CHAPTER 1

INTRODUCTION

In Thailand, there are many silk yarn and textile handicrafts factories that annually produce large amounts of waste (from unreel silk cocoon). Utilization of silk waste has drawn so many attentions to researchers in order to enhance its value which can represent a significant economic and social benefit [1, 2]. In this thesis, the separation of the constituents in silk waste from yellow Thai silk cocoon *Bombyx mori*, i.e. pigments and proteins was focused aiming to optimize the extraction conditions using various solvent systems. In addition, the total contents of the constituents were also determined.

1.1 Methods of separation

The separation of mixtures in a pure state is an important process in chemistry. In general, a mixture can be separated into its components by taking advantage of the differences in its properties e.g., particle size, solubility, effect of heat etc [3]. The following are methods usually employed for separation of the mixtures that can be divided according to states of the sample as illustrated in Fig. 1.1 and techniques of separation are summarized in Table 1.1.

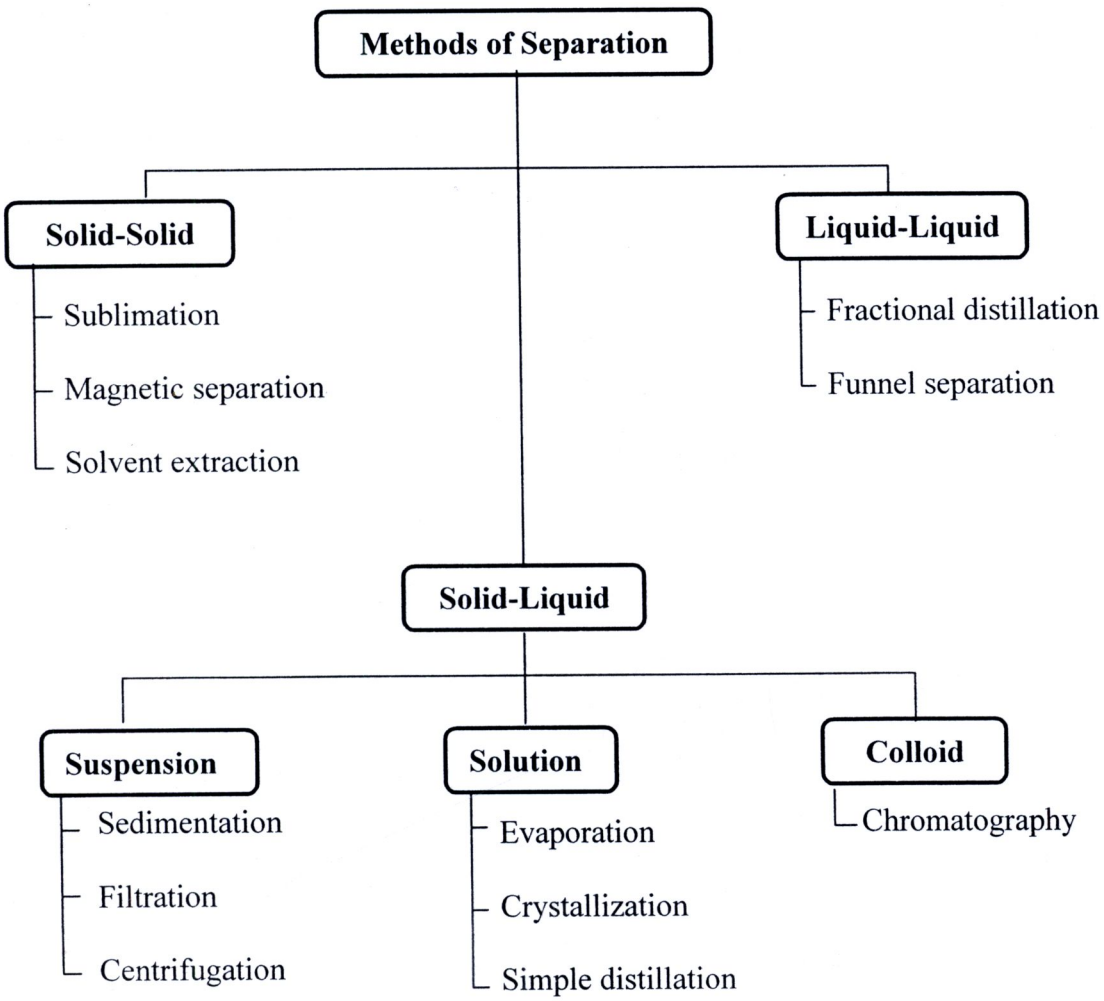


Fig. 1.1 Diagram illustrating methods for mixture separation.

Table 1.1 Common separation methods in chemistry laboratory [3-6].

Separation method	**Objects separated**	**Difference between objects**
Chromatography	Compounds in a solution with the same properties	Solubility with particular solvent
Crystallisation	Dissolved solids in a solution	State of object (solid and liquid)
Decantation	Insoluble solid and liquid	Size of particles
Evaporation	Solids that cannot decompose when heated in a solution	State of object (solid and liquid)
Filtration	Solids or group of solids and liquids in a mixture	Size of particles
Fractional Distillation	Mixture of miscible liquids	Boiling points
Funnel Separating	Immiscible liquids	Both are immiscible
Magnetic Separation	Magnetic substances from non-magnetic ones	Magnetism
Simple Distillation	Liquids in a solution	Boiling points
Solvent Extraction	Dissolved soluble component from solids	Solubility with particular solvent
Sublimation	Substances that sublime from two substances	Ability to sublime

1.1.1 Separation of a Solid-Solid Mixture

1.1.1.1 Sublimation

Sublimation is a process to separate a mixture of volatile from a non-volatile solid. The mixture is taken in a porcelain dish covered with a perforated filter paper on which an inverted glass funnel is placed to collect the vapors. Upon heating, the substance vaporizes and deposits on the walls of the funnel. The non-volatile substances are left in the dish. The picture of sublimation of ammonium chloride is illustrated in Fig. 1.2.

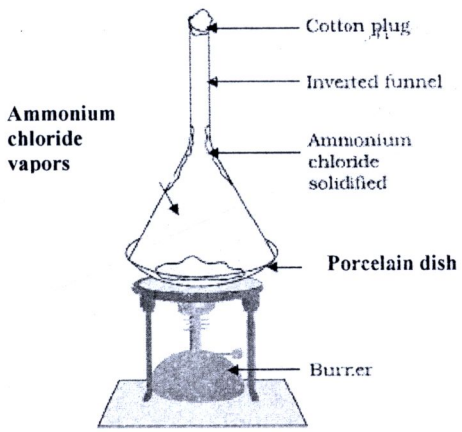


Fig. 1.2 Sublimation of ammonium chloride [7].

1.1.1.2 Magnetic Separation

Magnetic separation is used to separate a magnetic component from a mixture containing non-magnetic components. A magnet is moved over the mixture containing the magnetic substance e.g., iron filings. The substance get attracted to the magnet. The process is repeated until the magnetic material is

completely separated from the mixture. The separation of a magnetic substance by a magnet is shown in Fig. 1.3.

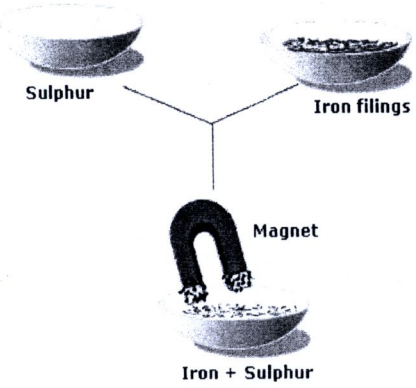


Fig. 1.3 Separation of a magnetic substance by a magnet [3].

1.1.1.3 Solvent Extraction

Solvent extraction is usually used to dissolve out the soluble component from solids using a solvent to separate it from a mixture and subsequently treating the solution to get the solid back. This method is widely used for removing interference and concentrating the sample before analysis. The process may be used either for the production of a concentrated solution of a valuable solid material, or to remove an insoluble solid, such as a pigment, from a soluble material with which it is contaminated. The method used for the extraction is determined by the proportion of soluble constituent present, its distribution throughout the solid, the nature of the solid and the particle size [4]. For details of solvent extraction, it will be discussed later.

1.1.2 Separation of a Solid-Liquid Mixture

A mixture of solid-liquid components can be categorized as suspension, solution and colloid.

1.1.2.1 Suspension

Suspension is a heterogeneous fluid containing solid particles that solute does not fully dissolve in solvent. Suspended particles are larger in size than molecules or ions of the liquid. The particles may be so large that they settle to the bottom of a container through gravity. Separation of the suspensions can be done by various methods.

1) Sedimentation or Decantation

Sedimentation is a process for separating an insoluble solid from a liquid which is suspended by allowing the solid particles to settle to the bottom of the container. If it involves pouring off the liquid, then leaving the solid behind, it is called decantation. The advantage of this method that it provides is the simplest and cheapest way of cleaning by using the gravity of the earth, but the disadvantage is on the difficulty of sediment examination due to the presence of excessive interference. An example of separating the mixture of coarse solid particles from a liquid e.g., muddy river water.

2) Centrifugation

The centrifugation using the sedimentation principle can be used for separating particles and molecules having different masses or densities. They will settle to the bottom of a tube at different rates. The remaining solution is properly called as supernatant. The supernatant is then either quickly decanted from the tube without disturbing the precipitate, or withdrawn with a pipette. Centrifugation can be divided into differential centrifugation and rate-zonal centrifugation. Separation through centrifugation is shown in Fig. 1.4 [5].

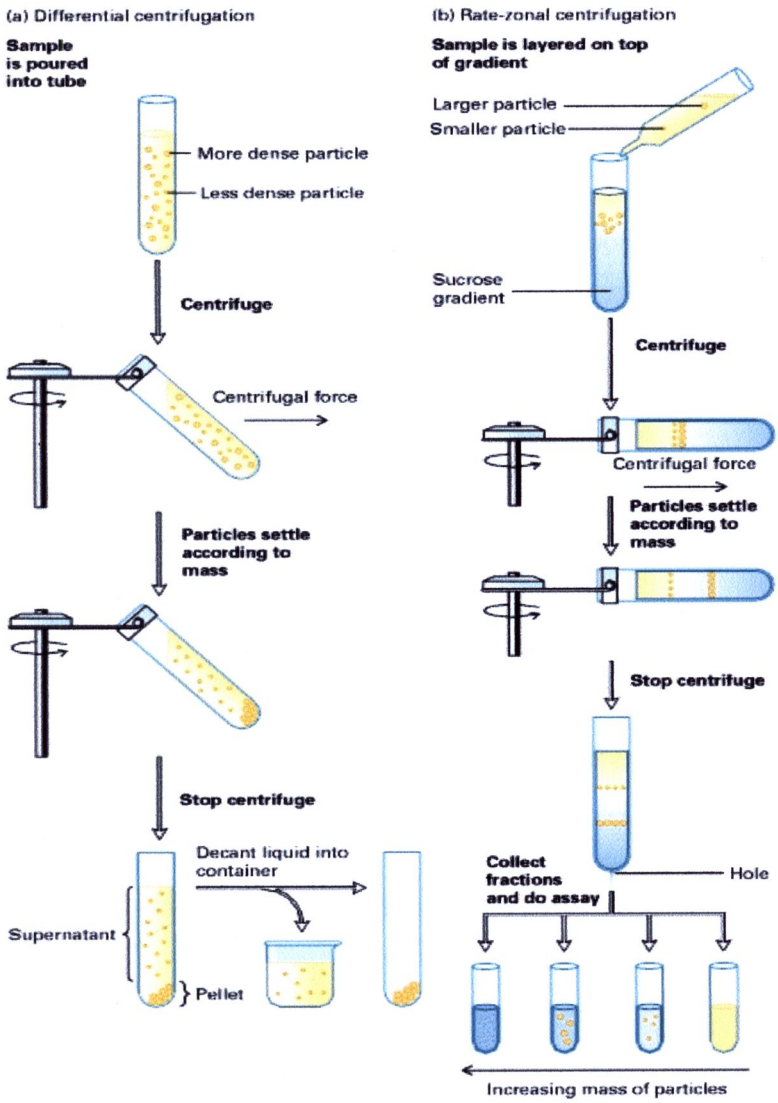


Fig. 1.4 Centrifugation method for separating particles; (a) differential centrifugation and (b) rate-zonal centrifugation [8].

3) Filtration

Filtration uses a filter paper or fine porous ceramic to separate an insoluble solid from a liquid. The solvent present in the solution can pass through the porous membranes such as filter paper, filter cloth etc. while the suspended particles cannot and are retained on the porous membranes e.g. sand from a mixture of sand and water.

