. 3.13.

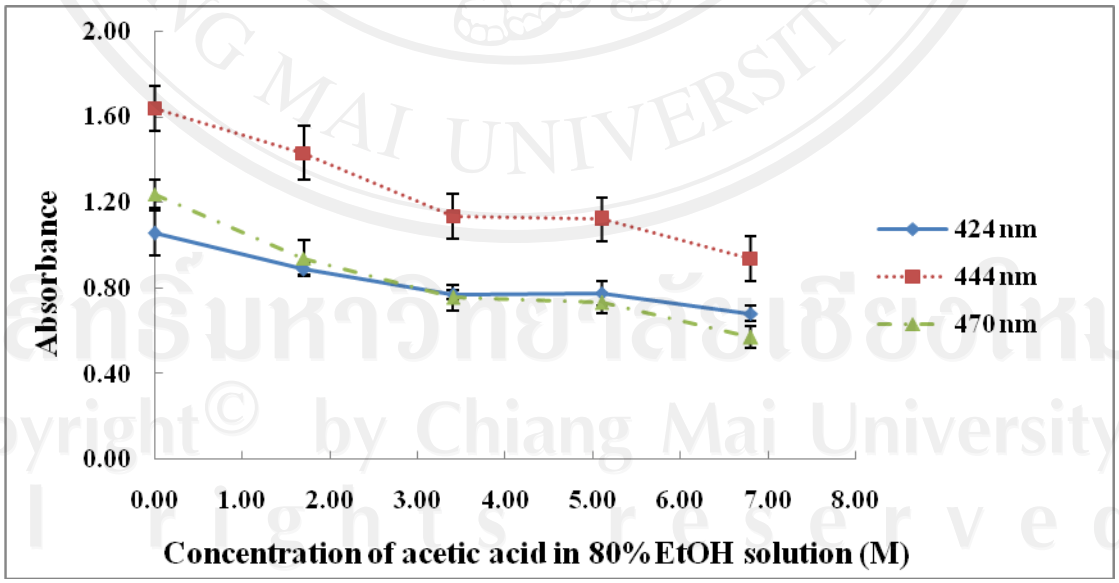


Fig. 3.13 Effect of the % acetic acid added in 80% v/v EtOH solution on pigment extraction (Absorbance readings done after 2.5 folds of dilution).

The presence of acetic acid affects on the extraction of pigment revealed from the decrease of the absorbance with increasing content of % acetic acid by decreasing pH values. But in the case of NaOH addition, the extraction became more efficient with the increase of NaOH concentrations (0.01-0.1 M in 80%EtOH which it is shown in Fig 3.14.

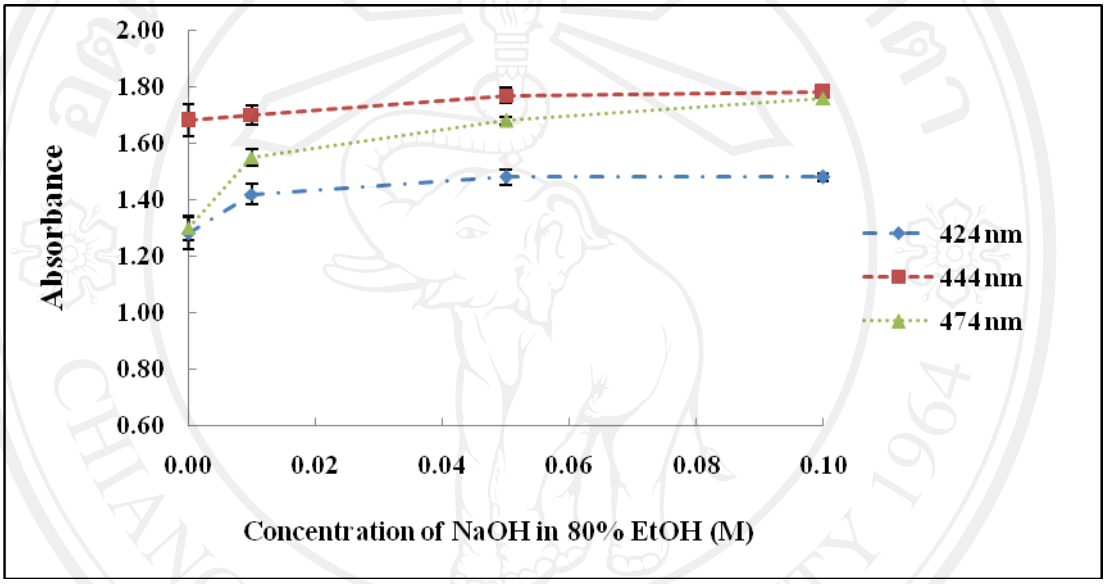


Fig. 3.14 Effect of concentrations of NaOH on pigment extraction (Absorbance readings done after 2.5 folds of dilution).

The results of NaOH addition indicate a slight enhancement of pigments removal from the cocoon due to the hydrolytic power of the alkaline medium acting on the proteins of the cocoon causing the rupture of pigment molecules from the cocoon protein. As silk fiber contains acidic groups (-COOH) and basic groups ($-NH_2$) in the molecule, therefore these polar functional groups tend to interact with the polar part of the pigment molecules. However, alteration of the pH of the solution often affects the polarity of the attracted species by breaking loose of

the pigments. It was also noticeable that the cocoon itself seemed to be destroyed and degraded in the alkaline solution when the NaOH concentration was higher than 0.1 M.

Moreover, by considering the structures of the major pigment present in the yellow silk, like lutein, $C_{40}H_{56}O_2$ (80% of total carotenoids), it consists of OH groups that can dissociate showing its acid property (Fig. 3.15) [123]. So the pigments will better react with the base and can be extracted in a greater extent. In addition, xanthophylls (lutein) are stable under alkaline treatments; thus, the use of ethanolic solutions of sodium hydroxide is a common method of saponification, which de-esterifies the pigment to free xanthophylls [124].

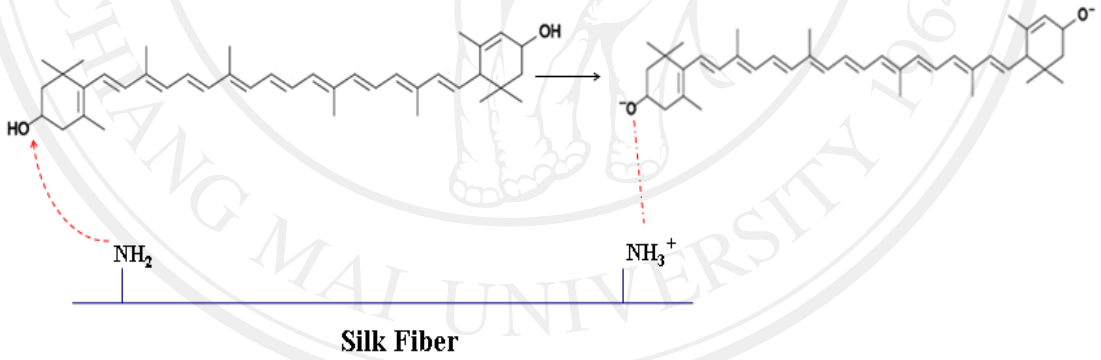


Fig. 3.15 The electrostatic interaction between lutein and silk fiber.

