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Research Paper

Isolation and purification of Dragline Silk protein from *Crossopriza lyoni* Web

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ABSTRACT

Spider silk is a robust biomaterial due to its desirable tensile strength, biocompatibility and biodegradability. It is composed of spidroins which are recently used in wide technical applications. At present, dragline spidroins from *Nephila clavipes* are heavily studied, thus, neglecting the presence of the protein in other species. The aim of this study is to isolate and purify dragline silk protein from *Crossopriza lyoni* web. Spider webs were pre-treated with 0.02 M of sodium carbonate and followed by spidroin extraction using 20% (w/v) SDS-ethanol extraction solution. Two-level full factorial design was applied to analyze the significant effect of parameters namely; temperature (°C), agitation speed (rpm) and incubation time (h) on maximum spidroin recovery. Optimization study was performed by Response Surface Methodology (RSM) through Box-Behnken Design (BBD). The crude sample was purified by gel filtration chromatography and characterized by one-dimensional SDS-PAGE for dragline spidroin presence. Agitation speed, incubation time and temperature posed significant effect on spidroin extraction with $p < 0.0500$ in decreasing order, respectively. From the optimization study, the highest spidroin concentration (1210.78 ± 0.974 µg/ml) was obtained at 90°C, 102.5 rpm and 4.5 h with a fitted model at p value of 0.0001. Validation experiment was conducted and the result showed that the model was good to fit to the experimental data with percentage error less than 1%. Purification of the crude sample resulted in the dragline silk protein putative peaks on the chromatogram at 280 nm with 0.1 ml of column volume. SDS-PAGE analysis further confirmed the presence of the dragline silk protein with a band of molecular weight of more than 250 kDa. Isolation and purification of dragline spidroin in the present study is anticipated to bridge the gap of knowledge in the dragline spidroins from non-araneoid species. This would be an initiative to expand the spider silk technology at national level in the near future.

Key words: Spider silk, Spidroin, *Crossopriza lyoni*, screening, optimization, purification.

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INTRODUCTION

Different species of spiders produce various kinds of silk throughout their life cycle which depends on the lifestyle of the spider. In the current research of fibre, spider dragline silk has been shown to display great potential as a remarkably strong and highly versatile material. It is largely involved in the production of extraordinary and high-performance fibre through the spider silk technology such as bullet-proof clothing and wear-resistant lightweight clothing (Powers, 2013; Singh et al., 2012).

In ancient times, spider silk has been the common material used to stanch wound from bleeding and even for fishing line or net (Hardy and Scheibel, 2009). Many efforts have been put forward by researchers to explore the potential applications of spider silk since it has not been harvested and commercialized in many applications.

Crossopriza lyoni (Blackwall) is known as tailed cellar spider or tailed daddy long-legs spider where its legs are very long and fragile. It produces different types of silk

fibre in different silk glands depending on the species and environment (Hardy et al., 2008).

Spider dragline silk, an impressive protein polymer is produced in major ampullate gland that is entirely made up of two main proteins which are the major ampullate spidroin 1 (MaSp1) and major ampullate spidroin 2 (MaSp2) (Powers, 2013; Singh et al., 2012; Yang et al., 2016). Normally, the large irregular web can be discovered around the human structures especially at the corner of building.

Currently in Malaysia, although there is a rise in spider silk technology but the technology has little or no improvement to enable the use of spider dragline silk in order to bring more benefits to the society. Low tenacity, low mechanical strength and low elasticity of rolled silk from silkworm have allowed spider dragline silk to be selected as the raw material in recent research. This has motivated indirectly many researchers to continue carrying out the studies to manufacture more silk-based products which is environmentally friendly.

Normally, native spider dragline silk protein is very hard to obtain in the pure form and sufficient quantities (Jastrzebska et al., 2016). Farming spiders is not a practicable way because they are very territorial and cannibalistic in nature where they eat one another in close quarters (Bain, 2016).

The webs cannot be harvested easily in huge amount for the silk protein extraction. Thus, selection of suitable methods of isolation and purification are essential in order to obtain highest concentration of pure dragline silk protein.