1.1.2.2 Solution

Solution is a homogeneous mixture composed of only one phase. In such a mixture, a solute is dissolved in another substance, known as a solvent. Separation of a solution can be done through evaporation, crystallization and also simple distillation.

1) Evaporation

Evaporation is used to separate the soluble solids from the solution by evaporating the solvent that does not decompose on heating. The solvent is lost into the surroundings. Evaporation can also be done using a device called rotary evaporator for efficient and gentle removal of solvents from samples at a reduced pressure. This promotes the rapid removal of excess solvent from less volatile samples and often done to isolate a product from a chromatographic separation or a solvent extraction. The picture of rotary evaporator is shown in Fig. 1.5.

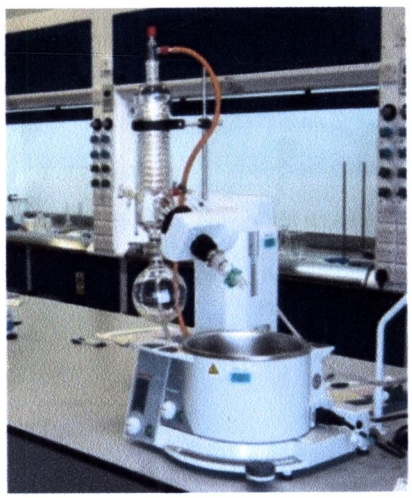


Fig. 1.5 Rotary evaporator [9].

2) Crystallization

Crystallization is a sophisticated form of evaporation technique in which crystals of the solute are encouraged to develop during the process of "dissolving out" from the solution as the solvent evaporates.

3) Simple Distillation

Simple distillation is the process of heating a solution containing soluble solids to form vapor of the liquid and then cooling the vapor to get the liquid back. For example, a distillation of salt water, the process can take place because water has a much lower boiling point than table salt, it is more volatile. If a solution of salt and water is boiled, the more volatile water evaporates and the salt is left behind. The water vapor is converted back to liquid form on the walls of the condenser as shown in Fig. 1.6.

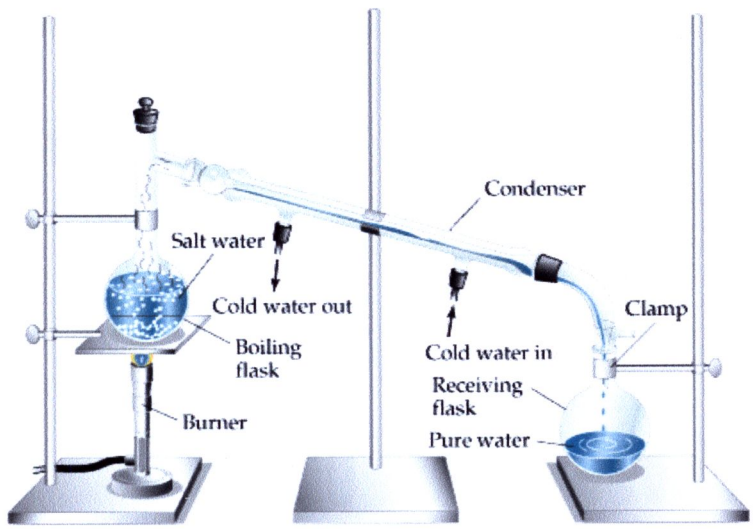


Fig. 1.6 Simple distillation [10].

1.1.2.3 Colloid

Colloid is a substance microscopically dispersed evenly throughout another substance and can pass though filter paper. Separation of the colloidal dispersion can be carried out using chromatographic technique.

Chromatography is an advanced technique of separation in which individual components of a mixture are separated from each other using the property of differential migration (different rates of flow). Here, a mobile phase, carrying the mixture, is passed through a selectively adsorbing stationary phase, which can retain the components of the mixture to different degrees. It is mostly applied in identifying mixtures that are colored or pigments [6]. Example of column chromatography can be seen the Fig 1.7.

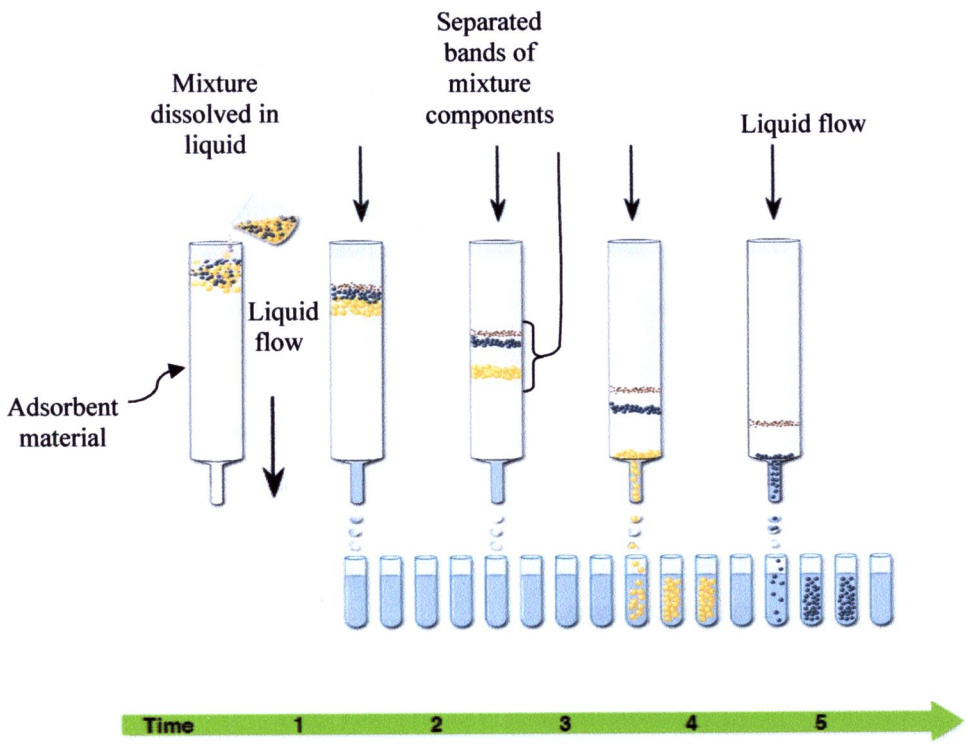


Fig. 1.7 Column chromatography [11].

1.1.3 Separation of a Liquid – Liquid Mixture

1.1.3.1 Fractional Distillation

Fractional distillation is a process of separating two or more miscible liquids by a modified distillation which compounds with different boiling points can be separated by controlled heating. The liquid with a lower boiling point is expelled out first and the vapor passes through the condenser condensing into a container as liquid. After that the liquid with higher boiling point evaporates and again is collected in a separate container. All the solutions are separately collected in the same manner. The fractional distillation apparatus is shown in Fig. 1.8. Example of this process is a separation of water and ethanol. The boiling point for water is 100 °C while for ethanol is 79 °C.

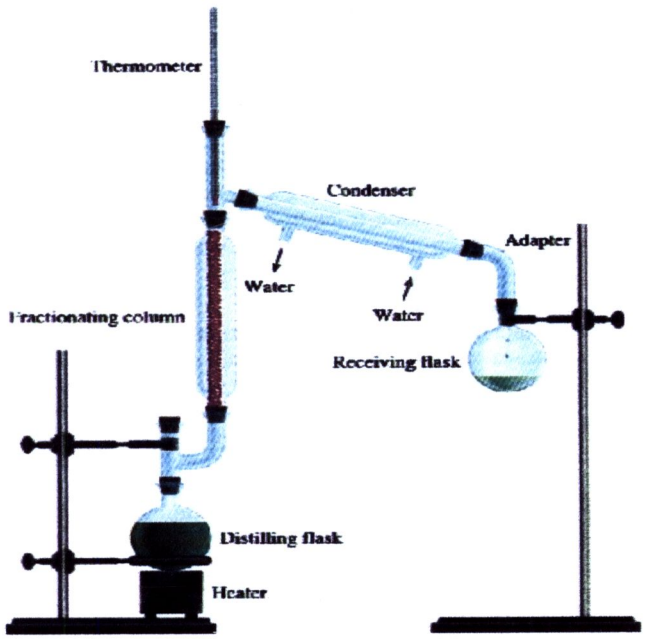


Fig. 1.8 Fractional distillation [12].

1.1.3.2 Funnel Separation

Separation with funnel is used to separate two immiscible liquids with different densities. The liquids are placed in a separating funnel and a container is put underneath. The liquid with lower density floats on top and the one with higher density lies below such as oil and water. Applications of this process include removal of vitamins from aqueous solutions and aromatic compounds from crude oil fractions. The funnel separation is commonly seen as illustrated in Fig. 1.9.

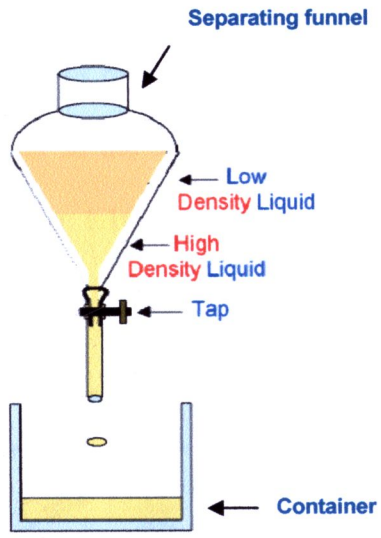


Fig. 1.9 Funnel separation [13].

1.2 Solvent Extraction Method

Besides those previously mentioned methods, a large number of extraction methods have also been developed. The most frequently used extraction methods are including heat reflux, Soxhlet and more recently ultrasound-assisted, microwave-assisted and subcritical water extractions [14, 15].

1.2.1 Heat Reflux Extraction (HRE)

HRE is performed when extraction of essential compounds or components from organic raw material is needed. In most cases, the extraction can be performed over many hours to achieve the targeted substance without the loss of essential components such as solvents. This kind of extraction is done by fixing a reflux condenser on the top section of the extraction flask. During refluxing, vaporization of the boiling mixture occurs followed by condensation of the volatile compounds on the inner wall of a condenser. The condenser is kept cool by passing cold water around the outer jacket of the condenser. The condensate falls back into the solution, so they are not lost in the surrounding atmosphere. The heat reflux extraction apparatus is shown in Fig. 1.10.

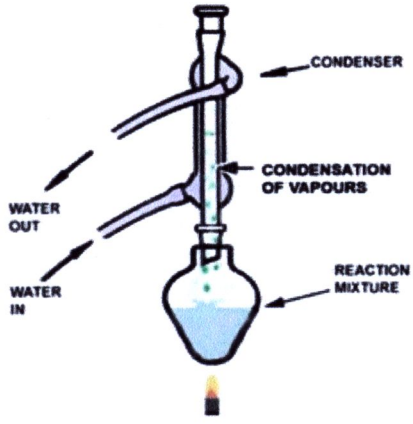


Fig. 1.10 Heat reflux extraction [16].

1.2.2 Soxhlet Extraction (SE)

SE involves bringing a material to be extracted (usually in solid form) into contact with the extraction solvent for a period of time, followed by separation of the solution from the solid debris. In this method, the material to be extracted is placed in a thimble made of cellulose or cloth in a central compartment. The solvent

is boiled in the reservoir, the vapors pass into the condenser and condense to liquid which drips into the thimble. Then the extraction of the sample occurs along the passage of dripping solvent which flows back down to the reservoir. The operation is repeated until complete extraction is achieved [17, 18]. Fig 1.11 shows the soxhlet extraction apparatus.

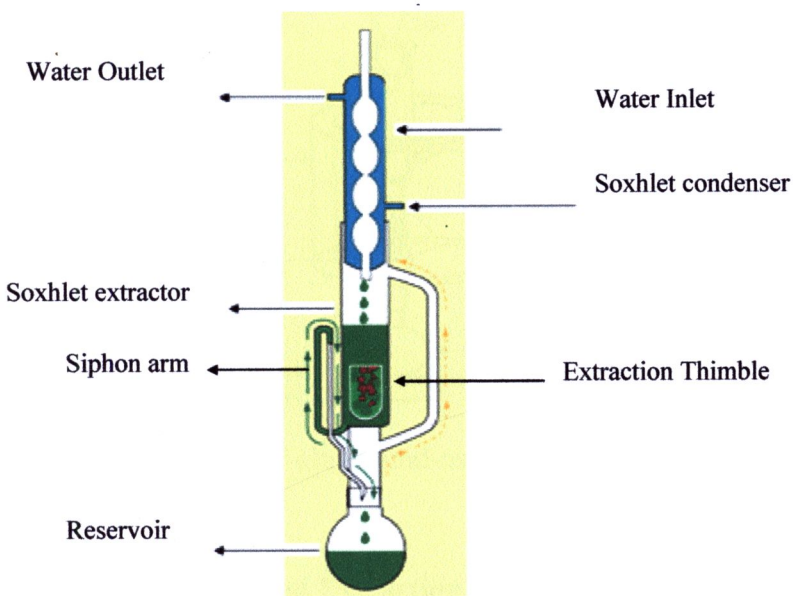


Fig. 1.11 Soxhlet extraction [4].