3.1.2.5 Effect of ionic strength

During the course of extraction that deals with polar molecules or charged particles, the ionic strength of the solution plays an important role on the interactions that can occur among species. Therefore, in this work, the ionic strength of solution were adjusted using four types of salt; KCl, CH_3COONa, $CaCl_2$ and $AlCl_3$ at 1.0 M. The pH value of each solution is shown in Fig 3.16.

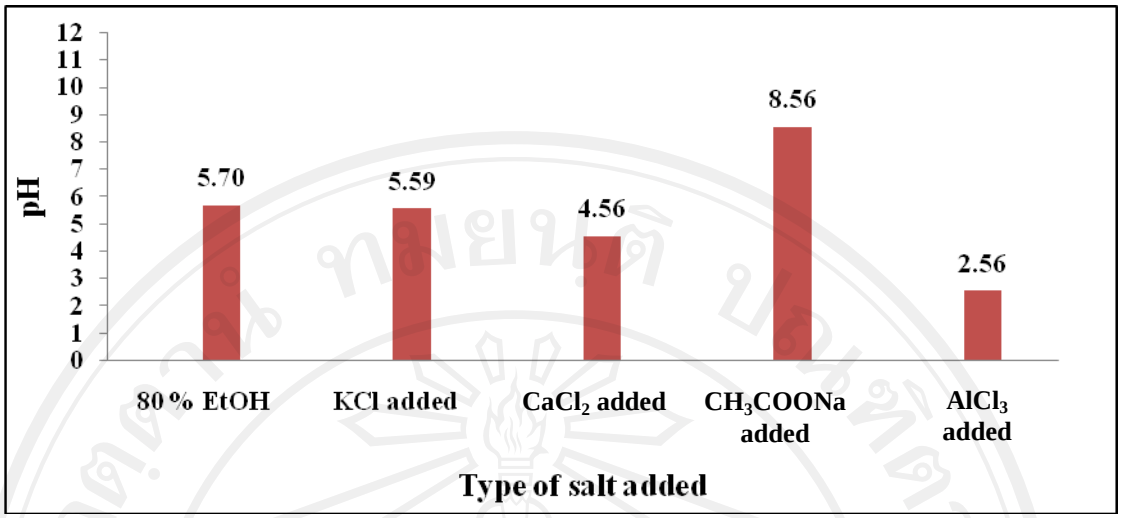


Fig. 3.16 The pH value of each salt containing solution.

The variation in the absorbance values of the pigment extracted when various kinds of salts were added is shown in Fig 3.17.

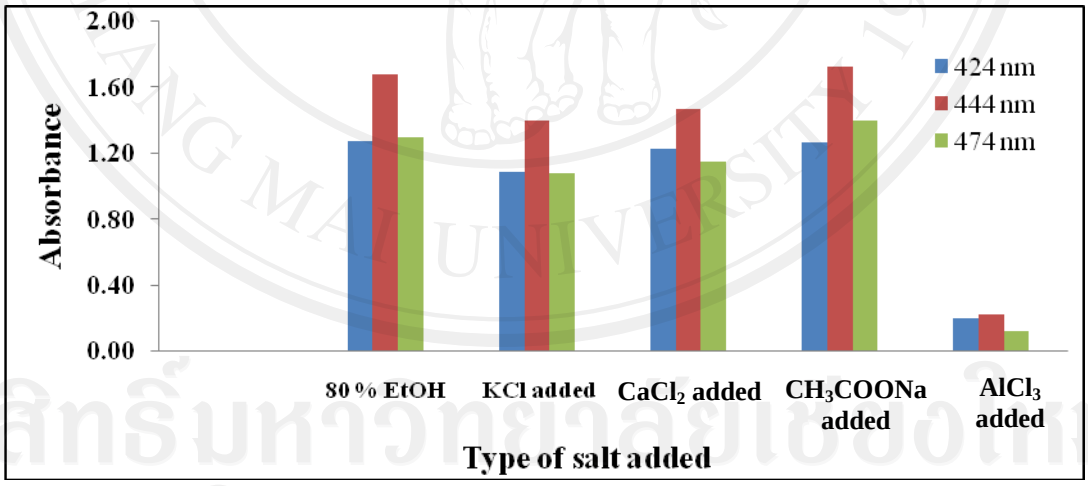


Fig. 3.17 Effect of salt addition on pigment extraction (Absorbance readings done after 2.5 folds of dilution).

It was found that the absorbances decreased with the addition of neutral salts (KCl and $CaCl_2$) and acid salts ($AlCl_3$), but increased slightly with the addition of basic salts (CH_3COONa). This observation confirms that the optimal condition for pigment extraction should be alkaline medium. However, the effect of ionic strength is not that prominent to pose some effect on extraction efficiency. For example the extractant containing either $CaCl_2$ or $AlCl_3$ did not yield any higher pigments removal, on contrary, the one modified with CH_3COONa which gave rise to a basic extracting medium showed better extracting power for pigments. Thus, the effect of extractant's basicity is more important than the ionic strength. Considering the effect of salt concentration that present in the extracting solution, (Fig 3.18), it was found that higher concentration yielded better extraction.

