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KINETIC MODEL OF ULTRASONIC-ASSISTED EXTRACTION WITH CONTROLLED TEMPERATURE OF C-PHYCOCYANIN FROM *S. PLATENSIS*

Phirunrat Thaisamak[1], Somkiat Jaturonglumlert[1,*], Jaturapatr Varith[1], Farah Saleena Taip[2] and Chanawat Nitatwichit[1]

[1] Faculty of Engineering and Agro-Industry, Maejo University, Chiang Mai 50290, Thailand; [2] Faculty of Engineering, 43400 Universiti Putra Malaysia, Serdang, Selangor, Malaysia

*Corresponding Author, Received: 10 June 2018, Revised: 14 July 2018, Accepted: 10 Oct. 2018

ABSTRACT: C-phycocyanin (CPC) from *S. platensis* has been reported as a great natural compound, which can be used as a natural colorant in food industries. The aims of this work were to demonstrate the potential of the ultrasonic-assisted extraction with controlled temperature (UAET). The UAET was used to improve the extraction efficiency, in terms of time, concentration and yield of the CPC. The operating parameters were investigated that are ultrasonic frequencies (28, 45 and 100 kHz) and controlled extraction temperatures (40, 45 and 50°C), and the best parameter values were identified. The first-order kinetic model was used to describe the mechanism of extraction under these processing parameters. The highest values of the concentration and yield were recorded by the UAET method that is 2.55 $mg.ml^{-1}$ and 127.70 $mg.g^{-1}$, respectively. The optimal extraction conditions were achieved at 30 min and 45°C of controlled temperature, with 100 kHz of ultrasonic frequency, and the solid-liquid ratio of 1:5 w/v. The kinetic parameters namely extraction rate constant, effective diffusivity and activation energy were proposed in this work that is 0.09 to 0.32 min^{-1}, 1.11×10^{-12} to 4.33×10^{-12} $m^2.s^{-1}$ and 28.76 to 47.39 $kJ.mol^{-1}$, respectively. This study has demonstrated the efficiency of the UAET, by providing an efficient method to produce the CPC extracts, with reduced time and energy, which has the potential for applications in the plant extractions.

Keywords: *C-phycocyanin (CPC), Ultrasonic, Kinetic model, S. platensis*

1. INTRODUCTION

Spirulina platensis (S. platensis) or *Arthrospira platensis (A. platensis)* is a microalga consisting of several valuable compounds, such as chlorophyll-a, carotenoid, and phycobiliproteins. CPC is a major group of phycobiliproteins, which are brightly colored pigments and soluble in water. It is commonly used as a natural colorant in food and cosmetic industries [1]. It is also the major phycobiliprotein in *S. platensis* (arranged in groups of cyanobacteria) that gives effect to the strong fluorescence. This compound has been utilized for many applications, including as a fluorescence marker in the medical field, food colorant, and substance in cosmetic and pharmaceutical products since it is a natural pigment and thus, are safe for the consumers compared to the synthetic dyes. The extraction process plays an important role in optimizing the concentration of the CPC.

The solid-liquid extraction process involves the use of a solvent, to dissolve a soluble fraction from an insoluble permeable solid, and is affected by the transfer of solute, from inside the solid matrix to the solvent [2]. During the extraction, one of the assumptions of the mass transfer is to consider the external mass transfer resistance as negligible, which is important and depends on the nature of the extraction. The concentration of solute inside the solid varies, leading to various conditions that can affect the extraction rate, which can be expressed in terms of the mass of solute leached per unit time [1].

CPC extraction from dried and wet *S. platensis* represents the important factor in the CPC production [3-5]. Various methods have been used for the purpose of extraction, where most of them involve the assistance of solvents, such as phosphate buffer, acetate buffer, sodium chloride, calcium chloride and ammonium chloride. On the other hand, some authors combined the solvent assistance with mechanical methods, i.e. freeze-thaw, sonication, homogenization, continuous mixing and rotary shaker [6-8]

Temperature is a significant parameter in the CPC extraction, as it affects the pigment and protein contents during the process. Su et al. (2014) explored the influence of temperature on the CPC extraction and found that the extraction rate increased with temperature. Whereas, the equilibrium concentration of the CPC was found to be decreasing with high temperature, as the CPC degrades due to the heat applied during the extraction [1].

