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Extraction of sericin from Thai native silk (Nangsew) yarn using mulberry (cv. Buriram 60) leaves extracts as a greener solvent

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Abstract

In the present study, Mulberry (cv. Buriram 60) leaves extracts is being used as a greener solvent for sericin extraction from Thai native silk (Nangsew) yarn. The extraction parameters such as extraction time, extraction temperature and solvent concentration were optimized. The optimal conditions included an extraction time of 30 min, an extraction temperature of 80 °C and a solvent concentration of 1% w v^{-1}. Under these conditions, the maximal yield of sericin was 3.72 ± 0.35% w w^{-1}. Moreover, the sericin extraction on different size of silk (mai 1, mai 2 and mai 3) have been studied. The results showed that the yield of sericin differed depending on the size of silk. The effect of mulberry leaves extracts solvent on the yield and structural characteristics of sericin and physical properties of silk were examined and compared with conventional solvents (hot water, commercial base for silk degumming and sodium carbonate). The extraction of sericin with a commercial base for silk degumming and sodium carbonate exhibited the high yield but produced the impurity sericin. The tensile strength of silk was decreased at about 150 MPa, indicating partial harmful damages of silk molecules after extraction. On the other hand, mulberry leaves extracts solvent generated purified sericin and tensile strength of silk was not decreased. Furthermore, sericin extracted with mulberry leaves extracts solvent showed the highest DPPH radical scavenging activity (63.37 µmol TE g^{-1}) and high level ABTS antioxidant activity (656.40 µmol TE g^{-1}) compared with other solvents. The finding of this research expected that the sericin extracted with mulberry leaves extracts solvent could be utilized in applications without further purification.

Keywords: Sericin; Extraction; Mulberry leaves

1. Introduction

Silk is natural fibrous protein which was spun from silkworm and composed of two proteins namely fibroin and sericin [1]. Silk manufacturing of Thailand focuses on utilization of silk fiber (fibroin) especially to fabricate the silk cloth. Sericin was removed from silk to waste water in reeling and degumming process. However, sericin has wide applications in medical, pharmaceutical and cosmetic [2] because of it is moisture absorption property and a lot of biological activity such as antioxidant, tyrosinase activity inhibition and anticancer activity [3]. Moreover, sericin enhances bioavailability of Zn, Fe, Mg and Ca in rats, and it has been suggested to use in food products [4]. Therefore, sericin was considered in this study.

The approach for extraction sericin from silk has several method such as using hot water [2], acidic solvents (tartaric acid, citric acid, formic acid), soap, alkaline solvent, enzymes, high temperature high pressure, ultrasound and infrared [5 – 10]. The extraction of sericin with sodium carbonate has been

used frequently in silk manufacturing but the extraction solution contains a high level of sodium salt that is difficult to separate from sericin [11]. Moreover, it released a large volume of wastewater during extraction process. Therefore, greener solvent is needed for sericin extraction. The mulberry (cv. Buriram 60) leaves extracts have been selected as a solvent to replace chemical solvents because of it is best antioxidant and medicinal properties [12] such as enhance cerebrovascular and coronary blood flows, regulate arrhythmia, soften angiosclerosis, decrease sugar and fat, high antioxidant and anticancer activity [13], as a result, sericin extracted from this solvent has better antioxidant activity than conventional solvent. In addition, it free from chemical to wastewater. Therefore, it is cleaner solvent and environmentally friendly process for sericin extraction.

In this study, sericin was extracted using mulberry (cv. Buriram 60) leaves extracts as a solvent and explored the optimum condition. The effect of solvent on the yield and quality of sericin had been compared.