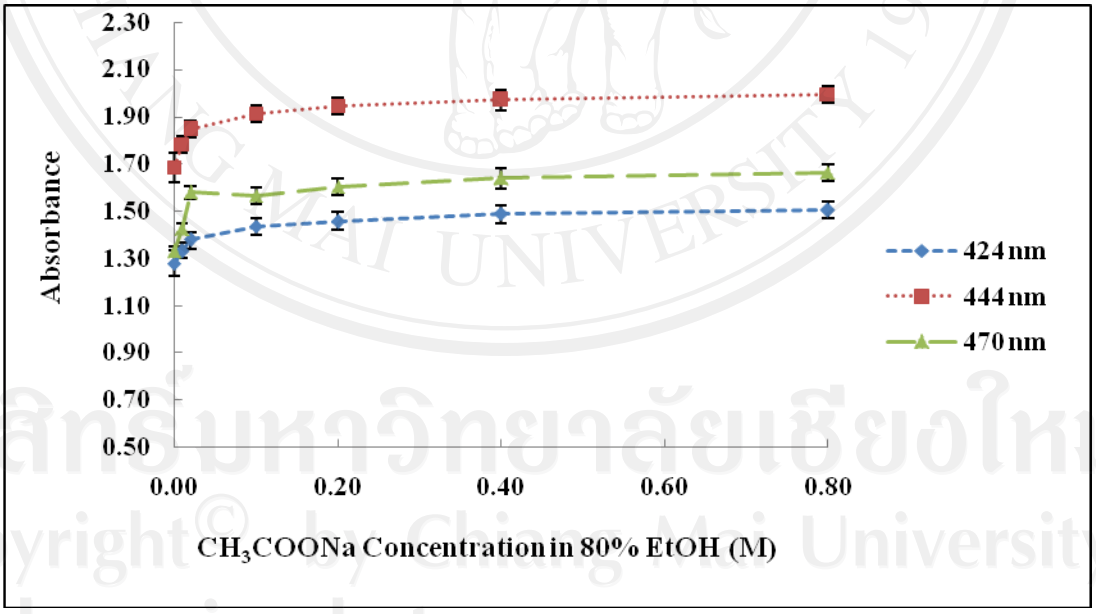


Fig 3.18 Effect of CH_3COONa concentrations on pigment extraction (2.5 folds dilution before taking reading).

Since the pigment is removed from the silk fiber through the acid-base reaction, the pigment molecules will be surrounded by the salt ions. The more ions are present in the solution, the better the hindrance effect the ions impose to the pigments. Thus it prevents the pigments to be re-adsorbed to the fiber. Therefore, 0.8 M CH_3COONa in 80 %v/v EtOH was selected as a suitable solvent for pigments extraction.

3.1.3 Determination of total pigments

Once the optimal extractant was chosen as 0.8 M CH_3COONa in 80 %v/v EtOH, the total pigments was extracted out of the cocoon consecutively. It was found that the contents of pigment could be exhaustively extracted within 4 consecutive runs at 80 °C for 30 min each. The final extract appeared to be a clear solution, suggesting that the yellow color of the raw material was completely removed as shown in Fig. 3.19.

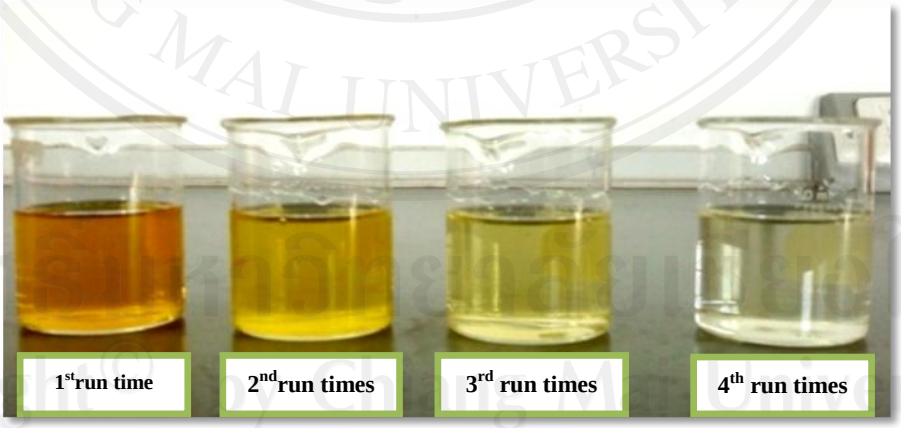


Fig. 3.19 Pigment solution obtained in each extraction.

The contents of total pigments were measured spectrophotometrically for all the samples which are summarized in Table 3.1.

Table 3.1 The absorbances of total carotenoid extract in Thai yellow silk cocoon, var. *Nangnoi Si Sa Ket* (Nan province) in each extracted batch.

No. of repeated batch	Absorbance		
	424 nm	**444 nm**	**470 nm**
1[a]	1.503*	1.993*	1.664*
2[b]	0.893	1.087	0.881
3[b]	0.178	0.209	0.169
4[b]	0.047	0.056	0.044

[a]Extraction with 0.8 M CH_3COONa 200 mL, 80 °C, 30 min
[b]Extraction with 0.8 M CH_3COONa 150 mL, 80 °C, 30 min
*2.5 folds dilution before taking reading

Due to the difficulty of obtaining the standard pigments, therefore it is not possible to determine the contents of the extracted pigments spectrophotometrically. So the quantification have to be done gravimetrically by recovering the extract as powder in a rotary evaporator, followed by removing the precipitated salt and freeze-drying to obtain pigment powder. The process involving pigment extraction of silk cocoons till obtaining the pigment powder is illustrated in Fig. 3.20. The pigment powder obtained by freeze-drying method appears to be a yellow powder as illustrated in Fig. 3.21.

For the weights of total pigment extracted from Thai yellow silk cocoon, var. *Nangnoi Si Sa Ket* obtained from Nan and Chiang Mai provinces are 2.40 ± 0.06 and. 2.45 ± 0.04 mg/g of dry weight, respectively. This result reveals that the amounts of pigment extracted from those two samples can be regarded as not different at all (See Table 3.2).