Ultrasonic-assisted extraction (UAE) method has been proven to be effectively used to damage or break cell walls, reduce particle size, and rate of mass transfer [9]. Hadiyanto et al. (2016) have been reported that the ultrasonic irradiation is an efficient

tool to improve the performance of the CPC extraction [10].

However, studies involving the UAE of CPC can still be hardly found, especially to understand the effects of significant variables, such as extraction temperature and ultrasonic frequency. In order to extract the CPC, the selection of ultrasonic frequency is important as it affects the yield of the CPC [11]. Thus, it is essential to find the extraction method that can efficiently increase the CPC yield. The aims of this work were to demonstrate the potential of the UAET. The operating parameters were investigated that are ultrasonic frequencies (28, 45 and 100 kHz) and controlled extraction temperatures (40, 45 and 50°C). The first-order kinetic model was used to describe the mechanism of extraction under these processing parameters.

2. MATERIALS AND METHODS

2.1 Microorganism and Culture Medium

S. platensis was supplied by the Faculty of Fisheries Technology and Aquatic Resources, Maejo University, Thailand. The culture medium was supplemented with 16 $g.l^{-1}$ sodium bicarbonate ($NaHCO_3$), 2.5 $g.l^{-1}$ sodium nitrate ($NaNO_3$), 1 $g.l^{-1}$ sodium chloride (NaCl), 0.5 $g.l^{-1}$ di-potassium hydrogen phosphate (K_2HPO_4) and 0.2 $g.l^{-1}$ magnesium sulfate ($MgSO_4$) [12].

2.2 Culture and Harvesting

All the experiments involved the utilization of *S. platensis* that was cultivated by a smart control algae system [12]. The growth of *S. platensis* was evaluated by measuring the optical density of biomass using a spectrophotometer (Spectro SC, U.S.A.) at the absorbance wavelength of 680 nm. The initial optical density of the *S. platensis* was recorded, while harvesting was only carried out when the optical density of about 1.2 has been reached. Freshly harvested biomass was thoroughly washed twice using the ozone water, to remove all the culture media components.

2.3 Influencing Parameters in the CPC Extraction Process

The aim was to observe the significant parameters, which can reduce the non-significant parameters in the extraction process. All experiments involving CPC extraction were conducted using 0.1 M sodium phosphate (pH 7.0) as the solvent [11]. All the chemicals used in this study were analytical grade.

2.3.1 Crude S. platensis

The crude *S. platensis* was evaluated by comparing between the dried and wet *S. platensis*. For the dried crude, the harvested *S. platensis* was oven-dried at 50°C for about 7 h. For the wet crude, the harvested *S. platensis* was desiccated and packed for storage at -10°C. Extraction was conducted via repeat freeze-thaw (RFT) as both of them were weighted and put into a test tube, then mixed with sodium phosphate as the solid-liquid ratio of 1:5 w/v. The process was then repeated for 3 cycles [11].

2.3.2 Effect of UAE on the solid-liquid ratio

This experiment has investigated the effect of UAE on different of solid-liquid ratio. Crude *S. platensis* obtained was studied via UAE as following: *S. platensis* was weighted and put into a test tube, then mixed with sodium phosphate as desired ratios of 1:5, 1:10 and 1:15 w/v, then the experiments were conducted with UAE under 100 kHz of ultrasonic frequency for 30 minutes.

2.3.3 Effect of UAET

This experiment has investigated the effect of ultrasonic assisted with controlled temperature in extraction. *S. platensis* was weighted and put into a test tube, then mixed with sodium phosphate as the solid-liquid ratio of 1:5 w/v. The experiments were performed at various controlled temperatures (40, 45 and 50°C) and ultrasonic frequencies (28, 45 and 100 kHz**;** all in continuous mode).

2.4 Studies of UAET on the CPC Extraction and Kinetics

The kinetic model of UAET was carried out in this section. *S. platensis* was weighted and put into a test tube, then mixed with sodium phosphate as the solid-liquid ratio of 1:5 w/v. One of the solvent tubes was placed inside an ultrasonic bath (Honda, W-113, Japan) with a controlled water temperature. The mixture tube was then placed in the ultrasonic bath as the temperature of the solvent reached the desired temperature. Fig.1 has presented a schematic diagram of the UAET.