2. Materials and methods

Materials

Thai native silk (Nangsew) yarns and mulberry (cv. Buriram 60) leaves were obtained from local textile industry of Buriram province. The silk yarn samples were kept in air-dried room until use. Standard sericin *Bombyx mori* (silkworm), Coomassie Brilliant Blue G-250, Bovine serum albumin, sodium carbonate, potassium persulfate, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), 2,2-azinobis-(-3-ethylbenzo thaizoline-6-sulfonoc acid) (ABTS) and 6-hydroxy-2,5,7,8-tetramethychlorman-2-carboxylic acid (trolox) were purchased from Sigma Aldrich. Sodium phosphate dibasic and potassium phosphate dibasic were sourced from Ajex Finechem. Phosphoric acid and sodium chloride were purchased from Chemikit. Ethanol was obtained from Merck. Double-distilled water was used throughout this study.

Preparation of mulberry (cv. Buriram 60) leaves extracts

Mulberry (cv. Buriram 60) leaves were cleaned in double-distilled water and dried at room temperature. Then, it was blended and weighed to yield 100 g before soaking in1000 mL of water (10% w v^{-1}). Extraction was carried out at ambient temperature for 24 h. The extract was filtered through 2.50 μm pore size filter paper from Whatman. The light yellow-green extract solution was kept at 4 °C in the refrigerator until use.

Sericin extraction and optimization

Silk yarns (2g) were extracted in 100 mL of mulberry leaves extracts with different extraction parameter i.e. extraction time for 10, 20 and 30 min, extraction temperature for 60 and 80 °C and solvent concentration for 1, 5 and 10% w v^{-1}. The sericin yield was determined following by Bradford assay.

Sericin determination by Bradford assay

Bradford solution was prepared according to Bradford [13]. Coomassie Brilliant Blue G-250 (100 mg) was dissolved in 50 mL of 95% ethanol. Then, this solution was added with 100 mL 85% w v^{-1} phosphoric acid. The solution was further diluted to a final volume of 1 liter. The sericin extracted solution (0.10 mL) was added to 5 mL of Bradford solution and incubated at room temperature for 10 min. Bovine Serum albumin (125, 250, 500, 1000 and 2000 mg L^{-1}) was used as a standard reference protein. The absorbance of sample was measured at 595 nm with UV-Visible spectrophotometer (PG Instruments, model T60)

Effect of extraction solvent

The effects of four different extraction solvents (mulberry leaves extracts, hot water [2], commercial base for silk degumming [14] and sodium carbonate [6]) were studied for the yield and quality of the sericin extracted using different extraction conditions (Table 1). Silk yarns (2g) were added in 100 mL of extraction solutions.

Table 1 Summary of the sericin extraction conditions used in this study

Solvent	Concentration	pH	Temperature (°C)	Time (min)
Mulberry leaves extracts	1% w v^{-1}	6.0	80	30
Hot water	-	7.2	80	30
Commercial base	0.5% w v^{-1}	11.4	80	30
Sodium carbonate	0.5% w v^{-1}	11.5	80	30

Effect of size of silk yarn

The different size of silk yarns (mai 1, mai 2 and mai 3) were obtained using hand-reeling technique and measured in denier term, which is presented as a weight in grams of a 9000-meter length of silk yarn [15]. The denier of mai 1, mai 2 and mai 3 are 219 ± 17, 258 ± 32 and 940 ± 173, respectively. Each report value is the average value from 15 measurements.

Scanning Electron Microscope (SEM) studies

The extraction was qualitatively assessed by the observation of the silk extracted fibers quality. The morphology of silk fibers was examined with SEM (JEOL, model JSM-6460LV).

Physical properties

The tensile properties were measured with auto tensile tester (Labthink, model PRAMTM XLW (PC)) as described elsewhere [8], for which the gauge length was 10 cm. All tests were conducted at strain rate of 50 mm min^{-1}. Each value reported is the average value of 7 measurements.

Characterization of sericin powder

The solvent obtained from extraction was evaporated by tray drying [9], the sericin extracted solution was kept in glass tray, in a hot air oven (Memmert, model UF110) at 40 °C till a dry powder was obtained. The structural information of sericin powder was characterized by Fourier transform infrared spectroscopy (FTIR) (Perkin Elmer, model Spectrum one).

