CHAPTER 3

RESULTS AND DISCUSSION

3.1. Extraction of the native Thai silk worm pupa oil

3.1.1. Extraction and physico-chemical stability of oil extracted from Soxhlet and maceration method

Figure 30 showed Thai native varieties silkworm pupa and **Figure 31** indicated the appearances of the native Thai silkworm pupa oil. The percentage yields of the oils from the variety of the native Thai silkworm pupa (Keaw Sakol, Nangnoi Srisaket, Nang Leung, Sam Rong, and None Ruesee varieties) by the Soxhlet and maceration extractions were 24-29 and 4-7%, respectively (**Table 24).** The Soxhlet extraction gave about 7 times higher yields than that obtained from the maceration extraction. The oil was soluble in ethanol and gave the pH range of 6.34-6.91. All oils were not stable and precipitated in acids (10% HCl and 10% CH_3COOH), bases (10% NaOH, 10% NH_4OH and 10% CH_3COONa), reducing agent (10% $FeCl_3$) and oxidizing agent (10% H_2O_2).

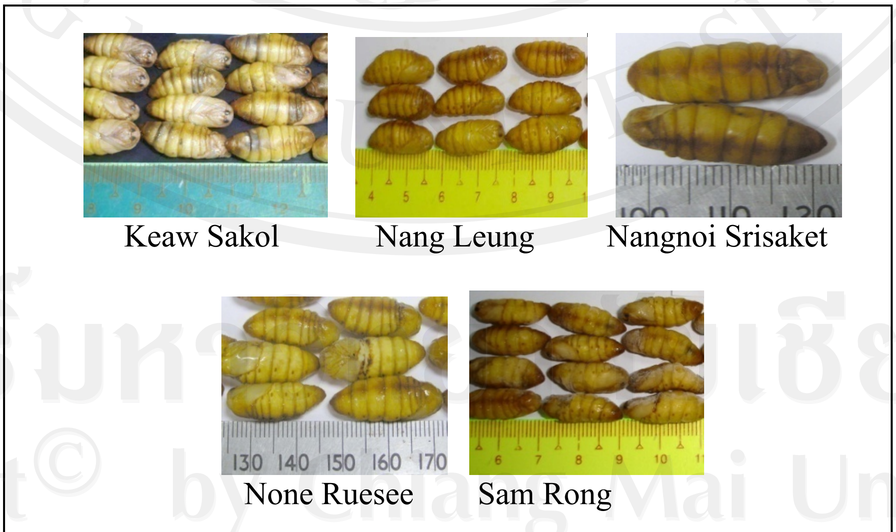


Figure 30 Thai native silkworm pupa variety silkworm pupa

Figure 31 The appearances of the native Thai silkworm pupa oil (A_1A_2= Keaw Sakol, B_1B_2= NangnnoiSrisaket, C_1C_2= Sam Rong, D_1D_2= Nang Leung and E_1E_2= None Ruesee varieties) ($A_1B_1C_1D_1$ E_1 =maceration method and A_2 B_2 C_2 D_2 E_2 = Soxhlet method)

Table 24 The percentage yields of the Thai native silkworm oil extracted by Soxhlet and maceration

Silk varieties	% yield *	
	Soxhlet extraction	Maceration extraction
Nangnoi Srisaket	24.00 ± 1.60 [a]	3.81 ± 0.02
Nang Leung	28.75 ± 1.52	7.00 ± 0.45
None Ruesee	24.18 ± 1.22	5.10 ± 0.67
Keaw Sakol	28.98 ± 1.65	5.77 ± 0.04
Sam Rong	27.22 ± 1.75	5.42 ± 0.89

* Values were means ±S.D. of the three experiments: n=3.

3.1.2 Physico-chemical characteristics of the oil

3.1.2.1 The linoleic acid contents

The percentages (% w/v) of linoleic acid from Keaw Sakol, Nangnoi Nang Leung, Sam Rong, and None Ruesee oils extracted by Soxhlet extraction were 2.19, 1.60, 5.89, 5.27 and 4.63, respectively, whereas those of Keaw Sakol, Nang- noi

Srisaket, Nang Leung, Sam Rong, and None Ruesee oils extracted by maceration extraction were 2.10, 2.25, 2.57, 2.02 and 2.50, respectively. Intact, the total lipid extracted from silk worm pupa has been reported to mainly consist of triacyglycerol, phosphatidyl ethanotl amine and phosphatidylcholine, while the quantities of linoleic acid in triacylglycerol and the total lipid were very small (Eiichi et al., 2002).

3.1.2.2 Fatty acid contents

Fatty acids were separated from the silkworm pupa oil by Soxhlet and maceration extractions. The fatty acid compositions in the oils determined by Gas Chromatography (GC) were shown given in **Figures 32A, 32B, 32C, 32D and 32E.** It demonstrated that silkworm pupa oil composed of both saturated and unsaturated fatty acids. Unsaturated fatty acids including alpha linolenic acid and linoleic acid which were essential fatty acids that found in maceration extracts more than the Soxhlet extracts. This may be due to the no heat involvement of the maceration extraction, thus the compounds were not destroyed and more stable than those extracted from the heated Soxhlet method. The silkworm pupa oils obtained from maceration extraction of Non Ruesee variety contained the highest oleic acid which is the main component at 30.17 % (but less than that in the avocado oil) and 42.31 % of alpha linolenic acid was found in the oil. The oil from the Soxhlet extraction of Keaw Sakol variety contained the highest oleic acid at 38.82 % and alpha linolenic acid at 32.06 % more than avocado oil of 1.44 % (Alicia et al., 2003). These two fatty acids were essential for humans. The lack of fatty acid would affect the growth rate and causes skin ulcer. Human can not produce linoleic acid and therefore have to get from foods (Sappayatosok, 1988). Silkworm pupa contained oil more than that in soybean which

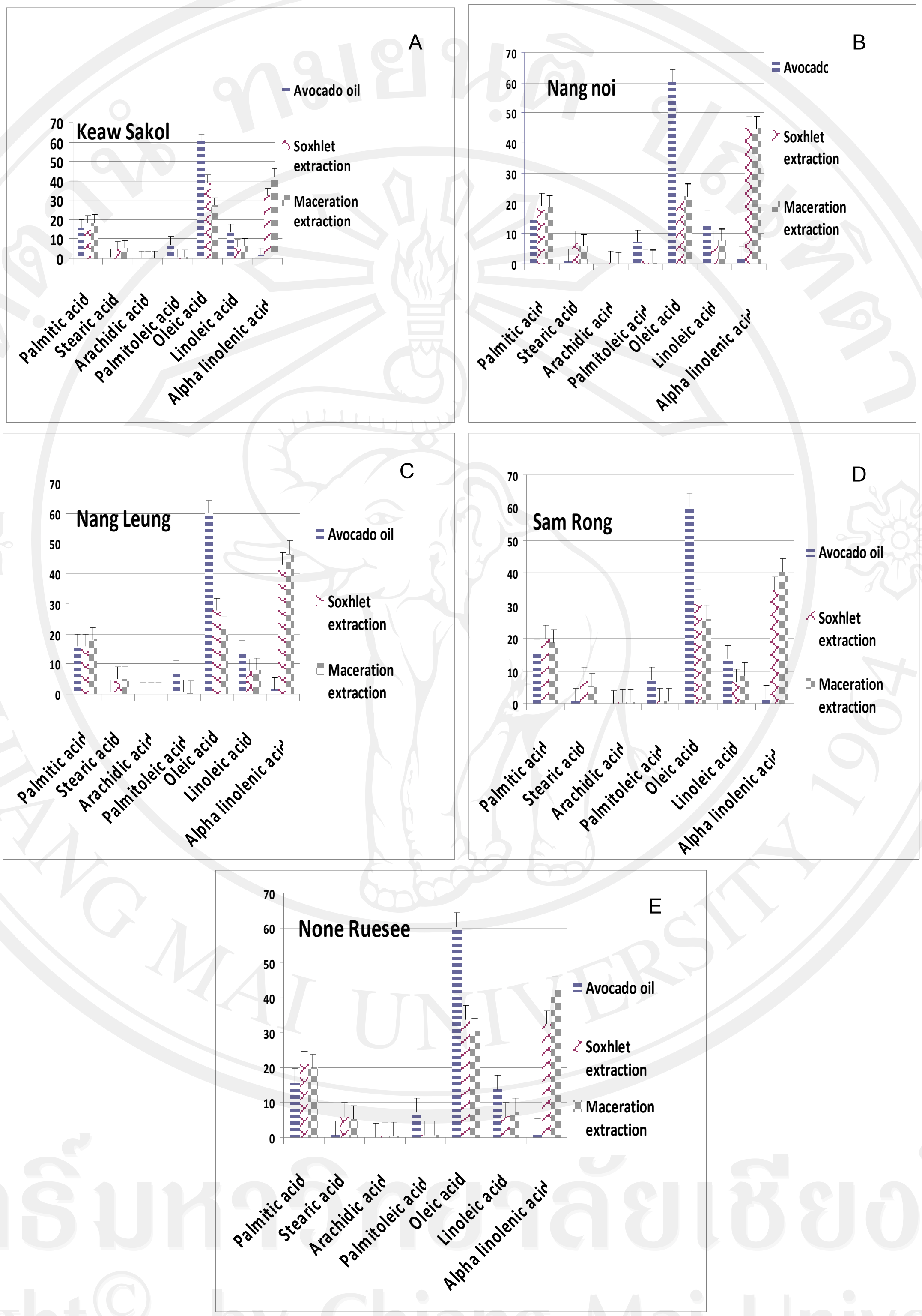


Figure 32 The percentages of fatty acids in the native Thai silkworm oil extracted by Soxhlet and maceration methods (32A= Keaw Sakol, 32B= Nangnoi Srisaket, 32C= Nang Leung, 32D = Sam Rong and 32E = None Ruesee varieties)

has only 18.7 %(w/v) (Manit et al., 2000). However, soybean has 51 % (w/v) of linoleic acid which was more than that in the silkworm pupa oil. But, soybean has 7% of linolenic acid which was less than that in the silkworm pupa oil. Thus, silkworm pupa can be a good source of the functional fatty acid, α-linolenic acid, Thus, it has the potential to develop as functional foods and cosmetics.

3.1.2.3 Tocopherol and cholesterol contents

The amounts of vitamin E in the oils of silkworm pupa varieties, Sam Rong and None Ruesee prepared by the Soxhlet method were 16.65 and 14.54 mg/ml, respectively (**Table 25),** whereas those obtained from the maceration extraction were only 9.32 and 7.31 mg/ml, respectively. The maceration produced very high quality oil with very low levels of acidic and oxidative products whilst retaining the vitamin E contents (Eyres et al., 2001). Vitamin E plays a role in aiding skin appearance and it is interesting and logical to speculate that a healthy oil diet containing this vitamin will assist to have a good complexion. The oil from silkworm pupa varieties, Nang noi, Srissaket and Nang Leung from the maceration method contained the high cholesterol contents at 752 and 608 mg/ml respectively, while those extracted by the Soxhlet extraction were 161.96 and 170.9 mg/ml, respectively. However, this value was smaller than total the cholesterol found in bovine liver (273.9 mg/ml) and common sausage (262.1 mg/ml) as revealed by Rowe et al. (1997). Vitamin E concentrations in the stratum corneum of exposed skin areas such as the forehead and the cheek are 20-fold higher than those in unexposed areas such as the upper arm (Shapiro and Saliou., 2001). Thiele et al. (1999) suggested the sebum as an alternative mode for the delivery of vitamin E to the upper layers of the skin.

Table 25 Vitamin E (mg/100g) and cholesterol contents (mg/100g) determined by HPLC of oil extracted from Thai native silkworm varieties by Soxhlet and maceration extraction methods

Silk varieties	Vitamin E(mg/100g) *		Cholesterol(mg/100g) *	
	maceration extraction	Soxhlet extraction	maceration extraction	Soxhlet extraction
Nangnoi Srisaket	9.32± 0.03	5.54± 1.03	752± 0.17	134.33± 0.07
Nang Leung	9.53± 0.98	7.91± 0.15	608± 0.25	87.82± 0.14
None Ruesee	14.54± 0.77	7.15± 0.11	173.52± 0.21	170.9± 0.11
Keaw Sakol	7.06± 0.23	6.85± 0.59	442± 0.32	71.69± 0.27
Sam Rong	16.65± 0.84	4.72± 0.07	165.03± 0.41	161.96± 0.31

* Values were means ±S.D. of the three experiments: n=3.

3.1.2.4 Physical and chemical contents

The chemical and physical properties including density, refractive index, acidic value, saponification value, unsaponification value, iodine value, and peroxide value and stability to oxidation using rancimet apparatus of all silkworm pupa oil by Soxhlet and maceration extractions were shown in **Table 26**. It was found that the oil from Sam Rong and None Ruesee silkworm pupa varieties extracted by Soxhlet method gave the highest induction time of autooxidation at 64.59 and 53.51 hours, respectively. Thus, it is potential useful oil to be used as antioxidants in cosmetic industry. The oils extracted by maceration method sustained the autooxidation for only a short period at 33.12 and 26.71 hours, respectively at 110 $^{\circ}$C with the low flow rate of 10 L/hours. The amounts of vitamin E in Sam Rong and None Ruesee silkworm pupa varieties extracted by maceration extraction were 16.65

and 4.53 mg/100g, respectively, whereas those obtained by the Soxhlet method were only 4.72 and 7.15 mg/100g, respectively. The oil from Nangnoi Srisaket and Nang Luang silkworm pupa varieties obtained from the maceration contained high cholesterol at 752 and 608 mg/100g, respectively, while those extracted by Soxhlet method were 134.33 and 87.82 mg/100g, respectively.

3.1.3 Biological activities of oil

3.1.3.1 Free radical scavenging activity

The free radical scavenging activities of the oils were assayed by DPPH method (Jung et al., 2006).The IC_{50} values (mg/ml) of the native Thai silkworm pupa oil were shown in **Figure 33.** The IC_{50} (mg/ml) of the Keaw Sakol, Nangnoi, Nang Leung, Sam Rong, and None Ruesee oil extracted by Soxhlet method were 14.81, 18.25, 19.40, 18.22 and 17.01, respectively. The SC_{50} values of Keaw Sakol, Nangnoi Srisaket, Nang Leung, Sam Rong, and None Ruesee oil extracted by the maceration method were 17.96, 18.77, 18.22, 12.51 and 10.08, respectively. The None Ruesee oil extracted by the maceration method showed higher DPPH scavenging activity than oils from other native Thai silkworm pupa oils. However, the IC_{50} values of the native Thai silkworm pupa oils were higher than the standard vitamin C, vitamin E and BHT which gave 0.42, 0.54 and 0.53, respectively, but lower than the standard linoleic acid. The native Thai silkworm pupa oil contained antioxidant and tocopherol (Pises et al., 2006). Therefore, the oil samples might also

Table 26 Physical and chemical characteristics of the native Thai silkworm oils extracted by Soxhlet and maceration methods

Characteristics	Avocado oil**	Soxhlet extraction					Maceration extraction				
		Nangnoi Srisaket	Sam Rong	None Ruesee	Keaw Sakol	Nang Leung	Nangnoi Srisaket	Sam Rong	None Ruesee	Keaw Sakol	Nang Leung
Density	0.9100	0.9640±0.02*	0.9675±0.04	0.9662±0.05	0.9666±0.10	0.9779±0.07	0.9641±0.02	0.9665±0.09	0.9671±0.03	0.967±0.07	0.978±0.06
Refractive index (25 °C)	1.466	1.4466±1.12	1.4459±1.02	1.4462±0.98	1.4458±0.97	1.4471±0.54	1.4669±1.07	1.4669±0.95	1.4692±1.04	1.4567±1.24	1.4695±0.76
Acid value (mg KOH/g)	0.65	0.83±0.51	0.95±0.25	0.99±0.28	0.47±0.67	0.65±0.23	1.27±0.34	1.27±0.18	0.97±0.19	0.69±0.09	1.10±0.12
Saponification value (mg KOH/g)	273	127.85±0.08	131.92±0.06	134.64±0.05	130.98±0.07	132.15±0.08	115.84±0.02	140.25±0.03	131.21±0.05	114.4±0.02	104.72±0.07
Unsaponification matter (%)	Not detected	0.1767±0.98	0.4338±1.14	0.0208±0.95	0.0843±1.26	0.0948±1.11	0.3372±0.97	0.4701±0.09	0.3214±1.13	0.2513±0.96	0.3184±1.23
Iodine value (mg iodine/g)	82.1	122.38±1.12	104.97±0.98	107.69±1.23	100.2±0.95	128.01±0.98	53.87±0.10	55.02±0.96	49.18±0.98	47.23±1.06	50.11±1.09
Peroxide value (mg/g)	10.68	4.44±0.04	4.51±0.06	4.52±0.10	4.47±0.09	4.49±0.11	4.27±0.08	4.15±0.05	3.91±0.12	4.01±0.98	4.42±1.03
Oxidative stability (110°C,10 l min/3g)	Not detected	1.34±0.04	64.59±0.05	53.51±0.02	22.2±0.08	20.1±0.04	2.34±0.07	33.12±0.02	26.71±0.04	11.12±0.06	10.5±0.08
Vitamin E (mg/100g)	Not detected	5.54±0.01	16.65±0.02	14.54±0.04	6.85±0.10	9.53±0.40	9.32±0.03	4.72±0.02	7.15±0.07	7.06±0.12	7.91±0.15
Cholesterol (mg/100g)	Not detected	134.33±0.02	161.96±0.03	170.9±0.09	71.69±0.10	87.82±0.30	752±0.04	165.03±0.12	173.52±0.11	442±0.09	6.08±0.21

* Values were means ± S.D. of the three experiments: n=3.