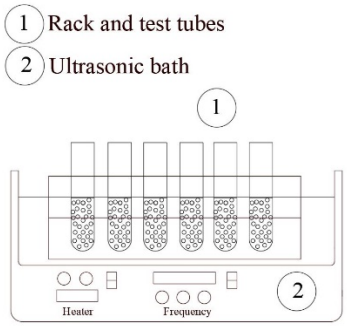


Fig. 1 Schematic diagram of the UAET.

The kinetic of UAET were evaluated by collecting the samples at every 10 to 60 min. The data were analyzed and evaluated to identify the performance of the extraction methods by kinetic parameters.

2.5 CPC Analysis

All the extracted CPC samples were analyzed according to the procedure carried out by Boussiba & Richmond (1979). The supernatant obtained was analyzed at the wavelength of 620 and 652 nm, using a spectrophotometer (Spectro SC, U.S.A.). The concentration and yield of the CPC were estimated using Eq. (1) and Eq. (2), respectively [11].

$$CPC = \frac{A_{620} - 0.474A_{652}}{5.34} \quad (1)$$

$$Yield = \frac{CPC \times V}{D} \quad (2)$$

Where, CPC is the CPC concentration (mg.ml^{-1}). Yield is the yield of CPC (mg.g^{-1}). A_{620}, A_{652} is the absorbance wavelength at 620 and 652 nm. V is the volume of the solvent (ml). and D is the dry weight (g) of the biomass.

2.6 Determination of Kinetic Parameters

In order to identify the kinetics that can better describe the CPC extraction from *S. platensis*, the concentration values were plotted as a function of time. The extracted CPC concentration as a function of time can be adequately predicted using Eq. (3), which represents the first-order kinetics, considering the boundary conditions of $t = 0$ to t, and $CPC_t = 0$ to CPC_t [13]:

$$CPC_t = CPC_s\left(1 - e^{-kt}\right) \quad (3)$$

Where, CPC_t is the extraction concentration at any time (mg.ml^{-1}), CPC_s is the extraction concentration at equilibrium (mg.ml^{-1}), and k is the extraction rate constant (min^{-1}). Both CPC_s and k values were calculated using the non-linear regression.

The above considerations regarding diffusion were applied by following these assumptions:

1. The CPC was uniformly distributed within the solid particles.
2. The particles were considered to be spherical in shape.
3. Diffusion remained constant through the extraction process.
4. The solution was perfectly mixed, as the energy was dissipated by the ultrasonic waves.
5. Resistance to mass transfer was negligible in the liquid phase.
6. The transport of CPC from solid particles to liquid occurred through diffusion.

Based on the aforementioned assumptions, a one-dimension at non-steady state diffusion model, i.e. derived from the Fick's second law, was used to describe the mass transfer of the solute from the spherical particles:

$$\frac{\partial CPC}{\partial t} = D_{eff}\left(\frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\partial CPC}{\partial r}\right)\right) \quad (4)$$

The initial condition for solving the diffusion equation is shown below:

$$CPC(r,0) = CPC_i \quad r \geq 0 \quad (5)$$

In the meantime, the boundary conditions are described as Eq. (6) and Eq. (7):

$$\frac{\partial CPC(0,t)}{\partial r} = 0 \quad t > 0, \quad (6)$$

$$CPC(r,t) = 0 \quad t > 0$$

(assuming no solubility limitation) (7)

Where, CPC is the solute concentration in the solid particles (mg.ml^{-1}), D_{eff} is the effective diffusivity coefficient (m^2.s^{-1}), r is the radius of solid particles (m), and t is the time (min).

Based on Fick's second law, the mathematical expression that linked between the CPC_t and D_{eff} of the solute can be described as follows:

$$\frac{CPC_t}{CPC_s} = 1 - \frac{6}{\pi^2}\sum_{n=1}^{\infty}\frac{1}{n^2}\exp\left(-\frac{D_{eff}n^2\pi^2 t}{r^2}\right) \quad (8)$$

Where, n is the positive root of the Bessel function of the first kind of order zero. However, following the short extraction period, only the first

term of the series solution is considered to be significant. Then, the linearized form is achieved by applying the natural logarithm function on both sides, as follows:

$$\ln\left(\frac{CPC_s}{(CPC_s)-(CPC_t)}\right)=\ln\frac{\pi^2}{6}+\frac{D_{eff}\pi^2 t}{r^2} \quad (9)$$

The dependence D_{eff} on the temperature follows a first-order rate process, generally described by the Arrhenius equation.

$$k=k_0\exp\left(\frac{-E_a}{RT}\right) \quad (10)$$

Where, E_a is the activation energy (J.mol^{-1}), T is the temperature (K), k_0 is the pre-exponential factor (min^{-1}), and R is the gas constant (8.314 J.K^{-1}.mol^{-1}). Therefore, activation energy can be graphically determined, as the straight line obtained by plotting the $\ln k$ as a function of $1/T$ would have a slope that is equal to $-E_a/R$.