Generally, there is less study on the silk fibre from other species of spider especially tailed cellar spider. Silk fibre from *Nephila clavipes* has been widely used for the research purposes among the araneoid species. *C. lyoni* that has adapted well in the human habitats is selected because high availability of spider silk can be obtained from the environment. It is believed that the protein structure of silk fibre is very unique which is not observed from other species. Therefore, the aim of this research is to isolate and purify the dragline silk protein from *C. lyoni* web.

METHODOLOGY

Sampling of raw material

Webs were collected in the presence of the spiders using broomstick to spool up the web around the area of Unicity Alam Campus, Sungai Chuchuh in order to avoid sampling the wrong web for other closely related species.

Pre-treatment of silk fibre

The collected spider webs were washed and dipped in water overnight followed by drying process at 60°C. In degumming process, boiling aqueous solution of 0.02 M sodium carbonate (Na_2CO_3) were used to pre-treat the dried spider webs in 1: 2 ratio for 20 min with continuous stirring (Sah and Kumar, 2010). The degummed silk fibres were dried overnight in hot air oven and readily used for spidroin extraction.

Primary extraction of dragline silk protein

20 mg of pre-treated silk fibres were dissolved in 20 ml of 20% (w/v) SDS-ethanol extraction solutions where the ratio of SDS to ethanol is 10: 3. The extraction process was performed in the ratio 1:1 at fixed condition of 70°C, 200 rpm for 2.5 h (Sah and Kumar, 2010; Mirghani et al., 2012). Thereafter, silk protein solution was filtered to separate the silk fibre from the extracted solution. Potassium dihydrogen phosphate was added to remove the extraction solutions. Then, the silk protein solution underwent centrifugation at 4000 rpm for 4 min. The concentrated supernatant was transferred into 15 ml of centrifuge tubes and stored in the chiller at -20°C prior to use. Meanwhile, the undissolved silk fibres were rinsed with distilled water severally and the dry weight measured after drying them in hot air oven.

Screening for significant factors for Dragline silk protein extraction

Screening study of parameters namely temperature, agitation speed and incubation time was performed by two-level full factorial design as the parameters may affect the spidroin recovery at high concentration. The range of each parameter was set at high and low levels using 70°C, 200 rpm and 2.5 h from the preliminary extraction process as the reference. Spidroin extraction was performed in different conditions according to the data obtained from the design. Each experiment was carried out in triplicates to obtain the average value. Thereafter, the protein concentration was measured by Bradford protein assay for each set of data. Parameters are considered as being significant to spidroin extraction ($p < 0.05$). The best extraction solution that produced the highest protein concentration was subjected for optimization study.

Statistical optimization of extraction condition

The optimization study of the significant parameters was carried out by Response Surface Methodology (RSM) through Box-Behnken Design (BBD). Range value for each significant parameter was narrowed down according to the best extraction condition with the highest protein concentration obtained from two-level full factorial design as the reference. Spidroin extraction was performed

Table 1: Effect estimate summary of each run in two-level full factorial design.

Factors	Effect estimate	Sum of squares	Percentage of contribution (%)
A	112.88	25481.53	9.84
B	174.00	60552.00	23.38
C	148.00	43808.00	16.92
AB	225.88	1.020E+005	39.40
AC	-8.37	140.28	0.054
BC	-66.50	8844.50	3.42

according to the 17 randomized runs of experiment for data acquisition proposed from BBD by applying similar procedures with screening process.

Validation of the optimized extraction model

A validation experiment was performed to ensure the accuracy and fitness of the model to the experimental data obtained. The experimental and predicted values were compared to confirm the validity of the optimized conditions.

