

Optimization of extraction conditions on yield, crude protein content and emulsifying capacity of mucilage from *Talinum paniculatum*

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Abstract

This study was conducted to investigate the influence of extraction conditions i.e. water:fronds ratio (0.5:1 – 12:1), temperature (25 – 90°C) and pH (3 – 11) on extraction yield, crude protein content and emulsifying capacity of mucilage from *Talinum paniculatum* fronds. Response surface methodology with a face centered-central composite design was applied to optimize the extraction conditions. With 20 experimental runs, extraction yield, crude protein content and emulsifying capacity of the mucilage were recorded to be 2.32 – 4.90%, 15.05 – 30.97% and 8.05 – 37.93%, respectively. Response surface analyses showed that increases in mucilage yield were mainly due to significant ($p < 0.05$) quadratic effect of pH and also synergistic effect between water:fronds ratio and pH. In contrast, significant ($p < 0.05$) quadratic effect of temperature and its synergistic effect with water:fronds ratio led to increase in emulsifying capacity of the mucilage. Furthermore, linear effect of pH seemed to significantly ($p < 0.05$) increase the crude protein content, in addition to significant ($p < 0.05$) synergistic effect between water:fronds ratio and pH. Experimental data for each response were best fitted with a quadratic model, having high coefficients of determination ($R^2 = 0.81 - 0.98$) and no lack-of-fit. The optimum conditions for mucilage extraction from *T. paniculatum* were obtained at water:fronds ratio of 8.4:1, temperature of 90 °C and pH of 8, providing 3.44 % yield, 29.35 % crude protein content and 34.00 % emulsifying capacity of *T. paniculatum* mucilage.

Keywords: Mucilage, *Talinum paniculatum*, Extraction conditions, Optimization, Emulsifying capacity

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Introduction

Talinum paniculatum or commonly known as Fame Flower or Jewels of Opar and popularly known in Asia as ‘Javanese Ginseng’, is a leafy vegetable originally from tropical America. *T. paniculatum* is widely spread throughout South-East Asia including

Malaysia and Indonesia. The fronds which are young leaves and stems of this crop are traditionally consumed by local communities with several traditional cuisines and also as a medicinal herb which significantly contribute in dietary intake of the communities. According to Liliwirianis et al. (2011), *T. paniculatum* has the compound that could be



introduced as antimicrobial and antioxidant sources. In addition, it has been suggested by Thanamool et al. (2013) that root and leaf extracts of this crop possess estrogenic activity and anti-fertility effect attributable to phytosterols. Moreover, fronds (young leaves and stems) of *T. paniculatum* is known to contain complex polysaccharide or mucilage in an appreciable amount that this plant could be regarded as a potential source of hydrocolloid for food industry. However, very few related studies have been conducted especially on extraction and characterization of the mucilage.

Hydrocolloids were widely used in the food industries as stabilizers, thickeners, texture modifiers, gelling agents, crystallization inhibitors and encapsulating agents. There is a large increase in use and demand for new sources of hydrocolloids in food industry. According to the safety, availability and low process cost, plant mucilage including *T. paniculatum* mucilage, have a good potential as a new source of hydrocolloid. The chemical and functional properties of hydrocolloids however could be influenced by the extraction conditions.

Generally, the efficiency of the extraction of complex polysaccharide from plant source is influenced by multiple parameters such as solvent types, extraction temperature, pH, time and water to raw material ratio, and their effects may be either interactive or independent to one another (Samavati, 2013; Lai and Liang, 2012; Lan et al., 2011). There are various extraction conditions which would affect extraction yield, emulsifying capacity and protein content of mucilage which depends on sources of mucilage. For example, according to a study by Lai and Liang (2012), sodium bicarbonate extract for the mucilage extraction from fronds of *Asplenium australasicum* (J. Sm.) Hook generally showed higher yield than water extract, and mucilage yield increased with increasing extraction temperature. In another study, Koocheki et al. (2009a) reported that protein content and stabilizing effect of mucilage from *Lepidium perfoliatum* seed gum decreased with increases in temperature and time. Nazir et al. (2017) concluded that temperature and water to seed ratio gave a pronounced effect on the extraction yield compared to extraction time which gave a minor impact on the yield. The study done by Samavati (2013) showed that the higher the temperature, the higher the increasing yield of the mucilage of *Abelmoschus esculentus*. Besides, these studies and some other studies have also documented on an optimized extraction conditions in order to obtain mucilage/ gum with desirable yield and

quality.