** Moreno, et.al. 2003

contain some fatty acids which were more potent antioxidants than linoleic acid (Eiichi et al., 2002). This could explain that the silkworm pupa oil containing phospholipids and tocopherol, may play an important role in protecting the lipids against oxidation and carotenoids (such as lutein and neoxanthin) which might act as antioxidants.

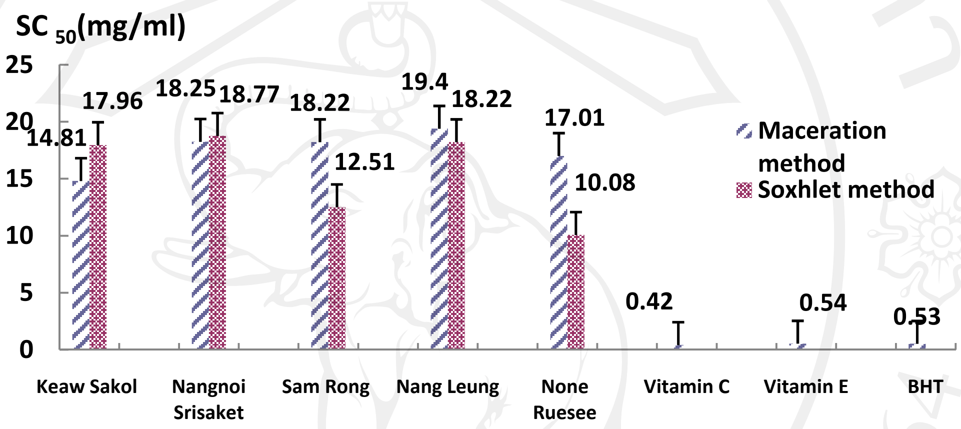


Figure 33 SC_{50} (mg/ml) values by DPPH scavenging assay of oil from various Thai native silkworm pupa prepared by the Soxhlet and maceration method

3.1.3.2 Tyrosinase inhibition activity

The tyrosinase inhibition activities of oils were assayed by the modified dopachrome method using tyrosine as a substrate (Long et al., 2000) The SC_{50} values (mg/ml) of the native Thai silkworm pupa oils were shown in **Figure 34.** The IC_{50} (mg/ml) of the None Ruesee oil extracted by Soxhlet method and Nang Leung, Sam Rong, and None Ruesee oils extracted by maceration method were 7.36, 31.56, 16.01, and 26.02, respectively. However, oil from Keaw Sakol, Nangnoi

Srisaket, Nang Leung, Sam Rong extracted by Soxhlet method, and Keaw Sakol and Nangnoi Srisaket extracted by maceration method showed no activity. This study indicated that the native Thai silkworm pupa oil extracted by maceration method showed higher tyrosinase inhibition activity than that obtained from the Soxhlet extraction since the substances in the oil that inhibited tyrosinase activity might be decomposed by heat. However, the IC_{50} of the native Thai silkworm pupa oils were higher than the standard vitamin C and kojic acid (0.36 and 0.15, respectively). From the analysis of the oil properties, the high contents of fatty acids, such as α -linolenic acid which was as high as 32-47 % were observed.

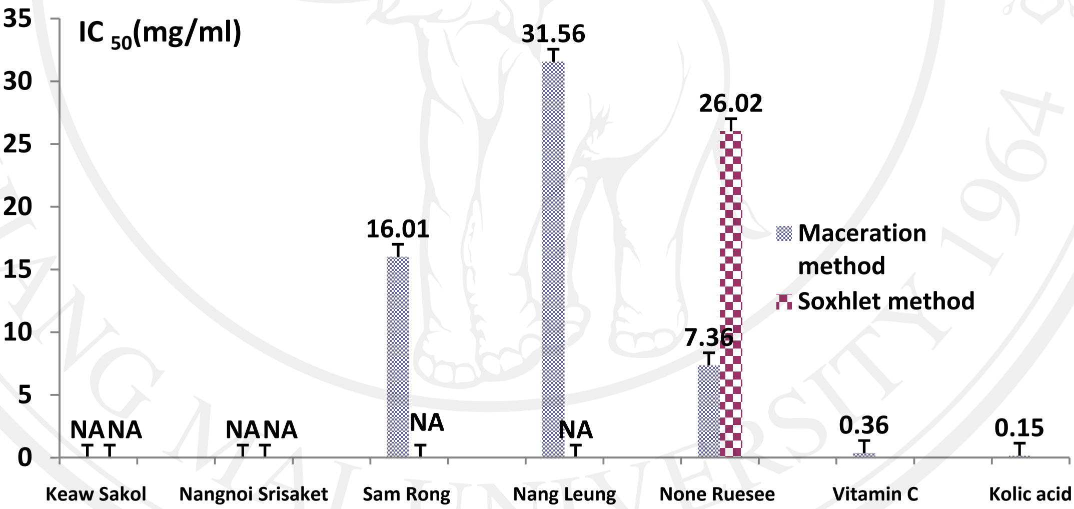


Figure 34 IC_{50} (mg/ml) values by tyrosinase inhibition activity assay of oil from various Thai native silkworm pupa prepared by the Soxhlet and maceration process

This fatty acid is an essential fatty acid that can not be naturally synthesized in the body but must be acquired from the nutrition. The quality of the oils was found to be the same as the standard oil and fat for consuming (Industrial Standards Institute. 1994. 670,TISI). The selection of the suitable varieties of silk as source of protein and fat is important for the development as food and cosmetic products. Thus, it is possible to use the natives Thai silkworm oil as food supplements for the balance of human nutrition, and as an alternative antioxidant and whitening agent for cosmetic industries. The efficiency of oil extraction by the Soxhlet and maceration extraction using petroleum ether were in the range of 24-29 % and 5-7 % respectively, The None Ruesee oils extracted by the maceration method were selected for the further studies.

3.2 The native Thai silk sericin protein

3.2.1. Extraction and physico-chemical stability of sericin extracted from the native Thai silk cocoons by the autoclave and alkaline method

3.2.1.1 Characterization of sericin extracted from the Thai native silk cocoon by the autoclave and alkaline method

Figure 35 showed the physical appearance of Thai native silk cocoons from the five difference varieties including Nangnoi Srisaket, Sam Rong, None Ruesee, Keaw Sakol and Nang Leung by autoclave and alkaline extraction. The yields of sericin yield using various extraction methods were listed in **Table 27.** For all silk cocoon varieties, sericin extracted by the autoclave method had higher yields compared with that extracted with alkaline extraction, at 28.93–35.80% and 22.57–28.34 %, respectively. This result was consistent with that reported by Aramwit P., (2010) who showed that the high-pressure extraction method gave higher yield. Sericin was soluble in hot water and had the pH range of 6.88–7.82. Although heat can remove a significant amount of carotenoids and flavonoids from the cocoon shells, minor amounts of both compounds were still present.

Table 27 Appearances, color values and % protein contents of silk protein powder prepared by the autoclave and alkaline extraction methods

Method	Varieties	Characteristics	Yield (%)	Color			Protein (%w/v)
				L*	a*	b*	
Autoclave	NNS	fine powder, homogenous, glitter	33.21 ± 0.11	74.79^{b}	6.55^{a}	29.85^{a}	28.58 ± 0.21
	NL	fine powder, homogenous, glitter	28.93 ± 0.17	74.66^{b}	6.51^{a}	29.75^{a}	26.44 ± 0.17
	NR	fine powder, homogenous, glitter	35.20 ± 0.21	74.85^{b}	6.53^{a}	29.81^{a}	27.08 ± 0.14
	KS	fine powder, homogenous, glitter	34.28 ± 0.22	74.75^{b}	6.38^{a}	29.78^{a}	28.77 ± 0.23
	SR	fine powder, homogenous, glitter	34.72 ± 0.27	74.85^{b}	6.49^{a}	29.69^{a}	26.85 ± 0.24
Alkaline	NNS	fluffy powder, glitter	28.34 ± 0.15	76.65^{a}	3.11^{b}	20.42^{c}	15.22 ± 0.13
	NL	fluffy powder, glitter	22.57 ± 0.22	76.45^{a}	3.18^{b}	20.35^{c}	16.98 ± 0.17
	NR	fluffy powder, glitter	27.56 ± 0.23	76.84^{a}	3.14^{b}	20.28^{c}	18.72 ± 0.22
	KS	fluffy powder, glitter	25.24 ± 0.19	76.39^{a}	3.09^{b}	20.32^{c}	17.87 ± 0.23
	SR	fluffy powder, glitter	24.58 ± 0.21	76.55^{a}	3.16^{b}	20.28^{c}	19.10 ± 0.24

Note: Means value in the column followed by different superscript are significantly different ($P < 0.05$), NNS =Nangnoi Srisaket, NL= Nang Leung, NR= None Ruesee, KS= Keaw Sakol, SR=Sam Rong

(a) (b)

Figure 35 Appearances of silk protein (None Ruesee) powder prepared by (a) autoclave and (b) alkaline methods.

The sericin from the Thai native silk cocoon extracted by autoclave and alkaline method was bright yellow powder with the contents of total nitrogen, moisture, ash, pH, water solubility, total plate count and the *E. coli* value in the range of 14.43-16.74 %, 3.85-4.10 %, 1.28-1.54 %, 6.88-7.73, 93-99 %, < 30 CFU/g and negative result, respectively as shown in **Table 28.** When compared with the commercial silk protein powder, it was indicated that the sericin protein from this study contained higher total nitrogen and less moisture and ash than those of the commercial product. The similar results have been reported by Wu J-H et al., 2007.

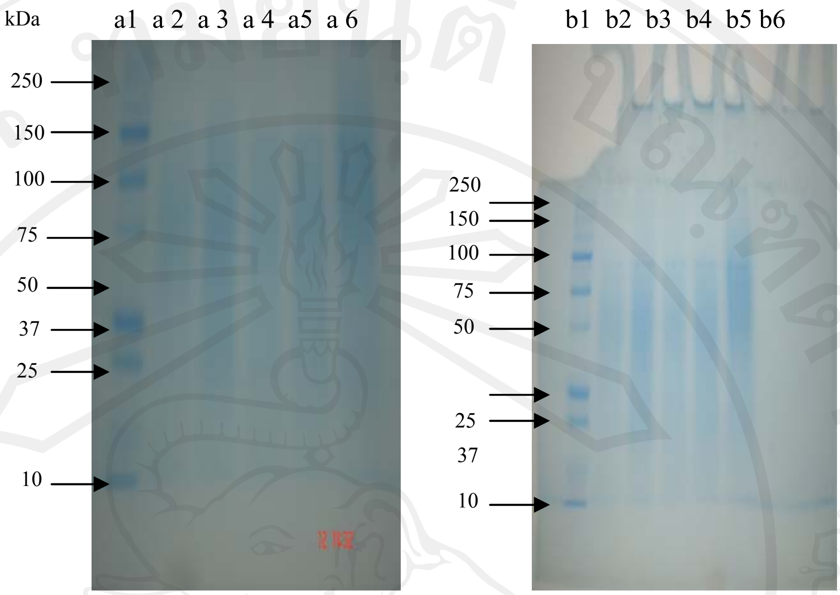
3.2.1.2 Gel electrophoresis of the sericin from the Thai native silk cocoon

The molecular weight patterns of the sericin hydrolysate by the alkaline and autoclave methods of the five varieties were accessed by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and coomassie staining (Pharmacia: EPS 300) and showed in **Figures 36 (a) and (b),** Lanes a (1) and b(1) were the standard protein with the molecular weight of 10 to 250 kDa (Gibco Co.). Lanes a (2) to a (6) and b (2) to b (6) were the protein samples from various silk cocoons varieties prepared by the autoclave and alkaline methods respectively. The average molecular weights of the silk sericin, by the alkaline and autoclave methods were approximately 50-150 kDa. The average molecular weight of sericin extracted by alkaline method gave the wide range of 75-150 kDa. The protein by autoclave method gave the molecular weight in the range of 50-100 kDa. None Ruesee variety by the alkaline extraction method gave the molecular weight in the range of 40-75 kDa.

Table 28 Characteristics of sericin protein from *Bombyx mori* Linn. (Native Thai silk) and the commercial sericin product

Characteristics	Commercial product (Promois®)	Alkaline extracted sericin protein					Autoclave extracted sericin protein				
		NNS	NL	NR	KS	SR	NNS	NL	NR	KS	SR
Total nitrogen (% w/w)	13.0	16.57 ± 0.19	15.98 ± 0.17	16.53 ± 0.15	16.47 ± 0.16	16.74 ± 0.17	14.57 ± 0.21	14.65 ± 0.22	14.43 ± 0.18	14.47 ± 0.20	14.54 ± 0.17
Loss on drying (%w/w)	10.0	4.06 ± 0.22	4.02 ± 0.25	3.95 ± 0.27	4.00 ± 0.24	3.89 ± 0.19	4.02 ± 0.21	3.99 ± 0.22	3.85 ± 0.25	4.08 ± 0.28	4.10 ± 0.24
Ash (% w/w)	3.0	1.37 ± 0.11	1.35 ± 0.17	1.28 ± 0.15	1.31 ± 0.17	1.30 ± 0.19	1.38 ± 0.18	1.54 ± 0.20	1.37 ± 0.169	1.41 ± 0.15	1.40 ± 0.17
pH	5.55 – 7.50	7.82 ± 0.11	7.58 ± 0.10	7.61 ± 0.12	7.58 ± 0.09	7.62 ± 0.28	7.12 ± 0.11	7.15 ± 0.12	7.02 ± 0.13	6.88 ± 0.17	7.11 ± 0.16
Water solubility (%)	Soluble	97 ± 0.22	97 ± 0.21	98 ± 0.23	96 ± 0.25	98 ± 0.26	93 ± 0.27	94 ± 0.22	95 ± 0.28	94 ± 0.29	95 ± 0.22
Total plate count (CFU/g)	no report	< 30	< 30	< 30	< 30	< 30	< 30	< 30	< 30	< 30	< 30
E. coli	no report	negative	negative	negative	negative	negative	negative	negative	negative	negative	negative

Note; NNS =Nangnoi Srisaket, NL= Nang Leung, NR= None Ruesee, KS= Keaw Sakol, SR=Sam Rong



(a) Autoclave method (b) Alkaline method

Figure 36 Separation of protein by the SDS-PAGE method at 12.5% gel and dyeing by comassie brilliant blue R-250 a(1): Standard protein: a(2) Sam Rong: a(3) Nang Luang a (4) Keaw Sakol; a(5) Nangnoi Srisaket; a (6) None Ruesee; b(1):Standard protein: b(2) Sam Rong: b(3) None Ruesee; b(4)Keaw Sakol; b(5),Nang Lueng: b(6)Nangnoi Srisaket

Meanwhile, the protein from Nang Luang variety by the autoclave method was in the range of 50-100 kDa. Then, the molecular weight of the protein from extraction by alkaline was smaller than that by the autoclave. This method was consistent with the research report on the molecular weight of the protein by the enzyme immobilization method by Yu-Qing Zhang et al, (2004). Extraction of the silk protein by the alkaline method at 0.5 N sodium chloride would gave the molecular weight in the range of 30-50 kDa. Our findings were in agreement with those reported by Sprague et al, (1975), which indicated that sericin is a mixture of at least 15 different polypeptide chains, ranging in the size from 20 to 220 kDa.