2.7 Statistical Analysis

The results were expressed as the mean ± standard deviation (mean ± SD) of triplicate data set, from an independent experiment. Statistical analysis was performed using the analysis of variance (ANOVA), followed by Duncan's multiple range test (DMRT). The differences were considered significant if $p < 0.05$ (95 % confidence interval).

The root means square error (RMSE) and coefficient of determination (R^2) was also calculated, to statistically evaluate the accuracy of the mathematical model used to simulate the extraction kinetics.

3. RESULTS AND DISCUSSION

3.1 Influencing Parameters in the CPC Extraction

3.1.1 Effects of crude S. platensis

For dried spirulina, the spirulina was oven-dried at 50°C for about 7 h. For wet spirulina, the moist spirulina was used for the extraction. The CPC from both dried and wet spirulina were extracted using the repeat freeze-thaw method, using a solid-liquid ratio of 1:5 w/v. The results are shown in Table 1.

CPC extracted from the wet *S. platensis* showed higher concentration and yield values, which agrees with the results of previously works involving the effects of drying method on the CPC content in *S. platensis*, i.e. Sarada et al. (1999) who has been studied the effect of drying method to the CPC content in the *S. platensis* which has been proposed that significant loss of CPC in the dried crude, due to its sensitivity with extremely temperature (approximately 50 %) during the oven-dried process, i.e. 1.09 mg.ml^{-1} as compared to 0.24 mg.ml^{-1}, for wet and dried *S. platensis*, respectively [3].

Table 1 Comparison of the concentration and yield of the extracted CPC from dried and wet crude *S. platensis.*

Crude *S.platensis*	CPC	Yield
Dried	1.60±0.11^{a}	79.91±5.77^{a}
Wet	2.03±0.18^{b}	101.41±9.14^{b}

The data are represented as mean±SD of three replicates. $^{a-b}$ means in the same column with different letters are significantly different ($p<0.05$)

Thus, the wet crude has been chosen as the suitable form of *S. platensis*, to be used for the CPC extraction.

3.1.2 Effect of UAE on the solid-liquid ratio

One of the important influencing parameters is the interaction between the solvent and solid. The volume of solvent should be sufficient to permit a good hydration and swelling of the solid phase, leading to a better yield of the extracted compound. The effects of UAE on the concentration and yield of the CPC were studied under UAE at 100 kHz at various solid-liquid ratios of 1:5, 1:10 and 1:15 w/v.

Table 2 Comparison of the concentration and yield of the extracted CPC using different solid-liquid ratio.

Ratio	CPC	Yield
1:5	2.40±0.18^{a}	119.56±8.99^{a}
1:10	1.07±0.02^{b}	53.71±1.23^{b}
1:15	0.46±0.05^{c}	22.92±2.74^{c}

The data are represented as mean±SD of three replicates. $^{a-b}$ means in the same column with different letters are significantly different ($p<0.05$)

The results are shown in Table 2. The UAE was found to enhance mass transfer, together with the suitable of solid-liquid ratio that indicates strongly influence the concentration and yield of the CPC. The highest values were obtained using the 1:5 w/v ratio, which is parallel with the findings reported in a previous study [11]. These results are superior to that reported by Minkova et al. (2003) and Yan et al. (2011), which involved the extraction of CPC from fresh biomass of *S. fusiformis* (1.28 mg.ml^{-1}) and *S. platensis* (90.10 µg.ml^{-1}), respectively [14, 15].

Hence, by using the smaller solid-liquid ratio, the extraction buffer is sufficient to be used in the

extraction of the CPC, as there is already liquid present in the wet *S. platensis*. On the other hand, if a larger solid-liquid ratio is being used, more impurities will be outflow from the cells, which can increase the CPC dissolution and also decrease its purity [16].

3.1.3 Effect of UAET

The influence of ultrasonic application on the extraction process and temperature are significant parameters of CPC extract, where the phenomenon is known as acoustic cavitation and the accelerating mass transfer by temperature occurred. The cavitation force able to successively accelerate the heat and mass transfer rate, disrupt the cell walls and facilitate the release of the extractable compounds. The results are shown in Table 3.