Antioxidant activity

Antioxidant activity by DPPH assay

The DPPH radical scavenging activity of sericin powder was measured by method described by Giraporn et al. [16] with a slightly modified. The 2 mL of sample solution was added with 2 mL of a freshly prepared 0.20 mM DPPH solution dissolved in ethanol. The sample was mixed and incubated in the dark at room temperature for 30 min. The decrease in absorbance of DPPH was measured at 517 nm using UV-Visible spectrophotometer (PG Instruments, model T60). Inhibition (%) of DPPH = $(A_{control} - A_{sample}) \times 100 / A_{control}$. The trolox was used as a reference standard and the results were expressed as mmol trolox per gram of sericin (μmol TE g^{-1})

Antioxidant activity by ABTS assay

The trolox equivalent antioxidant capacity (TEAC) of sericin has been analyzed by using ABTS methods. The ABTS assay was conducted as previously described by Chen et al. [17] with a slightly modified method. The stock solution of $ABTS^{\cdot+}$ was prepared by reacting a 7 mM aqueous solution of ABTS with 2.45 mM potassium persulfate, and this mixture was allowed to stand in the dark room temperature for 12 – 16 h. The solution was then diluted with phosphate buffer (pH 7.40) to have an absorbance of 0.70 ± 0.02 at 734 nm. This solution was used as the working solution. In addition, 100 μL of 1 mg mL^{-1} sample were completely mixed with 3 mL of working solution. The mixture was incubated at room temperature for 1 h, and the absorbance at 734 nm was immediately recorded. The trolox solutions (in the range from 100 to 1000 μM) were used to obtain the calibration curves. The results are expressed as mmol equivalents of trolox per gram of sericin (μmol TE g^{-1}).

Statistical analysis

All results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA). The significant differences ($p < 0.05$) between the

mean values were determined using the Least Significant Difference test. All analyses were carried out using SPSS software.

3. Results and discussion

The optimization of sericin extraction

Fig. 1 shows the yield of sericin obtained at different temperatures, times and solvent concentrations of extraction, using mulberry leaves extracts as solvent. Maximum yield of sericin ($3.72 \pm 0.35\%$ w w^{-1}) was obtained after 30 min extraction at 80 ºC with 1% w v^{-1} of mulberry leaves extracts. The results show that the increase of the extraction temperature and extraction time causes higher yield of sericin. In case of different solvent concentrations, the results indicated that it has no significance to the extraction yield.

Effect of size of silk yarn

Fig. 2 presents the effect of silk yarn size and the extraction solvent on the extraction yield of sericin. The size of silk used in this study are mai 1 (219 ± 17 denier), mai 2 (258 ± 32 denier) and mai 3 (940 ± 173 denier), and the SEM image of the different size of silk are shown in Fig. 3. Base on Fig. 2, it can be seen that the small size of silk can cause the lower yield. This phenomenon is in accordance with the research conducted by Mondal *et al.* [18]. The authors reported that the outer layer of the silk cocoon had the higher sericin content. Mai 3 is silk yarn that reeled from the outer layer of silk cocoon as a result of the higher yield of sericin.