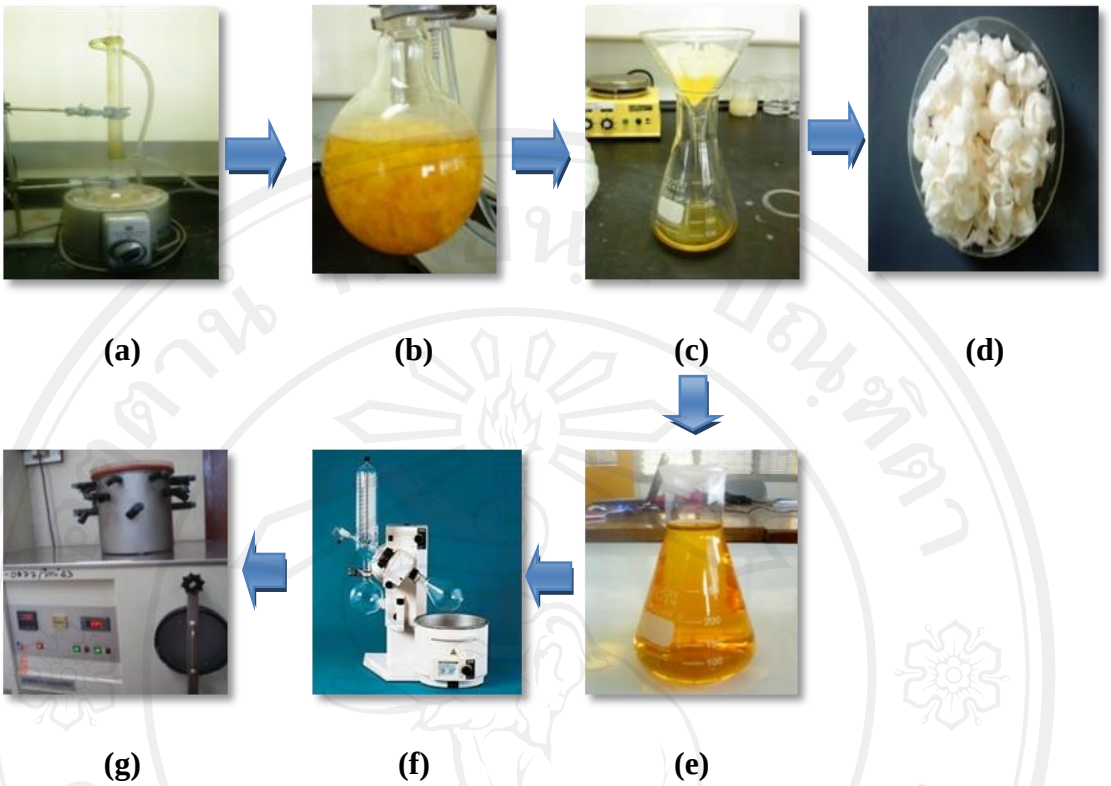


Fig. 3.20 Process of pigment extraction: (a) refluxing, (b) extracting, (c) filtering, (d) cocoon residue, (e) pigment solution (f) rotary evaporating and (g) freeze-drying.

Fig. 3.21 The freeze–dried pigment powder.

Table 3.2 The weight of total pigment in Thai yellow silk cocoon, *var*. *Nangnoi Si Sa Ket* (Nan and Chiang Mai provinces).

Source	**Trial**	**Total pigment**	
		mg/g	**(%w/w) W_1**
Nan	1	24.2	2.42
	2	24.6	2.46
	3	23.0	2.30
	4	24.3	2.43
	Mean ± SD	24.0 ± 0.6	2.40 ±0.06
Chiang Mai	1	24.8	2.48
	2	24.5	2.45
	3	23.8	2.38
	4	24.8	2.47
	Mean ± SD	24.5 ± 0.4	2.45 ± 0.04

From Table 3.2, it can be observed that the total pigment in silk cocoon was about 24.4 mg/g dry weight. This quantity is regarded as high content when compared with those reported for the amount of carotenoids and flavonoids (0.7 and 5.1 mg/g dry weight, respectively) in Yellow Thai silk waste [2] and carotenoid 0.52 mg/g dry weight in Yellow silk cocoons [125]. The differences that occur among those reported are due to the incomplete removal of salt and impurity during the purification process. Thus the amount of pigment in this study is therefore higher than those obtained from other studies.

3.2 Extraction and Determination of the sericin and fibroin proteins

Since the degumming is defined as a process involving the sericin extraction prior to spinning at controlled conditions that intended to have little or no effect on the underlying fibroin or dyeing in the silk industry. The principle of degumming process is to break the peptide linkage of amino acid in sericin structure into a small molecule such as amino acids and its oligomer with hydrolysis reaction. On the other hand, fibroin is insoluble in alkaline while sericin is soluble. In this study, silk cocoon were swelled with water and exploded under high temperature, thus external sericin was easily removed from fibroin.

For the sericin extraction, the silk cocoon, after removing the pigment, was degummed by alkaline buffer and surfactant solutions (0.1M Na_2CO_3, 0.1M $NaHCO_3$ and 2% SDS (pH~10) [126]. The action of alkaline buffer is imposed on the deprotonation of carboxylic acid side chain of the sericin. In addition, high solubility of sericin is generally obtained at pH values between 9.5 and 10.5 [127]. Moreover, the sodium dodecyl sulphate (SDS) solution was added in degumming solvent to create the mutual effects of hydrophobic alkyl chains and surface charges or electrostatic interaction surrounding SDS chains. This can enhance protein recovery. The degumming solution was obtained after boiling silk cocoons in each degumming solvent at 95 °C for 30 min [126]. Then, the degummed silk cocoon was dried at 70 °C in the oven for 24 h and weighed.

After that sericin was precipitated from the degumming solution by adding 5% acetic acid as a precipitant. Sericin was precipitated by this solvent due to the existence of an isoelectric point. Sericin, like other proteins, has an isoelectric point about 4 of which its solubility is the lowest. The sericin was then separated out by centrifugation and purified further by dialysis. Once the sericin solution was put in the dialysis tube (Molecular weight cutoff = 10000) which was placed in dynamically flow distilled water for 24 h, the sericin which has higher molecular weight (15000-310000) is therefore retained in the dialysis tube while smaller molecule can pass through the membrane. After freeze-drying, the sericin powder was obtained in Fig 3.22 appears to be a white powder. The extraction process of silk cocoons for sericin is illustrated in Fig. 3.23.

Fig. 3.22 The freeze-dried sericin powder.