Table 3 Comparison of the concentration and yield of the extracted CPC based on different extraction methods.

Extraction method	CPC	Yield
RFT	2.03±0.29[a]	101.62±1.44[a]
UAE	2.40±0.18[b]	119.56±8.99[b]
UAET	2.55±0.14[c]	127.70±0.22[c]

The data are represented as mean±SD of three replicates. [a-b] means in the same column with different letters are significantly different ($p<0.05$)

The highest values of the concentration and yield were recorded by the UAET method at 45°C, 30 min at 100 kHz, that are 2.55 $mg.ml^{-1}$ and 127.70 $mg.g^{-1}$ which were higher than RFT about 25.62 %. These results are superior to that reported by Doke et al. (2005), who did the CPC extraction from wet biomass of *S. platensis*, and obtained yields of 86.30 $mg.g^{-1}$ for repeated freeze-thaw method and 82.10 $mg.g^{-1}$ for homogenization method [4], and superior to that reported by Manirafasha et al. (2017), who did the CPC extraction from wet biomass, and obtained CPC yield of 12.2 $mg.g^{-1}$ for ammonium chloride solution [17]. The result was also related to that reported by Choi and Lee (2018) who did the CPC extraction from S. maxima and reported the ultrasonic extraction was better than agitation method [18].

This could be explained why the UAET has the potential to explode the cell walls [19] and effectively increase the mass transfer via a thermo process [1]. Moreover, the time taken for the entire process of the RFT was higher than the UAET by about 72 h, and thus, the energy consumption and cost of reagent might be excessive.

Conclusively, the UAET method using wet *S. platensis* with the solid-liquid ratio of 1:5 w/v, has been chosen for the CPC extraction. The UAET method was evaluated by varying the controlled temperatures and ultrasonic frequencies, while the kinetics of extraction were also investigated.

3.2 Studies of UAET on the CPC Extraction

It is well-known that the amount of ultrasonic energy transmitted to the solvent plays an important role in the extraction efficiency and rate in most cases of UAET. The results of various ultrasonic frequencies with controlled temperatures were shown in Fig 2.

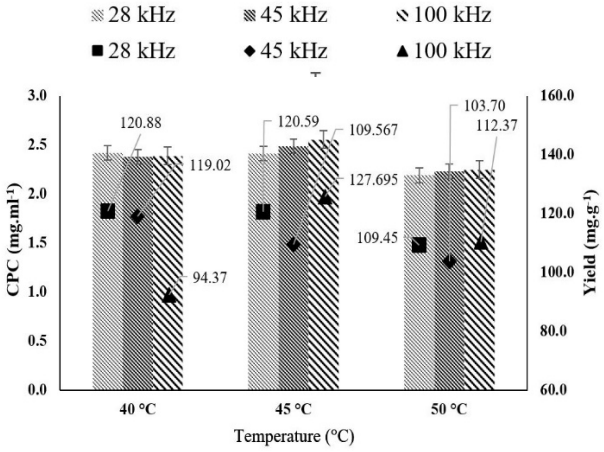
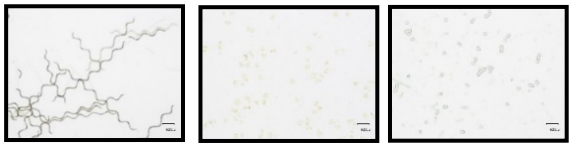


Fig. 2 The effects of UAET on the CPC and Yield (the patterns represent the CPC, while solid symbols represent the yield)

The maximum CPC concentration achieved at 45°C with 100 kHz of ultrasonic frequency. A higher extraction rate was achieved for the extraction with UA. However, a temperature higher than 50°C was found to give effect to the CPC stability, and along with the cavitation of the ultrasonic frequency, the diffusion of the solute to the solvent was enhanced. This result was related to the study by Su et al. (2014) who proposed that the extraction rate increases with increasing temperature, whereas the CPC concentration decreases with higher temperature. This indicates that the CPC was being degraded by the heat, during the extraction process [1].