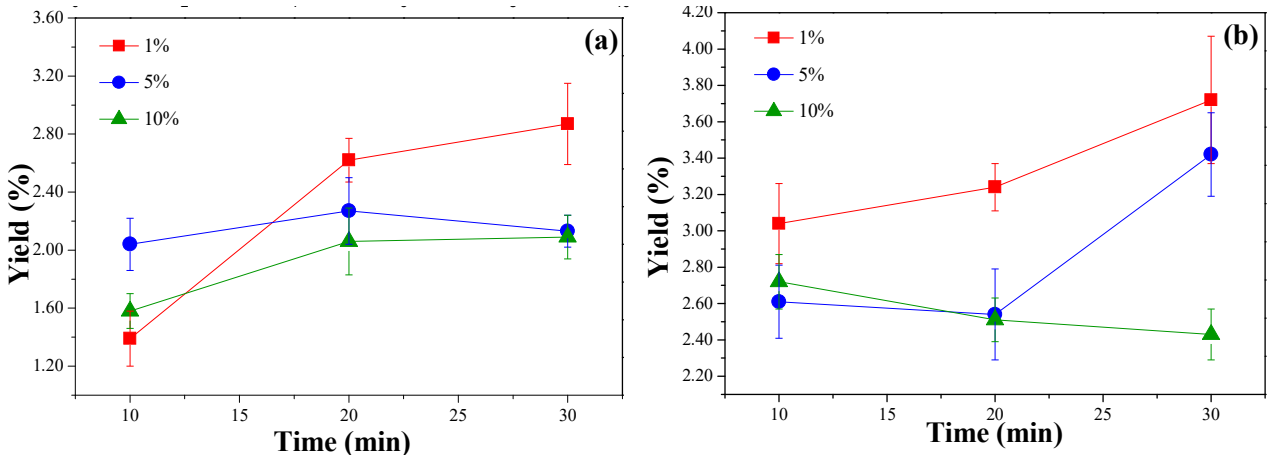

Fig. 1 Effect of time and mulberry leaves extracts concentration on yield of sericin extraction at 60 ºC (a) and 80 ºC (b)

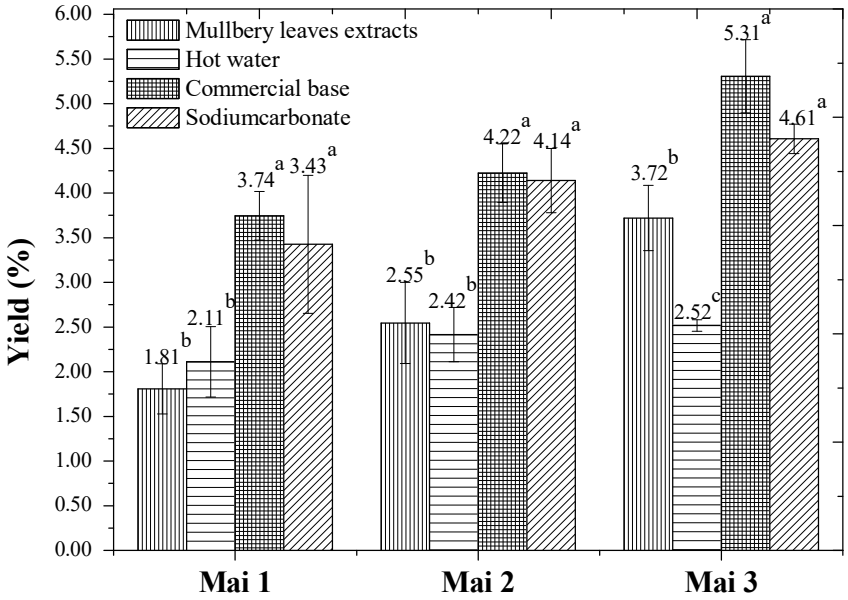

Fig. 2 Effect of extraction solvent and size of silk yarn on sericin yield. Results are expressed as mean ± SD (n = 3) with different letters represent significant different at $p < 0.05$ of each type of silk.

Effect of extraction solvent

In the case of extraction solvent, commercial base for silk degumming was found to be the most effective solvent for sericin extraction but it has not improve significantly to the extraction yield of sodium carbonate (Fig. 2). A similar result was observed by Yun et al. [6], sodium carbonate extraction produces more sericin than the extraction with hot water. Furthermore, these results correspond well with past studied [11] that have reported the acidic and strongly alkaline solvents produce a high yield of sericin more than neutral solvent.