1.2.3 Ultrasound-assisted Soxhlet Extraction (USE)

The USE principle is a modified Soxhlet extraction by applying acoustic vibrations with frequencies above 20 kHz to break the cell wall and matrices. This action will increase the surface contact between the sample and solvent and improves the extraction efficacy of the targeted compound. Luque-García & Luque de Castro, [19] combined UAE with conventional soxhlet extraction for the analysis of total fat in oleaginous seeds. A water bath was modified such that the Soxhlet chamber was located in it. The bath was sonicated by a probe to accelerate the extraction process.

The efficiency was similar to, or even better than, those of conventional Soxhlet extraction and saving both time and sample manipulation. The assemble of the ultrasound-assisted soxhlet extraction is shown in Fig. 1.12.

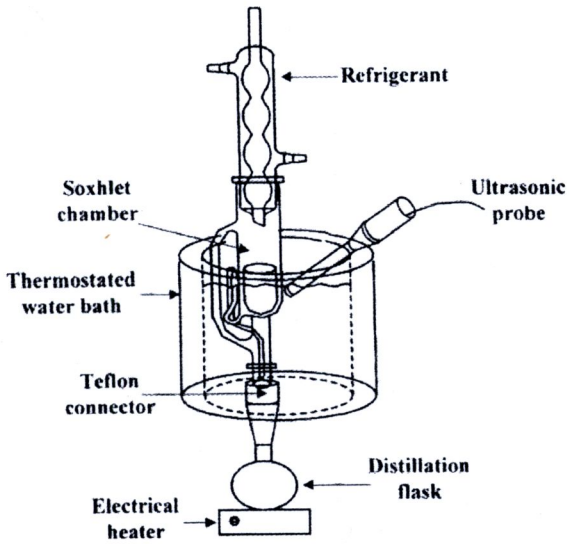


Fig. 1.12 Assemble of ultrasound-assisted Soxhlet extraction [19].

1.2.4 Microwave-assisted Extraction (MAE)

MAE is widely recognized as a versatile extraction technique, especially for solid samples. MAE utilizes electromagnetic radiation to desorb analytes from their matrices. The effect of microwave energy is strongly dependent on the nature of both the solvent and the solid matrix. Consequently, unlike classical conductive heating methods, microwaves heat the whole sample simultaneously. The main advantages of MAE are the usually high extraction rates due to the very rapid heating and the elevated temperatures, and the ease of instrument operation. A drawback is that the heating is limited to the dielectric constant of the sample/solvent [20]. The pictures of the heating principle of the classical conduction mode compared with the MAE is shown in Fig. 1.13.

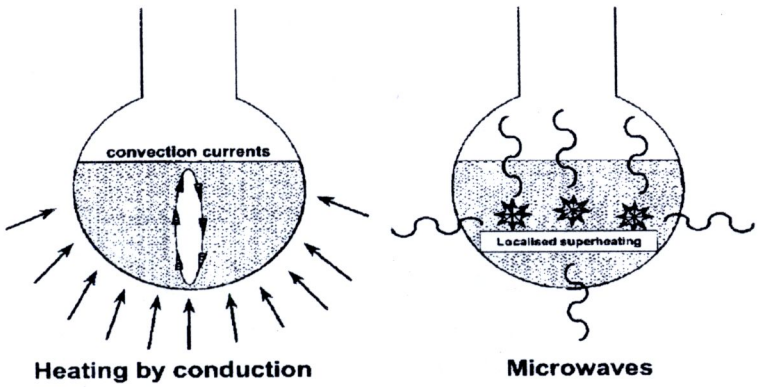


Fig. 1.13 The heating principles of classical conduction and the MAE [21].

1.2.5 Subcritical Water Extraction (SWE)

Subcritical water is the water being near the critical zone of which the temperature ranges are typically in between the boiling and critical temperatures. Water is held in its liquid state above its normal atmospheric boiling by application of pressure. The phase diagram of water as shown in Fig. 1.14 illustrates the critical temperature (T_c = 374 °C) and the critical pressure (P_c = 22.1MPa) of water, as well as supercritical zone and subcritical zone. By increasing the pressure of the gas above the critical point, it is possible to create liquid-like densities and solvating strengths. Near the critical point, the density of the gas will increase rapidly with increasing pressure; the solubility of many compounds is several orders of magnitude greater than that predicted from the classical thermodynamics of ideal gases. Recently, the use of water at sub- or supercritical state for extraction processes has been investigated by modifying pressure and temperature. It can result in a dramatic change of the dielectric value that may become similar to that of the polar solvents, such as the dielectric value of C_6H_{12} can be altered till it is similar to those of EtOH or

CH_2Cl_2 and ethyl acetate, and hence allowing the solubilization of a wide variety of moieties that normally dissolve only a little or undissolve in water [22].

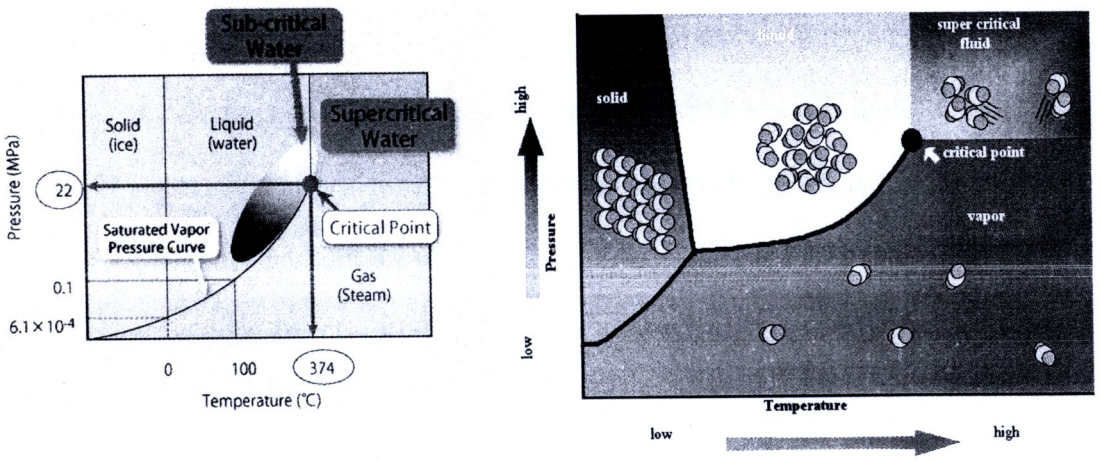


Fig. 1.14 The phase diagram of water [22].

Recently, Prommuak *et al.,* [2] studied the effect of solvent systems on efficiency of extracting carotenoids and flavonoids from yellow Thai silk waste. Different methods for extractions of these pigments were carried out using ethanol and subcritical water. For extraction of carotenoids, the ethanol was suitable extracting solvent and the amount of carotenoids increased with increasing temperature (50–79 °C) and extraction time (2–12 h). For flavonoids, the reaction was carried out in a closed batch reactor at temperature (80–150 °C) and extraction time (10–60 min). Subcritical water extraction was suitable at 120 °C for 10 min. But the amount of flavonoids decreased with increasing subcritical water temperature and extraction time due to decomposition at such conditions.

The selection of the condition for an extraction process is influenced by the factors which are responsible for limiting the extraction condition. Some effective parameters on extraction includes:

- **pH of the solution:** The pH becomes significant in metal and bio-extractions. pH value is maintained to improve distribution coefficient and minimize degradation of product.

- **Extraction time:** Extraction time is an important parameter in reactive extraction processes and in processes involving short-life components. Time have effect to extraction due to time affect on the diffusion of pigment from cell membrane into the solution.

- **Extraction temperature:** Temperature can be used as a variable to alter selectivity. Elevated temperatures are sometimes used in order to keep viscosity low and thereby minimizing mass-transfer resistance. In most cases, the solubility of the material which is being extracted will increase with temperature to give a higher rate of extraction. Furthermore, the diffusion coefficient will be expected to increase with the rise in temperature and this will also improve the rate of extraction.

- **Type of solvent:** The type of solvent will depend on the harmful chemicals present and the material being treated. A general principle in solvent extraction is "like dissolves like", which means that solvents only extract those samples which have similar polarity with the solvents

1.3 Purification method

In general, after separation or extraction, the analyte should be purified by some appropriate techniques such as precipitation or dialysis before performing further analysis.

1.3.1 Precipitation [23]

Precipitation can be used as concentration method of analyze and is ideal as an initial step in their purification before another analysis. For example, in the case of precipitating protein, due to the solubility of protein molecule in the solvent occurs by the distribution of charged groups (hydrophobic and hydrophilic) on its surface. The surface charged group will further interact with ionic groups in the solution. The schematic picture of hydrophobic group of general protein is shown in Fig. 1.15.

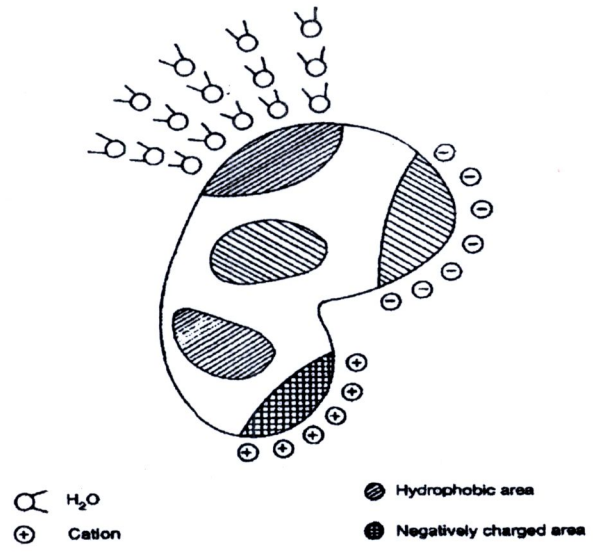


Fig. 1.15 Schematic representation of hydrophobic, negatively and positively charged areas of general protein [24].

Protein precipitation can occur by aggregation of protein molecules that be induced by changing pH and ionic strength or by adding miscible solvent. Precipitation can be recovered by filtration, centrifugation or washing with water or solvent. The protein precipitation can be done in many ways in order to purify the target protein.

1.3.2 **Dialysis** [25, 26]

Dialysis is one of the purification methods suitable for biomolecules. It is based on the separation of small molecules from large molecules by allowing diffusion of small molecule through the selective membrane. The dialyzed solution is placed in sealed dialysis tubing and immersed in a specific buffer. At equilibrium, the molecule, which is smaller than the pore of the membrane, will be removed and the larger is still retained. Applications of dialysis includes: removing salts, buffer exchange, fractionation, drug binding studies and drug release in cell models or organisms. The dialysis method is illustrated in Fig. 1.16.

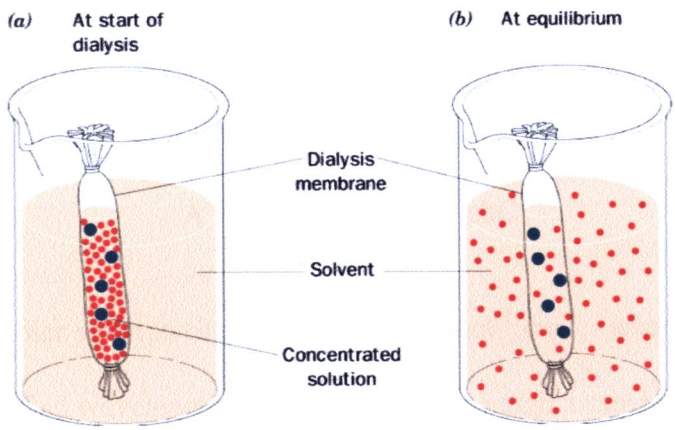


Fig. 1.16 Dialysis [26].

1.4 Experimental techniques

Many techniques have been used for determining the quantity of the sample and then characterizing its structure using various techniques e.g. Ultraviolet-Visible spectrometry (UV-Vis) and Fourier transform infrared spectroscopy (FT-IR).

1.4.1 Ultraviolet and Visible spectrophotometry

UV-Vis spectroscopy is one of the most important quantitative spectroscopic techniques. The wavelength range extends from about 190 nm to 750 nm which corresponds to electronic transitions of different origins. The ultraviolet region of the spectrum is generally considered to range from 200 to 400 nm and the visible region from 400 to 800 nm. The corresponding energies for these regions are about 150 to 72 and 72 to 36 kcal mol^{-1}, respectively [27].