3.2.1.3 Amino acid compositions

The amino acid composition of the sericin from the Thai native silk cocoons were shown in **Table 29**. The results indicated that sericin from the Thai native silk cocoon contained seventeen known amino acids, including all free amino acids of sericin extracted by difference methods for all five strains. The results indicated that from None Ruesee strain by the autoclave method contained high serine, aspartic and glycine content of about 34.93, 18.55 and 9.2 (mole %) respectively, while, Sam Rong strain contained high serine, aspartic and glycine contents of about 29.22, 18.62 and 8.79 (mole, %). Eighteen kinds of amino acids by the alkaline method. Moreover, the sericin amino acid compositions from the same strain varied according to the methods of the extraction.

The sericin protein was also studied for its amino acid profile. The similarity in amino acid profile of the protein to the commercial product was observed. The majority of the amino acids were glycine, alanine, serine and tyrosine. However, the differences were observed in that of the glycine and alanine contents in the prepared silk powder between the autoclave and alkaline method were rather smaller. The contents of other amino acids were much higher than those from the commercial sericin product. The amino acid compositions of the prepared silk powder may be affected by the different species of the silk. This result was consistent with that reported by Hemachantorn, K. (1987), indicating that the chemical compositions of the silk protein can be influenced by the silk species and feeds. Therefore, the amino acid pattern of the silk sericin can indicate the species of the silk.

Besides these, the silkworm having sericin quantity as high as approximately 33% and specific characteristic carbohydrate of approximately 3 % was result in the sericin to be capable in flocculation with keratin which was an important protein of stratum corneum and important mechanism in increasing good skin hydration and skin moisturizing with the capability in moisture absorption and release, creation of film in well protecting and entrapping the moisture, thus, making the skin moisturized tenderly mild, which, in this connection, film was flocculate in blended substance on the skin and can flocculate exceptionally well with water, thus, it was a valuable cosmetics substance in giving moisture in order to prevent the loss of water from the skin and function duty in skin protection. When applying sericin on the skin, it was create glutinous film and the skin can be protected after the film gets dried and that moisture was obviously and clearly be seen to be added to the skin (Pandamwa et al., 2004, Hua et al., 2002 and Chen et al., 2001), reduced the loss of water from the skin through epidermis and reduced wrinkle lines. On the capability in originating the films to sericin molecules, the more high quantity of molecules the better the creation of the films.

Total 29 The amino acid profiles of various sericin sample extracted by different methods from different species of *Bombyx mori* Linn (native Thai silk) and the commercial sericin product.

Amino acids[3]/	Type of native Thai silk										
	Alkaline method(0.05Na_2CO_3) (mole %) [1/]					Autoclave method (mole %) [1/]					The commercial product (mole %) [2/]
	KSD	SRD	NRD	NLD	NNSD	KSA	SRA	NRA	NLA	NNSA	
Aspartic acid	16.06	18.62	16.26	14.96	14.63	17.98	18.42	18.55	18.19	18	2.10
Serine	26.42	29.22	26.9	24.15	25.98	32.43	33.19	34.93	31.22	32.79	9.70
Glutamic aicd	6.41	6.4	7.19	6.89	6.23	5.56	5.51	6.25	5.56	6.18	1.50
Glycine	9.3	8.79	8.66	9.04	9.37	9.46	9.44	9.21	10.31	9.33	42.90
Histidine	1.67	1.59	1.51	1.39	1.36	1.84	1.75	1.79	1.63	1.76	Trace
Arginine	4.19	3.92	3.85	3.33	3.78	4.37	4.11	4.25	4.61	4.16	0.10
Threonine	6.98	6.83	6.12	5.55	5.9	8.67	8.36	8.43	8.98	8.09	0.90
Alanine	3.22	3.51	3.24	2.73	3.07	3.26	3.17	3.29	3.17	3.25	30.60
Proline	0.69	0.68	0.78	0.58	0.79	0.49	0.51	0.58	0.57	0.51	0.30
Cystine	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Trace
Tyrosine	4.21	4.04	3.41	2.93	3.48	4.81	4.72	4.79	5.29	4.56	4.90
Valine	2.65	2.71	2.44	2.13	2.32	3.08	3	3.06	3.05	2.93	2.60
Methionine	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Trace
Lysine	3.41	3.49	3.92	3.36	2.9	3.32	3.13	3.52	2.79	3.49	0.50
Isoleucine	0.56	0.63	0.61	0.45	0.85	0.62	0.63	0.66	0.63	0.65	1.00
Leucine	1.06	1.09	1.12	0.91	1.13	0.98	0.95	1.02	1	0.98	0.60
Phenylalanine	0.51	0.47	0.53	0.46	0.61	0.4	0.38	0.43	0.46	0.43	2.30

[1/]Silk powder (native Thai silk), [2/]Commercial product,[3]/In house method based on J. of AOAC.Vol.78,No 3,1995,D= Alkaline method and A=autoclave method

Note; KS= Keaw Sakol, SR=Sam Rong, NR= None Ruesee, NL= Nang Leung and NNS =Nangnoi Srisaket

3.2.1.4 FTIR spectra of sericin powders obtained from different extraction methods (high pressure and alkaline) were shown in **Figures 37 and 38**. The peak positions of amide I (C=O stretching), amide II (N-H deformation and C-N stretching) and amide III (C-N stretching and N-H deformation) of the sericin powders derived from autoclave and alkali processes were located at 1650 and 1523 cm^{-1} respectively. These amide bands contributed to the primary random coil structure of sericin (Chen, X. et al., 2001; Lee, K. et al., 2003). All sericin powder of the five strains obtained from autoclave extraction showed similar peak patterns except for the amide I characteristic peaks, which appeared at 1650 and 1624 cm^{-1}, indicating the presence of a random coil and β-sheet conformation respectively (Gil, E. S. et al., 2006).

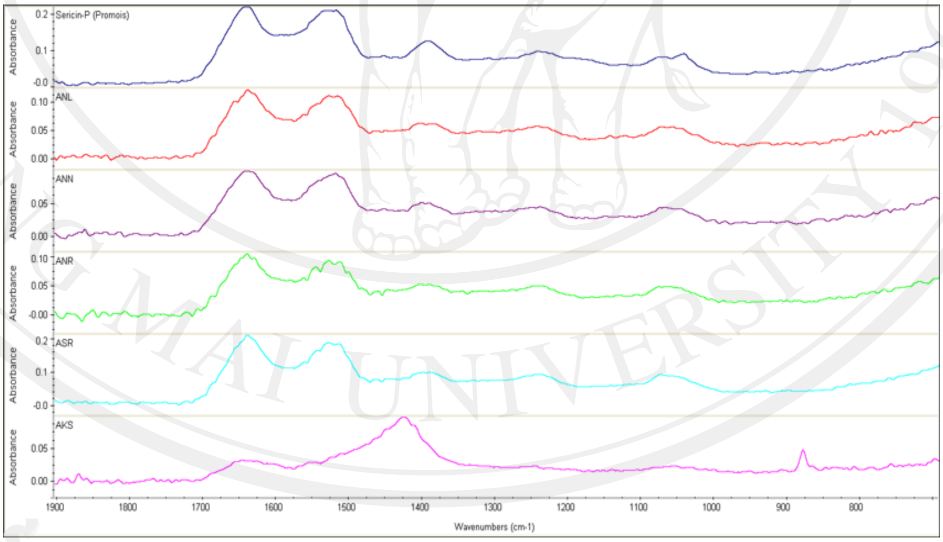


Figure 37 FTIR spectra of sericin from difference silk varieties obtained from autoclave extraction processes compared with Promois®.

(NNS= Nangnoi Srisaket, NL=Nang Leung, NR= None Ruesee, KS= Keaw Sakol, SR= Sam Rong and Sericin P (Promois®)

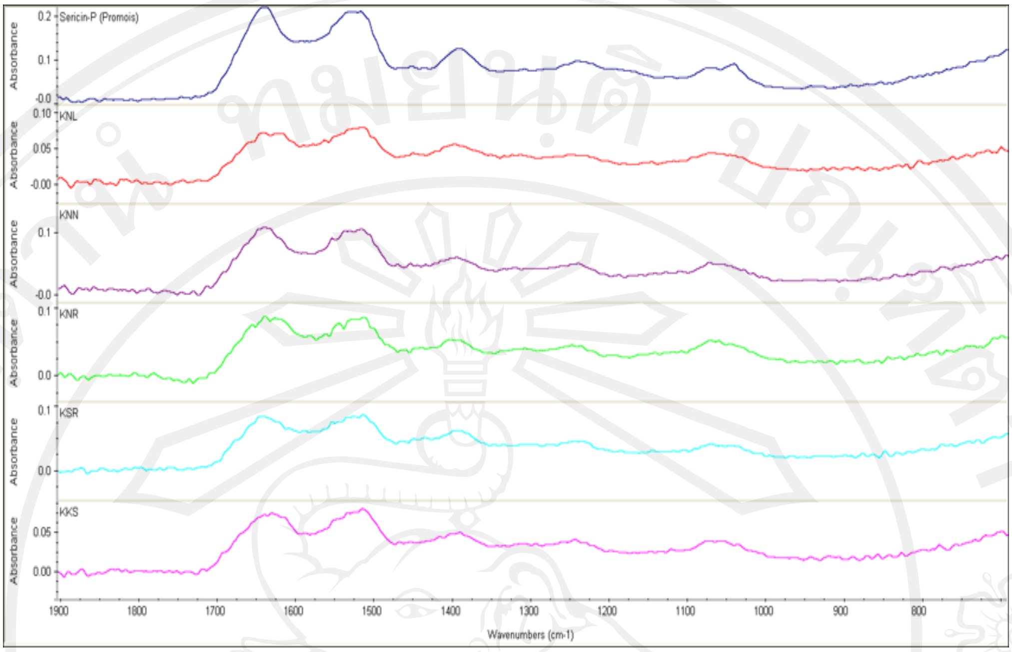


Figure 38 FTIR spectra of sericin from difference silk varieties obtained from alkaline extraction processes compared with Promois®.

(NNS= Nangnoi Sreisaket, NL=Nang Leung, NR= None Ruesee, KS= Keaw Sakol, SR= Sam Rong and Sericin P (Promois®)

All sericin powder of the five strains prepared by alkaline extraction appeared to have more similar absorption peaks than those extracted by the autoclave methods. The specific amide II peak was not clearly observed, while amide I occurred at 1525 and 1560 cm^{-1}, corresponding to a random coil and β-sheet respectively. (Abreu et al., 2005). The FT-IR spectra results revealed that the extraction method of sericin could affect the chemical structure of sericin. The similar FT-IR spectra implied that the molecular conformation of silk cocoon did not change even with different extraction method and a β-structure and random coil conformation were assumed.

3.2.2 Biological activities of sericin

3.2.2.1 Free radical scavenging activity

The SC_{50} (mg/mL) values of free radical scavenging activity of sericin from Keaw Sakol, Nangnoi Sreisaket, Nang Leung, Sam Rong, and None Ruesee were shown in **Figure 39** by the basic hydrolysis were 34.33 ± 0.97, 54.49 ± 0.59, 29.96 ± 0.34, 25.40 ± 0.08, and 35.44 ± 0.31, and those by autoclaving were 19.63 ± 0.09, 32.33 ± 2.06, 13.65 ± 0.20, 15.49 ± 0.19, and 24.44 ± 0.26, respectively. The autoclave process may be the appropriate methods to prepare sericin from Thai native silkworms, since sericin from these processes demonstrated higher free radical scavenging activity ($p < 0.05$). The antioxidative compounds in sericin may be heat labile. The SC_{50} values of sericin were higher than those of the standard antioxidants vitamin C, vitamin E, and BHT at 0.42 ± 0.04, 0.54 ± 0.09, and 0.53 ± 0.02 mg/mL, respectively), but lower than that of the standard linoleic acid (218.97±6.98 mg/mL) ($p < 0.05$). Wu et al. (2007) reported that the 50% scavenging activity by DPPH assay of sericin prepared from silk industry waste water was 31 mg/mL. Fan et al. (2009) have also reported that silk sericin did not show only DPPH radical scavenging activity, but also many antioxidative activities, such as hydroxyl and superoxide radical scavenging, lipid peroxidation of linoleic acid, reducing power, and ferrous ion chelating activity.

3.2.2.2 Tyrosinase inhibition activity

The IC_{50} (mg/mL) values of tyrosinase inhibition activity of sericin from Keaw Sakol, Nangnoi Sreisaket, Nang Leung, Sam Rong, and None Ruesee were shown in **Figure 40.** Sericin from the autoclaving process showed tyrosinase inhibition activities lower than those from the basic hydrolysis method (p < 0.05). Sericin from None Ruesee prepared by basic hydrolysis showed the highest tyrosinase

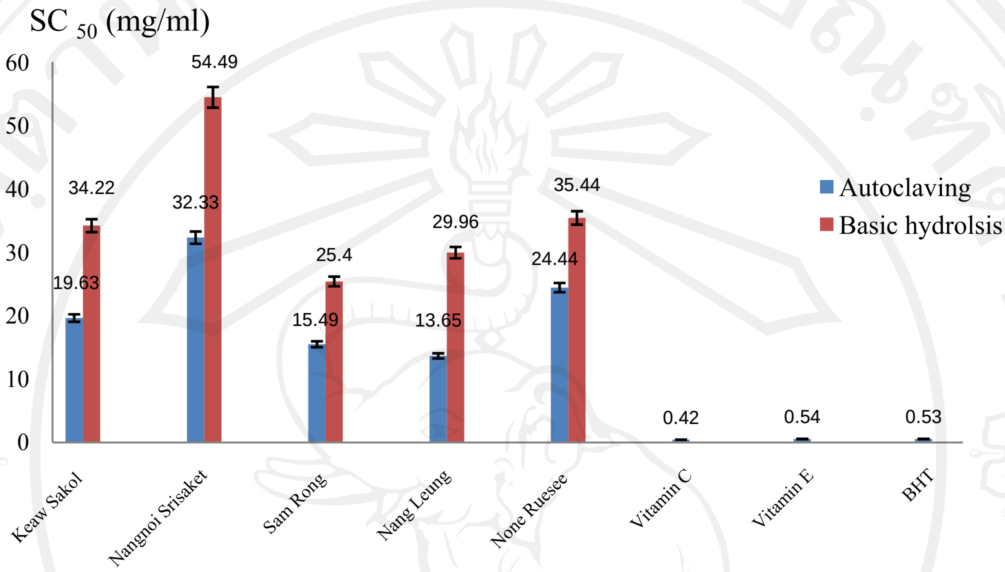


Figure 39 SC_{50} (mg/ml) values by DPPH scavenging assay of sericin from various Thai native silk cocoon prepared by autoclaving and basic hydrolysis in comparing To the standard antioxidants (vitamin C, vitamin E and BHT)

inhibition activity with an IC_{50} value of 1.20 mg/mL, but lower activity than the standards vitamin C and kojic acid of 3 and 8 times, respectively (p< 0.05). Some sericin prepared by autoclaving may be destroyed by heat, thereby decreasing the tyrosinase inhibition activity when compared with that prepared by the basic hydrolysis. Wu et al. (2008) reported that the IC_{50} value for the inhibition of mushroom tyrosinase activity by sericin prepared from silk industry waste water was 10 mg/mL.

In summary, for sericin, the extraction efficiency from the five Thai native silk cocoons by basic hydrolysis and autoclaving was similar. The basic hydrolysis process appeared to be the superior method since the tyrosinase inhibition activity of most sericins prepared by basic hydrolysis seemed to be higher than those from the autoclaving method. Sericin from None Ruesee by basic hydrolysis gave the highest tyrosinase inhibition activity with an IC_{50} value of 1.20±0.07 mg/mL, but lower than that of the standard vitamin C of about 3 times ($p < 0.05$). However, the highest free radical scavenging activity was shown in sericin prepared from Nang Leung by autoclaving with the lowest IC_{50} value of 13.65±0.20 mg/mL, although this was lower than that of the standard vitamin C about 33 times ($p < 0.05$).