As shown in the SEM micrographs (Fig. 4), sericin appears as a non-uniform coating on the surface of the silk fibers. The fiber surface of raw silk (Fig. 4 (a)) and extraction with mulberry leaves extracts (Fig. 4 (b)) are rugged with diverse granular sericin as in the extraction with hot water (Fig. 4 (c)). In addition, the fibroin fiber was not separated. In some part, silk yarn extracted with mulberry leaves extracts shown that the sericin layer broke away from fiber, revealed the clean fibroin fiber. Nevertheless, the SEM micrograph of silk extracted with commercial base (Fig. 4 (d)) and sodium carbonate (Fig. 4 (e)) show the clear extraction, the fiber surface was highly smooth and clean fibroin was appeared.

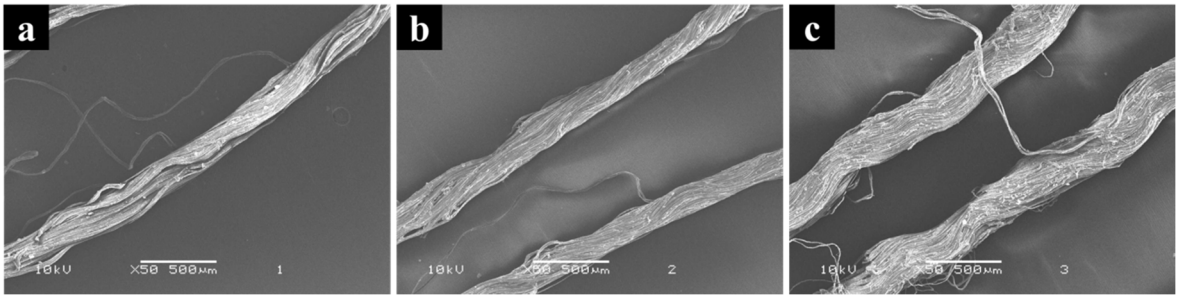

Fig. 3 SEM images of Mai 1 (a), Mai 2 (b) and Mai 3 (c). (50X.)

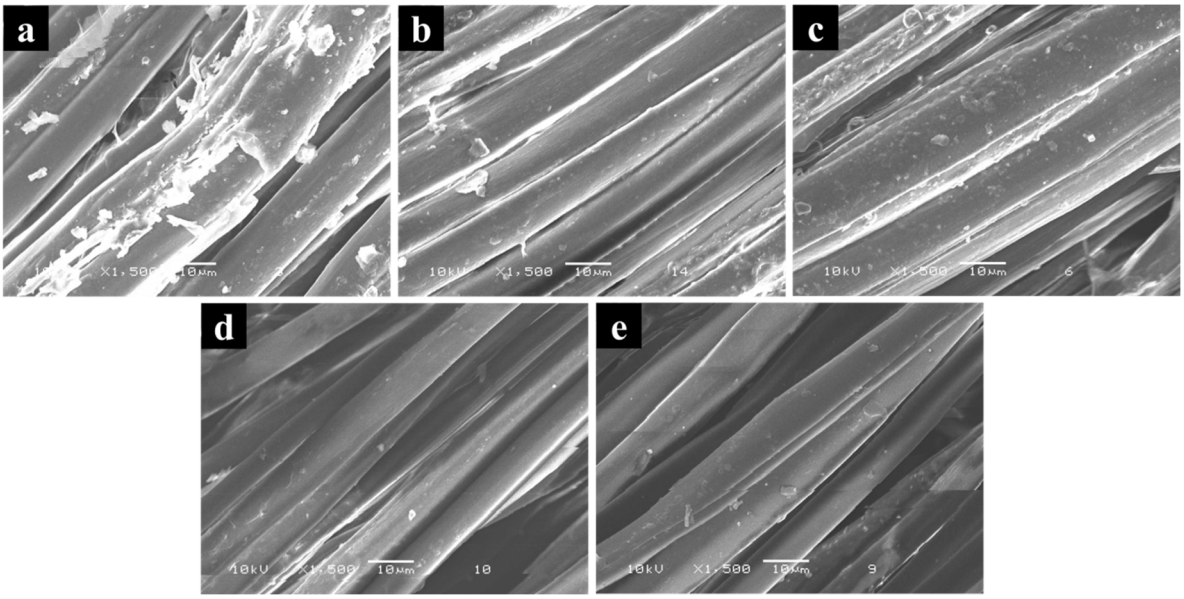

Fig. 4 SEM images of silk before (a), after extraction with mulberry leaves extracts (b), hot water (c), commercial base (d) and sodium carbonate (e). (3,000X.)