The general practice of determining the optimum extraction conditions is by varying one parameter while setting the other at an unspecified constant level. However, the major disadvantage of this single variable optimization is that it does not include interaction effects among the variables. Therefore, it does not depict the net effects of various parameters on the extraction efficiency. Response surface methodology (RSM) is an effective tool for optimizing the process in order to overcome this problem when many factors and their interactions might affect the desired responses. From a previous study, three responses (yield, protein content and emulsion stability) were determined for *L. perfoliatum* mucilage under optimum conditions of water to sample ratio (30:1), temperature (48.1°C), time (1.5 hours) and pH (8), respectively (Koocheki et al., 2009a). While two responses (yield and protein content) were determined for boat-fruited sterculia mucilage and *L. sativum* mucilage under optimized conditions of at least three of the same extraction parameters. Most recently, optimum extraction conditions for desired yield and functional properties of mucilage from *Plantago major* seed were obtained at 75°C, using 60:1 water to seed ratio at pH 6.8 (Behbahani et al., 2017).

As reported by previous studies, various conditions were applied during extraction of mucilage which were also expected to differently affect yield and properties of the *T. paniculatum* mucilage. Based on our screening study results (unpublished data), it has been shown that extraction temperature, pH and water to fronds ratio have significant influenced on the yield of *T. paniculatum* mucilage. Thus, the first objective of this study was to investigate the effect of these three extraction conditions on extraction yield, crude protein content and emulsifying capacity of *T. paniculatum* mucilage, which then led to determination of the optimum extraction conditions by means of RSM.

Material and Methods

Material

T. paniculatum fronds (young stems and leaves) used in this study were obtained from a controlled farm in Tumpat (Latitude: 6.23°, Longitude: 102.08°), Kelantan, Malaysia. Harvesting was done two months after the plants have grown with the stems were green in colour instead of brownish. After harvesting, the fronds were packed in plastic container and taken to the laboratory. The samples were cleaned manually to



remove all foreign matters. All chemicals used were of analytical grade.

Extraction of mucilage for optimization

Firstly, *T. paniculatum* fronds were weighed, trimmed and chopped and subsequently homogenized with distilled water at specified water to fronds ratio (w/w) (0.5:1-12:1) by using a blender (Waring 7011HG, Waring Inc., Waring and Co., USA). Upon extraction of mucilage, pH of the homogenate was adjusted using 0.1M NaOH/acetic acid for pH 3-11 while the temperature was set from 25–90°C and controlled within ±2.0°C using an adjustable temperature controlled water bath at a constant time (85 min). The extraction conditions were different based on run order of the experiment (Table 1). Distilled water was preheated to a designated temperature before added the samples. The samples were then filtered using four layers of muslin cloth. The filtered solution was mixed with three volumes of 95% ethanol (w/v) to precipitate the mucilage. The precipitated mucilage was dried in an oven (Memmert ULE 700, Germany) at 50°C for 5 hours and ground using a Waring blender (Modified from Singthong et al., 2009). The mucilage was placed

in an airtight container and stored at 4°C. The yield of mucilage was determined using the following equation:

$$\text{Yield of mucilage (\%)} = \frac{\text{Weight of dried mucilage (g)} \times 100\%}{\text{Weight of fronds used (g)}}$$

Crude protein analysis

Determination of protein content was carried out using Kjeltex system (2400 Kjeltex Analyser Unit, Foss Tecator AB, Hoganas, Sweden) based on a Kjeldahl method. Briefly a 2.0±0.5 g sample was digested in H₂SO₄ with the aid of Kjeltabs Cu 3.5 catalysts until a light green solution was formed. The solution was neutralized with NaOH solution before continuing with distillation process. The condensation yield was entrapped into a boric acid solution with the presence of a green bromocresol indicator. Finally, the mixture was titrated until the end point with formation of grey lavender colour.