Gel filtration liquid chromatography

Crude spidroin was purified by Sephacryl S-200 HR media on Fast Performance Liquid Chromatography system (Bio Rad; Model: 788-0001) at ambient temperature with maximum pressure of 3650 psi. Bio Rad ECONO glass column was used for the separation. 1 ml of protein sample was injected to the FPLC system using a syringe and connected to the FPLC system. Protein elution profile was shown on the screen monitor and peak fractions of the desired protein were determined. Single buffer of 0.02 M of Tris-HCl was used at a flow rate of 1 ml/min. The purified protein was collected in 2 ml using the fraction collector.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was performed to separate spidroins from silk protein dope solution based on their molecular weight. 10% of resolving gel and 5% of stacking gel were used respectively. For the sample preparation, 10 µl of sample solution was mixed with 10 µl of sample buffer in microcentrifuge tubes followed by the heating process at 95°C for 5 min in water bath. Gel electrophoresis was performed at a constant voltage of 200 V for about an hour. Thereafter, the gel was subjected to silver staining for spidroin visualization. The molecular weight of protein sample was identified by comparing with the protein standard.

Statistical analysis

Analysis of variance (ANOVA) analysis was performed using Design of Experiment (DOE) software. The experimental data was expressed in the form of mean and standard error. The significant value was set at $p < 0.05$ level to analyze the significance of each coefficient.

RESULTS AND DISCUSSION

Screening of significant parameters for dragline silk protein extraction

Table 1 shows the effect estimate summary for the two-level full factorial design. This includes the factors, effect estimate, sum of squares and percentage of contribution which are highly related to each other in the model. From the table, it can be inferred that interaction between temperature and agitation speed really dominated the process, accounting for 39.40% of the total availability. Main effect of agitation speed with 23.38% of contribution was highly significant among the main effects to the model. Among the factors, interaction between temperature and incubation time was less or insignificant due to its lowest percentage contribution of only 0.054%.

Table 2 shows the protein concentration of each run in the two-level full factorial design. The run number 1 obtained the highest protein concentration while run number 8 obtained the lowest protein concentration which was 636.75 ± 0.677 and 97.25 ± 0.870 µg/ml, respectively. Higher temperature, higher agitation speed and longer incubation time contributed to the maximum spidroin concentration of spider web (Addis and Raina, 2013). Thus, 80°C, 350 rpm and 3.5 h was opted for the subsequent study.

Based on Table 3, F-value of the model which was 307.50 with *p*-value less than 0.05 was considered significant. The terms of A, B, C and AB were also significant because their *p*-values were less than 0.05. The *p*-value was significant to determine which terms to keep in the regression model. The predicted R^2 of 0.9653 was in reasonable agreement with the adjusted R^2 of 0.9962. Adequate precision of 49.451 which was greater than 4 was desirable indicating an adequate signal to the model.

Statistical optimization of extraction condition

From the ANOVA analysis, model adequacy can be determined using the F-test and determination coefficient of R^2. The regression model was highly significant with *p*-value of 0.0001 and F-value of 26.27. R^2 value of 0.9717

Table 2: Experimental design matrix of two-level full factorial design.

Run	Temperature (°C)	Agitation speed (rpm)	Incubation time (h)	Experimental protein Concentration (µg/ml)	Predicted protein Concentration (µg/ml)
1	80	350	3.5	636.75 ± 0.677	640.94
2	60	350	1.5	216.50 ± 0.750	220.69
3	60	350	3.5	211.25 ± 0.661	207.06
4	80	350	1.5	468.50 ± 0.899	464.31
5	80	60	3.5	208.25 ± 0.750	212.44
6	60	60	3.5	424.75 ± 0.668	420.56
7	60	60	1.5	106.75 ± 0.901	110.94
8	80	60	1.5	97.25 ± 0.870	93.06

Table 3: ANOVA analysis of two-level full factorial design.

Source	Sum of squares	Mean square	F-value	*p*-value
				Prob > F
Model	2.588E+005	43137.10	307.50	0.0436
A	25481.53	25481.53	181.65	0.0471
B	60552.00	60552.00	431.65	0.0306
C	43808.00	43808.00	312.29	0.0360
AB	1.020E+005	1.020E+005	727.39	0.0236
BC	8844.50	8844.50	63.05	0.0798
ABC	18097.53	18097.53	129.01	0.0559

Note: A= Temperature (°C); B= Agitation speed (rpm); C= Incubation time (hour); Standard Deviation= 11.84; Mean= 296.25; Coefficient Variation % = 4.00; R^2= 0.9995; Adjusted R^2= 0.9962; Predicted R^2= 0.9653; Adequate Precision= 49.45. $p < 0.05$ is considered as significant.

indicated that 97.17% of the variability in the response could be predicted by the model. A, B, C, B^2, C^2 and AB were the significant terms of the model where the *p*-values were less than 0.05. In response surface methodology, the lack of fit was insignificant implying that the model fitted well. The proposed model fitted the experimental data and the independent variables had considerable effects on the protein concentration.