The UV-visible spectroscopy is a characterization technique in which the absorbance of the material is studied as a function of wavelength. Molecular absorption spectrophotometry is based on the measurement of the transmittance (T) or the absorbance (A) of solutions contained in transparent cells having a path length of b cm. Ordinarily, the concentration (C) of an absorbing analyte is linearly related to absorbance as represented by the equation:

$$A = -\log T = \log I_o/I = \varepsilon bC$$

where ε is the molar absorptivity with units of L mol^{-1} cm^{-1}. where I is the intensity of light at a specified wavelength λ that has passed through a sample (transmitted light intensity) and I_0 is the intensity of the light before entering the sample. This equation is a mathematical representation of Beer's law. A block diagram of the essential

components of a basic UV-Vis instrument is shown in Fig. 1.17. UV-Vis spectroscopy is one of the most useful and widely used tools available to the chemist for qualitative and quantitative analyses.

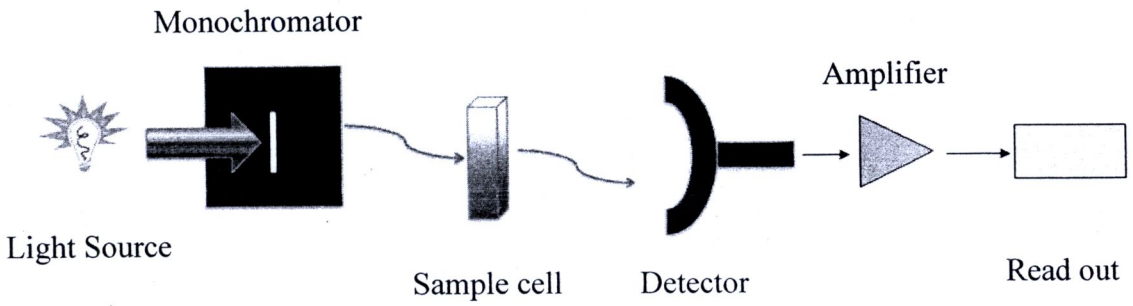


Fig. 1.17 Systematic diagram of UV-Vis instrument.

1.4.2 Fourier Transform Infrared spectroscopy (FTIR)

IR spectroscopy is a chemical analysis technique, which measures the infrared intensity versus wavelength (wave number) of light. Based upon the wavenumber, infrared light can be categorized as far infrared (4 – 400cm^{-1}), mid infrared (400 – 4,000cm^{-1}) and near infrared (4,000 – 14,000cm^{-1}). Infrared spectroscopy detects the vibration characteristics of chemical functional groups in a sample. When an infrared light interacts with the matter, chemical bonds will stretch, contract and bend. As a result, a chemical functional group tends to absorb infrared radiation in a specific wave number range regardless of the structure of the rest of the molecule.

Characteristic bands found in the infrared spectra of proteins and polypeptides exhibit several relatively strong absorption bands, which are approximately constant in frequency and intensity from one sample to another. These

bands are associated with the vibrations of the amide group, the structural unit common to these molecules [28, 29]. The polypeptide and protein repeating units give rise to nine characteristic IR absorption bands, namely, amide A, B, and I–VII. Of these, the amide I and II bands are the two most prominent vibrational bands of the protein backbone. Other amide vibrational bands are very complex depending on the details of the force field, the nature of side chains and hydrogen bonding, which therefore are of little practical use in the protein conformational studies [30]. Such bands are shown in Fig. 1.18 for N-methylacetamide, a model for the transpeptide group in proteins. The characteristic IR bands of the proteins and peptides are shown and also diagram of the essential components of infrared instrument is shown in Table 1.2 and Fig. 1.19, respectively.

Table 1.2 Characteristic infrared bands of peptide linkage [31].

Designation	**Approximate frequency (cm^{-1})**	**Description**
Amide A	3300	NH stretching
Amide B	3100	NH stretching
Amide I	1600-1690	C=O stretching
Amide II,	1480-1575	NH bending
Amide III	1229-1301	CN stretching,
Amide IV	625-767	OCN bending
Amide V	640-800	Out-of-plane NH bending
Amide VI	537-606	Out-of-plane C=O bending
Amide VII	200	Skeletal torsion

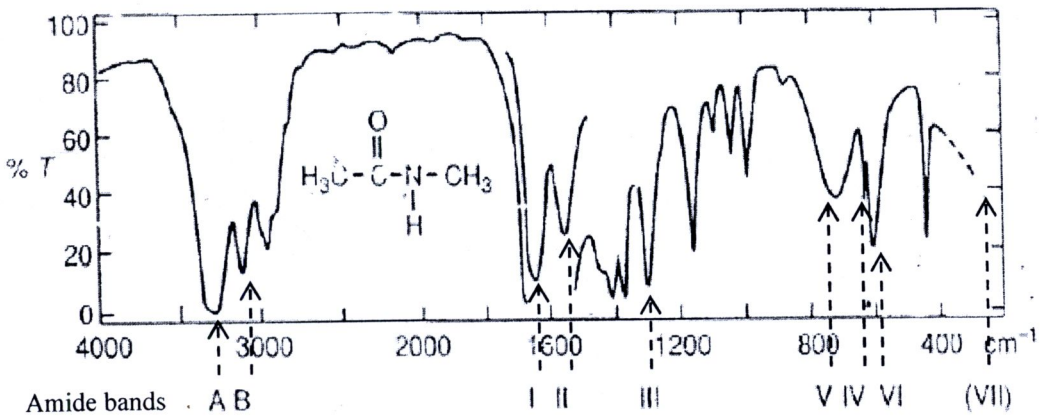


Fig. 1.18 Characteristic amide bands as exhibited by a capillary film of N- methylacetamide.

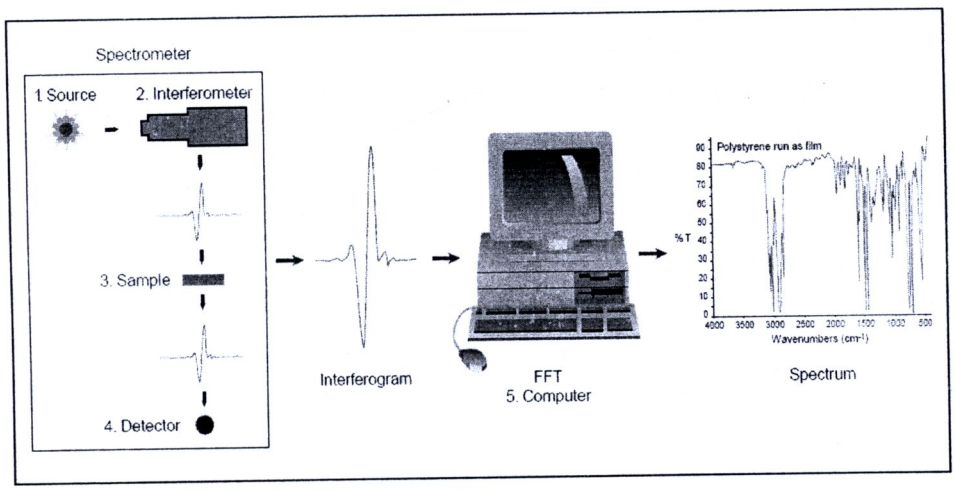


Fig. 1.19 Diagram of the components of infrared spectrophotometer [32].

Extraction techniques have been widely investigated to obtain such valuable natural compounds from plants for commercialization. From the conventional extraction methods involving room temperature extraction, HRE and SE are mostly based on the choice of solvents, heating or mixing to increase the solubility of materials and the rate of mass transfer. However, it is characterized by the

consumption of large volumes of solvent and lengthy extraction procedures [33]. UAE uses high-frequency sound to disrupt the target compound from the plant materials. MAE of biologically active compounds has many advantages over other extraction methods such as shortened extraction time and lower consumption of solvents [34]. However, one disadvantage of microwave extraction is its poor performance when either the target compounds or the solvents are non-polar, or when they are volatile. Furthermore, comparison of various extraction methods has been reported. Gao and Liu [35] studied an efficient method for extraction of flavonoids from cultured cells of *S. medusa* Maxim, with various extraction methods including ERT, SE, HRE, UAE and MAE. They were employed to extract flavonoids from dry cell cultures of *S. medusa* and obtained the maximum yield of flavonoids of 3.0%, 4.1%, 3.9%, 3.5% and 4.1%, respectively. MRE showed obvious advantages as the simplest and most efficient method that can be done in short duration for extracting flavonoids from *S. medusa* cultured cells.

In addition, the most appropriate method of extraction depends on the source and the type of sample to be extracted. The selection criterion of the best extraction method is based on the highest recovery, for which the degradation of the compounds due to the different process steps should be minimum. So along with the parameters involved, decision regarding the choice of extraction method also depends on the end product required [36]. There have been reports on study of parameters associated in the extraction of pigment. Xu *et al.* [37] studied the effect of various parameters such as temperatures, extraction times, concentrations of alcohol and material:aqueous ratios for ethanolic extraction of flavonoids from red raspberry.

They found that 75% EtOH solution (1:10 w/v sample to solvent ratio) is sufficient to obtain the highest yield. However the yield decreased after longer extraction time.

In this study, HRE is one choice for pigment extraction from silk cocoon that involves heating a reaction mixture to the boiling temperature of the reaction solvent and inducing the solvent to re-condense back into the reaction flask using a condenser. The advantage of this technique is very simple, cheap and efficient way to maintain a constant reaction temperature for a long period of time without the need to add more solvent or without any loss of volatile liquid. This technique is useful for performing chemical reactions under controlled conditions that require substantial time for completion. The parameters affecting the extraction including extraction time, temperature and acid/base condition of the extracting media was evaluated. The pigment extracts were analyzed by using UV-Vis technique and the percentage of pigment and protein in the silk cocoon was determined gravimetrically and characterizing the final product with FT-IR technique. For the importance of silk and its application are described as following.

1.5 Silks

Silks, called the "Queen of fibres", are generally defined as protein polymers of which their continuous protein fibre are produced while spinning for a cocoon. Silk includes a broad range of primarily protein-based high molecular weight polymers often associated with insects, silkworm, and orb weaving spiders to form their cocoons [38, 39]. There are mainly four varieties of silkworm e.g. Mulberry, Eri, Muga, and Tasar and each variety is reared by feeding on the leaves of

certain plants (Table 1.3). All the species of silkworm have four stages in their life cycles namely, the egg, larva, pupa and moth.

Table 1.3 The different species of silkworms and the types of leaves [40].

Silk	Type of worms	Types of leaves
Mulberry	Bombyx mori	Mulberry leaves
Eri	Samia cynthia ricini	Castor oil leaves
Muga	Antherea assamensis	Som (Machilus bombycina) and Sualu (Litsaea polyantha) leaves.
Tasar	Antherea mylitta	Arjun and Asan leaves

Silks have been gaining wide use in variety of applications as degradable biomaterials, biomedical and functional bio-membrane materials and fibers besides their traditional use as a textile raw material [41, 42]. Despite the triumphal advance of synthetic fibers, silk maintains its place in the raw material market, in the textile and clothing industries, and in retail trade because of its unique properties [43]. The use of such silks, depending on their origin, can be justified both by the combination of mechanical properties, non-toxicity, biocompatibility and biodegradability depending on source and treatment.

The production of Thai silk begins with the *Bombyx mori* (*B. Mori*) or mulberry silkworms and about 95 % of the world's production is of this species. *B. mori* is the most economic importance that emerging as an ideal molecular gene resource for solving a broad range of biological problems and produces massive amount of silk proteins during the final stage of larval development. [1, 44]

Commercially reared silkworms of the species *B. mori* are normally killed before the pupae emerge either by pricking them with a needle or dipping the cocoons into boiling water, thus allowing the whole cocoon to be unravelled as one continuous thread of great strength measuring from 500-1500 metres in length. Single filaments are too thin for utilization. For production purposes, several filaments are combined with a slight twist into one strand. This process is known as "silk reeling or filature". This allows a much finer cloth to be woven from the silk and have been highly valued as textile fibers [45]. Raw silk fiber can be prepared from cocoons by reeling and degumming process that is the removal of sericin layer (gum) prior to dyeing and finishing to get bright and lustrous fabrics. The source of silk and processes of making silk fiber are illustrated in Fig. 1.20.