Fig. 3 show that the breaking of cell wall has been enhanced by the application of UAET, which resulted in the high concentration and yield of the CPC. The concentration and yield of CPC obtained via the UAET method was higher than that of RFT. The breakdown of materials occurred following the application of ultrasonic frequency, due to the cavitation effect, which is effective in exploding the cell [5, 19]. The cavitation effect can increase the diffusion and mass transfer at the solid-liquid surfaces. Vinatoru (2001) described that the extraction mechanism involved two types of physical phenomena, i.e. diffusion through the cell wall, and washing out the cell content as the wall breaks down. [20].



(a) wet *S. platensis* (b) UAET (c) RFT

Fig. 3 Microscopic images of the *S. platensis* and its residues.

The microscopic images of the residues after two disruption methods. From Fig. 3(a), it can be seen that the fresh *S. platensis* has longer helical filaments, while in Fig. 3(b), following the UAET, the *S. platensis* was broken into shorter pieces, which can be assumed as spherical in shape. The *S. platensis* cell was reduced in size into smaller pieces and without color, which indicated that the cell wall of the *S. platensis* has been broken and the intracellular protein substance, e.g. protein and chlorophyll, can be out flown from the *S. platensis* cell. Fig. 3(c) shows the residues after the extraction with RFT, where the cell wall was broken into shorter pieces, but the color of some *S. platensis* cells were undisrupted, due to that the cells are still green and their cell walls were still remained intact, which indicated that some of the *S. platensis* cannot flow out from the protein and intracellular substances. This result was related to that reported by Tavanandi et al. (2018) who proposed the combination method resulted in increased extraction efficiency by using only one method [21].

In summary, it was hard to find a bigger cell debris when using a microscope, which indicated that the efficiency of the UAET was very high. This result is in parallel with the concentration and yield of the CPC.

3.3 Determination of Kinetic Parameters

The kinetic parameters were determined and statistically analyzed, as presented in Table 4, by using the non-linear regression. The results indicate the effect of UAET on the extraction rate constant, which was found to be in the range of 0.09 to 0.32 min^{-1}, for all the ultrasonic frequencies, used. For, the effective diffusivity, the results were found in a range of 2.80×10^{-12} , 2.69×10^{-12} and 4.33×10^{-12} $m^2.s^{-1}$ (at 45°C, using 28, 45 and 100 kHz, respectively) These indicate that the UAET can enhance the diffusion of the extraction.

The first-order kinetic model is seen to be in good agreement between the predicted and the experimental data, due to R^2 that fell within the acceptable range, and the low RMSE. This model is to be well fit for UAET which was the result of RMSE were presented in a range of 0.03 to 0.14. Together with the R^2, the values are also shown similar to RMSE values, the values were in the range of 0.87 to 0.99 that fell within the acceptable range.

Table 4 Kinetic parameters determined for the UAET of CPC, using the first-order kinetic model.

Frequency	Temp	Eq. (3)		Eq. (9)	Eq. (10)
		k	CPC_s	$D_{eff} \times 10^{-12}$	E_a
28 kHz	40	0.09	2.71	1.41	
	45	0.15	2.50	2.80	43.68
	50	0.32	2.26	2.33	
45 kHz	40	0.09	3.20	1.11	
	45	0.11	2.64	2.69	47.39
	50	0.24	2.23	2.78	
100 kHz	40	0.14	2.38	1.54	
	45	0.15	2.51	4.33	28.76
	50	0.24	1.95	3.44	

The experimental data and modeled extraction kinetics of UAET were shown in Fig. 4, these have shown the efficiency of the UAET, which can improve the aqueous solution, i.e. lower its viscosity and minimize the mass transfer resistance, with the higher temperature having significantly greater efficiency than the lower temperature. On the other hand, the temperature cannot be increased beyond certain limits, as this has been proven to be detrimental to the CPC, by inducing its thermal degradation [22]. The evaluations of the CPC concentration at various temperatures were shown in Fig 4. A faster extraction rate was obtained with higher temperature. This may be the characteristic of the UAET, which increased the diffusion coefficient of the solute at a higher temperature.

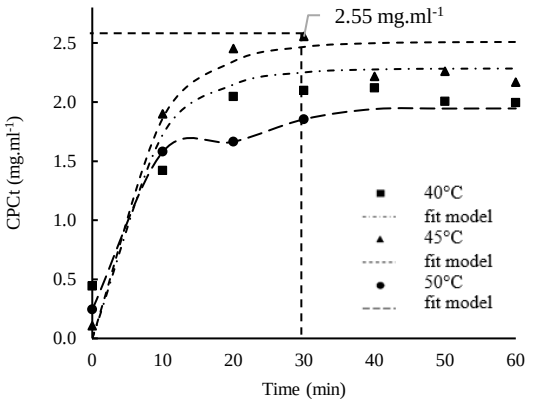


Fig. 4 Non-linear regression between the CPC_t and time values during the UAET extraction at 100 kHz.