For estimating and predicting the optimum dragline spidroin extraction condition, a second order polynomial model was fitted to correlate the relationship between the three significant parameters and protein concentration. The model was regressed by considering only the significant terms as shown in Equation 1:

$$Y= 4055.58 - 72.91 * A + 10.12 * B - 1125.19 * C – 0.07 * A * B - 0.01 * B^2 + 156.30 * C^2 \quad (1)$$

Where Y represents the response or protein concentration (µg/ml), while A, B and C represent the temperature (°C), agitation speed (rpm) and incubation time (h), respectively.

Reciprocal effect of significant parameters on optimization study

Figure 1 illustrates the reciprocal interaction between the temperature and agitation speed on protein concentration. Protein concentration was directly proportional to the temperature and agitation speed where the highest protein concentration was obtained at 90°C and 205 rpm, respectively. Higher temperature is needed to degrade the beta sheet structure of silk protein by breaking down the interlinking bonds between the spidroin for dissolution of the silk (Hardy et al., 2008).

Figure 2 demonstrates the effect of temperature, incubation time and their reciprocal interactions on protein concentration. The protein concentration reached maximum value at 90°C and 4.5 h, respectively. Figure 3 shows 3D surface plot of the protein concentration as a function of agitation speed and incubation time. Protein concentration was maximum when the agitation speed and incubation time were 102.5 rpm and 4.5 h, respectively between the spidroin for dissolution of the silk fibre (Hardy et al., 2008).

Validation of the optimized extraction model

The optimal extraction condition suggested by the Box-Behnken design was 89.88°C, 135.25 rpm and 4.49 h, respectively (Table 4). The result obtained was shown in Table 5. The validation was performed in duplicates and the protein concentration obtained were 1222.53 and 1232.34 µg/ml, respectively. The average protein concentration of validation process was 1227.44 µg/ml. From the table, the percentage error was 0.11% which was