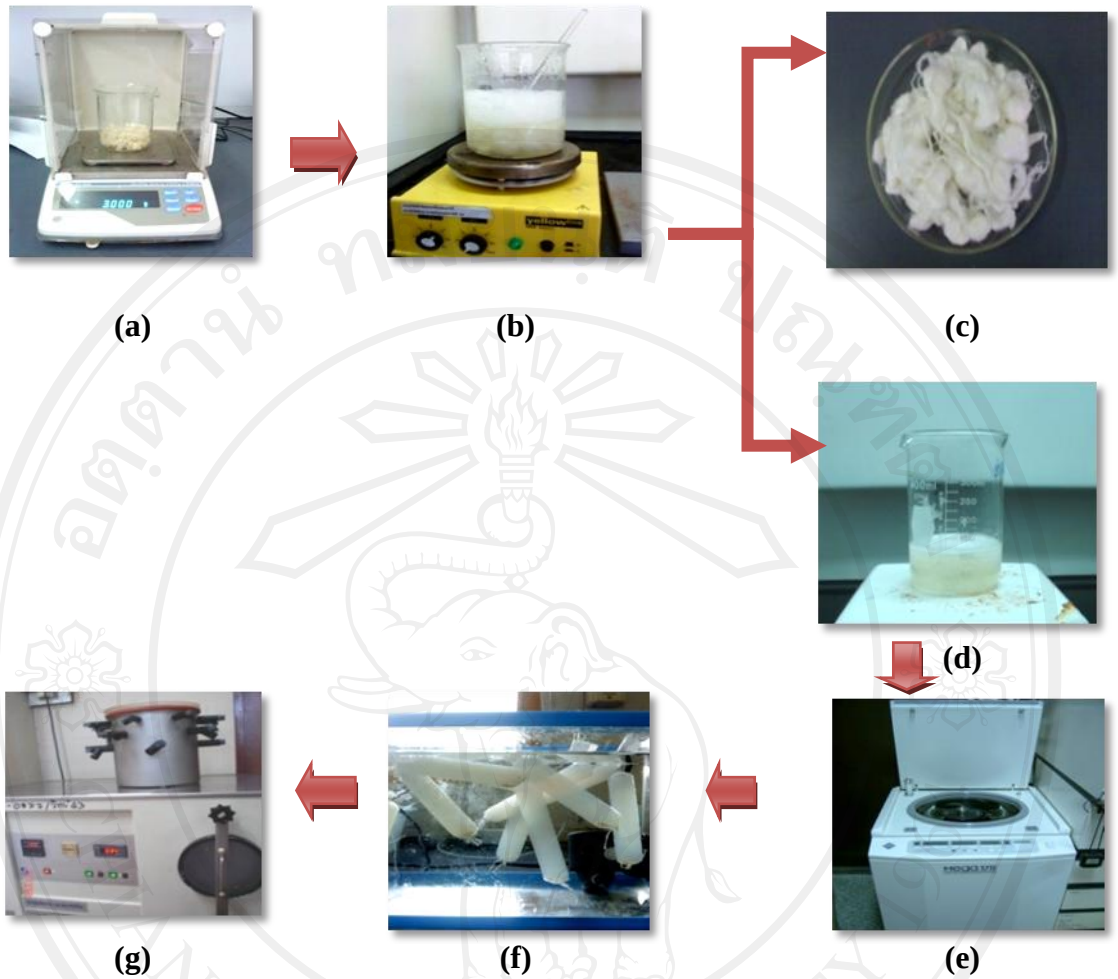


Fig. 3.23 Process of sericin extraction: (a) silk cocoon after pigment removal, (b) silk degumming (c) degummed cocoon, (d) degumming solution and precipitated sericin, (e) centrifugation, (f) dialysis and (g) freeze-drying.

The degummed silk cocoon (Fig. 3.23 (c)) was dissolved in a mixed solution of $CaCl_2$, ethanol and water, then purified by dialysis for 3 days. After that the fibroin solution was dried by freeze-drying to obtain fibroin powder. All processes mentioned above are illustrated in Fig. 3.24 (a)-(d), respectively.

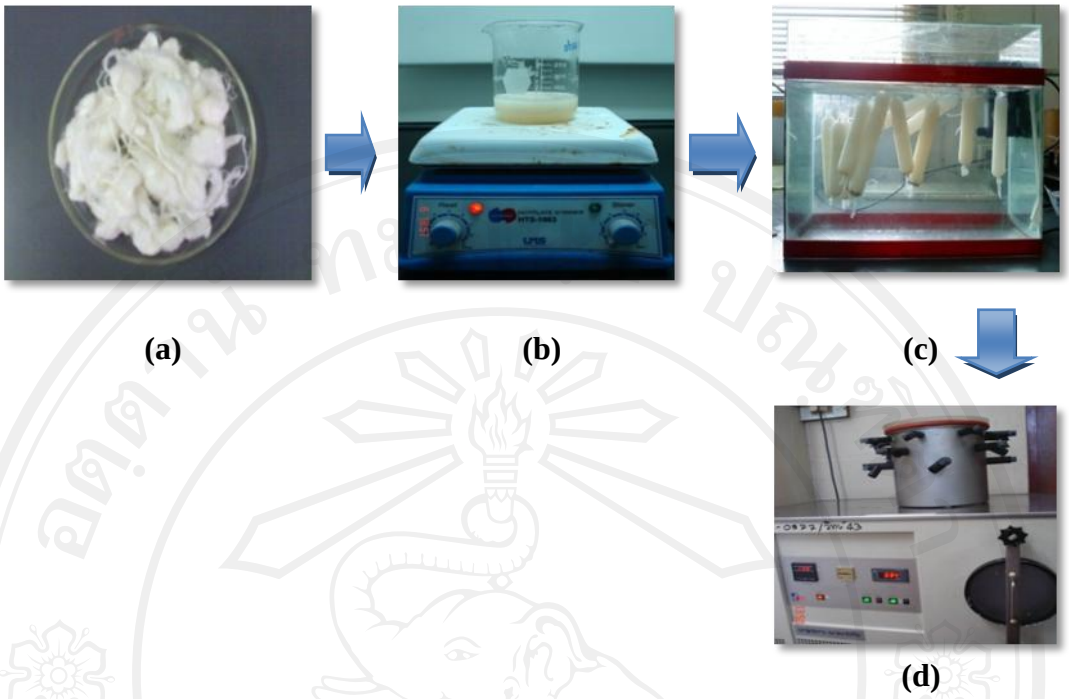
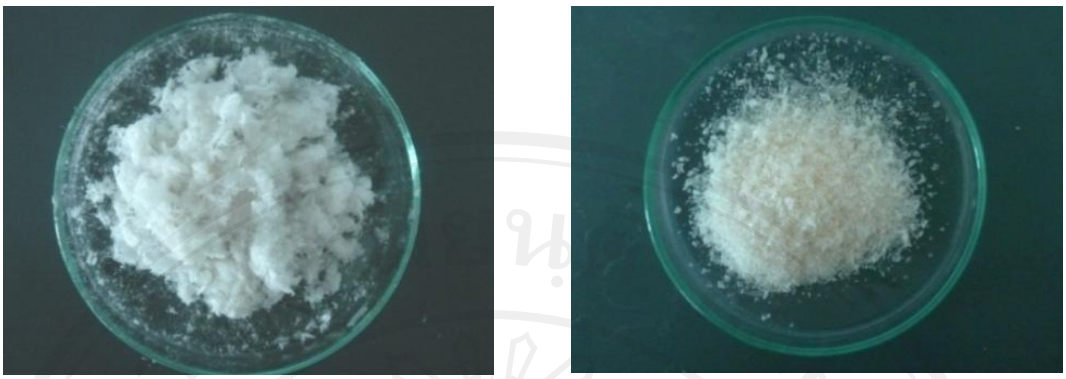


Fig. 3.24 Processes of preparation of fibroin powder: (a) degummed silk, (b) dissolving, (c) dialysis, (d) freeze-drying.

Referring to the step of dissolving, it was found that, at the temperature below 105°C, the degummed SF fiber hardly dissolved in neutral salt solvent and fibroin powder obtained is insoluble in water. And at temperature above 115°C, the more bubbles occurred in the solution and also high evaporation rate of solvent were observed that affected the dissolving of SF fiber. Therefore, degummed silk fiber was dissolved in the neutral salt solvent at range of temperature at 110 ± 5°C because the dissolvability of degummed silk fiber was increased and also loss of solvent was reduced. The SF powder obtained by using temperature at 110 ± 5°C appears to be a white powder as illustrated in Fig. 3.25.



(a) **(b)**

Fig.3.25 The SF powder prepared by using different temperature in the process of dissolving SF fiber (a) temperature at 110 ± 5°C, (b) temperature below 105°C.