Table 1: Response surface central composite design and results for yield, crude protein content and emulsifying capacity of *T. paniculatum* mucilage.

Run Order	Ratio ¹	Temperature (°C)	pH	Yield (%)	Crude protein content (%)	Emulsifying capacity (%)
1	12:1	57.5	7	3.53	26.28	15.48
2	6.25:1	57.5	11	3.70	17.65	8.05
3	6.25:1	57.5	3	2.52	17.31	9.10
4	0.5:1	57.5	7	2.32	20.16	14.46
5	6.25:1	25.0	7	2.90	27.36	31.82
6	6.25:1	90.0	7	3.15	30.97	32.95
7*	6.25:1	57.5	7	2.70	22.11	25.29
8*	6.25:1	57.5	7	2.73	19.47	27.06
9	0.5:1	90.0	11	2.77	20.51	13.33
10	12:1	90.0	3	3.01	18.66	13.10
11	0.5:1	25.0	3	2.59	25.44	25.00
12*	6.25:1	57.5	7	3.04	24.94	30.68
13	12:1	25.0	3	2.81	15.05	10.59
14	12:1	25.0	11	4.90	24.22	8.79
15*	6.25:1	57.5	7	3.11	27.46	34.15
16	12:1	90.0	11	4.57	25.66	15.12
17*	6.25:1	57.5	7	2.95	28.48	37.93
18	0.5:1	25.0	11	3.03	19.27	15.48
19*	6.25:1	57.5	7	3.03	27.38	30.59
20	0.5:1	90.0	3	2.69	23.75	15.48



The percentage of protein was calculated by using a protein conversion factor of 6.25 as follows (AOAC, 2000):

$$\text{Crude protein content (\%)} = \frac{(T - B) \times 14.007 \times 6.25 \times 100\%}{\text{weight of sample (mg)}}$$

Where;

T = volume of HCl used for titration of sample (ml)

B = volume of HCl used for titration of blank (ml)

N = normality of HCl

Determination of emulsifying capacity

Firstly, 10% of mucilage dispersion was prepared using deionized water at 80°C with continuous mixing for 2 hours. The dispersion was then held at room temperature (25°C) for overnight (to ensure a complete hydration) prior to use for emulsion preparation. Oil-in-water emulsions (O:W, 1:1 w/w) were prepared by adding 1 g of orange oil into hydrated gum and homogenized using Ultra-Turrax T-25 homogenizer (IKA Instruments, IKA-Werke GmbH and Co., Germany) at 6000 rpm for 1 min, followed by 10,000 rpm for 1 min and 14,000 rpm for 30 s at room temperature. Emulsions were then centrifuged at 800 rpm for 10 min at room temperature using a centrifuge (Hettich Zentrifugation D-78532 Tuttlingen, Hettich, Andreas Hettich GmbH and Co., Germany). Emulsifying capacity was evaluated based on the following equation (Modified from Sciarini et al., 2009):

$$\text{Emulsifying Capacity (\%)} = \frac{\text{Height of separated emulsion layer (cm)} \times 100\%}{\text{Height of total emulsion (cm)}}$$

Experimental design and statistical analyses for optimization

A central-composite design (CCD) (face-centered) with three variables was used to study the response pattern and to determine the optimum combination of variables. The extraction was carried out under various conditions, according to the design which provided a total of 20 runs of extraction conditions. The effect of three independent variables which were water: fronds ratio, X_1 (0.5:1-12:1), temperature, X_2 (25-90 °C), and pH, X_3 (3-11) were determined. The independent variables were coded to three levels of -1, 0, +1. The dependent variables (responses) involved were the yield (Y_1), protein content (Y_2) and emulsifying capacity (Y_3), which represent the quality of the

mucilage. The centre points were replicated six times for an error estimation. Response surface analyses were conducted to provide analysis of variance (ANOVA) outputs, regression coefficients and statistical significance of model terms, which finally allowed for regression modelling on the experimental data for all responses studied. The behaviour of the response surface was investigated for each response using the generalised polynomial regression equation as follows;

$$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad (1)$$

The response function (Y_i) was the independent variables which were extraction yield, protein content and emulsifying capacity. The coefficients of the polynomial model were represented by β_0 (constant term), β_1 , β_2 and β_3 (linear effects), β_{11} , β_{22} and β_{33} (quadratic effects) while β_{12} , β_{13} and β_{23} were the interaction effects and these represented the polynomial coefficients. In addition, contour/ surface plots were generated to better visualize any significant interaction effects (or curvatures) of independent variables on each response.