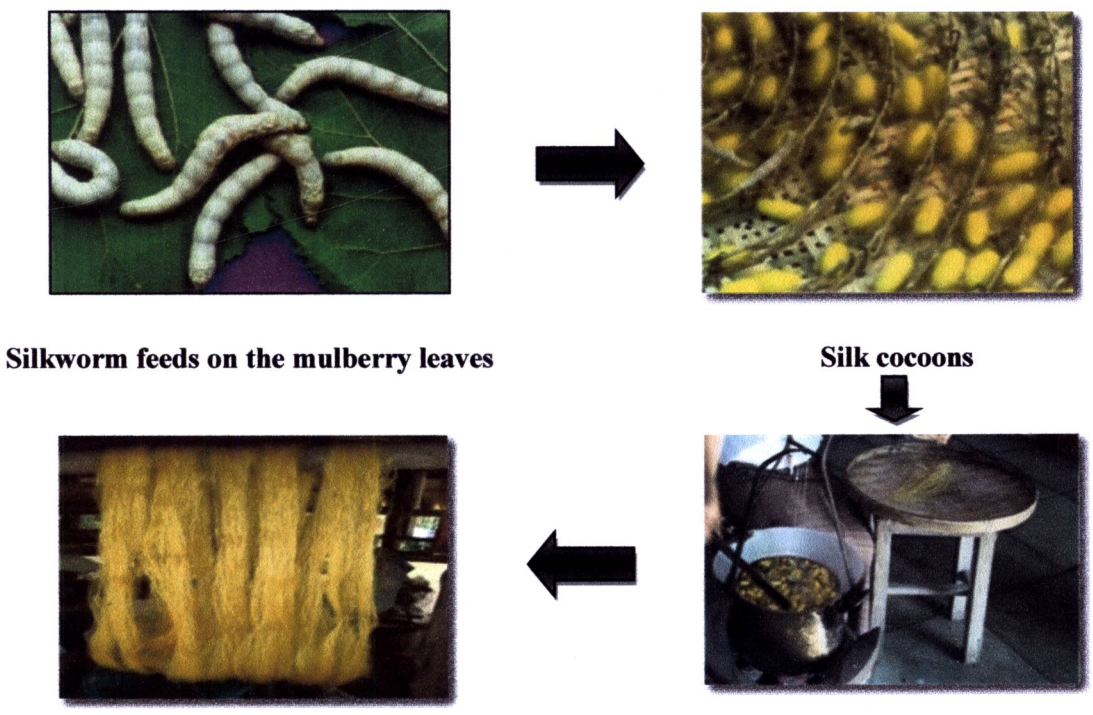


Fig. 1.20 Source of silk and processes of making silk fiber.

1.5.1 Composition of raw silk cocoon

Silk fibre is a product of silkworm while spinning to form cocoons. It consists of two major proteins, namely fibroin and sericin. The silk sericin (glue-like protein) envelops the fibroin fiber (core protein) with successive sticky layers to help in the formation of a cocoon case to protect the growing worm. It is the silk sericin and other impurities that mask the luster of the silk fibroin and cause hardness and coarseness of silk fibers. Fig. 1.21 shows each of silk fiber, the two cores are coated and wrapped together with sericin.

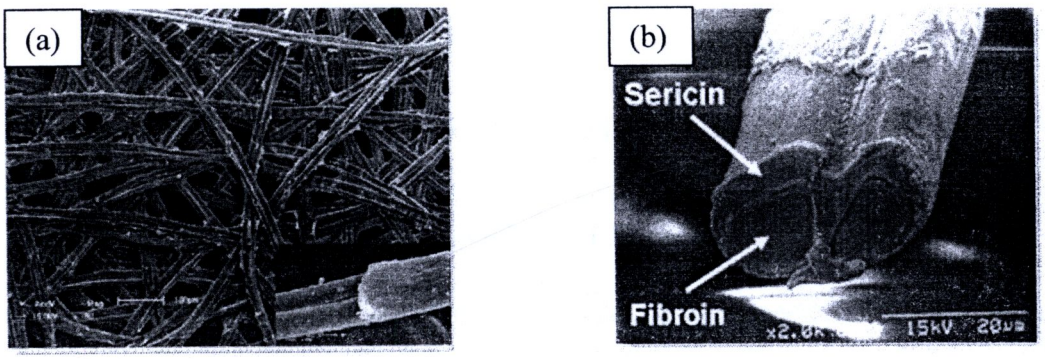


Fig. 1.21 SEM images of (a) the structure of a silk cocoon and (b) cross sectional structure of silk fiber showing the fibroin-sericin co-existence [46].

Fibroin and sericin proteins have been found to have numerous applications. For fibroin protein, it can also be found in various forms, such as gels, powders, membrane gels, sponges, powders, artificial ligaments and scaffolds depending on its applications [47, 48]. Recently, researchers have investigated the potentiality of silk fibroin as a candidate material for biomedical applications and functional biomaterial especially for tissue engineering. While sericin protein has been found to have antioxidant, antibacterial, UV resistant, tyrosinase-activity inhibiting and moisture absorbing and desorbing properties. Thus, because of such properties, sericin can be

properties, sericin can be applied in many fields as cosmetics, polymer and pharmaceutical products as well as for biomaterials manufacturing as indicated in Fig. 1.22 [44, 49]. It represents significant economic and social benefit if this sericin protein is recovered and recycled.

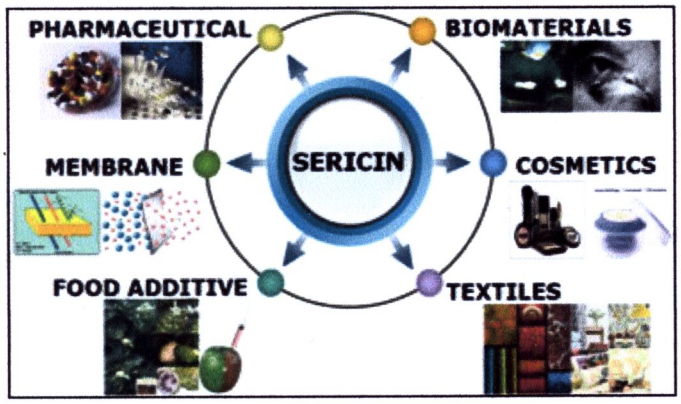


Fig. 1.22 Application of sericin in various industries [50].

The chemical compositions of raw silk are shown in Table 1.4, variations in the composition contents depend on the species, origin, and culture conditions of the raw silk.

Table 1.4 The chemical composition of silk.

Sample	Composition (*%w/w*)								
	Fibroin	Sericin	Fat & wax	moisture	Carbohydrates and Starches	Inorganic matter	Mineral salts	Pigment	Ref.
Silk cocoon shell	72-81	19-28	0.8-1.0	-	-	-	-	1.0-1.4	[45]
Raw silk	70-80	20-30	0.4-0.8	10-11	1.2-1.6	0.7	-	0.2	[51]
Raw silk	~75	23	1.5	-	-	-	0.5	-	[52]

Besides, the existence of fibroin and sericin proteins as important components in the silk cocoon, especially the native silk like yellow *Nangnoi Si Sa Ket,* that have been cultivated widely in Thailand, it also contains a small amount of pigments, which are mostly associated with carotenoids and flavonoids. Both of these pigments are remarkably reported as highly efficient antioxidants [53]. Carotenoids are the most widespread pigments in nature which are responsible for the unique yellow color of Thai silk. Other silk pigments include phenolic compounds particularly flavonoids which come from mulberry leaves, the sole food for *B. mori* larvae. The content of silk cocoon coloring component varies depending on the *B. mori* strain [54, 55]. The major carotenoid in the yellow colored silk was reported to be lutein about 80–90% of total carotenoids, whereas the β-carotene and α-carotene are minor components. Lutein itself has been reported as being an excellent agent in protecting against vision loss and reducing the risk of chronic disease, such as heart disease and cancer. [56, 57] For flavonoids, they have several pharmacological activities such as cardiotonic, anti-inflammatory, and anti-microbial activities [58]. Therefore, attentions on the study in order to enhance its value by separating the pigment, sericin and fibroin can bring up a significant economic and social benefit.

1.5.2 Pigments in silk cocoon

In general, pigments are natural coloring matter made by modification of materials from living organisms. The pigments occur in leaves, flowers and fruits; they are also present in skin, eyes and other animal structures; and in bacteria and fungi. Pigments have attracted widespread interest because of their general health and safety image and other important properties. Thus, natural

safety image and other important properties. Thus, natural pigments have been used in medicines, foods, clothes, furniture, cosmetics and in other products.

Since the color caused by the pigment arises when a molecule of pigment absorbs certain wavelengths of visible light and transmits or reflects others. The pigment can change the color of transmitted or reflected light as the result of wavelength-selective absorption [59, 60]. Chromophore is the part of a molecule responsible for its color where the energy difference between two different molecular orbitals falls within the range of the visible spectrum. Visible light that hits the chromophore can thus be absorbed by exciting an electron from its ground state into an excited state. Chromophores almost always arise in one of two forms: conjugated pi systems (also known as resonating systems) and metal complexes [61]. In the conjugated chromophores, the electrons jump between energy levels that are extended π orbitals, created by a series of alternating single and double bonds, often in aromatic systems, for examples, lutein (carotenoid) and quercetin (flavonoid) found in yellow silk cocoon. The conjugated double bonds that form the chromophore of the molecule are shown in Fig. 1.23 and highlighted in red.

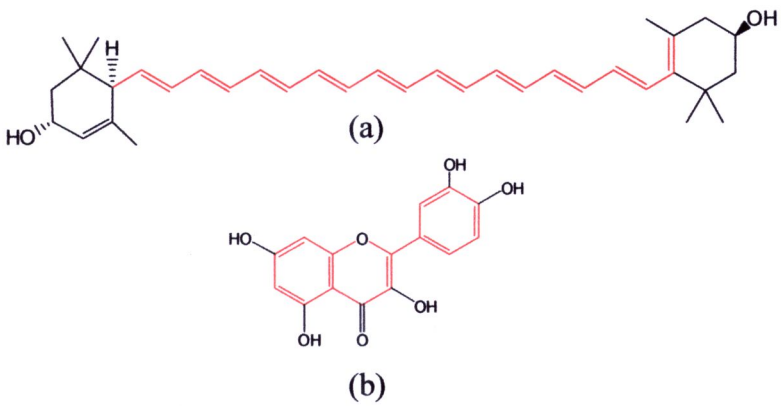


Fig. 1.23 Chemical structure of (a) lutein and (b) quercetin.

Pigment of yellow silk cocoon is a characteristic feature particular to the species. It is the presence of pigments derived from mulberry leaves appeared only in sericin layers, which coats the fibroin base. These pigments are absorbed from dietary leaves, transferred from the midgut to the silk gland via the hemolymph, and accumulated in the silk fiber [62].

There are many varieties of the silkworm *B. mori*, and they produce brilliantly colored yellow, pink, golden-yellow, flesh, sasa (yellowish green) and green cocoons. The pigments in yellow, pink, golden-yellow and flesh cocoons are derived from carotenoids, while those of sasa and green are from flavonoids. The silk cocoon with different colors are shown in Fig. 1.24.

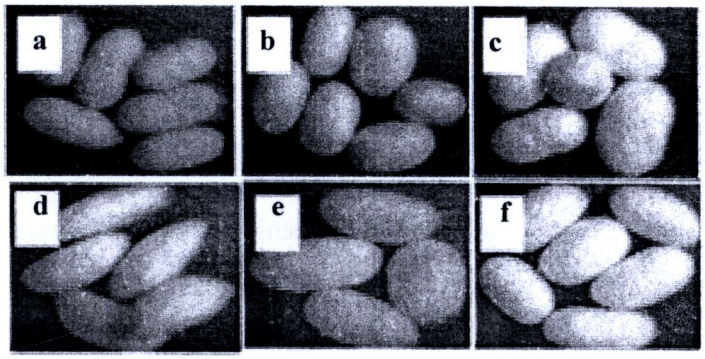
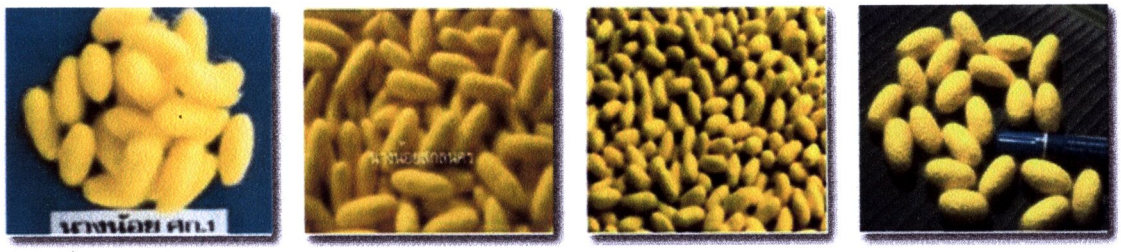


Fig. 1.24 Silk cocoon with different colors (a) pink cocoon; (b) yellow; (c) whitish sasa; (d) deep sasa; (e) golden-yellow; (f) white [63].