The weights and percentages of pigment, sericin and fibroin obtained from the silk cocoon extraction are summerized in Table 3.3.

Table 3.3 Weights and percentages of pigment, sericin and fibroin powder.

Source	**Trial**	**Pigment**		**Sericin**		**Fibroin**	
		(mg/g)	***%w/w***	**(mg/g)**	***%w/w***	**(mg/g)**	***%w/w***
Nan	1	24.2	2.42	68.7	6.87	581	58.1
	2	24.6	2.46	70.8	7.08	522	52.2
	3	23.0	2.30	71.8	7.18	547	54.7
	4	24.3	2.43	59.5	5.95	552	55.2
	Mean±SD	24.0 ± 0.6	2.40 ±0.06	67.7 ± 4.9	6.8 ± 0.5	550 ± 21	55.0 ± 2.0
Chiang Mai	1	24.8	2.48	84.3	8.43	626	62.6
	2	24.5	2.45	85.5	8.55	585	58.5
	3	23.8	2.38	71.5	7.15	550	55.0
	4	24.7	2.47	70.0	7.00	543	54.3
	Mean±SD	24.4 ± 0.4	2.45 ± 0.04	77.8 ± 7.1	7.8 ±0.7	576 ± 33	57.6 ± 4.0

From Table 3.3, the total amount of pigments extracted from silk cocoons cultivated in Nan and Chiang Mai are 24.0 ± 0.6 mg/g and 24.4 ± 0.4 mg/g, respectively. It was found that the amounts of pigments, sericin and fibroin in silk cocoon from different sources are slightly different. When comparing the amounts of sericin in silk cocoons obtained from Nan and Chiang Mai, it was found that the amount of sericin from Chiang Mai's samples (77.8 ± 7.1 mg/g) was higher than the one from Nan (67.7 ± 4.9mg/g). Similarly, the amount of fibroin from Chiang Mai silk cocoon (576 ± 33 mg/g) was slightly more than Nan silk cocoon (550 ± 21 mg/g).

3.3 Structural determination of sericin fibroin and pigment powders

FTIR spectroscopy is useful in structural analysis of silk fibroin and silk sericin, because the position and intensity of the amide bands are sensitive to the molecular conformation. The advantages of FTIR over other techniques are that spectra can be obtained for proteins in a wide range of environments, requiring less time and sample, and direct correlations between the IR amide I band frequencies and the secondary structure components can be found [31].

In general, the characteristic absorption bands of amides in crystalline state occur at the specific region as shown in Table 3.4. *Amide I* provides the most intense absorption band in proteins. It is directly related to the backbone conformation and the hydrogen bonding pattern that leads to stretching vibrations of the C=O bond of the amide. *Amide II* is more complex than amide I. and it derives mainly from in-plane N-H bending of the potential energy (Fig. 3.26). *Amide III* is very complex bands dependent on the details of the force and resulting from a mixture of several coordinate [128]. The following frequency ranges can be considered as having

presence of the amide III: random coil (1315-1260 cm^{-1}), disorder structure (1255-1245 cm^{-1}) and β-sheet structure (1242-1230 cm^{-1}).

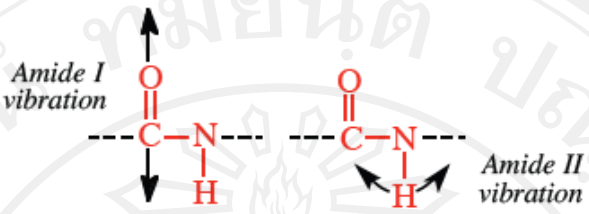


Fig. 3.26 The vibrations responsible for the amide I and amide II bands [28].

Table 3.4 Frequency ranges of characteristic absorption bands of amides in crystalline state [129].

Absorption bands	**Frequency ranges (cm^{-1})**	**Vibration types**
Amide I	1597-1672	C=O stretching (70-85%)
Amide II	1480-1575	N-H blending (40-60%)
Amide III	1229-1301	C-N stretching (18-40%).

For FT-IR spectra of sericin and fibroin were dramatically differed in absorption bands. While fibroin has a high degree of crystallinity due to stacked β-sheets, sericin has a more random structure [130]. This accounts for the differences in the main spectral features which relate to vibrations of the peptide backbone moieties [131].

From IR spectrum, protein conformation is determined by the peak positions of amide I, II and III corresponding to C=O, N-H and C-N. IR spectra of fibroin powder obtained from extraction of both Nan and Chiang Mai samples show similar patterns in Figs 3.27 and 3.28. Both fibroin powders identically show the N-H stretching bands at 3308 and 3313 cm^{-1}, C=O stretching peak at 1656 cm^{-1} (amide I), N-H bending peak at 1541 and 1537 cm^{-1}(amide II), C-N stretching peak at 1237 cm^{-1} (amide III). These peaks were those of β-sheet and random coil, amide I and amide II conformation, respectively.

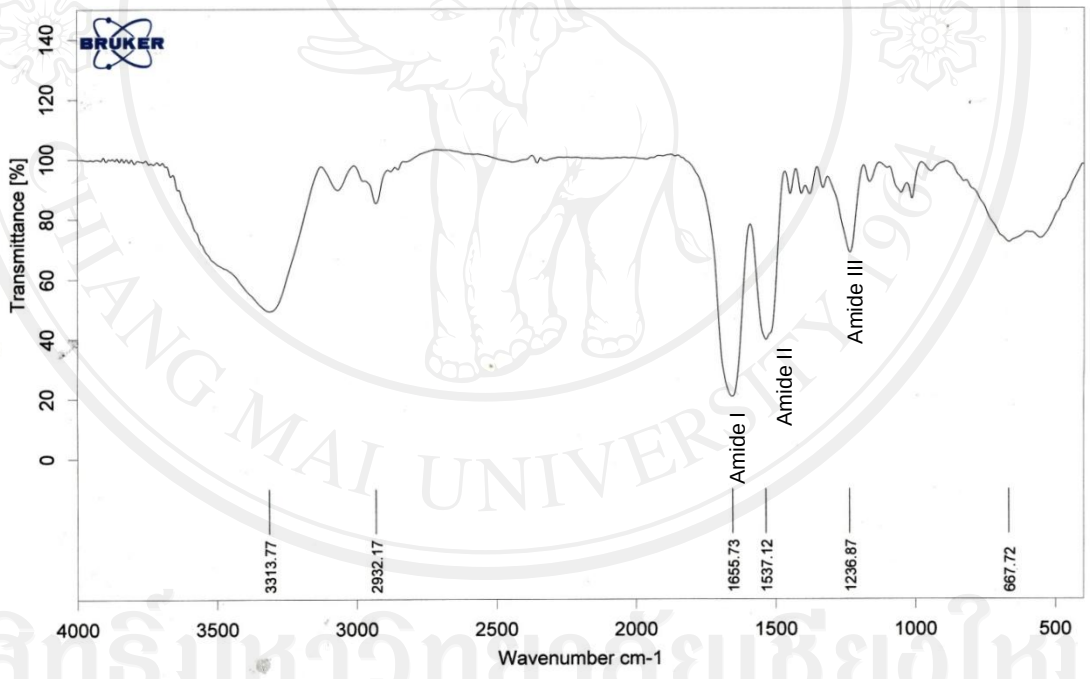


Fig. 3.27 FT-IR spectra of fibroin powder (NAN).