A numerical optimization were carried out by the response optimizer using the software to determine the exact optimum levels of individual and simultaneous multiple response optimizations leading to the desirable (D) response goals. A new extraction conditions were obtained based on the optimization. Finally the model was validated by comparing the actual values of mucilage yield, protein content and emulsifying capacity with the respective predicted values by the model equation. One sample t-tests were applied to determine significant difference between the predicted and actual values of each response. The experimental design, data analyses and contour/ surface plots were developed/ run using Minitab statistical software (Minitab Release 14, Minitab Inc, USA). The level of confidence used was at α -0.05.

Results and Discussion

Diagnostic interpretation of the response surface analysis

The influence of three independent variables (water to fronds ratio, temperature and pH) on extraction yield, protein content and emulsifying capacity were further



investigated using a CCD-face centered. The experimental data for yield, protein content and emulsifying capacity of the extracted mucilage under different extraction conditions are presented in Table 1. Second-order polynomial model was generated by fitting the data using the regression analysis and analysis of variance (ANOVA) to examine the significance of the effects and curvature.

As stated by Myers et al. (2016), lack of fit indicates the failure of a model which represents the experimental data at which points were not included in the variations or regression in the models cannot be accounted for random error. Based on the result, the model showed a non-significant lack of fit ($p > 0.05$) for all responses which implied the model adequately described the functional relationship between the main effects and the response (Table 2). The analysis of variance of different models showed that adding terms up to quadratic were significantly improved the model, therefore a full quadratic model is the most appropriate model for the three responses (with reduced terms).

The models gave a good R^2 (i.e. coefficient of determination) value for all of the responses which higher than 0.8. The R^2 values were 0.982, 0.810 and 0.967 for extraction yield, protein content and emulsifying capacity, respectively. These high R^2 values indicated that the variation found in the three responses could be well explained by the factors (i.e. water to fronds ratio, temperature and pH) in the model. However, a high value of R^2 does not always means that the regression model is good. R^2 will always increase by adding a variable to the model although the additional variable is statistically significant or not. Therefore, adjusted R^2 (adj- R^2) is better to be used in order to evaluate the model adequacy. Table 2 shows that R^2 and adj- R^2 values for the model did not differ greatly. The adj- R^2 values for extraction yield, protein content and emulsifying capacity were found to be 0.969, 0.673 and 0.930, respectively. A higher adj- R^2 indicated that non-significant terms have not been included in the model. Both R^2 and adj- R^2 values indicated that the accuracy and general availability of the polynomial model were adequate for optimization purpose.

Effect of extraction conditions on yield

The mucilage yield obtained was in the range of 2.32 - 4.90% (Table 1). Based on analysis of variance, the p-value of the model was significant ($p < 0.05$) (Table

2). The p-values were used in order to check the significance of every coefficient of estimation as the smaller the magnitude of p, the more significant is the corresponding coefficient of estimation (Koocheki et al., 2009a). The p-values less than 0.05 indicate model terms are significant. From the model of extraction yield, a quadratic effect of three factors was significant ($p < 0.05$). The quadratic model gave the equation as follows:

$$\text{Yield (\%)} = 2.747 - 0.024X_1 - 0.005X_2 - 0.092X_3 + 0.001X_2X_2 + 0.012X_3X_3 + 0.017X_1X_3 - 0.001X_2X_3 \quad (2)$$

The linear terms namely water to fronds ratio (X_1), temperature (X_2) and pH (X_3) were not significant. However, the quadratic effect of pH (X_3X_3), and interaction effects of water to fronds ratio-pH (X_1X_3), and temperature-pH (X_2X_3) significantly ($p < 0.05$) affected the mucilage yield. The interaction effect between water to fronds ratio and pH had the strongest influence on mucilage yield in which positively increased (i.e. synergistic effect as expressed by a positive coefficient of estimation) the extraction yield of *T. paniculatum* mucilage. Contour and surface plots showing this effect could be referred to Figure 1. It is seen that when the temperature was held at 57.5°C, increasing in water to fronds ratio seemed to result in further increase in extraction yield of mucilage but to a certain extend depending on the pH. The extraction yield of mucilage was the highest at water to fronds ratio of 12:1 and pH 11 (Table 1).