Thai silk is one of the mulberry silkworm (*B. mori*) silks but it differs somewhat in the appearance. It is yellower in color, the filaments are coarser. These characteristics cause Thai silk to have its own style after weaving. Thai silk products are mainly produced by domestic industries in the northern and north-eastern parts of Thailand [64]. The yellow silk cocoon can be found, such as those varieties

Nangnoi Si Sa Ket, Nangnoi Sakon Nakhon, Udonthani and *Ubon Ratchathani 60-35* or *Dokbua*, which the color, shape, size and wrinkle of the cocoon differs according to silkworm variety, rearing season and harvesting conditions. Some example of silk cocoons found in Thailand are shown in Fig. 1.25.



Nangnoi Si Sa Ket ***Nangnoi Sakon Nakhon*** ***Udon Thani*** ***Ubon Ratchathani 60-35 (Dokbua)***

Fig. 1.25 Some example of silk cocoons found in Thailand [65].

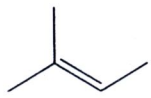
As carotenoids and flavonoids are present in the silk cocoons, their characteristics are considerably worth of interest as follows.

1.5.2.1 Carotenoids

Carotenoids are natural pigments, normally widespread in nature, especially living organisms. Carotenoids are responsible for the bright red, orange, or yellow coloration of many fruits and vegetables. There are several dozen carotenoids in the foods that we eat, and most of these carotenoids have antioxidant activity.

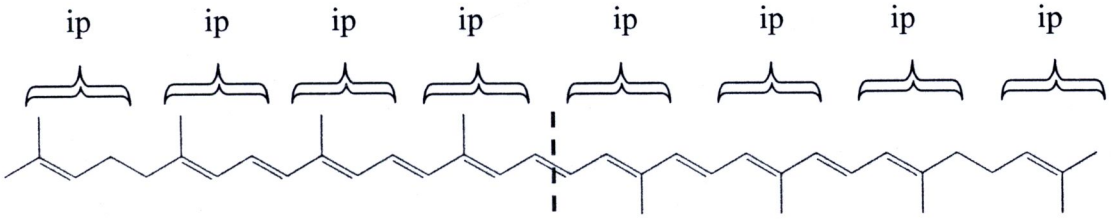
In general, carotenoids are isoprenoid compounds comprised of 40-carbon in eight isoprene units as shown in Fig. 1.26. Where its conjugated double bonds responsible for their characteristic colors and their photochemical properties. Carotenoids are classified by their chemical structures as: (i) carotenes that are

carbon, hydrogen, and additionally, oxygen. Carotenoid behavior is derived substantially from their highly delocalized polyene backbone. The central carbon chain remains the same, but the groups at either end ring differ slightly. Depending on the degree of substitution, these rings can strongly influence the overall properties of the carotenoid [66, 67].



ip = isoprene group

C_{40} Carotenoid = 8 isoprene units



Center of the molecule

Lycopene

Fig. 1.26 Basic structure of carotenoid [68].

Carotenoids may be classified as hydrocarbon carotenes and xanthophylls, which are oxygenated derivatives of carotenes. Representative examples of carotenes include β-carotene, α-carotene, and lycopene. Examples of xanthophylls include lutein, astaxanthin, capsanthin, zeaxanthin, and capsorubin.

More than 700 naturally occurring carotenoids have been identified [69]. Some carotenoid structures are presented in Fig. 1.27.

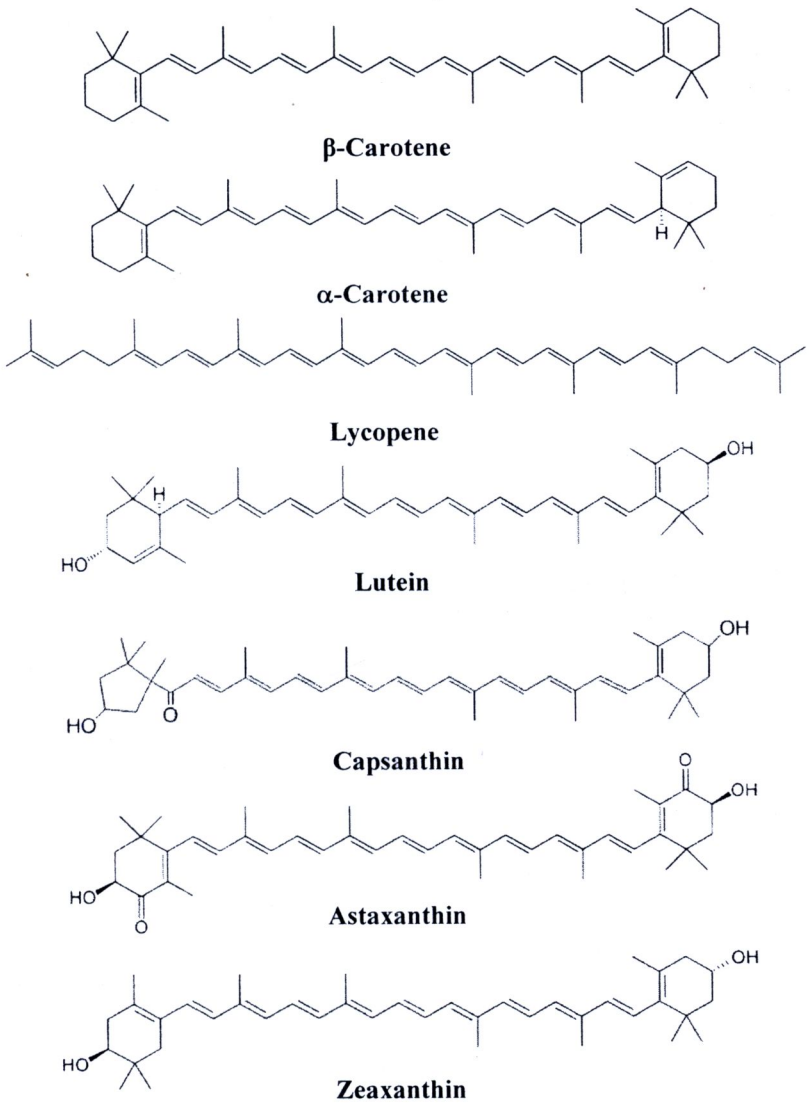


Fig. 1.27 Chemical structure of some carotenoids [70].

In addition, their colors ranging from yellow, through bright orange, to deep red, is directly linked to their structure. The double carbon-carbon bonds interact with each other in a process called conjugation. As the number of double bonds

increases, the wavelength of the absorbed light increases, giving the compound an increasingly red appearance.

Carotenoids found in the human diet are primarily derived from plants. Animals cannot synthesize carotenoids, so their presence is due to dietary intake. The carotenoids can also be found in vegetables, fruit and flowers. Apricots, cantaloupe, carrots, pumpkin and sweet potato are sources of α-carotene and β-carotene; pink grapefruit, tomatoes and watermelon are sources of lycopene, ζ-carotene and β-carotene. Mango, papaya, peaches, prunes, squash and oranges are sources of lutein, zeaxanthin, β-cryptoxanthin, α-, β- and ζ-carotene, whereas green fruits and vegetables such as green bean, broccoli, Brussels sprout, cabbage, kale, kiwi, lettuce, pea and spinach are sources of lutein, zeaxanthin, α- and β-carotene. Carotenoid concentrations in fruits and vegetables vary with plant variety, degree of ripeness, time of harvest, and growing and storage conditions [71].

Carotenoids have a broad of functions, especially in relation to human health. Carotenoids are widely used an antioxidant and they are thought to be responsible for the beneficial properties of fruits and vegetables in preventing human diseases including cardiovascular diseases (CVD), cancer and other chronic diseases [72, 73] because of important role in health maintenance by helping to reduce the impact and minimize the oxidative damage caused by surplus free radicals [74]. The consumption of lutein and zeaxanthin reduces 40 % of the age related macular degeneration [75]. They are also dietary sources of Pro-vitamin A which is important for human vision, sperm generating, bone and tooth generating, tissue restoration and skin nourishing [76]. For lutein and zeaxanthin are thought that these carotenoids play an important role in protecting the macula from light-induced damage (blue

light), scavenging free radicals formed in the photoreceptors and protecting eyes from the sun [56]. So, carotenoids are used as food colorants, cosmetics or pharmaceuticals. The role of carotenoids in the prevention of chronic diseases and their biological actions are summarized in Fig. 1.28.

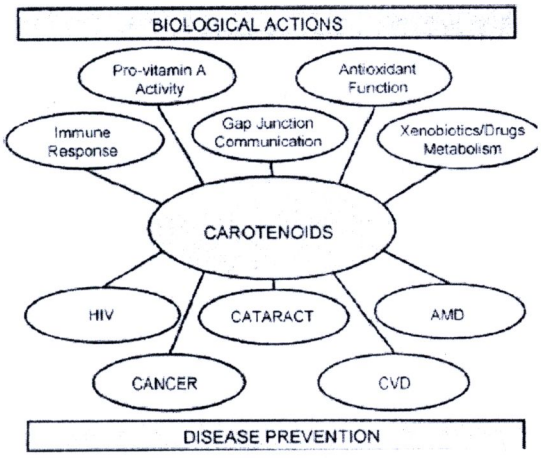


Fig. 1.28 Role of carotenoids in the prevention of chronic diseases [77].

2) Flavonoids

Flavonoids constitute a group of phenolic substances found in many terrestrial or floating plants, but not aquatic plants. Flavonoids are of particular interest because of their high prevalence in foods such as fruits, vegetables and tea [60]. The functions of flavonoids in plants are believed to be as protective agents against UV radiation, antioxidants and also free radical scavengers [78].

The basic structure of flavonoids comprises of 15 carbons (C6–C3–C6), arranged in the form of two benzene rings (A and B), which are connected by an oxygen-containing pyrine ring(C). The basic flavonoid structure is illustrated in Fig. 1.29.

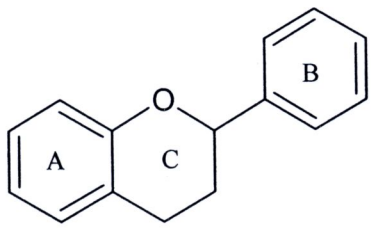


Fig. 1.29 Basic flavonoid structure [67].

Flavonoids containing a hydroxyl group in positive C-3 of the ring are classified as flavonol and anthocyanidins, and those lacking it as flavanone and flavones. They are different in their properties depending on number and position of hydroxyl groups and sugar substitutions. It has been shown in radical absorbance assays that the antiperoxyl radical activities increase with the number of hydroxyl groups. More than 5000 subclasses of flavonoids were identified by Harborne [78]. Chemical structures of some flavonoids are shown in Fig. 1.30.

Flavones and flavonols have yellowish colors. The colors are sensitive to pH. The yellow becomes much deeper in solutions of high pH. The colors of flavones tend not to fade in strong light as flavonols do, but they are paler. The yellow color of onion skins is due to a mixture of flavones and flavonols consisting of quercetin, kaempferol and quercetin-3-glucoside [72].

Anthocyanins are the most highly colored substance among the flavonoids. They are responsible for the scarlet, red, violet and blue in many flowers, fruits and vegetables. Anthocyanins are soluble in water and easily extracted into weakly acidic solution. However, the color is pH dependent.