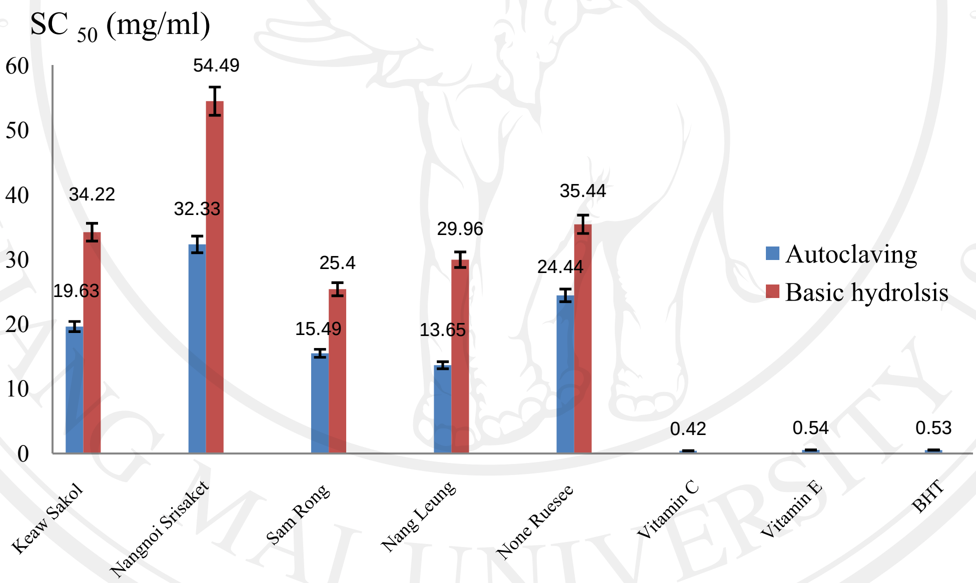


Figure 40 IC_{50} (mg/ml) values by tyrosinase inhibition activity assay of sericin from various Thai native silk cocoon prepared by autoclaving and basic hydrolysis in comparing to the standard antioxidants (vitamin C and kojic acid)

Thus, sericin from None Ruesee strain extracted by autoclave method was selected for the further studies.

3.3 Preparation of niosomes entrapped with sericin and oil extracted from None Ruesee silkworm

3.3.1 Physical stability of blank niosome formulations prepared by chloroform film method with sonication (CFS)

The physical appearances including vesicular size, the polydispersibility index (PDI), pH value, color and dispersibility of the blank niosome (Tween 61/ cholesterol at 1:1 molar ratio) kept at different temperatures (4 ± 2, 30 ± 2 and 45 ± 2 °C) for 3 months were shown in **Table 30**. The vesicular sizes and pH values of all blank niosomes prepared by CFS after kept at 4 ± 2, 30 ± 2 and 45 ± 2 °C for 3 months were increased due to the aggregation. The sedimentation of all blank niosomes stored at 45 ± 2 °C was less than those stored at 4 ± 2 and 30 ± 2 °C (This blank niosome Tween 61/ cholesterol at 1:1 molar ratio) in distilled H_2O were selected for the entrapment of sericin and oil extracted from None Ruesee silkworm. The physical appearances of blank niosomes (Tween 61/ cholesterol at 1:1 molar ratio) prepared by CFS after kept at 30 ± 2 °C for 3 months were shown in **Table 31.**

Table 30 The physical appearances of the blank niosomes (Tween 61/ cholesterol at 1:1 molar ratio) prepared by CFS after kept at 4, 25 and 45 °C at initial, 1, 2 and 3 months.

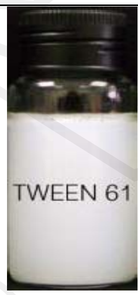
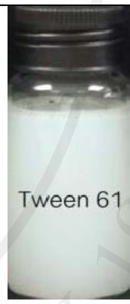
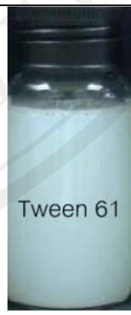
Temperature	Physical appearances of blank niosomes			
	At initial	1 month	2 months	3 months
4 ± 2 °C				
25± 2 °C				
45± 2 °C				

Table 31 The physical properties including vesicular size, the polydispersibility index (PDI), pH, color and dispersibility of blank niosomes (Tween 61/ cholesterol at 1:1 molar ratio) by CFS kept at different temperatures for 3 months

Formulations prepared by CFS Tween 61 cholesterol, 1:1	at initial				after 3 months			
	Z-average (nm)	The polydispersibility index (PDI)	pH	Physical appearances	Z-average (nm)	The polydispersibility index (PDI)	pH	Physical appearances
4 ± 2 °C	61.46 ±0.33	0.27±0.03	6.20 ± 0.15	translucent, white, no sedimentation	101.46±25.33	0.34±0.07	6.24 ±0.02	translucent, white, no sedimentation
30 ± 2 °C	61.46 ±0.33	0.27±0.03	6.20 ± 0.15	translucent, white, no sedimentation	104.11 ±28.73	0.41±0.11	6.54 ±0.03	translucent, white, no sedimentation +1
45 ± 2 °C	61.46 ±0.33	0.27±0.03	6.20 ±0.15	translucent, white, no sedimentation	97.36 ±18.74	0.57±0.10	6.62 ±0.02	translucent, white, no sedimentation +2

Note: degree of sedimentation from low to high (+1 to +5)

3.4 Characteristics of the niosome formulations entrapped with the sericin and oil silkworm

3.4.1 The vesicular size determination

Niosome formulations entrapped with the sericin and oil from silkworm prepared by the CFS were smaller in the rage of 92-800 nm, with broad size distribution as showed in **Table 32**. They were still in the nanosize range which will be useful for the tranepidermal application. However, niosomes with smaller size distribution can be prepared by ultrasonication (Chattopadhyay and Gupta, 2002).

3.4.2 Morphology of the vesicles

The morphology of niosomes prepared by CFS technique was the mixture of large unilamellar vesicles (LUVs) and unilamellar and multilamellar vesicles (MLVs) (**Figures 41 and 42**). This may be due to the different preparation techniques which have different mechanisms of vesicle formation (Manosroi et al., 2008).

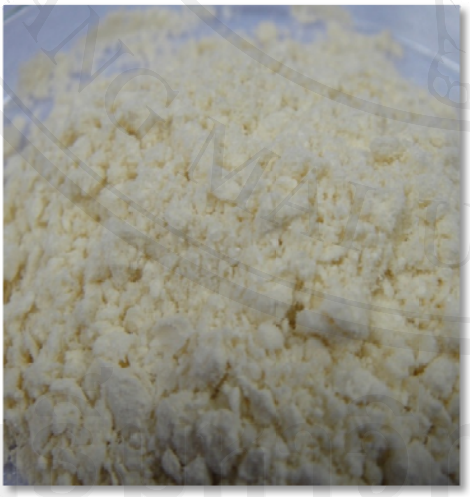


Figure 41 Niosomes entrapped with sericin and oil from None Ruesee silkworm by Canon EOS450D

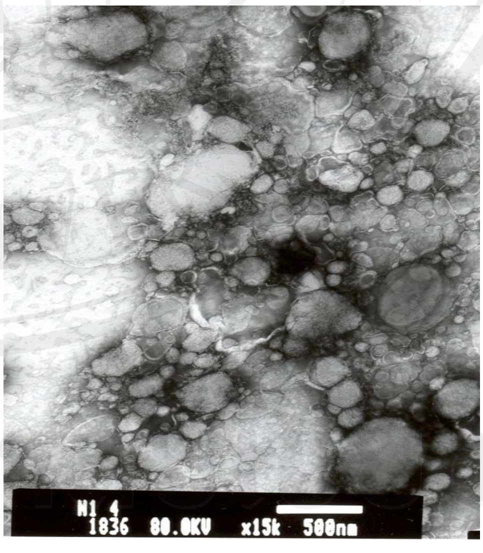


Figure 42 Morphology of niosomes entrapped with the sericin and oil silkworm prepared by CFS investigated by TEM with the magnification of ×15.k

Table 32 Physical appearances (vesicular size, pH and physical appearances) of niosomes (Tween 61/ cholesterol at 1:1 molar ratio) entrapped with sericin and oil at 1 and 1 % w/w respectively, of the niosomal forming materials kept at various temperatures for 3 months

Temperatures	Vesicular size (nm)			pH			Physical appearances		
	At initial	1 month	3 months	At initial	1 month	3 months	At initial	1 month	3 months
4 ± 2 °C	92.42 ± 85.17	620.7 ± 188.40	653.8 ± 236.14	6.20 ± 0.01	6.28 ± 0.02	6.41 ± 0.02	translucent, white,no sedimentation	translucent, white,no sedimentation	translucent, white, sedimentation+1
30 ± 2 °C	92.42 ± 85.17	647.1 ± 245.58	669.8 ± 299.81	6.20 ± 0.01	6.34 ± 0.02	6.68 ± 0.02	translucent, white,no sedimentation	translucent, white, sedimentation+1	translucent, white, sedimentation+1
45 ± 2 °C	92.42 ± 85.17	612.5 ± 261.01	797.1 ± 238.88	6.20 ± 0.01	6.58 ± 0.02	7.15 ± 0.02	translucent, white,no sedimentation	translucent, white, sedimentation+1	translucent, white, sedimentation+2

Note: pH of distilled water = 6.20

The reasons for selecting 1% of sericin and 1% of silkworm oil for entrapped in niosomes by using the basis derived from physical quality test (Hao et al., 2002) through the observation on the change occurring as a results of room temperature condition by taking the observation on the brightness, sedimentation, acid-base and measurement on the size of the particles to determined how their change and then from that point, selection on the percentage on the minimum change or non-change was adopted for further study

The floating particles will be stable when zeta potential value was more than +30 mV or lesser than -30 mV which, in this connection, zeta potential value will demonstrate the level of impulsion between the adjoining charges, therefore, the colloids having positive zeta potential value can flocculate well with the skin and absorb well, however, they may cause high irritations, but, the colloids having negative zeta potential value will not flocculate well with the skin, however, they will cause lesser irritations (Bayindir and Yuksel, 2010). Therefore, positive zeta potential value is yet another option for use in the selection of the well stable formula

The size of niosome after keeping for a period of 3 months was found to be bigger. The aggregation of niosome with the other substance may be arisen when kept for a period of times and will cause the sedimentation (Sophie et al.,1998) and with the weight of the rather large size sericin molecules (50-150 Kda), it may results in an aggregation. But, sericin was amino acid, having serine as enormous compositions, thus, making it able to entrap water in the skin and create films for coating

3.3.3 Entrapment efficiency determination

The entrapment efficiencies of a substance in the bilayer vesicles depend on its polarity as well as the lamellarity and size of the vesicles. The entrapment efficiencies of the sericin and oil of the silkworm entrapped in niosomes by the CFS method were shown in **Table 33**

Table 33 The entrapment efficiencies (%EE) of niosomes entrapped with None-Ruesee strain silkworm extracts containing sericin and oil prepared by the CFS method

None Ruesee strain silkworm extracts	Entrapment efficiency(%)[a] by CFS
Sericin	33.76
oil (in the form of linoleic acid)	43.71

Note: Sericin and linoleic acid contents were determined by HPLC and GC, respectively in comparing to their standards

The entrapment efficiencies (%EE) of the None Ruesee strain silkworm extracts containing sericin and oil niosomes entrapped with prepared by the CFS method, were 33.76 % for sericin and 43.71%. of oil (in the form of linoleic acid). Niosomes prepared by CFS yielding the mixture of unilamellar and multilamellar vesicles gave the entrapment efficiencies of oil, higher than sericin, owing to the more intercalation between the lipophilic silkworm oil and the membrane of niosomes (Guinedi et al., 2005). The entrapment efficiencies in niosomes of sericin which was hydrophilic compound gave higher value than the water soluble compounds.

The result of the entrapment found that the percentage of the entrapped oil in niosomes was not 100%. This was due to the degradation of oil during the entrapment process of the oil in niosomes. Also, the oil may be destroy by the high frequency of sonication. Therefore, the new methods of niosomes preparation should be used, such as supercritical carbon dioxide in order to avoid heat and high frequency from sonication (Shin et al., 2001).

The sericin was capable to dissolve in water at the temperature of 50 $^{\circ}$C and gave the pH of 7.0-7.5, while the deionized water gave the pH of approximately 6.2. Sericin when dissolved in the polar solvent had the molecular weight in the range of 20-70 Kda (the hydrolyzed sericin). Therefore, when sericin was entrapped in niosomes, it was entrapped in the aqueous layers of niosomes.

Since sericin in deionized water had the positive charge, it can also be adsorbed on the surface of the niosomal membrane which had the negative charge from the hydroxyl group of cholesterol. The neutral niosomal membranes composed of Tween 61/cholesterol at 1:1 molar ratio have been reported to have negative zeta potential (**Table 33**). Thus, the entrapment of sericin in this niosomal dispersion was higher (33.76%) than 10-20 % which was the normal entrapment efficiency of water soluble compounds (Manosroi et al., 2010).

3.3.4 The antioxidative activity of niosomes entrapped with None Ruesee sericin and oil prepared by the CFS method

The DPPH radical scavenging and tyrosinase inhibition activities of niosomes entrapped with None Ruesee strain silkworm sericin and oil were listed in **Table 34**. It was found that the free radical scavenging activity and tyrosinase inhibition activities of niosomes entrapped with oil and sericin were lower than that of the unentrapped oil and sericin of about 14.2-45 times. Moreover, the niosomes showed lower activity than that of the standard antioxidants (vitamin C, vitamin E and BHT) and the standard tyrosinase inhibition (vitamin C and kojic acid) of about 100-10,000 times, but higher than that of linoleic acid of about 20-700 times.

Table 34 Tyrosinase inhibition activities (IC_{50}) and DPPH radical scavenging activity (SC_{50}) of niosomes entrapped with None Ruesee strain silkworm sericin and oil extracts prepared by CFS

Samples	DPPH radical scavenging $SC_{50} \pm SD$ (mg/ml)	DPPH radical scavenging $SC_{50} \pm SD$ (mg/ml)		Tyrosinase inhibition activities $IC_{50} \pm SD$ (mg/ml)	Tyrosinase inhibition activities $IC_{50} \pm SD$ (mg/ml)	
Niosomes entrapped with None Ruesee strain silkworm sericin and extracts		sericin	oil	Niosomes entrapped with None Ruesee strain silkworm sericin and extracts	sericin	oil
62.46± 0.85		24.44 ± 0.75	10.08 ± 0.84	4.51± 0.77	1.20 ± 0.75	26.02 ± 0.81
Blank niosome	886.81 ± 0.85	ND	ND	632.47± 0.85	ND	ND
Vitamin C	0.03± 0.85	0.42 ± 0.57	0.42 ± 0.78	0.24± 0.95	0.36 ± 1.11	0.36 ± 1.09
Vitamin E	0.24± 0.17	0.54 ± 0.45	0.54 ± 0.55	ND	0.15 ± 0.65	0.15 ± 1.07
BHT	0.07± 0.28	0.53 ± 1.17	0.53 ± 1.19	ND	ND	ND
Kojic acid	ND	ND	ND	0.050± 1.25	ND	ND
Std. linoleic acid	9295.16 ± 2.57	ND	ND	ND	ND	ND

Note; CFS= chloroform film with sonication

Blank niosomes prepared by CFS method gave some DPPH radical scavenging activities and tyrosinase inhibition activities, due to the hydroxyl groups or double bonds in the chemical structure of cholesterol which can act as hydrogen donating radical scavengers (Valenzuela et al., 2003). Niosomes entrapped with None Ruesee strain silkworm mixed sericin and oil extracts gave higher DPPH radical scavenging activity and tyrosinase inhibition activities than the blank niosome of about 14.2 and 45 times, respectively, resulting from the effects of the hydrophilic compounds sericin which were entrapped in the internal aqueous phase of niosomes. However, silkworm oil in the extract which was a hydrophobic compound entrapped in niosomes gave less radical scavenging activity and tyrosinase inhibition activities than Blank niosome of about 14 times. (Venditti et al., 2008). However, the entrapment of oil and sericin in niosomes will be beneficial for cosmeceutical uses since the niosomes show higher activity than the standard linoleic acid.

Niosomes entrapped with oil and sericin that possesses special property of amino acids named serine which consist of hydroxyl group in high quantity (approximately 33%) and carbohydrate with the specific characteristics (approximately 3%), have the capability to well flocculate with keratin that was the important protein of stratum corneum by blending together to become the same substance of the film on the skin and capable to exceptionally flocculate with water. The capability of the film depends on sericin molecule mass. The higher the quantity of molecule mass, the better the origination of the film. The results of the test by applying oily cream in water containing sericin at the concentration of 0.35% or 70 mg/cm^3 on the skin twice a day in the area of crow's feet in a certain number of the volunteers for 21 days, have found that small size wrinkl declined and the deep wrinkle line became somewhat shallower (Voegeli, 1993).