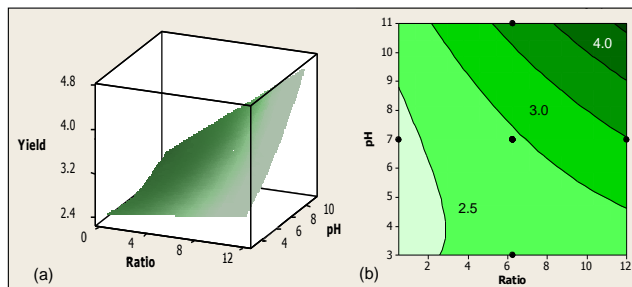


Figure 1: Surface (a) and contour (b) plots for effect of water to fronds ratio (ratio) and pH on yield (%) of *T. paniculatum* mucilage

The increase of mucilage yield with higher water to fronds ratio was due to the availability of more liquid which increased the driving force of mucilage out of the fronds.

Table 2: Estimated coefficients and Analysis of variance (ANOVA) for polynomial models evaluated for prediction of responses in *T. paniculatum* mucilage

Source	Extraction yield (%)				Crude protein content (%)				Emulsifying Capacity (%)			
	DF	Coefficient	Sum of squares	<i>p</i> *	DF	Coefficient	Sum of squares	<i>p</i> *	DF	Coefficient	Sum of squares	<i>p</i> *
Model	7	2.747	7.413	0.000	7	17.400	293.589	0.003	9	7.450	1769.81	0.000
Linear	3		5.801	0.155	3		11.787	0.009	3		58.64	0.000
Ratio* (X_1)		-0.024		0.103		-0.338		0.615		2.177		0.016
Temperature (X_2)		-0.005		0.470		-0.443		0.024		-0.975		0.001
pH (X_3)		-0.092		0.163		5.772		0.002		13.003		0.000
Quadratic	2		0.292	0.002	3		200.044	0.001	3		1625.26	0.000
X_1X_1		-		-		-0.050		0.306		-0.302		0.000
X_2X_2		0.001		0.133		0.004		0.017		0.007		0.001
X_3X_3		0.012		0.013		-0.462		0.001		-1.024		0.000
Interaction	2		1.320	0.000	1		81.758	0.004	3		85.91	0.040
X_1X_2		-		-		-		-		0.014		0.021
X_1X_3		0.017		0.000		0.139		0.004		0.065		0.141
X_2X_3		-0.001		0.018		-		-		0.011		0.163
Residual error	11		0.143		11		69.292		9		61.12	
Lack-of-Fit	7		0.130	0.053	7		59.048	0.133	5		23.19	0.774
Pure error	4		0.0123		4		10.244		4		37.93	
Total	19		7.890		19		365.487		19		1831.44	
R^2		0.982				0.810				0.967		
R^2 (adjusted)		0.969				0.673				0.930		

*Ratio = Water to fronds ratio; $p < 0.05$ is considered significant at 95% confidence level.

This result was in agreement with some reported studies, whereby a greater mucilage yield obtained from Iranian *A. esculentus* pods (Samavati, 2013), *Alyssum homolocarpum* (Koocheki et al., 2009b) and *Opuntia* spp. seeds (Sepúlveda et al., 2007), as the volume of water relative to raw materials was increased. This effect became stronger with increased pH due to alkaline condition which had promoted hydrolysis of insoluble constituents (e.g. cellulose) that originally associated with mucilage and thus increased the extractability of the mucilage (Karazhiyan et al., 2011). Differently, at water to fronds ratio of 6.25:1, the extraction yield of mucilage increased at higher pH and this effect was less affected by increases in temperature (Figure 2). Likewise, Somboonpanyakul et al. (2006) found that the highest extraction yield of Malva nut gum was obtained in the

alkaline solution with insignificant influence of temperature. However, it is contrary to the results found by Koocheki et al. (2009a) and Wu et al. (2007) who reported the minor effect of pH on extraction yield of polysaccharides with increased temperature. In addition, extraction temperature did not give significant ($p > 0.05$) effect on yield of mucilage is also in parallel with the findings reported by Karazhiyan et al. (2011) for *Lepidium sativum* seed gum.