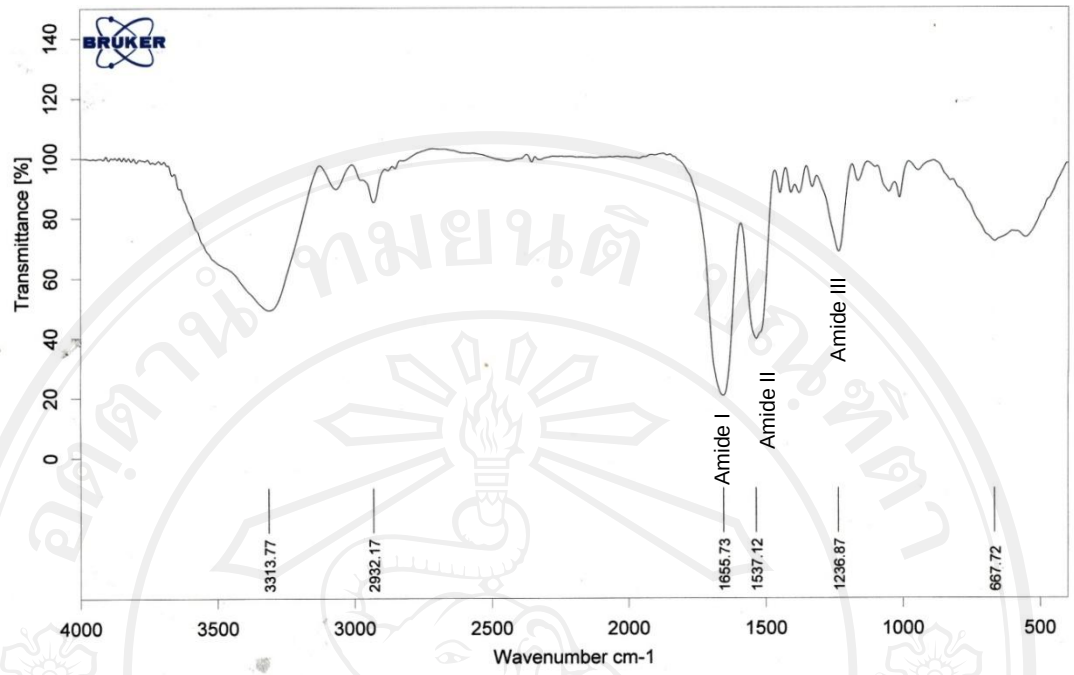


Fig. 3.28 FT-IR spectra of fibroin powder (Chiang Mai).

FTIR spectra of sericin obtained from the samples from Nan and Chiang Mai also show similar patterns in Figs 3.29 and 3.30. Again sericins from both sources identically show N-H stretching band at 3308 cm^{-1}, C=O stretching band at around 1659 cm^{-1} (amide I), N-H bending at 1535 cm^{-1} (amide II) and C=O symmetry stretching band at around 1395 cm^{-1} and the peak at 1191 cm^{-1} appear but the peak for amide III (C-N stretching) disappears. These are a further signature peak for sericin at two absorbances and it was also found that the absorption band of amide III had gone. The prominence of these features may be ascribed to the relatively high content of carboxylic acid and alkyl hydroxyl containing amino acid side chains in sericin. Associated water and the raised hydroxyl content together also cause the overlap to the broad absorption band at around 3300 cm^{-1}.

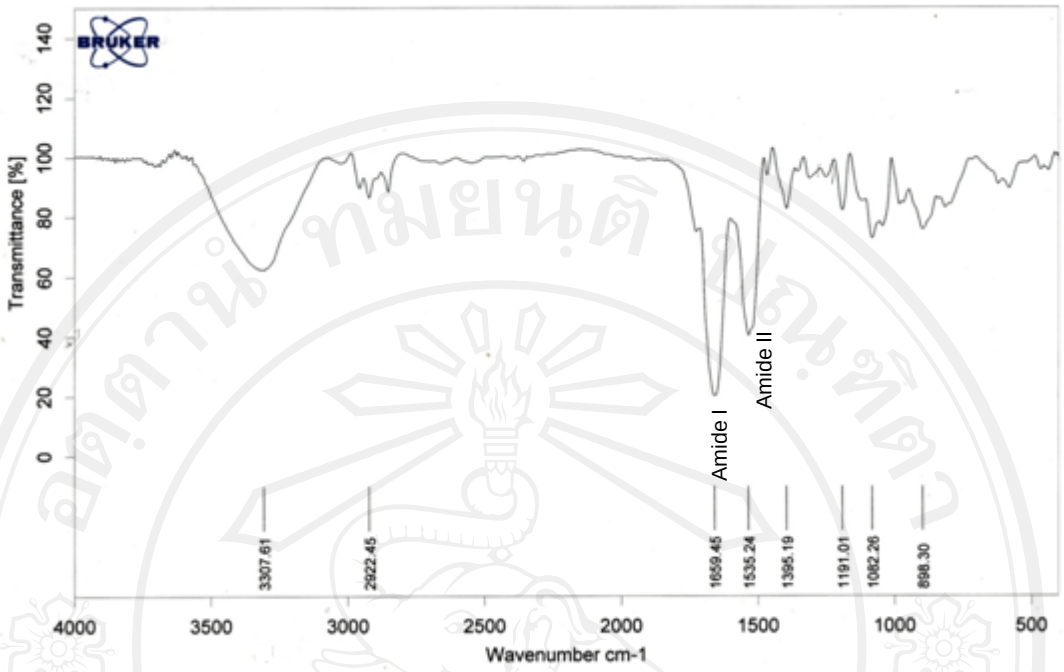


Fig. 3.29 FT-IR spectra of sericin powder (NAN).