3.5. Development of the serum containing of niosomes entrapped with oil and sericin from silk

3.5.1 Selection of the suitable serum base

3.5.1.1 Quality of the serum products available in the market.

Anti-wrinkle serum products available in the market were in the group of face skin nourishing product with the consumers target group who wanted to reduce wrinkles and take care of the skin problems of not smooth and no moisture.

In checking the physical and chemical quality of the anti-wrinkle serum products available in the market of the 5 brands (A, B, C, D and E) as shown in **Table 35.** All brands gave the pH values in the range of 4.17- 6.01. The color L*a*and b* values of all 5 brands indicated statistically significant difference. Results on checking the quality of those prototype products were used as the criteria for the selection of the developed serum formulations.

Table 35 Color and pH quality of ant wrinkle serum products available in the market

Brand	Color value			pH
	L*	a*	b*	
Brand A	22.02 ± 1.28^{d}	63.98 ± 1.33a	13.26 ± 1.48^{c}	5.98 ± 0.99^{b}
Brand B	34.48 ± 2.27^{b}	32.84 ± 2.29^{c}	6.83 ± 2.65^{d}	4.17 ± 0.78^{d}
Brand C	46.57 ± 1.24^{a}	17.96 ± 1.32^{d}	1.94 ± 1.47^{e}	5.73 ± 1.01^{c}
Brand D	22.26 ± 2.32^{c}	62.96 ± 2.47^{b}	15.67 ± 2.51^{b}	6.01 ± 1.12^{b}
Brand E	46.66 ± 1.58^{a}	10.73 ± 1.67^{e}	24.84 ± 1.77^{a}	7.01 ± 0.94^{a}

Note: $^{a-d}$ Mean values in the same column followed by different superscript were significant difference ($p \geq 0.05$)

3.5.1.2 Selection of the serum base formulations

The selected serum base formulations were selected and modified from the formulations of firming and moistening care (www.seppic.com, 2006), O/W moisturizing cream PC49031/5 (www.eac.com. 2008), anti - wrinkle cream gel 0-0014 (www.noveon.com. 2003), firm up firming cream ST-50 (www.arch.com, 2005) and skin-firming (www.croda.com. 2006) as illustrated their compositions in Table 20 and **Figures 23-28**.

Table 36 showed the appearance of the five anti-wrinkle serum products **and Table 37** indicated the physical and chemical quality of anti-wrinkle serum products. The pH range was 3.50-7.77, the viscosity value range was 2,501-5,482 Cp, and the color value was in the system CIE L*a*b*.All anti- wrinkle serum products were homogenous with high viscosity and had white appearance. When examined for the brightness value (L*), the product were in 33.47-51.10 range. The a* value or index value showed red and green. a* had the positive value indicating that the product had red color. If the a* value was negative, the product had green color.

In the measurement, it was found that the color of the products was yellowish white. Thus, a* value which gave positive value within the range of 11.50-32.58 and the b* indicated yellow and blue. If the b* value was negative, indicated that the product had blue color. The product color was yellowish, Thus, b* had the color in positive in the range of 0.64-7.42. Each formula had a different color value with statistically difference at the confidence level of 95%.

The sensory quality test according to the hedonic scaling test of the 5 basic formulas, using 50 volunteer was shown in **Table 38.** It was found that formulas 3 and 5 gave the highest preference score in the moderate preference without the significant difference at the confidence level of 95% on the viscosity, dispersion, skin absorbency, stickiness, moisturizing and the overall preference.

Table 36 Appearances of the five anti-wrinkle serum products

Basic Formula	Appearance	Picture
1	White cream with moderate viscosity and homogenization	
2	White cream with high viscosity and homogenization	
3	White cream with high viscosity and homogenization	
4	White cream with moderate viscosity and homogenization	
5	White cream with low viscosity and homogenization	

Table 37 Physical and chemical quality of the five anti-wrinkle serum products

Basic formula	Color value			pH	Viscosity
	L*	a*	b*		
1	48.82 ± 2.31^{b}	16.25 ± 1.35^{c}	2.15 ± 2.21^{d}	7.77 ± 1.25^{a}	$5,482\pm 2.35^{a}$
2	51.10 ± 2.44^{a}	15.71 ± 1.58^{d}	0.64 ± 2.56^{e}	3.50 ± 1.58^{e}	$4,457\pm 2.44^{b}$
3	41.80 ± 2.58^{c}	19.65 ± 1.69^{b}	3.92 ± 2.74^{b}	5.88 ± 1.67^{b}	$3,369\pm 2.65^{c}$
4	33.47 ± 2.61^{e}	32.58 ± 1.57^{a}	7.42 ± 2.69^{a}	5.17 ± 1.77^{c}	$2,501\pm 2.47^{d}$
5	39.93 ± 2.38^{d}	11.50 ± 1.94^{e}	3.74 ± 2.87^{c}	3.61 ± 1.58^{d}	$2,536\pm 2.58^{d}$

Note: $^{a\text{-}d}$ Means values in the same column followed by different superscript were significant difference ($p \geq 0.05$)

However, after testing the feeling in various features by the just-about-right method as shown in **Table 39.** It was found that the 5 serum products gave the feeling on the thickness of the serum substance of the feeling scores. The basic formula no. 3 was in good level or gave the highest percentage of feeling score at 58, 62, 50, 60 and 66 of 5 attributes respectively, of disperse of serum, skin absorption, sticky feeling, moisturizing and overall preference.

Table 38 Scores of preference by the volunteer with the different characteristics of the five anti-wrinkle serum products

Characteristics	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5
Viscosity of the serum content (before applying)	6.44 ± 2.35[a]	6.58 ± 2.81[a]	7.02 ± 2.58[a]	6.40 ± 2.65[a]	6.56 ± 2.87[a]
Distribution of the serum (while applying)	6.26 ± 3.14[b]	6.76 ± 3.25[ab]	7.06 ± 3.25[a]	6.48 ± 3.10[ab]	6.88 ± 3.54[ab]
Skin absorbency (while applying)	6.12 ± 3.21[c]	6.56 ± 3.35[abc]	6.86 ± 3.47[ab]	6.44 ± 3.65[bc]	7.14 ± 3.78[a]
Stickiness (after applying)	5.98 ± 4.11[c]	6.52 ± 3.98[abc]	6.66 ± 3.57[ab]	6.32 ± 3.74[bc]	7.02 ± 3.52[a]
Skin moisturizing (after applying)	6.20 ± 2.47[b]	6.46 ± 2.68[ab]	6.80 ± 2.68[a]	6.08 ± 2.78[b]	6.86 ± 2.64[a]
Overall preference	6.30 ± 1.87[b]	6.60 ± 1.98[ab]	7.02 ± 1.65[a]	6.36 ± 1.58[b]	7.12 ± 1.58[a]

Note: [a-c] Mean values in the same column followed by different superscript were significant difference ($p \geq 0.05$)

Thus, the anti-wrinkle serum basic formula no. 3 was selected for the further development, as the testers had given the intensity of the 6 characteristics in the good level and the highest percentages of satisfaction compared with the basic formula no.5.

Table 39 Percentages of feeling score (frequency) of the volunteers on various characteristics of anti-wrinkle serum basic formulas 1-5

Characteristics	Formula No.	Percentages of feeling score (frequency) of the volunteers				
		much too weak	slightly to weak	just-right	slightly to strong	much too strong
Lotion viscosity (before applying)	1	0	12	52	30	6
	2	0	10	50	38	2
	3	2	2	58	34	4
	4	0	22	36	42	0
	5	4	18	54	22	2
Lotion dispersion (before applying)	1	0	22	56	22	0
	2	2	20	44	34	0
	3	0	8	62	28	2
	4	2	20	40	38	0
	5	2	8	50	38	2
Skin absorption (while applying)	1	0	26	52	22	0
	2	0	32	44	24	0
	3	0	12	50	38	0
	4	2	28	46	24	0
	5	0	10	46	44	0

Table 39 Percentages of feeling score (frequency) of the volunteers on various characteristics of anti-wrinkle serum basic formulas 1-5(continued)

Characteristics	Formula No.	Percentages of feeling score (frequency) of the volunteers				
		much too weak	slightly to weak	just-right	slightly to strong	much too strong
Sticky feeling (after applying)	1	0	32	40	26	2
	2	0	32	50	16	2
	3	2	18	60	20	0
	4	0	30	50	20	0
	5	2	32	50	16	0
Skin moisturizing (after applying)	1	4	18	68	10	0
	2	0	24	66	10	0
	3	0	14	66	20	0
	4	0	32	58	10	0
	5	4	22	60	14	0

3.5.2. Optimization of the serum formulations

3.5.2.1 Quality of the basic serum formulas that had passed the selection

The serum product best formula no.3 the quality value of can be summarized the quality of the basic value formulas comparable to the market products. The clinical quality of the basic formulas in the same range with the commercial products was the elasticity value, while the chemical and physical qualities of the basic formulas arranged in the same range with the market products. The pH and viscosity were lowers than the products in the market. Meanwhile, the sensory quality of the basic formula as shown in **Tables 40 and 41** in the features of skin elasticity, viscosity, skin absorption, stickiness, skin moisturizing, softness and firmness were not the quality within the range of product in the market as well. Thus, the characteristics that are below the market range may be improved further.

Table 40 Summary of the product quality value of the commercial product compared with the basic formula serum

Quality factor	Quality value range	
	Commercial products	Basic formula
1.Quality clinical value		
1.1 Value of skin elasticity		
Factor that shows skin elasticity Ua/Uf	0.908-0.928	-
Ur/Ue *	0.962-1.080	0.984
Uv/Ue *	0.423-0.656	0.487
1.2 Increased ability to retain water on the skin (g/hm^2)	18.2-22.4	
2. Chemical and physical quality value		
2.1 pH	4.10-7.01	3.5
2.2 Viscosity (cP)	ND	4,457

Table 40 Summary of product quality value of the commercial product compared with the basic formula serum (continued)

Quality factor	Quality value range	
	Commercial products	Basic formula
3. Sensory quality value (intensity score)		
3.1 Fineness of cream ns	11.07-12.6	8.34
3.2 Elasticity	5.0-8.5	4.89
3.3 Viscosity	5.2-7.0	7.22
3.4 Dispersion ns	8.87-9.38	8.8
3.5 Skin absorbency	8.20-9.3	6.86
3.6 Slippery skin while massaging ns	6.11-7.28	6.5
3.7 Sticky	5.3-6.25	6.66
3.8 Oiliness after massaging ns	4.3-4.7	4.27
3.9 Skin moisturizing	6.63-7.6	6.4
3.10 Smoothness	8.10-9.77	7.5
3.11 Glossiness ns	0.3-1.9	2.2
3.12 Skin firmness	0.9-4.3	2.54

Note: ns Means value no statistically significant difference at the confidence level ($p \geq 0.05$)

Table 41 Quantitative descriptive analysis (QDA) of the basic serum formula compared with the market products

Formula	Sensory characteristic (Intensity score)											
	Fineness ^{ns}	Elasticity	Viscosity	Dispersion^{s}	Skin absorbency	Slippery^{ns}	Sticky	Oiliness after massaging^{ns}	Moisturizing	Smoothness	Glossiness^{ns}	Firmness
formula 1	12.33±0.79	5.63±0.74^{cd}	6.70±0.79^{a}	8.73±0.58	7.73±0.70^{cd}	6.83±0.82	5.97±0.71^{a}	4.30±0.60	8.77±0.79^{a}	8.43±0.67^{bc}	0.87±0.73	1.5±0.68^{cde}
formula 2	12.53±0.77	7.43±0.88^{ab}	5.47±0.85^{c}	8.87±0.73	7.83±0.64^{bcd}	6.93±0.80	6.60±0.85^{a}	4.50±0.63	6.7±0.48^{c}	8.83±0.58^{bc}	0.53±0.35	2.03±0.68^{b} c
formula 3	12.6±0.83	5.53 ±0.85^{d}	5.77±0.82^{bc}	8.97±0.51	7.50±0.68^{d}	6.77±0.55	6.07±0.79^{a}	4.37±0.70	7.17±0.88^{bc}	8.77±0.67^{bc}	1.13±0.64	2.83±0.61^{b}
formula 4	12.47±0.71	6.17±0.66^{cd}	7.5±0.74^{a}	9.17±0.58	8.17±0.79^{bcd}	6.87±0.48	5.73±0.58^{a}	4.67±0.50	8.5±0.58^{ab}	8.40±0.89^{c}	0.90±0.69	3.93±0.60^{a}
formula 5	12.52±0.81	5.63±0.75^{cd}	7.00±0.75^{abc}	8.97±0.69	7.67±0.68cd	6.83±0.44	6.30±0.69^{a}	4.77±0.58	7.17±0.64^{bc}	8.17±0.57^{c}	0.73±0.63	1.87±0.80^{c} d
Market A	12.73±1.25	6.90±0.75^{bc}	5.4±0.83^{c}	9.17±0.61	8.57±0.79^{b}	6.77±0.73	5.90±0.50^{a}	4.6±0.70	7.43±0.79^{bc}	9.23±0.76^{ab}	1.07±0.70	0.9±0.59^{e}
Market B	11.07±0.75	7.67±0.84^{ab}	6.17±0.70^{abc}	9.38±0.62	8.37±0.46^{bc}	6.63±0.66	6.10±0.62^{a}	4.5±0.71	7.57±0.80^{abc}	9.73±0.85^{a}	1.17±0.74	4.37±0.69^{a}
Market C	12.43±0.81	8.43 ±0.9^{a}	5.87±0.73^{abc}	9.20±0.65	9.47±0.33^{a}	6.47±0.62	3.47±0.39^{b}	4.40±0.89	7.10±0.67^{c}	9.77±0.61^{a}	0.40±0.46	1.17±0.80 d^{cde}
Market D	11.97±0.49	6.73±0.84^{bcd}	7.30±0.83^{ab}	9.17±0.44	8.17±0.85^{bcd}	6.63±0.86	6.40±0.73^{a}	4.73±0.55	7.67±0.56^{abc}	8.37±0.76^{c}	0.67±0.82	1.00±.45de
Market E	12.37±0.53	5.63±0.63^{cd}	6.43±0.67^{abc}	9.22±0.68	8.20±0.79^{bcd}	6.40±0.85	6.23±0.62^{a}	4.43±0.62	6.63±0.59^{c}	8.90±0.80^{bc}	0.93±0.82	1.93±0.72^{d} e

Note: ^{ns} Means values with no statistical significant difference at the confidence level ($p \geq 0.05$)

$^{a\text{-}dj}$ Mean values in the same column followed by different superscript were significant difference ($p \geq 0.05$)

Then, the selected basic formula no.3 was improved in terms of viscosity, texture of gel and spreadability. The types of emulsifier used were Seppic gel 305, Simugel EG and CDRM 2051 and were added to the serum formula at 0.5%. physical, chemical and sensory qualities were evaluated and the results were showed in **Table 42.** The pH was higher than the commercial products, while the viscosity value found that Seppic gel gave higher viscosity value than Simugel EG and CDRM 2051 with statistically significant difference at the confidence level at 95%.

Upon testing the sensory quality of serum with an ingredient of viscosity thickeners, CDRM 2051, Simugel EG and Seppic gel 305 at the level of 0.5% by the 9-point Hedonic scale method with the volunteers who had not pass the 50 volunteers with the results in **Table 42,** the volunteers gave the rate of liking score in terms of viscosity, gel fineness and oiliness on the skin after massaging. It was found that there were no statistically significant differences ($p \geq 0.05$) between the samples.

From the basic formulation test, it was found that viscosity, absorbability, spreadability and oiliness needed to be improved. Various thickener agents were added into the basic formulation at 0.5% w/w. The results were shown in **Tables 42 and 43.** There were significant difference in the term of viscosity, pH, stickiness, absorbency and overall liking ($p \geq 0.05$). The just-about- right test showed that most volunteers gave just-right level to the sample using CDRM 2051 for stickiness and absorbency attributes at 61% and 62%,respectively.

Table 42 Quality value of the basic formula serum added with Seppic gel 305, Simugel EG and CDRM 2051 at the level of 0.5% w/w.