Effect of extraction conditions on crude protein content

According to Table 1, *T. paniculatum* mucilage obtained in this study was characterized as a protein-polysaccharide polymeric complex with high crude protein content ranging from 15.05 to 30.97%.



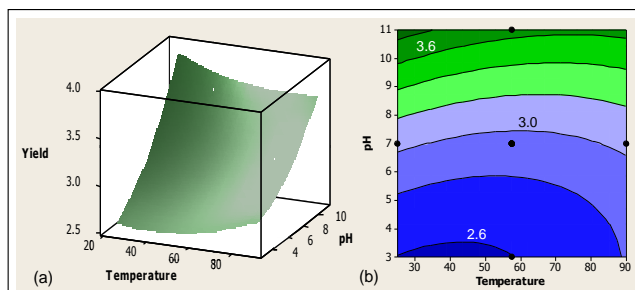


Figure 2: Surface (a) and contour (b) plots for effect of temperature (°C) and pH on yield (%) of *T. paniculatum* mucilage

The protein is believed to be attached to carbohydrate (i.e. sugar constituents) groups of the mucilage as glycoproteins. The values were well expected for such crude mucilage since extraction condition applied in this study was unable to efficiently hydrolyse covalent bonds within the glycoproteins. Glycoproteins are abundantly exist as integral membrane cell of plants and thus often could be co-extracted with complex polysaccharides including gums and mucilage. However, further purification of gums and mucilage using ionic solution like saturated barium hydroxide and trichloroacetic acid might efficiently hydrolyse the covalent bonds and thus could reduce the protein content (Sébastien et al., 2014), but this is not the case for the present study since further purification was not applied.

The highest protein content was observed at water to fronds ratio of 6.25:1, temperature of 90°C and pH of 7 in which the protein content reached a level of 30.97% (Table 1). This value was much higher than those reported by Sepúlveda et al. (2007) for *Opuntia ficus indica* mucilage (7.3%) and Somboonpanyakul et al. (2006) for Malva nut gum (8.4%). Nevertheless, the range of protein content in *T. paniculatum* mucilage was comparable with the protein content in crude polysaccharides of Tamarillo (Gannasin et al., 2012), boat-fruited sterculia seeds (Wu et al., 2007) and *P. flexuosa* mucilage (Ibañez and Ferrero, 2003) with the values of 21.18%, 20% and 21.9%, respectively.

The results in Table 2 indicated that all linear and quadratic effects of extraction temperature (X_2) and pH (X_3) were significant ($p < 0.05$) for crude protein content whereas the effect of water to fronds ratio (X_1) was not significant ($p > 0.05$). The regression equation representing the relationship between the protein content of *T. paniculatum* mucilage and the test variables derived from RSM is as follows:

$$\text{Crude Protein content (\%)} = 17.400 - 0.338X_1 - 0.443X_2 + 5.772X_3 - 0.050X_1X_1 + 0.004X_2X_2 - 0.462X_3X_3 + 0.139X_1X_3$$

(3)

Among the quadratic terms, only temperature (X_2X_2) and pH (X_3X_3) gave significant ($p < 0.05$) effect on the crude protein content. According to Equation 3, the linear effect of pH (X_3) showed the strongest positive influenced on this response. Besides, the result also showed that interaction effect between water to fronds ratio and pH (X_1X_3) was highly significant ($p = 0.004$) (Table 2) with a positive coefficient of estimation. Figure 3 further demonstrates the occurrence of curvature due to this synergistic interaction with maximizing effect on crude protein content of mucilage at intermediate pH range across all water to fronds ratio (when the temperature was set at 57.5°C (centre point).