The tensile strength and strain (%) of silk extracted with different solvents are present in Table 2. The tensile strength and strain (%) of raw silk was 333 ± 78 MPa and 14.0 ± 4.20 %, respectively. After extraction with mulberry leaves extracts and hot water, the tensile strength was slightly decreased and has no significant differences with raw silk. Whereas, the tensile strength of silk was extremely decreased at about 150 MPa when extracted with commercial base and sodium carbonate, indicating partial harmful damages of silk fiber after extraction with both solvents. The results were in accordance with the recent report by Jiang et al. [19]. The strain (%) of silk extracted with all solvents has no significant differences with raw silk.

Table 2 Effect of solvent on Tensile strength of silk

Solvent	Tensile strength (MPa)	Strain (%)
Raw silk	333 ± 78^{a}	$14.0 \pm 4.2^{a,b}$
Mulberry leaves extracts	297 ± 56^{a}	17.9 ± 5.5^{a}
Hot water	298 ± 47^{a}	17.0 ± 1.9^{a}
Commercial base	158 ± 41^{b}	12.3 ± 1.7^{b}
Sodium carbonate	181 ± 26^{b}	12.1 ± 1.6^{b}

Data are represented as the mean ± SD (n = 7); different letters with same column are significantly different at $p < 0.05$.

FT-IR analysis of sericin powder

The FT-IR spectra of sericin powder extracted from four solvent are shown in Fig. 5. Standard sericin powder (Fig. 5 (a)) showed that FT-IR absorption has strong peak at 1652 cm^{-1} confirming the amide I absorption which arises mainly from the C=O stretching vibration [9]. The peak at 1527 cm^{-1} (amide II) [20] assigned to random coil structure and 1238 cm^{-1} (amide III) [1] attributed to β-sheet structure which generates from C-N stretching vibration coupled to the N-H plane blending vibration. In addition, the signature peak for sericin at 1400 cm^{-1} was found in case of all samples in Fig. 4. It was indicated that the structure of sericin powder extracted from mulberry leaves extracts (Fig. 5 (b)) and hot water (Fig. 5 (c)) has no difference with standard sericin powder. Furthermore, sericin powder

extracted from commercial base (Fig. 5 (d)) and sodium carbonate (Fig. 5 (e)) showed the absorption peak at 1650 cm^{-1} (amide I) which is a useful peak for confirming structure of proteins. Aside from this peak, sericin powder extracted from both solvents showed the absorption band at 1440 cm^{-1} and 880 cm^{-1} which originated from asymmetric stretching and out-of-plane blending of CO_3 in Na_2CO_3 [21] and similar as Na_2CO_3 powder spectrum (Fig. 5 (f)). This result indicates that sericin powder extracted from commercial base and sodium carbonate contains a high amount of sodium carbonate that is difficult to separate from sericin powder [11]. Therefore, it is not suitable to use in applications without further purification.