Quality value	Thickener agents		
	Seppic gel 305 0.5%	Simugel EG 0.5%	CDRM 2051 0.5%
1.Chemical and physical quality value			
Viscosity (cP))	15294.67 ± 5.27[a]	3302.00 ± 5.31[a]	3321.00 ± 6.27[b]
pH	8.12 ± 3.14[a]	8.06 ± 3.58[c]	8.16 ± 3.69[b]
2.Sensory quality value (liking score)			
Viscosity [ns]	6.10± 2.85	6.08± 2.65	5.58± 2.58
Gel Softness [ns]	6.96± 4.15	6.90± 3.85	6.80± 2.98
Stickiness	6.38± 2.37[a]	5.70± 2.58[b]	6.12± 2.23[ab]
Absorbency	6.52± 4.01[a]	5.98± 4.22[b]	6.32± 4.38[ab]
Oiliness [ns]	6.52± 3.36	5.96± 3.78	7.36± 3.58
Overall liking	6.66± 1.51[a]	6.24± 2.05[b]	6.22± 1.99[b]

Note: [a-c] Mean values in the same column followed by different superscript were significant difference ($p \geq 0.05$)

[ns] Means values with no statistical significant difference at the confidence level ($p \geq 0.05$)

Table 43 Just-about-right score of the volunteers with the serum added with CDRM 2051, Simugel EG and Sepigel 305 at the level of 0.5% w/w.

Sensory characteristics	Thickener agent	Percentage (%)				
		much too weak	slightly to weak	just-right	slightly to strong	much too strong
Viscosity	CDRM 2051	18.0	36.0	40.0	6.0	0.0
	Simugel EG	6.0	42.0	34.0	18.0	0.0
	Sepigel 305	12.0	28.0	52.0	8.0	0.0
Softness	CDRM 2051	0.0	8.0	66.0	22.0	4.0
	Simugel EG	0.0	10.0	68.0	22.0	0.0
	Sepigel 305	0.0	14.0	66.0	16.0	4.0
Stickiness	CDRM 2051	5.0	12.0	61.0	14.0	8.0
	Simugel EG	2.0	18.0	34.0	34.0	12.0
	Sepigel 305	4.0	16.0	38.0	28.0	14.0
absorbency	CDRM 2051	2.0	14.0	62.0	16.0	6.0
	Simugel EG	4.0	34.0	48.0	11.0	3.0
	Sepigel 305	0.0	26.0	52.0	14.0	8.0
Oiliness	CDRM 2051	0.0	12.2	61.2	22.4	4.1
	Simugel EG	2.0	10.0	52.0	30.0	6.0
	Sepigel 305	2.0	12.0	64.0	20.0	2.0

The results from skin elasticity test were shown in **Table 44.** It was found that the Ur/Ue value of the sample with CDRM 2051 close to 1.0.This means that it had more water retaining ability than other samples.

Table 44 Elasticity of skin and the ability of the transepidermal water loss on the skin by the 3 formulas of the anti-wrinkle serum

Clinical quality factor	Quality value range			
	Commercial product	Seppic gel 305, 0.5 %	Simugel EG, 0.5 %	CDRM 2051, 0.5 %
1.1 Skin elasticity value				
Factors that showed skin elasticity Ua/Uf	0.908-0.928	-	-	-
Ur/Ue *	0.962-1.080	0.954	0.961	0.991
Uv/Ue *	0.423-0.656	0.553	0.529	0.492
Ur/Uf	0.498-0.636	-	-	-
1.2 Increased ability in retaining water at the skin (g/hm^2)	5.4	7.25	7.12	8.05

3.5.2.2 Optimization of the serum formations

In order to improve the performance of the product in terms of viscosity, softness, slipperiness and stickiness, C_{14-22} alkylalcohol and C^{12-20} alkylglucoside MONTANOVL) and C_{12-20} alkylglucoside and Cyclospentasiloxame, dimethicomol,dimethicone crosspolymer (and) phenyltrimethicone blend (DCCB 3031) were added to the selected formulation from section 3.4.2.1 at 0.5, 1, 1.5% and 1, 1.5, 2 %, respectively. Suitable levels of MONTANOV L were studied using 3x3 factorial experimental designs. The liking test was conducted with 50 volunteers and the results were shown in **Table 45.** If was found that overall liking score of the sample with 1 **%** MONTANOV L and 1.5 % DCCB 3031 was the higher compared with other samples.

Table 45 Preference scores on various features of the anti-wrinkle serum

Formula	Factor		Preference score						
	x1	x2	Viscosity^{ns}	Softness^{ns}	Absorbency^{ns}	Stickiness ^{ns}	Moisturizing^{ns}	Firmness	Overall liking
1	0.5	1	7.3	7.9	7.3	7	7.5	6.2	7.2
2	0.5	1.5	7.4	7.6	7.5	7.6	7.2	6.7	7.5
3	0.5	2	7.6	7.4	7.6	7.4	6.9	6.9	7.8
4	1	1	8.6	8.6	8.3	8.2	8.4	8.3	8.1
5	1	1.5	8.4	8.8	8.6	8.8	8.7	8.4	8.7
6	1	2	8.6	8.3	8.1	8.6	8.2	8.2	8.4
7	1.5	1	7.9	8.1	8.1	6.9	7.7	8	7.1
8	1.5	1.5	8.2	8.3	7.7	6.7	7.2	7.8	7.7
9	1.5	2	8.1	8	7.5	7.1	7	7.2	7.3

Note: x_1 the concentration of MONTANOV L (%), x_2 is concentration of DCCB 3031 (%)

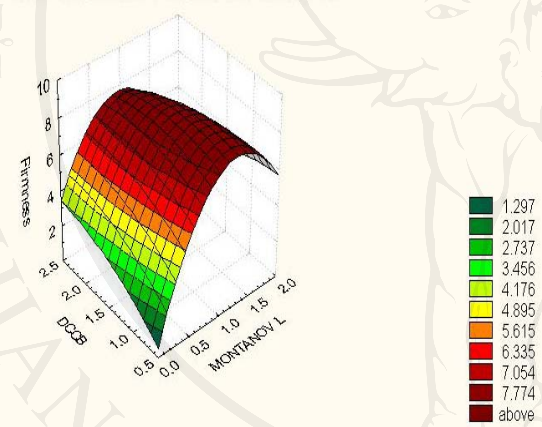
^{ns} the means with no statistical significant difference at the confidence level of 95% ($p \leq 0.05$)

There were significant difference between samples in the terms of firmness and overall liking. The regression equations showing relationships between firmness and thickening agents and overall liking as well as the thickening agents were developed as shown in **Table 46.** The response surface graphs was shown in **Figures 43 (a) and (b),** The equation showed that MONTANOV L and DCCB 3031 had significant effects on firmness and overall liking.

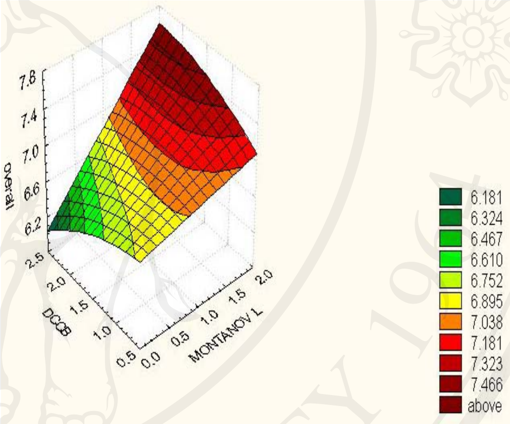
Table 46 Equations of the relations between the preference scores of serum and the production factors

Serum quality value	Mathematical simulation	Correlation value (R^2)
Firmness	$-0.972 + 12.650x_1 + 3.433x_2 - 1.500x_1x_2 - 4.667x_1^2 - 0.667x_2^2$	0.999
Overall Preference	$6.795 + 0.314x_1x_2 - 0.121x_2^2$	0.907

Note: x_1 the concentration of Montanov L (%), x_2 the concentration of DCCB 3031 (%)



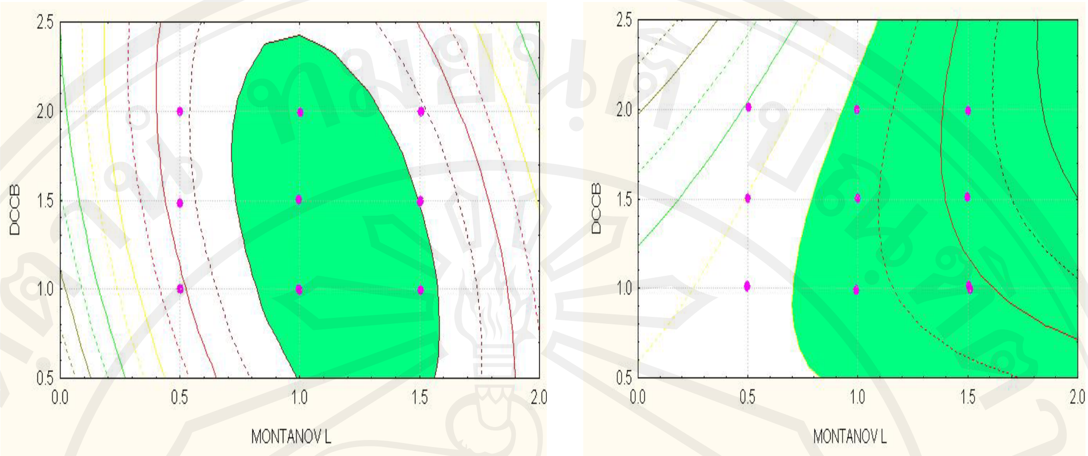
(a) Face Skin raised firmness



(b) Overall preference

Figure 43 Response surface graphs showing relations between the concentrations of Montanov L and DCCB 3031 with the effects on preference of skin firmness (a) and overall preference (b)

The contour plot was shown in **Figures 44 (a) and (b),** and the superimpose plot was shown in **Figure 45**. Form the contours plot, it can be seen that the optimized level of Montanov L and DCCB 3031 were 1.0 and 1.5 %, respectively. Thus, the Montanov L at 1.0% and DCCB 3031 at 1.5% were chosen to give the highest preference score compared with other formulas. Thus, the formula 5 was used further to develop the products.



(a) Firmness (b) Overall preference

Figure 44 contour plot graphs showing the score range of suitable preference in the productions of raised skin firmness such as preference on firmness (a) and overall preference (b).

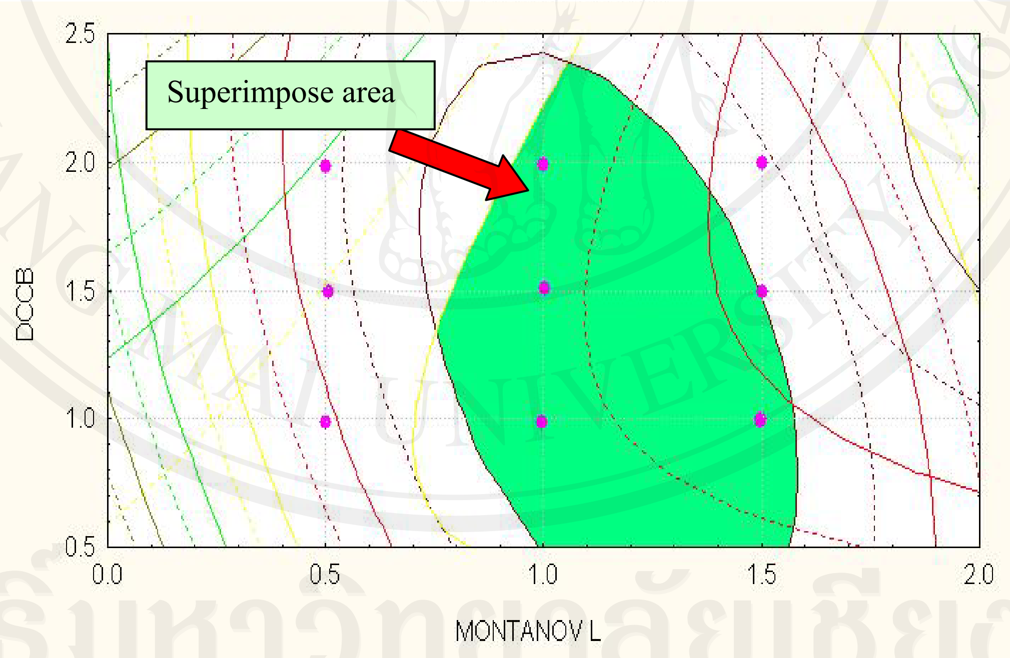


Figure 45 Contour plot graph of the preference score value on the raised skin face firmness and overall preference upon superimpose area derived of the green area which are Montanov L and the DCCB 3031 suitable amount.

3.5.3 Preparation of niosome serum

3.5.3.1 Elasticity study of niosome serum formulations using cutometer

Anti-wrinkle serum were prepared from the optimized formulations obtained from section 3.4.2.2 by adding niosomes containing oil and sericin from Thai native silkworm at 3,6 and 9% w/w. The suitable level of niosome was obtained from the results of free radical scavenging and tyrosinase inhibition activities test in section 3.3.4.

Five volunteers were used to evaluate the skin elasticity by cutometer MPA 580 device. The skin elasticity tests were shown in **Table 47.** It was found that there were significant differences between the samples.

The formula No. 3 of 6 % niosomes had the factor value of Ur/Uf which was higher than other formulas significantly, which showed the efficiency of the formula in increasing skin elasticity. Thus, the formula with niosome volume at 6 % was used further as the finished product to test for the long-term efficiency and acceptance of the consumers. Then, the serum formula with niosome at 6 % was tested with 4 volunteers by at home applying on the left and right face. They had to come back to check the skin condition every other week for 4 weeks. The results from the experiment found that the Ue and Ur factor value increased after using the product longer and had statistically significant difference at the confidence level of 95% as shown in **Table 48** and **Figure 46**.

Table 47 Comparisons of factor values used to explain skin elasticity before and after adding niosomes entrapped with oil and sericin from silk

Factor skin elasticity	Niosomes (%)	Before using	After using	Paired sample t-test	
				t	Sig (2-tailed)
Ur/Ue	3	0.9890	1.0683 [a]	-10.920	0.008*
	6	0.0890	1.0757 [a]	-422.857	0.000*
	9	0.0890	1.1027 [b]	-238.190	0.000*
Uv/Ue	3	0.6697	0.5847 [A]	-6.927	0.020*
	6	0.6697	0.5770 [A]	-3.831	0.062
	9	0.6697	0.5721 [B]	-22.655	0.002*
Ur/Uf	3	0.5719	0.6125 [e]	-3.287	0.081
	6	0.5719	0.6149[e]	-16.225	0.004*
	9	0.5719	0.6301 [f]	-22.558	0.002*

Note: * means statistical significant difference at the confidence level of 95% before and after using the product.

a-b means statistical significant difference at the confidence level of 95% of Ur/Ue

A-B means statistical significant difference at the confidence level of 95% of Uv/Ue

e-f means statistical significant difference at the confidence level of 95% of Ur/Uf

Table 48 Ur and Uf values of the serum with 6% niosomes entrapped with oil and sericin from silk after using for 0, 2 and 4 weeks.

Week	Ur	Uf
0	0.0633±1.87 [c]	0.0400±3.57 [c]
2	0.1100±2.54 [b]	0.1433±3.21 [b]
4	0.2400±2.21 [a]	0.1700±3.14 [a]

Note:[a-c] means statistical significant difference at the confidence level of 95% ($p \leq 0.05$)

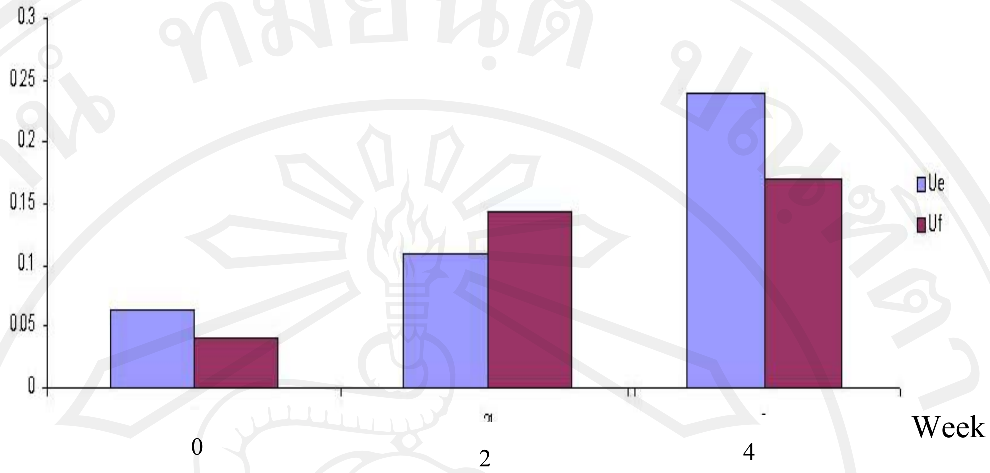


Figure 46 The Ur and Uf values of skin after using the serum base mixed with 6 %w/w niosomes entrapped with sericin and oil at 0, 2 and 4 weeks.