There were several studies on the optimization of extraction conditions on the protein content of gum or mucilage. Karazhiyan et al. (2011) have discovered significant linear effects of pH and extraction temperature on the protein content of *L. sativum* seed gum along with insignificant effect of water to seed ratio on the protein content of gum, which is slightly different with the result obtained in the present study. Jouki et al. (2014) similarly reported that water to seed ratio had no significant effect on the protein content of quince seed mucilage, in line with the effect of temperature. In contrast, the present findings recorded that linear increase in temperature could significantly reduce (i.e. negative coefficient of estimation) the crude protein content of *T. paniculatum* mucilage.

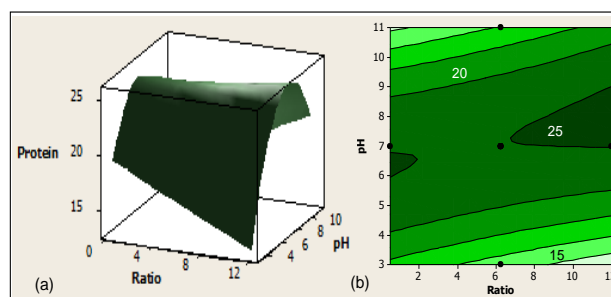


Figure 3. Surface (a) and contour (b) plots for effect of water to fronds ratio (ratio) and pH on crude protein content (protein) (%) of *T. paniculatum* mucilage

It has been suggested that extraction at too high temperature will lead to continuous reduction of the

protein due to denaturation (Mirhosseini and Amid, 2012) that might loosen the covalent bonds in glycoproteins.

Effect of extraction conditions on emulsifying capacity

As depicted in Table 1, the emulsifying capacity obtained for *T. paniculatum* mucilage was in the range of 8.05% to 37.93%. The emulsifying capacity of the mucilage might be attributed to its high protein content. The protein fraction of the mucilage is able to adsorb onto the surface of oil droplets during emulsification and thus create an oil-water interphase and at the same time the carbohydrate portion inhibits flocculation and coalescence by electrostatic repulsions and steric forces as previously suggested by Wang and Cui (2005). In fact, most polysaccharides have some proteins in their extracts which may give some surface activity for emulsion stability (Junior et al., 2013).

According to Table 2, all linear and quadratic terms for emulsifying capacity were highly significant ($p < 0.05$). The following equation is the regression model for emulsifying capacity of *T. paniculatum* mucilage:

$$\text{Emulsifying Capacity (\%)} = 7.450 + 2.177X_1 - 0.975X_2 + 13.003X_3 - 0.302X_1X_1 + 0.007X_2X_2 - 1.024X_3X_3 + 0.014X_1X_2 + 0.065X_1X_3 + 0.0108X_2X_3 \quad (5)$$

All linear terms showed significant ($p < 0.05$) effects on emulsifying capacity of the mucilage with water to fronds ratio and pH showed an increasing effects as opposed to temperature. As observed for the crude protein content, pH also gave the strongest desirable effect on emulsifying capacity. In addition all quadratic effects were also significant ($p < 0.05$) with both temperature and pH gave negative coefficient of estimation. This reflects that emulsifying capacity of the mucilage could be suddenly decreased when extreme high temperature or pH were applied during extraction.

Otherwise, the undesirable effect of extreme temperature seemed to be counteracted when certain water to fronds ratios were employed. This fact is proven by a significant interaction effect observed between water to fronds ratio and temperature (X_1X_2), which might synergistically increase the response. Based on Figure 4, at neutral (pH 7), the emulsifying capacity was found to be more than 32% at around $<40^\circ\text{C}$ and $>75^\circ\text{C}$, when water to fronds ratio ranged

of approximately 0.6:1 – 9.5:1 and 3:1 – 10:1, respectively.