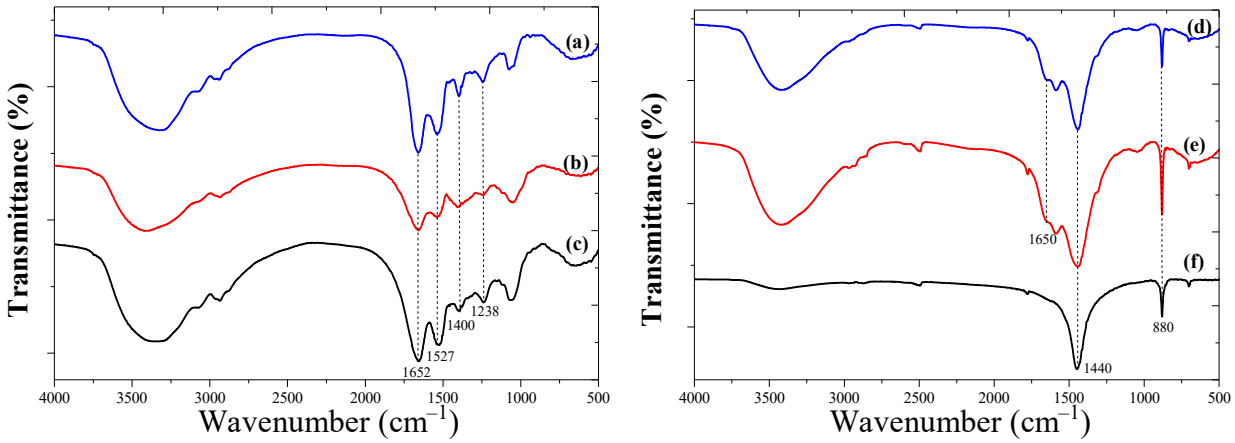

Fig. 5 FT-IR spectrum of standard sericin (a), sericin powder extracted from mulberry leaves extracts (b), hot water (c), commercial base (d), sodium carbonate (e), and standard sodium carbonate powder (f).

Antioxidant activity

Table 3 showed the antioxidant activity of sericin powder extracted with different solvent. Base on the extraction solvent, DPPH radical scavenging activities varied with the type of solvent. Sericin extracted with mulberry leaves extracts showed the highest DPPH scavenging activity. TEAC of all sample were analyzed by investigating their ability to scavenge the $ABTS^{\cdot+}$. The sericin extracted with mulberry leaves extracts exhibited the high level of TEAC value. It was indicated that, the sericin extraction using mulberry leaves extracts can improve the antioxidant activity properties of sericin because of the excellent antioxidant activities of mulberry leaves that postulated to be as a result of free radical compound such as carotenoids, flavonoids, moracins and others present in the leaves [22].

Table 3 Antioxidant activity of sericin powder extracted with different solvents.

Sericin powder extracted with different solvent	DPPH assay (μmol TE g^{-1})	ABTS assay (μmol TE g^{-1})
Standard sericin	3.46 ± 0.89^{b}	758.78 ± 3.90^{a}
Mulberry leaves extracts	63.37 ± 1.76^{a}	656.40 ± 5.42^{b}
Hot water	4.56 ± 0.81^{b}	545.34 ± 24.83^{c}
Commercial base	4.88 ± 1.28^{b}	242.96 ± 7.17^{d}
Sodium carbonate	4.91 ± 0.45^{b}	236.89 ± 3.44^{d}

Data are represented as the mean ± SD (n = 3); different letters with same column are significantly different at $p < 0.05$.

4. Conclusion

The extraction efficiency of sericin from Thai native silk (Nangsew) yarn using mulberry leaves extract was affected by extraction time and extraction temperature. An increase in extraction yield was observed with response to an increase of extraction time and extraction temperature. The obtained yield was comparable or better than extraction with hot water but significantly lower than extraction with the commercial base for silk degumming and sodium carbonate. The morphology of silk after extraction revealed the clean fibroin fiber when extracted with the commercial base for silk degumming and sodium carbonate. However, the tensile strength of silk was decreased after extraction with both solvents, indicating silk fiber was damaged. Furthermore, sericin powder extracted with mulberry leaves extracts showed the similar FT-IR spectrum with standard sericin powder. It was indicated that sericin from this method no have sodium salt and can be used without further purification. Compared to sericin from the commercial base for silk degumming and sodium carbonate where several steps such as separation, extraction and filtration are required to separate sericin from sodium salt. The sericin extracted with mulberry leaves extracts exhibited the strongest DPPH radical scavenging and high level of TEAC value. Finally, sericin extracted with mulberry leaves extracts is completely free from chemical so making it is a greener solvent.

5. Acknowledgement

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