3.5.3.2 Color and fragrance development of serum with niosomes entrapped with sericin and oil from the native Thai silkworm

A. Color study of niosome serum formulations

In the inquiry on color of serum mixed with niosomes, the most consumers wanted in the top 4 rankings were which creamy yellow, mild yellow, white and mild pink. They were tested to find the color. The consumers liked most by preference ranking, 1= like the most, and 4= like the least. They were used to analyze and interpret the test results by the Friedman's test with the results shown in **Table 49.** It found that the creamy yellow had the total value of the likeness in the early ranking and much less than other colors. It showed that most of the volunteers ranked the preference of creamy yellow in the first place due to the reason that this color was close to natural color. The preference was color of serum with niosomes in creamy yellow compared with other colors. The next preference rankings were mild yellow and white respectively, while pink gave the least preference. Thus, creamy yellow serum was chosen to be further developed on the fragrance.

Table 49 The total rank of the preference ranking on color of serum with niosomes entrapped with sericin and oil from the native Thai silkworm

	Creamy yellow	Mild yellow	White	Pink
Total preference rank	68 ± 2.54^{a}	102 ± 2.68^{b}	103 ± 2.47^{b}	127 ± 2.65^{c}

Note: $^{a-c}$ Means values in the same row followed by different superscript were significant difference ($p \geq 0.05$)

B. Fragrance study of serum formulations

In the experiment to find a suitable fragrance by using 3 types of fragrance including, Naomi, silk blossom and tea, which were the consumers liked the most from surveying fragrance in the market. By adding fragrance in the serum formula at 0.6 % w/w and tested for the sensory quality on the fragrance characteristics before, during and after using together with giving the score value on sense from testing by the just-about-right method. It was found the 3 fragrance characteristics upon inhaling before and after using had no statistically significant difference on the fragrance feature while inhaling and the overall preference. It was found that naomi, and silk blossom fragrance received the highest preference score while the tea fragrance received the lowest preference as shown in **Table 50**.

Table 50 Scores on fragrance preferences in the serum formula mixed with niosomes entrapped sericin and oil from the native Thai silkworm

Fragrance	Preference score		
	Silk blossom	Naomi	Tea
Fragrance before application [ns]	6.0± 1.69[ab]	6.2± 1.84 [a]	5.2± 1.71[c]
Fragrance during application	5.8± 1.92[ab]	6.3± 1.88[a]	5.1± 1.64[c]
Fragrance after application [ns]	6.3± 2.01[a]	6.5± 2.10 [a]	5.7± 2.03 [a]
Overall preference	6.4± 2.11[a]	6.6± 2.31[a]	5.4± 1.98[b]

Note: [a-c] Means values in the same row followed by different superscript are significant difference ($p \geq 0.05$)

[ns] Means statistical with no significant difference at the confidence level of 95% ($p > 0.05$)

In **Table 51,** the volunteers had the feeling on the intensity of the fragrance before and after using silk blossom at the level that was just right, or the highest percent preference score was 46.7 and 40.0% respectively. For the sense on fragrance during use it found that the volunteers had the feeling that the intensity of the scent was little high, or the highest percentage was at 50%. While for the naomi fragrance, it found that the volunteers had the feeling on the intensity of the scent before use little strong or the highest percentage was at 40.0%, and the fragrance during and after use found that the volunteers had the feeling of fragrance intensity at the just-right level, or the highest percentage was at 56.7 and 66.7% respectively. The value was higher than the silk blossom. However, the tea received the lowest score of all types and had the fragrance intensity before and during use little stronger. Thus, the fragrance with most suitability was naomi due to the volunteers had the feeling that its intensity was at the good level and was the highest percentage of the fragrance compared with other types. Moreover, the fragrance also received the preference score on the feature of fragrance during use with the highest preference.

Table 51 Fragrances of volunteers by the just-about-right method

Type of fragrance	Characteristics	Percentage (%)				
		much too weak	slightly to weak	just-right	slightly to strong	much too strong
Silk blossom	fragrance	0.0	16.7	40.0	43.3	0.0
	before using	0.0	3.3	46.7	36.7	13.3
	during using	0.0	3.3	36.7	50.0	10.0
	after using	0.0	13.3	40.0	36.7	10.0
Naomi	fragrance	0.0	16.7	33.3	50.0	0.0
	before using	0.0	3.3	40.0	46.7	10.0
	during using	3.3	0.0	56.7	26.7	13.3
	after using	0.0	6.7	66.7	20.0	6.7
Tea	fragrance	0.0	16.7	33.3	46.7	3.3
	before using	0.0	6.7	16.7	43.3	33.3
	during using	0.0	6.7	20.0	40.0	33.3
	after using	0.0	10.0	30.0	30.0	30.0

Then, 0.6% Naomi fragrance was used in the optimized the anti-wrinkle serum mixed with niosomes which was selected for the further studies. The serum formula composition with niosome and the selected fragrance was shown in **Table 52** and **Figure 47**.

Table 52 Ingredients of the serum products mixed and the selected fragrance

Part	Raw Material Used	Percentage (g/100 g)
A	Water	63.82
	Sodium EDTA	0.05
	Glycerin	3
	Carbopol® Ultrez 21 Polymer	0.2
	Triethanolamine	0.5
B	C_{14-22} alkylalcohol and C_{12-20} alkylglucoside	1
	Simusol 165	0.4
	Propylene glycol	1.5
	Florasun 90	2.3
	Silsense™ DW-18	3
	Finsolv TN	2
	Octyldodecanol	1.5
	Octyl palmitate	2
	Floramac® 10	1
	Tocopheryl acetate	0.2
	Panthanol	0.2
	Butylated hydroxytoluene	0.1
	Shea butter	0.4
	Methyl paraben	0.1
	Propyl paraben	0.1
C	Vitamin A	0.1
D	Germaben® II E	0.4
E	Cyclopentasiloxane, dimethiconol, dimethicone crosspolymer (and) phenyltrimethicone blend	1.5
F	Niosome	6
	Water DI	6
G	Sodium polyacrylate (and) dimethicone (and) cyclopentasiloxane (and) trideceth-6 (and) PEG/PPG -18/18 dimethicone	2
H	Naomi fragrance	0.6
	Musk	0.03
	Total	100

Figure 47 The serum containing niosomes entrapped with sericin and oil from the native Thai silkworm and the selected fragrance

3.5.3.3 Quality study of the serum containing niosomes entrapped with sericin and oil from silk

Table 53 showed the chemical, physical and micro-organism qualities of the serum containing niosomes entrapped with sericin and oil from None Ruesee silk and fragrance. It gave the pH of 6.97, color of L*, a* and b* of 88.4, 3.68 and 20.55 respectively, viscosity of 5200 cP, total plate count of $6.33x10^2$ and, the Ur/Ue on skin elasticity at 1.068 and transpidermal water loss on skin at 32.4 g/m^2

Table 54 showed the sensory quality of the serum product containing niosomes entrapped with sericin and oil extracted from None Ruesee silkworm. It was found that the sensory scores in all characteristics were in the same range as the product in the market as shown in **Table 54.**

Table 53 Chemical, physical properties and micro-organism contamination of the serum products containing niosomes entrapped with sericin and oil from silk and the selected fragrance

Quality Factor	Measured Value
1. pH	6.97±1.25
2. Chemical quality	
- color L*	88.4±2.44
- a*	3.68±0.98
- b*	20.55±2.87
Viscosity (cP)	5,200±2.57
3.Micro-organisms quality	
- Total plate count	$6.33X10^2$
- *Clostridium spp*. (colony/g)	Not found
- *Staphyloccus. aureus*	Not found
- *Streptococcus spp*	Not found
- *Salmonella spp*	Not found
- *Pseudomonas aeruginosa*	Not found
- Coliform bacteria (MPN/gram)	Not found
- *Escherichia coli*	Not found
-Yeast and Mold	Not found
4. Skin elasticity	
Ur/Ue *	1.068±0.97
Uv/Ue *	0.709±3.10
Ur/Uf	0.578±2.78
Tranepidermal water loss on skin. (g/m^2)	32.4±1.84
5. Irritation test	Non-irritation

Table 54 Sensory qualities of the serum products containing niosomes entrapped with sericin and oil from silk

Sensory characteristics	Average score
Color intensity	7.12 ± 0.85
Fragrance	8.64 ± 1.12
Viscosity	7.25 ± 0.96
Dispersion of the product	8.55 ± 1.17
Skin absorption	8.32 ± 0.75
Stickiness	7.58 ± 0.89
Skin moisturizing	8.15 ± 1.23
firmness after use	8.39 ± 0.77

3.5.3.4 Physical and chemical stability of the serum containing niosome entrapped with oil and sericin from silk

The developed serum product containing niosome was put in plastic bottles (50 g) and stored at the ambient temperature (30 °C) and the accelerated condition at 35 and 45 °C. The samples were drawn and tested the physical and chemical properties at every week for 8 weeks

A. Chemical stability of the serum containing of niosome entrapped with oil and sericin from silk

The value of the pH of the developed product serum stored at different temperatures increased when compared to initial. The pH value after storing 8 weeks at 3 temperatures were found to have an increased pH of to more than 50%

compared to the value at initial. The changes of pH of the serum stored at various temperatures were shown in **Figure 48**.

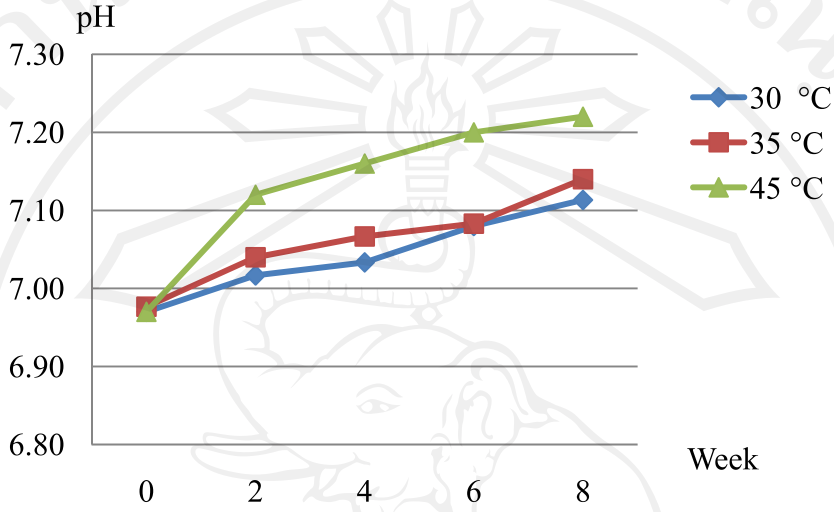


Figure 48 Changes of pH of the developed serum stored at 30, 35 and 45 °C for 8 weeks.

B. Physical stability of the developed serum

B 1. Viscosity value

The value of viscosity of the developed serum stored at the temperature increased when compared to at initial. It was found that the changes of viscosity of the serum when stored in an accelerated condition were statistically significant. The product stored at the controlled condition at the ambient temperature (30 °C) and the accelerated condition at the temperatures at 35 °C and 45 °C. It gave the increase viscosity during 4 week, Thus, stored at accelerated condition at high temperature (45 °C) resulted in a change of gel with an increase rate of viscosity higher than stored at room temperature and 35 °C, because of the increase rate evaporation of water in the formula at higher, and the change of viscosity value during storing at the various temperature as shown in **Figure 49**.

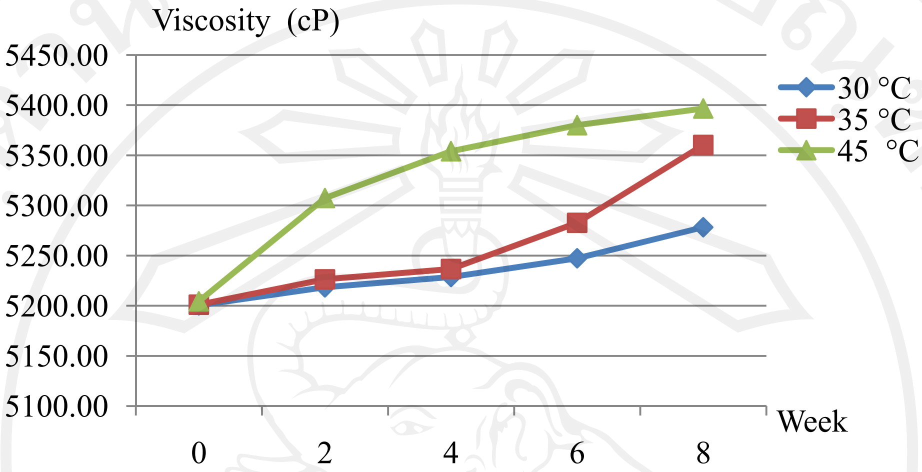


Figure 49 Viscosity changes of the developed serum stored at 30, 35 and 45 °C for 8 weeks.

B 2. Color value (L*a*b*)

L* value

Color of the developed serum was measured in the system of CIE, L*a*b*.The serum mixed with niosome had the creamy yellow color. When consider at L* value or lightness value of the serum, it has increased the trend with significance compared to initial. The ambient temperature (30 °C) and accelerated condition at 35 and 45 °C for 8 weeks, a trend of lightness value was slightly increased, or the product color was faded or milder **(Figure 50)**.

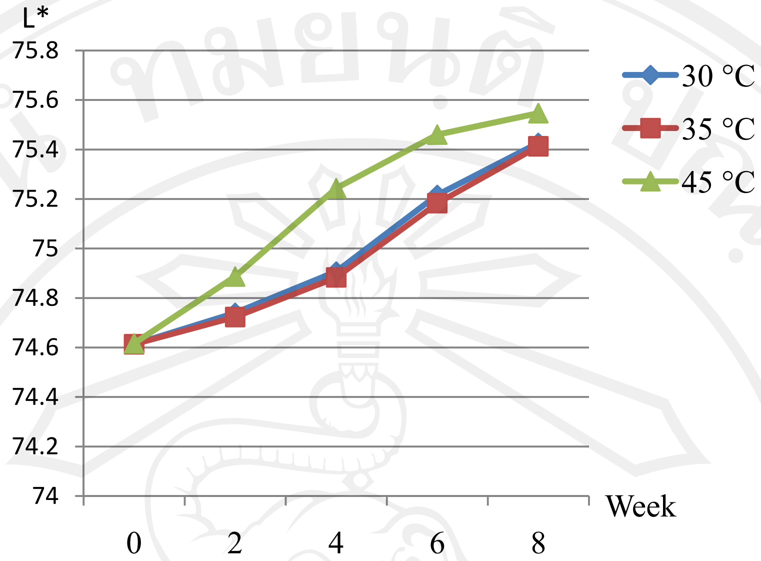


Figure 50 Change of the lightness value (L*) of the developed serum stored at 30, 35 and 45 °C for 8 weeks.

a* value

When consider a* value or a value that denoted red and green colors of the sample, if the a* value was positive, it showed that the product is red. If a* was negative, it shows that the product was green. The measured valued found that the color of serum raised firmness was creamy yellow. So, a* was positive value during stored at the accelerated conditions was at the temperatures of 35 and 45 °C for 8 weeks. The a* value of the raised firmness serum had decreased significantly from the 2nd week onward or the product had dark yellow or pale yellow. At the temperature of 45 °C, the a* value reduced greatly and showed the influence of excessive high temperature during stored indicating cause of changes in appearance on the product color to pale color. While stored in the controlled condition at room temperature (30 °C) for 8 weeks, it found that the value a* has no change or no significant difference. The a* value after passing 8-week stored had the value decreased no more than 50% compared to the starting value. The change in a* value during stored at various temperatures was shown in **Figure 51**.