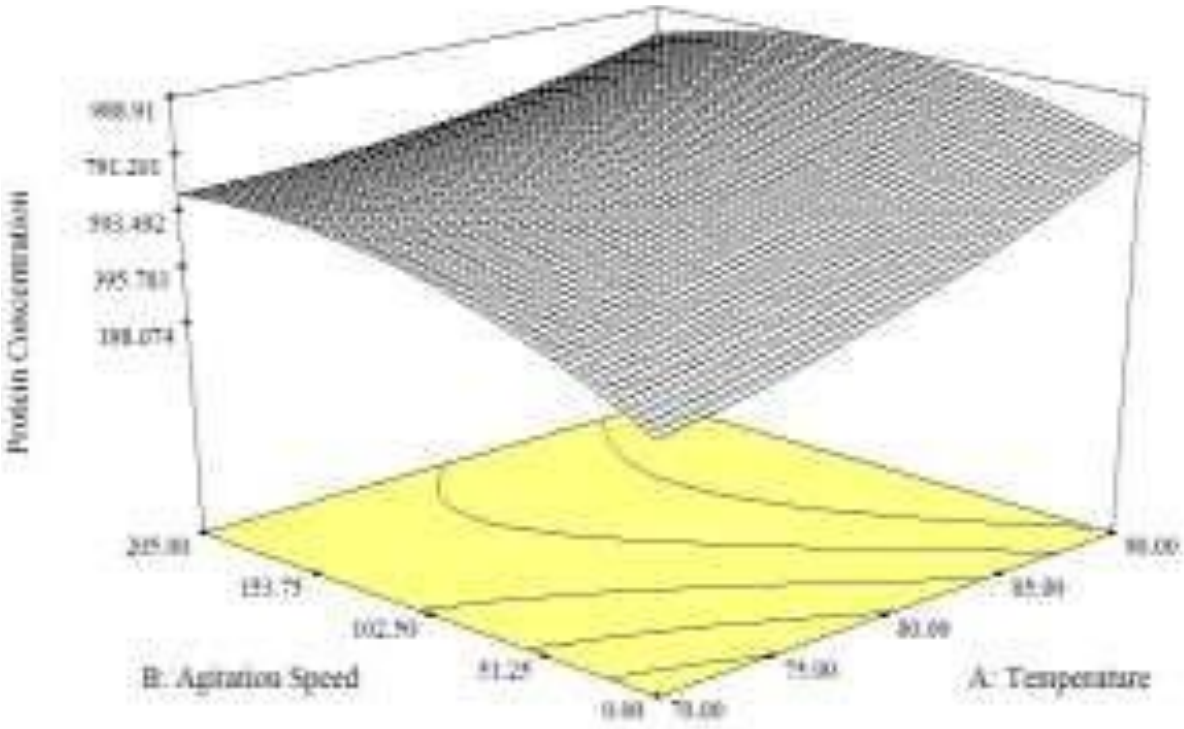

Figure 1: 3D surface plot of the protein concentration as function of temperature and agitation speed.

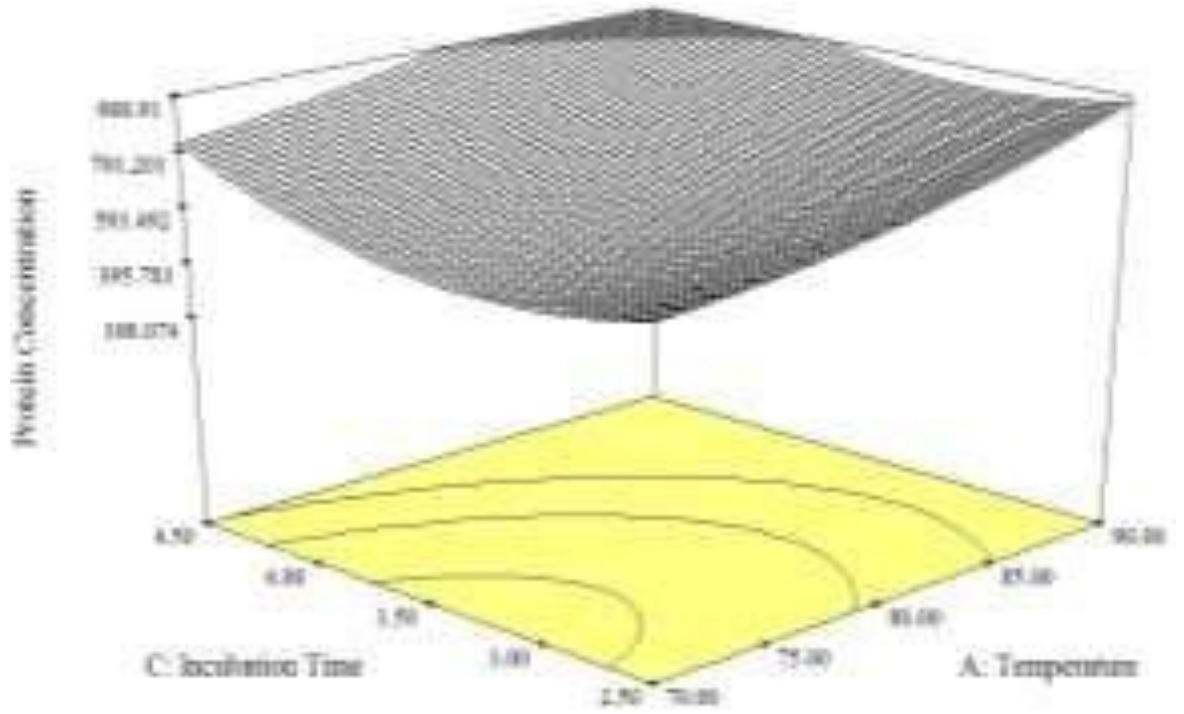

Figure 2: 3D surface plot of the protein concentration as function of temperature and incubation time.

