CHAPTER 4

CONCLUSION AND SUMMARY

The results from this study can be concluded as the followings:

1. The oils extracted from five native Thai silkworms varieties; Keaw Sakon, Nangnoi Srisaket, Sam Rong, Nang Leung and None Ruesee showed the yields from Soxhlet and maceration methods were 24-29% and 5-7%, respectively. Oils extracted from None Ruesee by the Soxhlet method, and those extracted from Nang Leung, Sam Rong , and None Ruesee by the maceration method showed free radical scavenging activity. Oil extracted from None Ruesee by the maceration method gave the highest free radical scavenging activity. Moreover, oil extracted by the Soxhlet extraction from None Ruesee gave the highest tyrosinase inhibition activity, but lower than that of the standard vitamin C and kojic acid. The silkworm pupae oil obtained from Soxhlet extraction had unsaturated fatty acids content in the range of 72-79 %, and alpha-linolenic acid content in the range of 32-44 %, whereas that obtained from the maceration extraction had the unsaturated fatty acid contents in range of 75-80%,and alpha-linolenic acid contents in the range of 40-46 %. The result was also demonstrated that the acid, saponification, un-saponification, iodine, peroxide and stability value to oxidation using rancimet apparatus of the silkworm pupa oil from Sam Rong and None Ruesee strains extracted by Soxhlet extraction showed sustain ability to the oxidation reaction for the longest period of time. Furthermore, vitamin E was also detected in Sam Rong and None Ruesee silkworm pupa oils extracted by Soxhlet extraction.

2. The extracting of sericin silk cocoon of all five native silk strains with autoclave pressure and alkali had effects on physical qualification, chemical elements, amino acid and the ability in free radical scavenging and inhibition of enzyme tyrosinase. The yields of sericin obtained by alkaline (0.5 N Na_2CO_3) and autoclave method (at 121 °C for three hours) were in the range of 3.6-7.2 and 5.2-8.2%, respectively. The prepared sericin silk cocoon derived from both methods had L* a* and b* color value differently, with the difference in the range of 60.98, 76.65, 3.11-6.59 and 20.42 – 29.85 respectively. Sericin extracted from Nangnoi Srisaket by alkaline and autoclave method gave the highest serine contents. Sericin extracted from None Ruesee and Nang Leung by alkaline gave the highest tyrosinase inhibition activity. Sericin extracted from Nang Leung and Sam Rong by autoclave method exhibited the highest free radical scavenging activity. The percentages of the protein contents were determined by the Lowry method. The average percentages of protein contents by autoclave and alkaline methods were in the range of 20.10-25.74 and 16.52-20.19, respectively. The molecular weight patterns of the sericin hydrolysate were accessed by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The average molecular weight of sericin extracted by alkaline method with the wide range from about 75-150 kDa, while that by autoclave method gave molecular weight in the range of 50-100 kDa. None Ruesee variety by alkaline method gave the molecular weight in the range of 40-75 kDa. FTIR spectra of sericin powders derived from autoclave and alkali processes were located at 1650 and 1523 cm^{-1} respectively. Different silk varieties contain distinct sericin with various amino acid compositions, which were significantly influenced by the extraction methods.

3. Oil and sericin of None Ruesee variety silkworm were entrapped in blank niosomes composed of Tween 61 and cholesterol at 1:1 molar ratio prepared by the chloroform film with sonication method. The blank niosomes were physical stable with uniform size and no sedimentation.

4. The entrapment efficiency of sericin and oil in niosome was 33.76 and 43.71 %, respectively. The average particle size of niosomes by zetasizer analyzer was 92-800 nm. The morphology of the prepared niosomes was in the mixture of unilamellar and mutilamellar vesicles (MLVs), and large unilamellar vesicles (LUVs).

5. The developed niosomes entrapped with sericin and oil from None Ruesee variety silkworm were stable after kept at 4 ± 2, 30 ± 2 (room temperature) and 45 ± 2 °C for 8 week. The chemical and physical stability of sericin and oil from None Ruesee variety silkworm seemed to be more when entrapped in niosomes and incorporated in the serum formulations.

6. The compositions of the developed anti-wrinkle serum containing niosomes entrapped with sericin and oil from native Thai silkworms with the selected fragrance were 0.15 Carbopol ®Ultrez 21 polymer, 1.5 C_{14-22} alkylalcohol and C_{12-20} alkylglucoside, 1.6 cyclopentasiloxane, dimethiconol, dimethicone crosspolymer (and) blend, 1 sodium Polyacrylate (and) dimethicone (and) cyclopentasiloxane (and) trideceth-6 (and) PEG/PPG -18/18 dimethicone, 6 niosome, (containing 1%w/w sericin and 1% w/w oil of None Ruesee silkworm), naomi 0.6 and light yellow color. The characteristics of the serum had the viscosity of 14,500 cP, pH of 6.97, light

yellow color with L* a* b* value of 75.57, 0.54, 28.19 with no phase separation. The total plate counts of bacteria and yeast/mold were less than 10 colony/g. The *in vivo* tests in human volunteers showed that this serum gave superior skin hydration determined by transepidermal water loss, and improved the skin elasticity significant after the 8-week treatment.

7. The anti wrinkle serum containing niosomes enrapped with sericin and oil from native Thai silkworms stored for 8 weeks at the ambient temperature (30 °C) and in the accelerated conditions at the temperatures of 35 and 45 °C gave no change of all qualities (physical, chemical and sensory) compared to at initial.

8. The testing of the acceptance of the consumers on the product containing niosomes entrapped with sericin and oil from native Thai silkworms showed that most consumers had the opinion (81.7%) that the developed products had good quality equal to the market products, with the product acceptance at 85.4%. Those rejected the product mostly gave the reason of too short time of moisturizing and wanted to reduce the scent and get more wrinkle reduction (13.42%). The developed serum product had the overall preference score in the medium level and medium satisfaction toward the feature of the product mostly at the satisfaction. They were interested to buy the product at 86.58%.

9. The estimated cost of oils and sericins from silkworm was 3,933.8 Bath/ kilogram and niosomes entrapped from sericin and oil from Thai native silkworm was 44,000 Bath/ kilogram and the developed anti-wrinkle serum containing niosomes

entrapped with sericin and oil from native Thai silkworms was 2963.2 Baht/unit (50g).

RECOMMENTATION

1. In testing the acceptance of consumers which involved too many people, the test was conducted by using only 1 product and the blank formula was not included in the test due to the reason that it was just the test on the acceptance of the consumers and the method requiring data where marketing assessment was conducted which will be used as guidelines in further improving and developing of the products.

2. In testing the chemical stability of the serum products containing niosomes entrapped with the silkworm oil and protein, this study has not performed. Only physical stability has been done. For the further development of the obtained product, the chemical stability of oil and protein containing in the products should be investigated.

3. The method selected for the physical stability test was not quite suitable since the storage temperatures used were very close (i.e. 30, 35 and 45 $^{\circ}$C) making such condition to be somewhat very close in the condition which may cause the observation on the changes of the products during storage period not to be remarkably different. Therefore, the conditions at 4, 30 and 40 $^{\circ}$C should be used. Also, it was recommended that more suitable test methods should be added, such as Freeze-thaw cycle and heating - cooling cycle etc.

4. The developed serum product containing niosomes entrapped with oil and sericin had two types of preservatives namely, methyl paraben and propyl paraben

which were added to oil while Germaben II E (propylene glycol (and) diazolidinyl urea (and) methyl paraben (and) propyl paraben) was added to the water phase in order to control and prevent the maturity of microorganisms.