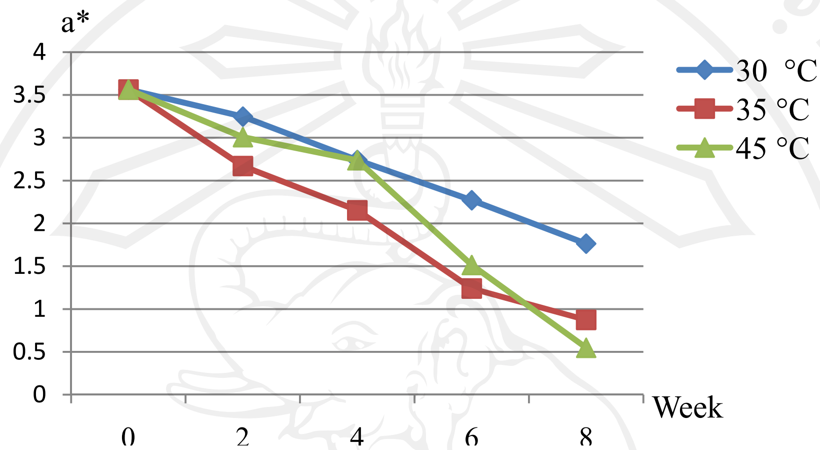


Figure 51 Changes of color value on the a* of the developed serum stored at 30, 35 and 45 °C for 8 weeks.

b* value

Upon considering b* or the value that denoted yellow and blue colors of the sample, if b* was positive, it showed that the product is yellow. If b* was negative, it showed that the product was blue. The results found that the serum product of raised the firmness after stored for 8 weeks had the trend of b* value increased from the starting significantly. The product had more yellowish color during stored at the ambient temperature (30 °C) and in the accelerated condition at the temperature 35 and 45 °C. The b* value increased of no more than 50% compared to the starting value. The changes of b* color value during storage at various temperatures were shown in **Figure 52**.

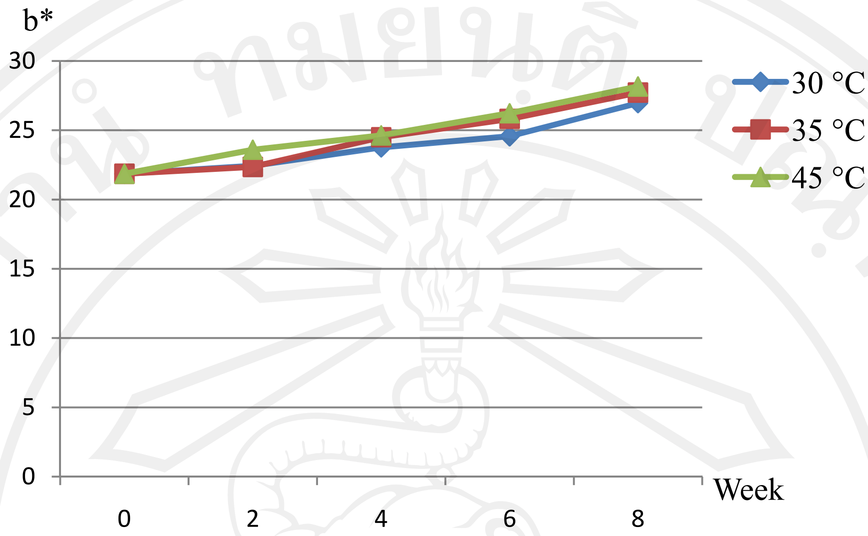


Figure 52 Changes of color value in b* of the developed serum stored at 30, and 45 °C for 8 weeks.

The physical and chemical qualities of the serum containing niosomes entrapped with oil and sericin from silk during stored at 8 weeks, found that the most overall quality value at the ambient temperature (30 °C) and the accelerated condition at the temperature 35 °C and 45 °C had a change of no more than 50 % compared with the starting value. In generally, to decide to launch the developed serum product to the market, the qualities of the products should be considered

For the serum stability, it was found that the alkalinity and acidity, color, smell and viscosity have changed as a result of the oxidation of the products from the environment; such as, light, temperature, heat and containers (Banks and Muir, 1985), particularly, the large quantity of lipid substances which may cause the more peroxidation reactions.

3.6. Cost estimation of the oil, sericin from the native Thai silk worm and anti-wrinkle serum mixed with of niosomes entrapped with sericin and oil from silk

3.6.1 Estimation cost of the products from Thai native silkworm

The cost calculation of raw material sericin and oil was shown in **Tables 55 and 56.** The costs of sericin, oil and the serum were 3,933.8, 7,338.2 and 44,000 Baht, respectively.

Table 55 The estimated costs of oil and sericin from the Thai native silkworm

Raw materials	Unit price /kg.	Unit price /grams	Quantity (grams/ 1000g)	Total costs of oil (Baht)	Total costs of sericin (Baht)
Silkworm pupa	120	0.12	100	12	-
Petroleum ether	758/2500 ml	0.303	750	227.25	-
Sericin cocoon	120	0.12	25	-	3
DI water	65	0.065	4,000	-	260
Filter paper	430	4.30	6	-	25.8
GF/A Filter paper	14,000/ 30ml	466.7	2 m	-	933.4
Power (volt)	-	2.5 unit	54,65 hour	135	162.5
				374.25	1384.7
Costs/1000 g of the initial raw material	5.10 % of the initial raw material (oil)				
	35.2 % of the initial raw material (sericin)				
Costs/1000 g the of product	(1000*375.25)/51.0 = 7338.2 (oil)				
	(1000*1384.7)/352.0 = 3,933.8 (sericin)				

Table 56 The estimated costs of niosomes entrapped with sericin and oil from Thai native silkworm

Raw materials	Unit price /kg.	Unit price /grams	Quantity (grams/1000g)	Total costs (Baht)
Sericin and oil	11,272	11.272	2	22.5
DI water	65	0.065	1700	110.5
Cholesterol	5,500/100g	275	5	1,375
Chloroform	800/2,500ml	0.32	1000	320
Tween 61	2,806/500g	5.612	8	44.9
Power (volt)	-	2.5 unit	115 hr.	287.5
				2,160.4
Costs/1000 g of the initial raw material	4.91 % of the initial raw material			
Costs/1000 g of the product	(1,000*2160.4)/49.1 = 44,000			

3.6.2 Estimation cost of the anti-wrinkle serum containing niosomes entrapped with sericin and oil from Thai native silkworm

According to the cost calculation of the product in the scale of 1,000 gram, as shown in **Table 57,** the price was about 2,963.2 Baht. Due to 10% weight loss during the production, the cost for 1,000 gram was about 296.3 Baht. The product size 50-gram bottle and the total cost of the product were about 3,013.2 Baht based on the raw material price, excluding VAT, in October 2009.

Table 57 The estimated costs of the developed anti-wrinkle serum containing niosomes entrapped with sericin and oil from Thai native silkworm

Raw Materials Used	Quantity Used (g/1000 g)	Cost / Unit (Baht/1000 g)	Total cost (Baht)
Water	698	50	33.95
Sodium EDTA	0.5	250	0.125
Glycerin	30	75	2.25
Carbopo®Ultrez21 polymer	2	1,000	2
Triethanolamine	5	150	0.75
C14-22 alkylalcohol and C12-20 alkylglucoside	10	750	10
Simusol 165	4	800	3.2
Propylene glycol	15	650	9.75
Florasun 90	23	800	18.4
Silsense™ DW-18	30	1,100	33
Finsolv TN	20	320	6.4
Octyldodecanol	15	280	4.2
Octyl palmitate	20	280	5.6
Floramac® 10	10	2,200	22
Tocopheryl acetate	2	900	1.8
Vitamin A	1	54,000	54
Panthanol	2	450	0.9
Butylated hydroxytoluene	1	1,100	1.1
Shea butter	4	550	2.2
Methyl paraben	1	220	0.22
Propyl paraben	1	240	0.24
Germaben® II E	4	750	3
Cyclopentasiloxane,dimethiconol,dimethicone crosspolymer (and) phenyltrimethicone blend	15	350	5.25

Table 57 The estimated costs of the developed anti-wrinkle serum containing niosomes entrapped with sericin and oil from Thai native silkworm (continued)

Raw Materials Used	Quantity used (g/1000 g)	Cost / Unit (Baht/1000 g)	Total cost (Baht)
sericin	14	5,000	70
Water	60	50	3
Sodium polyacrylate (and) dimethicone (and) cyclopentasiloxane (and) trideceth-6 (and) PEG/PPG -18/18 dimethicone	20	1,300	26
Niosomes	60	43,942.9	2,636.5
Perfume	6	1,542	9.252
musk	0.3	1,100	0.33
Total			2963.23

Note : Loss of weights during the production process (% weight loss) was equal to10%.

3.7 Consumer acceptance study on the serum containing of niosomes entrapped with oil and sericin from silk

The consumer acceptance study of the serum containing of niosomes entrapped with oil and sericin from silk was done by the Home Use Test method of 82 target female consumers aged during 36-65 years. Non-probability sampling was used in the study by distributing the survey sheets and product samples to the volunteers to use for 4 weeks. The result was following:

3.7.1 Demography data of volunteers

There are three age ranges of volunteers: 56.10% are aged 30-40 years, 28.0% were 41-50 years, 8.50% are 51-60 years, and 7.3% are over 60 years. The volunteers had different academic degrees that 42.7% had a Bachelor degree, 35.4% included the volunteers with a Master degree and the ones with a Doctorial degree. For occupations, 34.1% of the volunteers were employees of private organizations and 28% run their own businesses. Income rate was also varied that 31.7% earn during 30,001-40,000 Baht per month, and 29.3% earned 10,001-20,000 Baht per month. The most favorable type of anti-wrinkle moisturizer was in cream formula which was used by 56.1% of the volunteers. The secondary favorable type was in gel formula which was used by 37.8% of the volunteers. **Table 58** showed personal data of the volunteers.

3.7.2 Information of consumer acceptance study on anti-wrinkle serum containing niosomes entrapped with oil and sericin from silk

The volunteers had used the anti-wrinkle serum for 4 weeks and, after using, they were asked to fill the survey sheet for the outcome comparing to other anti-wrinkle moisturizers.

Most volunteers found that the anti-wrinkle serum sample had a strong particular smell because the smell lasts quite long after applying. They believed that more fragrance adding would help minimize the smell the compositions of the serum. That was one of the main reasons they found the serum unacceptable. The study also showed that the positive effect appeared too slowly probably due to the too short experimental period. They believed that the longer experimental period, i.e. 1-2 months, would make the positive effect noticeable. They also suggested that the serum should have natural color of the ingredients in the formulation. Most volunteers agreed that the serum was smooth and fine and it gave better results in smoothening and softening the skin, and nourishing skin moisture in comparing to the commercial products.

Table 58 Personal data of the volunteers in the consumer acceptance study

N=82

Survey data	Frequency (persons)	Percentage
1. Age		
30-40 years	46	56.1
41-50 years	23	28
51-60 years	7	8.5
Over 60 years	6	7.3
2.The highest obtained academic degree		
Lower than high-school	0	0
Primary high-school	0	0
Secondary high-school/vocational school	4	4.9
College diploma	14	17.1
Bachelor degree	35	42.7
Master degree or higher degree	29	35.4
3. Occupation		
Government or state enterprise officer	22	26.8
School student	0	0
Employee of private organization	28	34.1
College student	0	0
Professional employment	4.9	4
Business owner	23	28
Housewife	5	6.1

Table 58 Personal data of the volunteers in the consumer acceptance study (continued)

Survey data	Frequency (persons)	Percentage
4. Monthly income		
Less than 10,000 baht	8	9.8
10,001-20,000 baht	24	29.3
20,001-30,000 baht	15	18.3
30,001-40,000 baht	26	31.7
40,001-50,000 baht	9	11
Over 50,000 baht	0	0

The result of the study revealed that 81.7% of the volunteers suggested that the serum quality was as good as the commercial products. An amount of 13.4% thought that the serum quality was better than the commercial product. The acceptance of the serum quality was as high as 85.4% of the volunteers. However, there were several reasons that caused some volunteers to refuse the product, which 25% of volunteers found the serum possess strong smell of the chemical and the effect on wrinkle reduction was not noticeable due to the short experimental period. Also, 16.7% found greasy residue on the skin after applying and the whitening effect was not different the commercial products. **Table 59** showed the consumers acceptance study.

Consumer preference level was shown in **Table 60**. According to the preference level, color of the serum, application of the serum on skin, absorption to skin, skin moisture after using, softness and smoothness of complexion, reduction of deep wrinkles on the skin, and overall liking were like moderately whereas the satisfaction with the smell of the serum was neutral.

Table 59 Information obtained from the consumers acceptance study

Survey data	Frequency (persons)	Percentage
5. What type of anti-wrinkle moisturizer do you frequently use? (Choose only one answer.)		
Cream	46	56.1
Gel	4	4.9
Lotion	1	1.2
Cream gel	31	37.8
6. How do you think about the developed product was better comparing to typical products sold in the market?		
The developed product was better than the commercial products	11	13.4
This developed product was as good as others.	67	81.7
This developed product was not as good as others.	4	4.9
7. Product acceptance		
Accept (Go to question 10.)	70	85.4
Not accept (Continued on question 9.)	12	14.6
8. Reasons for not accepting the product [(1)]		
Slow absorption to skin	1	8.3
Greasy residue on skin after applying	2	16.7
Strong fragrance weak	3	25
Unsatisfactory color	1	8.3
Unnoticeable whitening effect	2	16.7
Unnoticeable wrinkle reduction	3	25

Note: [(1)] means the questions that allow volunteers to choose more than one answer and that were questioned to the respondents who did not accept the product.

Table 60 Preference levels of the volunteers on the characteristics of the anti-wrinkle serum containing niosomes

Characteristics	Mean	Preference level
1. Serum color	6.3	Like slightly
2. Serum smell	5.8	Like slightly
3. Application of the serum on skin	7.3	Like moderately
4. Absorption to skin	7.5	Like moderately
5. Skin moisture after used	7.5	Like moderately
6. Softness and smoothness of complexion after used	7.2	Like moderately
7. Effect of deep wrinkles reduction	6.3	Like slightly
8. Overall liking	7.4	Like moderately

The satisfaction level was shown in **Table 61**. According to the satisfaction level, the satisfaction characteristics of the serum were medium to high in almost categories. The smell was the only characteristic that got very low satisfaction

3.7.3 Decision to buy the developed product

The decision to buy the anti-wrinkle serum containing niosomes after trial showed that 86.58% of volunteers were interested in buying the product. Only a few volunteers refused to buy the product due to several reasons, that 36.4% of volunteers thought that the smell of the particular chemicals was too strong, 27.3% thought the skin moisture disappeared in very short time and the rest said that the effect on wrinkle reduction was not obvious, the serum left a greasy residue on skin after applying, and the whitening effect on the skin was unnoticeable with the percentages of 18.1, 9.1, and 9.1 respectively. For selling price of the product (size

50 grams), most volunteers, (52.4%) said that the price should be similar to the commercial product, and 45.1% say it should be lower than the commercial product shown in **Table 62**

Table 61 Satisfaction levels of the volunteers on the characteristics the anti-wrinkle serum containing niosomes

Characteristics	Mean	Satisfaction level
1. Color	4.25	High satisfaction
2. Smell	2.86	Very low satisfaction
3. Effect of wrinkles reduction	3.19	Moderately satisfaction
4. Skin moisture after use serum	4.12	High satisfaction
5. Skin firming after use serum	3.02	Moderately satisfaction
6. Whitening effect on skin after use	3.08	Moderately satisfaction

Table 62 Decision to buy the anti-wrinkle serum containing niosomes

Survey criteria	Frequency (persons)	Percentage
9. Will you buy the product if it is more expensive than other products in the market?		
Yes (Go to question 10)	71	86.5
No (Continue on question 11)	11	13.4
10.Reasons for not buying the product (2)		
Greasy residue after applying	1	9.1
Short-term moisturizing	3	27.3
Slow absorption to skin	0	0
Strong fragrance	4	36.4
Unsatisfactory color	0	0
Unnoticeable effect on wrinkle reduction	2	18.1
Unnoticeable whitening effect to skin	1	9.1

Table 62 Decision to buy the anti-wrinkle serum containing niosomes (continued)

Survey criteria	Frequency (persons)	Percentage
11.Suitable selling price for the product size 50 grams		
Lower than the market	37	45.1
Similar to the market	43	52.4
Higher than the market	2	2.4

Note: (2) means the questions that allow volunteers to choose more than one answer and that were questioned to the volunteers who did not accept the product.