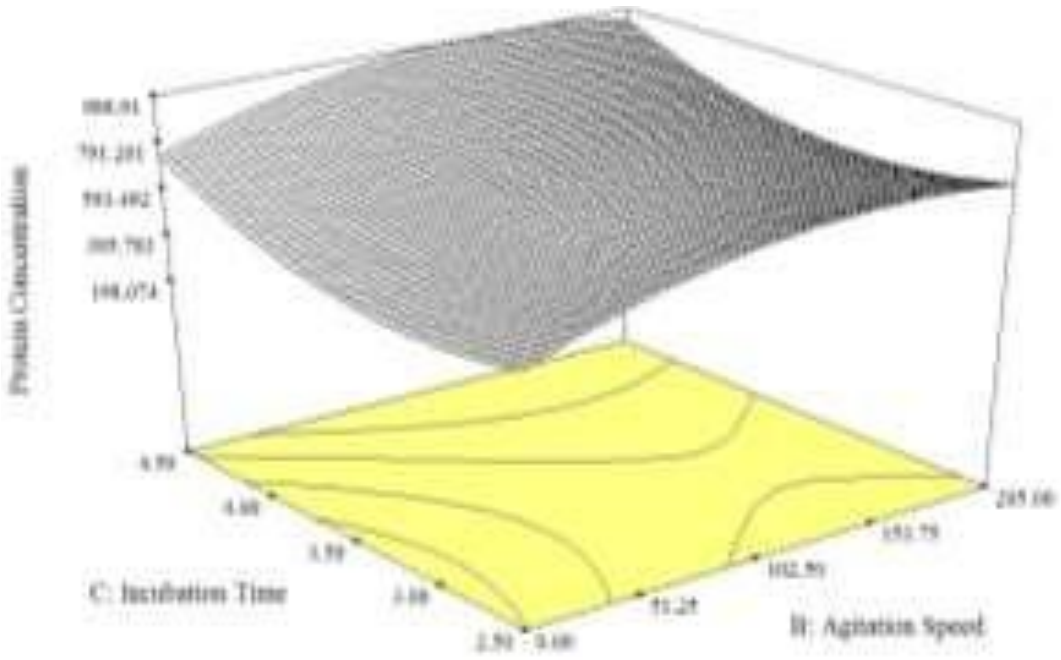

Figure 3: 3D surface plot of the protein concentration as a function of agitation speed.

Table 4: ANOVA analysis of Box-Behnken design.

Source	Sum of squares	Mean square	F-value	*p* value
				Prob > F
Model	7.102E+005	78907.41	26.27	0.0001
A	2.834E+005	2.834E+005	95.80	<0.0001
B	1.092E+005	1.092E+005	36.89	0.0005
C	1.081E+005	1.081E+005	36.53	0.0005
A^2	13988.28	13988.28	4.73	0.0662
B^2	75619.68	75619.68	25.56	0.0015
C^2	1.029E+005	1.029E+005	34.77	0.0006
AB	22782.08	22782.08	7.70	0.0275
AC	1627.62	1627.62	0.55	0.4824
BC	790.58	790.58	0.27	0.6211

Note: A= Temperature (°C); B= Agitation speed (rpm); C= Incubation time (hour); Standard Deviation = 54.39; Mean = 741.14; Coefficient Variation. % = 7.34; R^2 = 0.9717; Adjusted R^2 = 0.9352; Predicted R^2 = 0.6866; Adequate Precision = 23.862; Lack of fit [(F=2.56) and *p*= 0.1926]. $p < 0.05$ is considered as significant.

Table 5: The validation result for optimized extraction model.

Run	Protein concentration (µg/ml)	Average protein concentration (µg/ml)		Percentage error (%)
		Experimental	Theoretical	
1	1222.53	1227.44	1228.79	0.11
2	1232.34			

acceptable since it was less than 1%. Thus, the optimization process by RSM was valid and reliable to optimize the protein extraction. The relationship between the studied independent variables and response was satisfactory representing the present protein extraction process from silk fibre.

Gel filtration liquid chromatography

Figure 4 illustrates the chromatogram of the silk protein eluted out at corresponding column at 280 nm. The dragline spidroin putative peak was assumed as the presence of desired dragline silk protein in the sample.