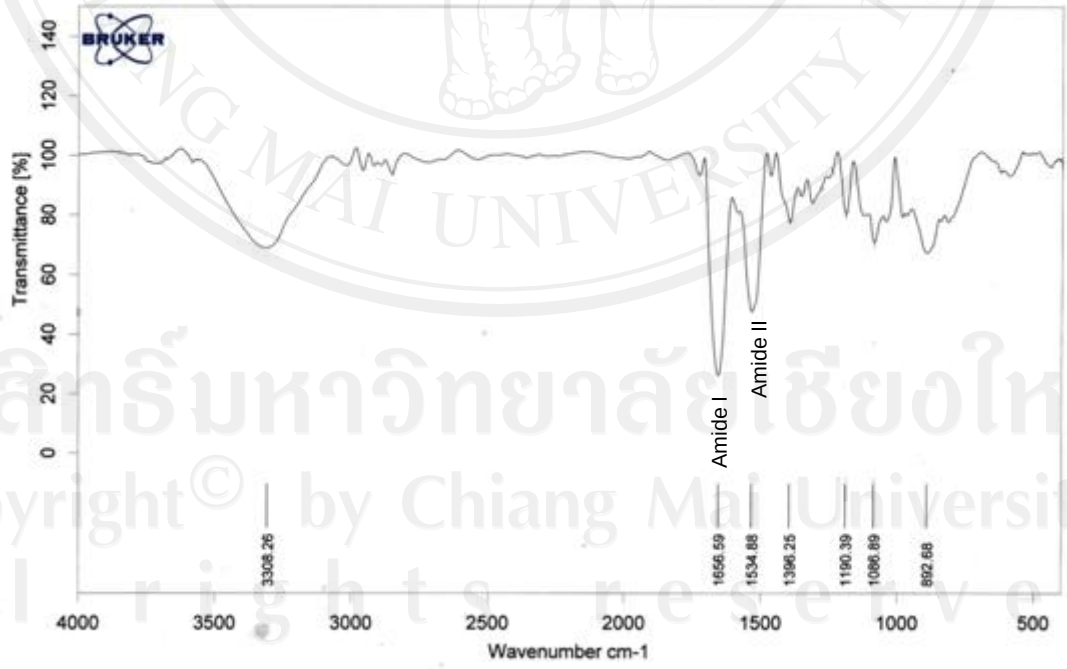


Fig. 3.30 FT-IR spectra of sericin powder (Chiang Mai).

These observed bands and their assignments are agreeable with the previous observation as shown in Table 3.5 and 3.6.

Table 3.5 Comparison of IR absorption bands of fibroin as amide I, amide II and amide III.

Identification	**Absorption band (this study) (cm^{-1})**		**Absorption band [a] (cm^{-1})**	**Absorption band [b] (cm^{-1})**
	Nan's	**Chiang Mai's**		
Amide I	1656	1657	1654	1655
Amide II	1537	1537	1558	1540
Amide III	1237	1237	1239	1235

[a]Baimark *et al* [132]

[b]Bayraktar *et al* [133]

Table 3.6 Comparison of IR absorption bands of sericin as amide I, amide II.

Identification	**Absorption band (this study) (cm^{-1})**		**Absorption band [a] (cm^{-1})**	**Absorption band [b] (cm^{-1})**
	Nan's	**Chiang Mai's**		
Amide I	1659	1657	1650	1655
Amide II	1535	1535	1530	1550

[a]Mei &Paul[131]

[b]Takeuchi et al [134]

The FTIR spectra of pigment obtained from silk cocoon is shown in Fig. 3.31. The pigment itself shows the O-H stretching band at 3384 cm^{-1}, C–H stretching band at 2919 and 2850 cm^{-1}, C=O stretching, C=C stretching, N–H bending band at 1625 cm^{-1}, C–H bending band at 1384 cm^{-1}. This kind of spectra is quite resemble to those found from the functional groups similar to lutein (carotenoid) [135].

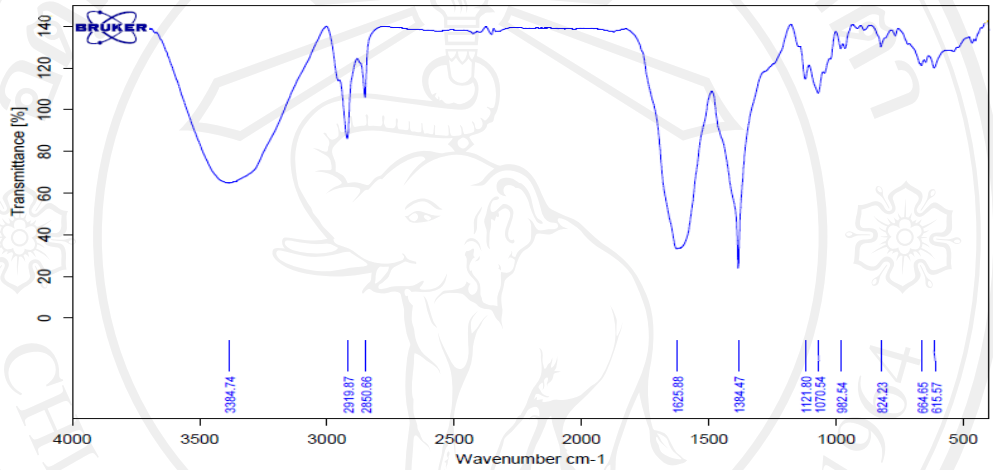


Fig. 3.31 FT-IR spectra of pigment powder.

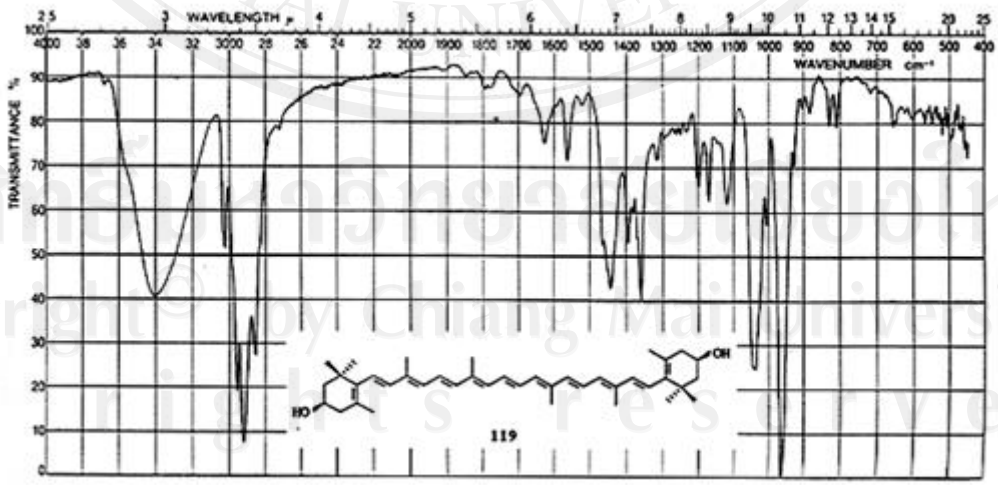


Fig. 3.32 FT-IR spectra of lutein [135].