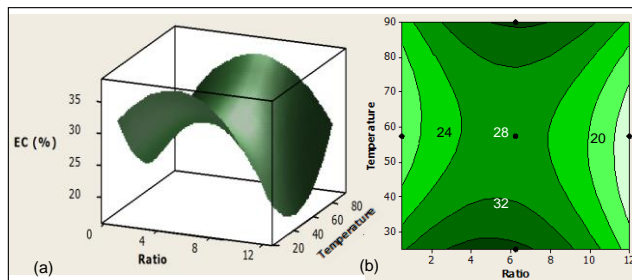


Figure 4. Surface (a) and contour (b) plots for effect of water to fronds ratio (ratio) and temperature ($^\circ\text{C}$) on emulsifying capacity (EC) (%) of *T. paniculatum* mucilage

Optimization of the extraction conditions and model validation

In the present study, response surfaces were plotted in order to study the effects of extraction parameters and their interactions on the yield of *T. paniculatum* mucilage, protein content and emulsifying capacity. Optimum condition for extraction of *T. paniculatum* mucilage was determined to obtain maximum extraction yield, protein content and emulsifying capacity. The goal for independent variables for water to fronds ratio (X_1), extraction temperature (X_2) and pH (X_3) had been set to be in the designated experimental range. In order to obtain final point of optimum conditions for optimum extraction yield, crude protein content and emulsifying capacity, several trials were done using a response optimizer in which the highest desirable value ($D = 1$) was selected. As a results, the optimum conditions obtained were water to fronds ratio of 8.4:1, extraction temperature of 90°C and pH of 8 with the optimum predicted values of mucilage yield, crude protein content and emulsifying capacity of 3.44%, 29.35% and 34%, respectively.

In order to validate the predicted results, four independent extractions ($n = 4$) were performed at the optimum conditions and the extracted mucilage were subsequently subjected to yield, crude protein content and emulsifying capacity determinations. The experimental data provided mean (\pm standard deviation) values of 3.43% (0.09) of extraction yield, 30.34% (0.69) for crude protein content and 34.67% (0.46) which were very much close to the predicted values. Based on one sample t-tests, there was no significant difference ($p > 0.05$ i.e. 0.063 – 0.799)

between the experimental value against the predicted value for each response, verifying that the model was adequate and valid to predict future responses for this particular extraction process.

Several previous studies have documented optimum extraction conditions for gums and mucilage involved temperature, water to raw material ratio and pH as factors of interest, similarly employed in the present study for *T. paniculatum* mucilage. For instance, Behbahani et al. (2017) reported that the optimum extraction conditions for *Plantago major* seed mucilage were at 75°C, using 60:1 water to seed ratio at pH 6.8 in order to obtain maximum yield (15.18%) and emulsion stability (67.40%) as well as desirable values for several other functional properties. Earlier, Amid and Mirhosseini (2012) suggested temperature of 85.0°C, water to seed ratio of 35.5:1 and pH 11.9 as the optimum conditions for durian seed gum with high extraction yield (56.4%) and good functional properties. Somehow, maximum yield (17.36%) with lowest protein content (2.84%) and acceptable values of other properties of *L. perfoliatum* seed gum have been predicted to be achieved at optimum conditions of 48.1°C, using water to seed ratio of 30:1 and pH 8 (Taherian et al., 2009). From the present findings and these three previous studies, the optimum extraction conditions seemed to be very much depended on the raw material itself and designated experimental domain for each factor as well as type of responses being examined.

Conclusion

This study demonstrated that significant linear effects of all independent variables i.e. temperature, water to fronds ratio and pH were only observed on emulsifying capacity of *T. paniculatum* mucilage. It was also found that pH being the most influential factor that linearly increased the crude protein content of the mucilage alongside its emulsifying capacity. Moreover, all responses seemed to be significantly ($p < 0.05$) increased due to quadratic and interaction effects (at least one for each) but the later effect was found to be stronger with higher magnitude of estimation coefficients. In particular, synergistic effect between water to fronds ratio and pH desirably increased the mucilage yield and its crude protein content while increase in mucilage emulsifying capacity was very much due to synergistic effect between water to fronds ratio and temperature. The use of RSM in providing valid quadratic models for future

data prediction, was also proven in this study, since optimum extraction conditions at 90°C, pH 8 and water to fronds ratio of 8.4:1 successfully provided experimental values for all responses that were not significantly ($p > 0.05$) difference from their respective predicted values.

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Contribution of Authors

Ibrahim NH: Designed the study, supervised the study and prepared the manuscript draft
Zakaria TNDT: Conducted the study and prepared the manuscript draft
Hamzah Y: Supervised the study and prepared the manuscript draft

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