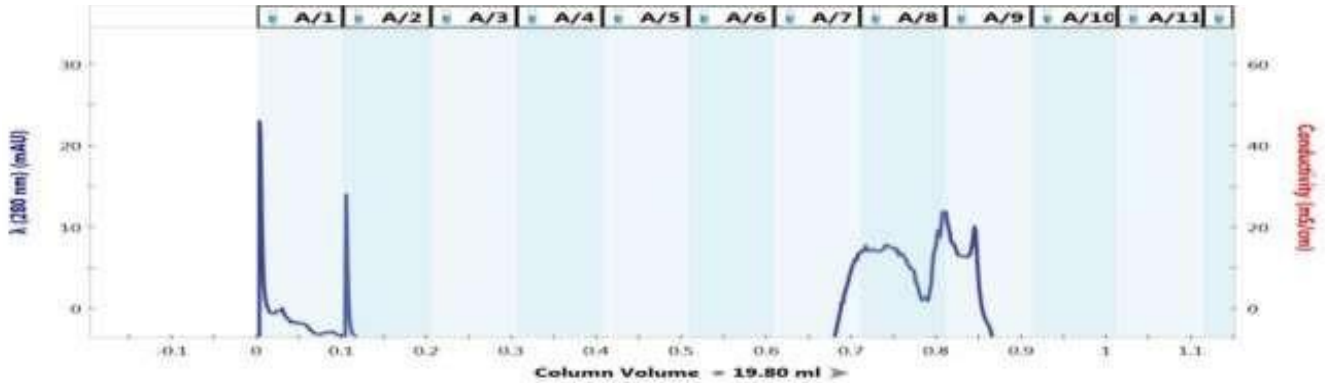

Figure 4: Chromatogram of silk protein peaks detected at 280 nm wavelength.

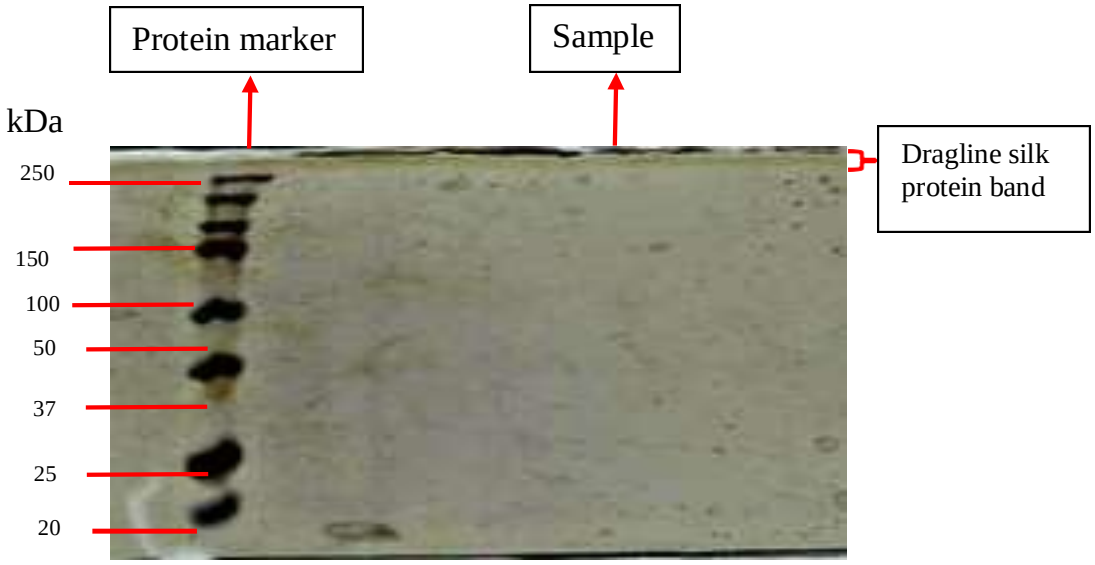

Figure 5: SDS-PAGE gel with band and marker.