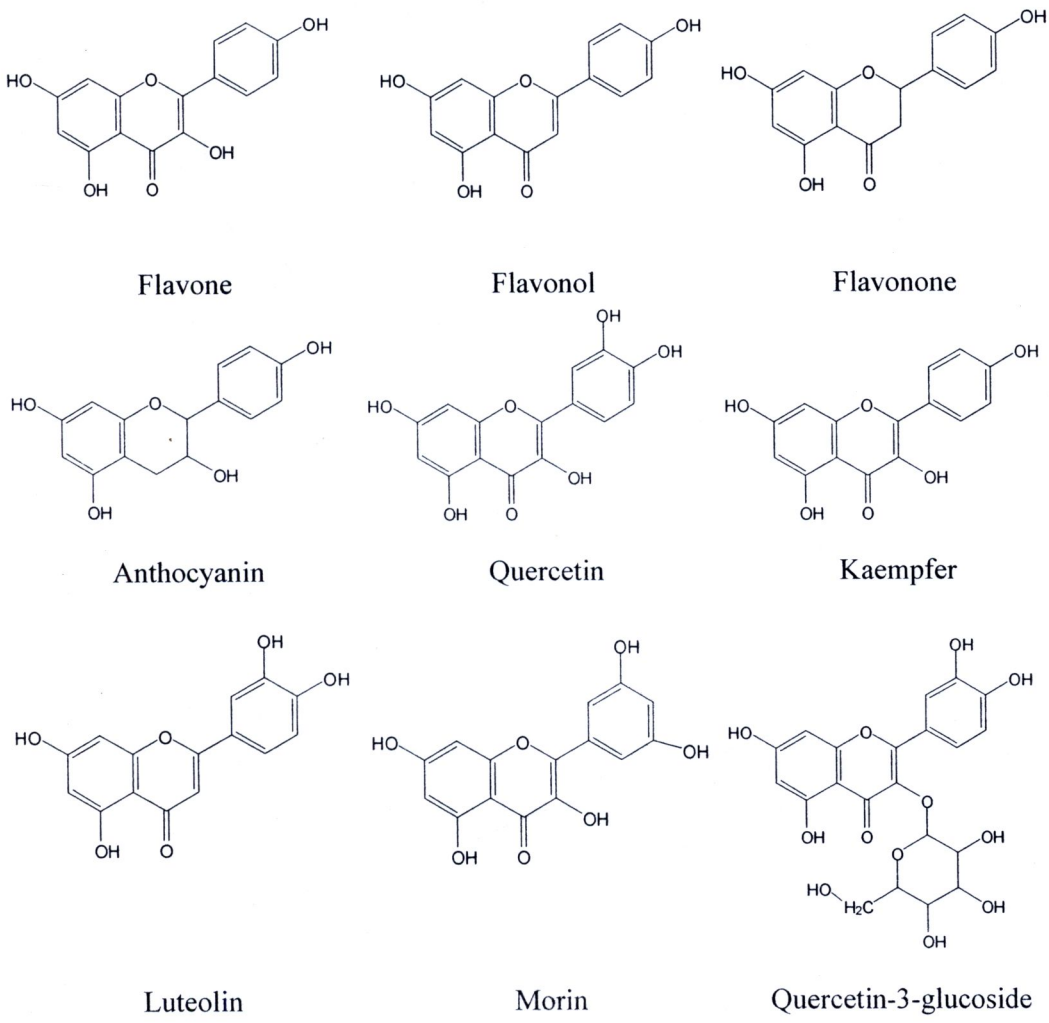


Fig. 1.30 Chemical structure of some flavonoids [79].

In plants, flavonoids occur mainly in leaves and in the outer parts of the plants. In vegetables, quercetin and its glycosides predominate but glycosides of kaempferol, luteolin and apigenin are also present [80]. Onions have been shown to contain large amounts of flavonoids, especially quercetin and its glycosides. Onion, apple skin, tea, grape and broccoli are sources of flavonol. Peel of citrus fruits is a source of flavanone. Celery and parsley are sources of flavones. Red wine is source

of flavonol. Cherry, raspberry, strawberry and grape are sources of anthocyanidins. Soybeans are sources of isoflavonoid [81].

Flavonoids are generally antioxidants and act as free radical scavengers as they are potential reducing agents and protected from oxidative reactions taking place inside the body. Flavonoids have a wide range of biological effects, such as inhibition of key enzymes in mitochondrial respiration, protection against coronary heart disease and anti-inflammatory, antitumor, and antimicrobial activities [81]. They are beneficial against many other health problems such as osteoarthritis [82] and ocular disorders [83] and act as phytoestrogen [84] and there is also some evidences from epidemiological and *in vivo* studies that their consumption is associated with a reduced risk of certain cancers and cardiovascular diseases [85, 86]. In addition to the proposed beneficial effects of flavonoids, mutagenicity and carcinogenicity have also been reported [87]. They occur in many fruits and vegetables which are a large part of a balanced human daily diet. Depending on the constituents of the diet, flavonoids intake can vary in the range of 50–800 mg per day [88].

1.5.3 Solvent extraction of pigments

Carotenoids are soluble in lipids or in nonpolar solvents, except when they form complexes with proteins and sugars. Hence, they are extracted with nonpolar solvents. At present, many organic solvents have been used for extraction of carotenoid. If the tissue is previously dried, then water-immiscible solvents are used such as petroleum or ethyl ether; with the fresh materials acetone or ethanol are used. Other solvents such as hexane, heptane, and isooctane are not so good for extraction,

but their other characteristics are favorable. On the other hand, it must be considered which compounds will be extracted: polar solvents (such as acetone, methanol, ethanol) are good with xanthophylls but not with carotenes. As a general rule, the extraction process consists of the removal of hydrophobic carotenoids from hydrophilic medium. The use of nonpolar solvents is not recommended because of penetration through the hydrophilic mass that surrounds pigments is limited, while slightly polar solvents dissolve poorly carotene in dried samples and solubility diminish in fresh samples. Thus, it was postulated that complete extraction can be reached by using samples with low moisture, and slightly polar plus nonpolar solvents [89].

Aramwit *et al.* [90] reported the procedures for extraction of flavonoids and carotenoids from colored silkworm cocoons with triplicately extraction in 70% EtOH (1 g of silk cocoon and 30 ml of ethanol) for 24 h at room temperature (25 °C) to remove all flavonoids and carotenoids.

Lin & Chen [91] studied the comparison of extraction efficiency for carotenoids in tomato juice. Five solvent systems were used, i.e., (1) ethanol–hexane (4:3, v/v), (2) acetone–hexane (3:5, v/v), (3) ethanol–acetone–hexane (2:1:3, v/v/v), (4) ethyl acetate–hexane (1:1, v/v) and (5) ethyl acetate (100%). The ethanol–hexane (4:3, v/v) was found to result in the highest yield of carotenoids extracted from tomato juice.

For flavonoids, in general, having aromatic rings containing polar substituent groups (hydroxyl, carboxyl, and methoxyl) and glycosyl residues that altogether produce a polar molecule. Consequently, they are more soluble in water than in nonpolar solvents, but depending on the media conditions, flavonoids could be

soluble in ether at a pH value where the molecule was unionized. The extraction using alcohols as a solvent is a conventional method to obtain many flavonoids from various natural sources. Among the alcohols, ethanol is the most favored due to the fact that it acts as an environmentally friendly and safe for food use while methanol has toxic effect [67].

In addition, Hernández *et al.* [92] studied the extraction, quantification and identification of flavonoids present in *Terminalia catappa* leaves. The pigments were extracted using hexane-acetone in the ratios from 0/100 to 100/0. Flavonoid concentration was quantified using a spectrophotometer at 370 nm. The results showed that flavonoids were extracted with high polarity solvent mixtures. Using 20:80 hexane-acetone, a maximum of 5.054 mg quercetin / 100 ml of extract was obtained.

In the work of Hirayama *et al.* [93], it was reported that yellow pigments from the cocoon shell sample had been extracted by MeOH–H_2O (2:1, v/v) at 60 $^{\circ}$C for 2 h. The extract was filtered and concentrated to a small volume under reduced pressure, after which H_2O was added. The aqueous solution was applied to a solid-phase extraction. Two flavonoids containing the L-proline moiety, 6-C-[(2S,5S)-prolin-5-yl] quercetin and 6-C-[(2S,5R)-prolin-5-yl] quercetin were then investigated spectroscopically.

1.5.4 Techniques for pigment quantification

Extracts from fruits and vegetables are usually analyzed by high-performance liquid chromatography (HPLC) in order to separate and quantitate various flavonoids or carotenoids [57, 67,]. The characterization of flavonoids or carotenoids is accomplished by combination of HPLC/UV/Vis with photodiode array detection, mass spectrometry (MS), nuclear magnetic resonance (NMR) spectroscopy and spectrophotometric methods [94-97].

There have been numerous reports relating to extraction and identification of pigments from silk cocoon. Kurioka & Yamasaki [55] identified three quercetin glycosides including quercetin 5-O-β-D-glucoside, quercetin 7-O-β-D-glucoside, and quercetin 4′-O-β-D-glucoside, and two kaempferol glycosides including kaempferol 5-O-β-D-glucoside and kaempferol 7-O-β-D-glucoside, along with their aglycones, quercetin and kaempferol, were isolated from an ethanolic extract of Sasamayu cocoon shells. The chemical structures were characterized by chemical and spectroscopic methods including UV spectroscopy and HPLC-ESI-MS.

Tamura *et al.* [51] identified three flavonoid 5- glucosides including quercetin 5-glucoside, quercetin 5,4' diglucoside, and quercetin 5,7,4'-triglucoside from the yellow-green cocoon shell of the silkworm race ''Multi-Bi''of Bombyx mori. Pigments were extracted from the cocoon shell by MeOH–H_2O (2:1, v/v) at 60 °C for 1 h and were then detected and identified by HPLC, UV-Vis, FT–IR, and NMR.

For this study, the contents of flavonoids and carotenoids from silk cocoon (silk waste) were determined gravimetrically and their identities were verified spectrophotometrically.

1.5.5 Structure of Silk protein

In general, the structure of protein can be classified in four types: primary, secondary, tertiary and quaternary structures and silk protein adapts various secondary structures, including α-helix and the β-sheet as shown in Fig. 1.31.

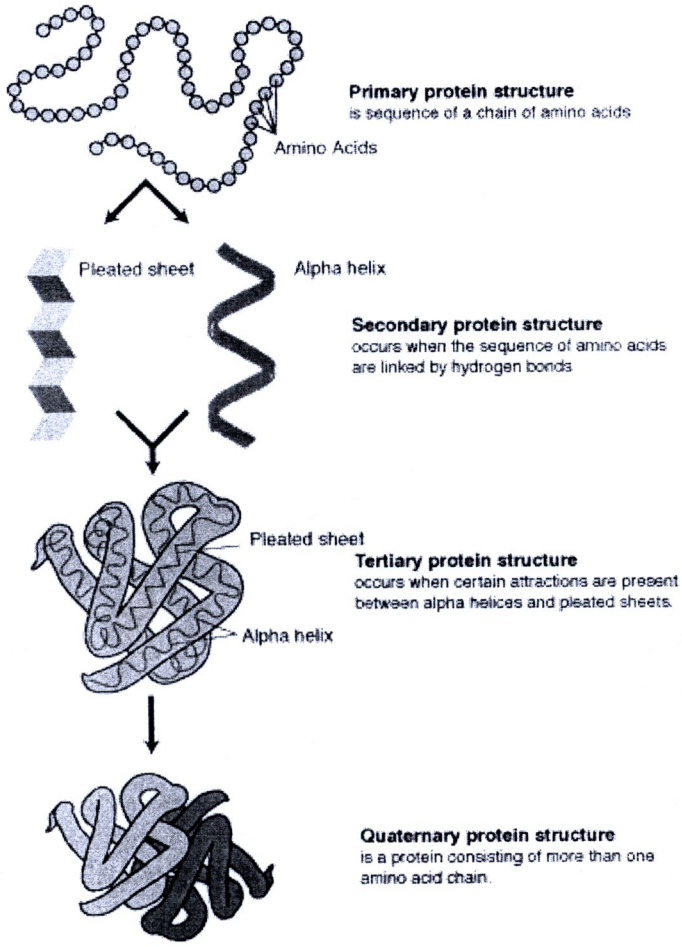


Fig. 1.31 Common structure of protein [98].

For the secondary structure of silk, it can be divided into two types, the α-helix and β-sheet. The α-helix is held together by hydrogen bonds between the hydrogen atoms of amino group and the oxygen atoms of carboxyl groups in polypeptide chain. Another form of secondary structure, the β-sheet, is caused by

hydrogen bonding. The β-sheet is caused by hydrogen bonding between the hydrogen atoms (amino group) and the oxygen atoms (carboxyl group) of amino acids on two chains lying side-by-side. Fig. 1.32 shows the projections of macromolecule segments forming the α-helix, β-sheet structures and other forming such as random coil and turn.

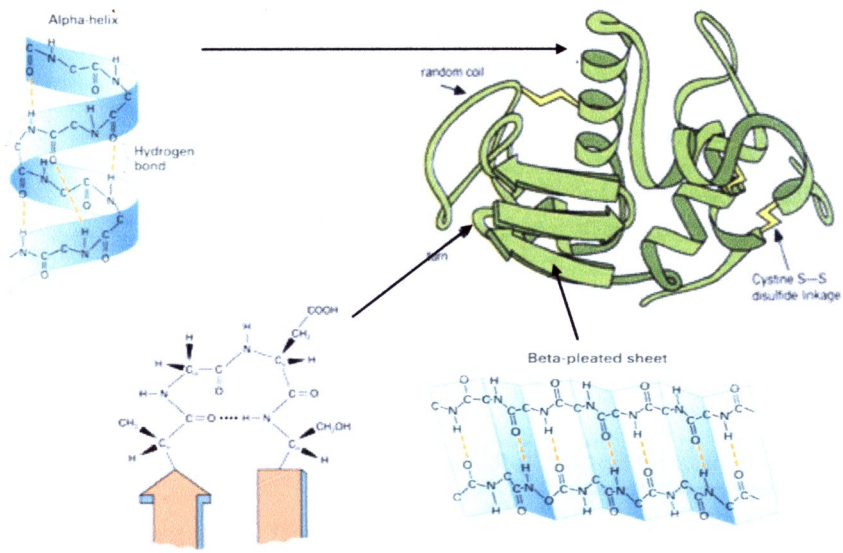


Fig. 1.32 Secondary structure of proteins include *random coil* (no structure), *alpha-helix*, the *beta*-sheet, and the *turn* [99, 100].