However, there were unknown peaks that might be present in the sample other than dragline silk protein. Initial sharp peak was located at 0.01 ml column volume, while a second peak at 0.1 ml column volume could be determined from the chromatogram indicating that the larger protein molecules were eluted from the column. Other peaks between 0.68 and 0.87 ml column volume were identified where smaller sizes of protein molecules were eluted, respectively. The fraction volume between 0.01 and 0.1 ml column volume was collected for further identification of the molecular weight of the silk protein.

SDS-PAGE Analysis of dragline silk protein

Figure 5 shows the SDS-PAGE gel after silver staining process. The first column indicated the band for the protein marker ranged from of 20 to 250 kDa. From the gel, a prominent band was obtained with molecular weight more than 250 kDa in reference to protein marker. This showed that the dragline silk protein was extracted successfully from *C. lyoni* spider web as the protein band had a molecular weight of more than 250 kDa.

Conclusion

Based on the findings of this research, the evaluation from protein concentration extracted from *C. lyoni* web was performed by two-level full factorial design and Box-Behnken design for screening purpose and optimization study, respectively. Purification techniques were applied to obtain highest protein purity. Through the screening process, three different parameters of temperature, agitation speed and incubation time showed significant effect on the spidroin extraction ($p < 0.05$). 80°C, 350 rpm

and 3.5 h contributed to the highest protein concentration and were used as reference for the optimization study. During optimization study, the range values of each parameter were narrowed down and then optimized by response surface methodology through Box-Behnken design. The highest protein concentration of 1210.78 ± 0.974 µg/ml was obtained at 90°C, 102.5 rpm and 4.5 h. R^2 of 0.9717 and insignificant lack of fit indicated that the model was good fitness to the experimental data. Validation of optimized model was carried out and the percentage error of less than 1% proved that the model was valid and reliable to the spidroin optimization study.

According to the chromatogram of gel filtration liquid chromatography at 280 nm, the dragline spidroin putative peaks represented the presence of the desired protein in the sample at column volume of 0.1 ml. Larger size of protein molecules were first eluted followed by smaller size of protein. Based on the SDS-PAGE analysis of the purified protein collected from FPLC, the protein was identified as the dragline silk protein since the molecular weight obtained was approximately more than 250 kDa. The dragline silk protein from *C. lyoni* web was successfully isolated and purified in the research.

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REFERENCES

Addis KT, Raina SK (2013). Dissolution properties of silk cocoon shells and degummed fibres from African wild silkmoths. J. Biol. Sci. 16(20): 1199–1203.

Bain M (2016). Synthetic spider silk could be the biggest technological advance in clothing since nylon. Quartz. pp.1–15.

Hardy JG, Scheibel TR (2009). Production and processing of spider silk proteins.

Hardy JG, Romer LM, Scheibel TR (2008). Polymeric materials based on silk proteins. Polymer. 49(20): 4309–4327.

J. Polym. Sci. Part A: Polym. Chem. 47(16): 3957–3963.

Jastrzebska K, Felcyn E, Kozak M, Szybowicz M, Buchwald T, Pietralik Z, Jesionowski T, Mackiewicz A, Dams-Kozlowska H (2016). The method of purifying bioengineered spider silk determines the silk sphere properties. Scientific Reports. 6(28106): 1–13.

Mirghani M, Kabbashi N, Elfaki F, Zulkifli M (2012). Bt-201: Investigation of the spider web for antibacterial activity. Malaysian International Conference on Trends in Bioprocess Engineering (MICOTriBE).

Powers A (2013). Spider Silk: Stronger than steel? nature's supermaterial. Berkeley Plann. J. 18(1): 45–49.

Sah MK, Kumar A, Pramanik K (2010). The extraction of fibroin protein from *Bombyx mori* silk cocoon: Optimization of process parameters. Int. J. Bioinform. 2(2), 33– 41.

Singh K, Maity S, Singha M (2012). Spinning and applications of spider silk. Front. Sci. 55(2): 92–100.

Yang YX, Qian ZG, Zhong JJ, Xia XX (2016). Hyper-production of large proteins of spider dragline silk MaSp2 by *Escherichia coli* via synthetic biology approach. Process Biochem. 51(4): 484–490.

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