1.5.6 Interactions between silk fibroin and silk sericin

The hypothetical intermolecular interactions between fibroin and sericin is shown in Fig. 1.33. In case that both fibroin and sericin are considered as the primary structure, each protein has a repeated amino acid sequence that is capable of forming the β -sheet structure. Since sericin has the hydroxyl groups regularly on one side of the peptide backbone (see the arrow), when fibroin and sericin approach each other, there are hydrogen bonds (dotted lines) formed between the oxygen of the C=O in the silk fibroin and the O-H and N-H hydrogens in silk sericin [44].

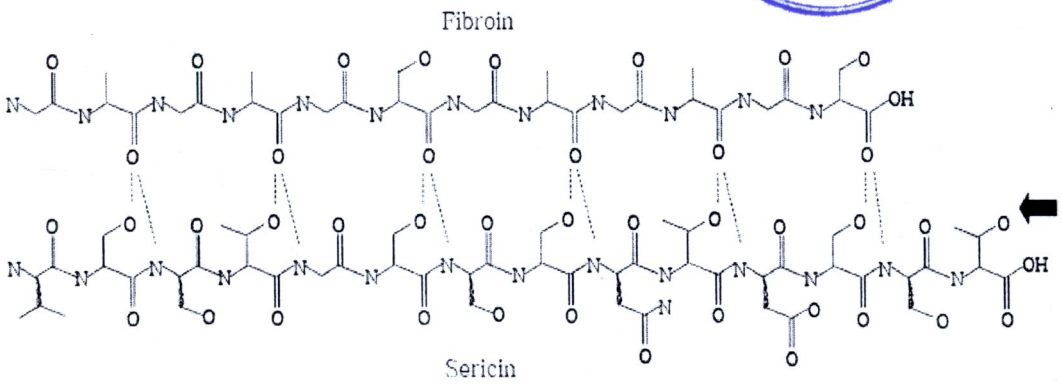
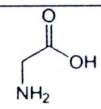
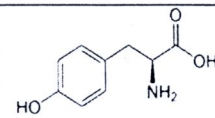
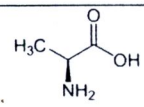
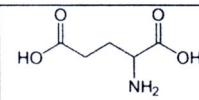
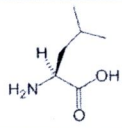
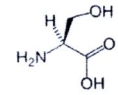
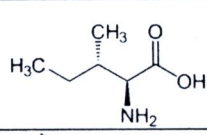
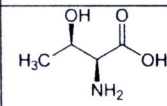
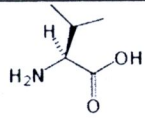
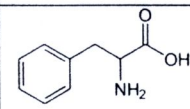
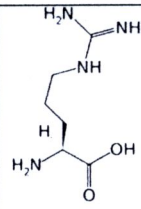
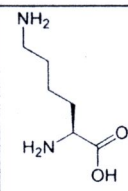
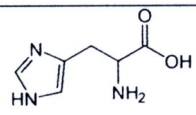
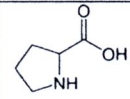
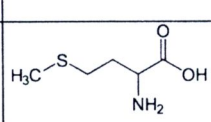
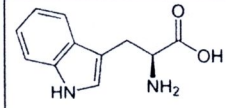
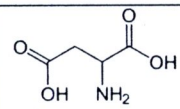
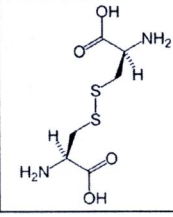


Fig. 1.33 Schematic representation of the intermolecular hydrogen bonding at the boundary of silk fibroin and silk sericin [101].

1.5.7 Amino acid composition of silk proteins

The silk of *B. mori* is composed of the proteins: fibroin and sericin, and other matters such as fats, wax, sand pigments plus minerals. Fibroin in the *B. mori* comprises a high content of the amino acids; glycine and alanine, respectively and the amino acid sequence of fibroin contains repetitive glycine-alanine-glycine-alanine-glycine-serine (GAGAGS) repeats which self-assemble into an anti-parallel β-sheet structure [45]. The key amino acids in sericin are serine, threonine, aspartic acid and glutamic acid is shown in Table 1.5.

Table 1.5 Amino acid composition of fibroin and sericin (Values are given as gram of amino acid per 100 g of protein) [45].

Amino acids	Structure	FB	SC	Amino acids	Structure	FB	SC
Glycine		42.8	8.8	Tyrosine		11.8	4.9
Alanine		32.4	4.0	Glutamic acid		1.7	10.1
Leucine		0.7	0.9	Serine		14.7	30.1
Isoleucine		0.9	0.6	Threonine		1.2	8.5
Valine		3.0	3.1	Phenylalanine		1.2	0.6
Arginine		0.9	4.2	Lysine		0.5	5.5
Histidine		0.3	1.4	Proline		0.6	0.5
Methionine		0.2	0.1	Tryptophan		0.5	0.5
Aspartic acid		1.9	16.8	Cystine		0.1	0.3

SC: sericin
FB : fibroin

1.5.8 Silk Sericin

Silk sericin is a kind of water-soluble protein, comprises a group of serine rich-proteins produced in the middle gland of silk worm. It will be removed as waste, before dyeing and finishing.

Sericin contains three major and two minor proteins of which molecular weights range from 20 to 400 kDa and remains in a partially unfolded state, with 35% β-sheet and 63% random coil, and with no α-helical content [102]. The major molecular conformation of easily soluble sericin is random coil, whereas the β-sheet structure is more difficult to dissolve. Therefore, the properties of sericin are different from fibroin filament in solubility, hydrophilicity and stickiness. Generally, fibroin can be changed to β-sheet structure by alcohol but sericin is not perfectly changed [103]. It is generally known that thermal treatment of sericin especially in the presence of organic solvents or water can affect the changing of sericin structure from random coil to β-sheet. Sericin changes into aggregated state (β-sheet structure), containing strong hydrogen bond by water and organic solvent [101]. The repeated moisture absorption makes aggregated structure of the molecule denser and forms more crystalline structure, which have reduced solubility. Swelling and reactivity are the lowest in the isoelectric region of pH 4 – 5 [104].

Sericin can be extracted by various methods, such as high pressure and high temperature (95-100°C) techniques, acid (such as sulfuric, hydrochloric, tartaric and citric acids), alkaline (such as sodium carbonate and sodium hydroxide), soap (such as Marseille soap), synthetic detergents solutions, or enzyme (such as papain) extractions. The size of the resulting sericin molecules depends on factors such as temperature, pH, and the processing time [105, 106]. Nowadays, soap is replaced by

synthetic detergent because it has some advantages over soap such as being low cost and helping in reduction of the extraction time and damage of the fiber. Disadvantages of acid and alkaline, they have a problem on the surface area of silk and lead to fiber degradation and subsequent loss in physical properties such as dull appearance, surface fibrillation, poor handle, drop in tensile strength. Enzymatic hydrolysis of sericin proteins can be used to solve this problem but it has some disadvantages such as a specific condition and expensiveness [105, 107].

Sericin protein is useful because of its special properties, i.e. being antioxidative, antibacterial, UV resistant, tyrosinase-activity inhibition and moisture absorbing and desorbing. Thus, because of its properties, sericin can be applied in many fields such *as* cosmetics, polymer and pharmaceutical products as well as for biomaterials manufacturing [44, 49].

Recent studies, Nishida *et al.* [108] examined the film, gel and sponge dosage forms of sericin containing high molecular fragments as a drug releasing material for potential use as a medical dressing film, ointment-based injectable gel, and implant respectively by measuring *in vitro* release properties of a charged model protein drug, fluorescei isothiocyanate–albumin (FA), for each dosage form using different molecular weights and concentrations of sericin. Fig. 1.34 shows the appearance of each sericin preparation.

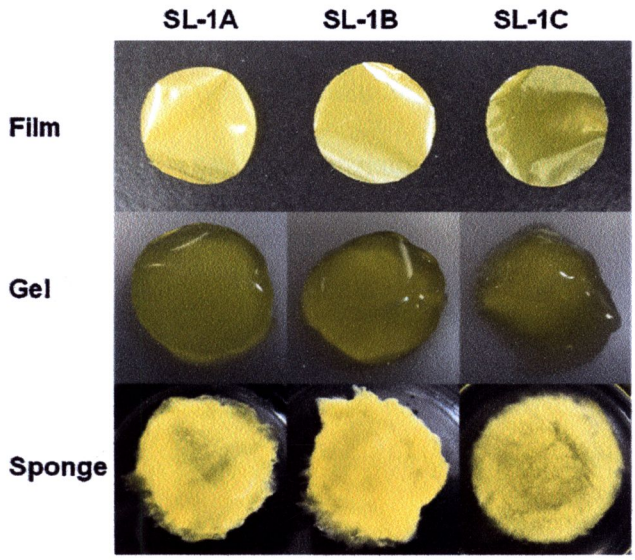


Fig. 1.34 Photographs of sericin film, gel and sponge comprised of various sericin solutions (SL-1A, SL-1B and SL-1C). Heating condition of SL-1A: 100 °C for 20 min; SL-1B: 100 °C for 60 min; SL-1C: 120 °C for 20 min. Scale bar: 10mm. FA: fluorescein isothiocyanate–albumin [108].

1.5.9 Silk fibroin

Silk fibroin (SF) is the major part of the silk protein obtained from the cocoon. The fibroin synthesized at the back gland of silkworm is highly oriented and become fibrosis during spinning, it is known to arrange itself in three structures, called silk I, II, and III. Silk I is the natural form of SF, as emitted from the *B. mori* silk glands before spinning. Silk II refers to the arrangement of SF molecules in spun silk, which has greater strength and is often used commercially in various applications. Silk III is formed principally in solutions of SF at an interface [109].

SF is a linear polypeptide whose major amino acid composition consists primarily of glycine (Gly), alanine (Ala) and serine (Ser) [110]. The primary structure arising from this characteristic of amino acid sequence has been proposed by

Yamane *et al.* [111] as $[Gly\text{-}Ala\text{-}Gly\text{-}Ala\text{-}Gly\text{-}Ser]_n$. It is a heterodimeric protein with a heavy chain (395 kDa) and two small subunits, P25 and L-chain, of 25 kDa. Its physical characters are semi-crystalline, consisting of two phases, one is the well-known, highly crystalline β-sheeted phase (insoluble in hot water) and the other is a lesser- or non-crystalline phase [112]. SF can also be found in various forms, such as gels, powders, membranes gels, sponges, powders, artificial ligaments and scaffolds depending on its applications [46, 47]. SF can be dissolved at elevated temperatures (60–110 °C) with neutral solutions of salts such as calcium chloride ($CaCl_2$), lithium bromide (LiBr), lithium thioisocyanate (LiSCN), or a mixture of $CaCl_2$:H_2O:CH_3CH_2OH (in a 1:8:2 molar ratio) following with dialysis against distilled water to remove the salt residue and giving aqueous silk solutions that can be processed into powder, films, gels homogeneous membrane [38].

SF has been studied as a starting material for non-textile applications including biosensor, cosmetic, food production, and biomaterials. Recently, researchers have investigated the potential of silk fibroin as a candidate material for biomedical applications. Advantages of fibroin are good biocompatibility, tensile strength and elasticity, good oxygen permeability, water vapor permeability, biodegradability, microbial resistance and minimal inflammatory reaction [113]. It has been widely used as a substrate for enzyme immobilization [114]. Moreover, these applications, fibroin proteins have found an effective application as an ingredient in cosmetic formulations including skin and hair care products due to their moisturizing effect. Therefore, in recent years, the range of possible end-users of fibroin has considerably expanded with emphasis on its use as finishing agent for

natural or man-made textiles requiring properties of moisture absorption, antistatic properties, softness, and comfort.

1.6 Research Objectives

1 To optimize the extraction conditions using different solvent systems in order to obtain the maximum amount of pigments in the Thai yellow silk cocoon *(var. Nangnoi Si Sa Ket)*.

2 To quantify the pigments, fibroin and sericin in order to correlate their contents in the Thai yellow silk cocoon *(var. Nangnoi Si Sa Ket)*.