In order to obtain the quantitative data pertaining to the effect of temperature on the extraction rate, the Arrhenius equation was used. The estimated activation energy values at 28, 45 and 100 kHz for the UAET were 43.68, 47.39 and 28.76

$kJ.mol^{-1}$, respectively. Thus, the UAET could be accelerated by the solubility of the CPC into the extraction solvent, and consequently increased the extraction rate, as compared to when only using temperature in the extraction.

4. CONCLUSIONS

UAET was found to considerably improved both the kinetics and quantitative extraction of the CPC in *S. platensis*. The optimal extraction conditions were achieved at 30 min and 45°C, with an ultrasonic frequency of 100 kHz, which values of the CPC and yield were recorded as 2.55 $mg.ml^{-1}$ and 127.70 $mg.g^{-1}$, respectively. A first-order kinetic model was successfully developed for describing the mechanism of UAET. The model provides the prediction of CPC concentration during the extraction and the kinetic parameters. This study has demonstrated the efficiency of the UAET, by providing an efficient method to produce the CPC extracts, with reduced time and energy, which has the potential for applications in the plant extractions.

5. ACKNOWLEDGMENTS

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6. REFERENCES

[1] Su C.-H., Liu C.-S., Yang P.-C., Syu K.-S., and C Chiuh.-C., Solid-liquid extraction of phycocyanin from Spirulina platensis: Kinetic modeling of influential factors, Separation and Purification Technology, Vol. 123, 2014, pp. 64-68.

[2] Chaiklahan R., Chirasuwan N., Triratana P., Tia S., and Bunnag B., Effect of extraction temperature on the diffusion coefficient of polysaccharides from Spirulina and the optimal separation method, Biotechnology and Bioprocess Engineering, Vol. 19, 2014, pp. 369-377.

[3] Sarada R., Pillai M. G., and Ravishankar G. A., Phycocyanin from Spirulina sp: influence of processing of biomass on phycocyanin yield, analysis of the efficacy of extraction methods and stability studies on phycocyanin, Process Biochemistry, Vol. 34, Issue 8, 1999, pp. 795-801.

[4] Doke Jayant M., An Improved and Efficient Method for the Extraction of Phycocyanin from Spirulina sp, International Journal of Food Engineering, Vol. 1, 2005, pp. 1-11.

[5] Chaiklahan R., Chirasuwan N., Loha V., Tia S., and Bunnag B., Separation and purification of phycocyanin from Spirulina sp. using a membrane process, Bioresour Technol, Vol. 102, 2011, pp. 7159-7164.

[6] Silveira S. T., Burkert J. F., Costa J. A., Burkert C. A., and Kalil S. J., Optimization of phycocyanin extraction from Spirulina platensis using factorial design, Bioresour Technol, Vol. 98, Issue 8, 2007, pp. 1629-1634.

[7] Zhu Y., Chen X. B., Wang K. B., Li Y. X., Bai K. Z., Kuang T. Y., A simple method for extracting C-phycocyanin from Spirulina platensis using Klebsiella pneumoniae, Appl Microbiol Biotechnol, Vol. 74, 2007, pp. 244-248.

[8] Suman S., Neha P., Nakuleshwar D. J., Manoj K., and Sharma G., Optimization purification and characterization of phycocyanin from *Spirulina platensis*, International Journal of Applied and Pure Science and Agriculture, Vol. 2, Issue 3, 2016, pp. 15-20.

[9] Karabegovic I., Nikolova M., Velickovic D., Stojicevic S., Veljkovic V., and Lazic M., Comparison of antioxidant and antimicrobial activities of methanolic extracts of the Artemisia sp. recovered by different extraction techniques, Chinese Journal of Chemical Engineering, Vol. 19, Issue 3, 2011, pp. 504-511.

[10] Hadiyanto, Suttrisnorhadi, Heri Sutanto, and Meiny Suzery, Phyocyanin extraction from microalgae Spirulina platensis assisted by ultrasound irradiation: the effect of time and temperature, Songklanakarin J. Sci. Technol., Vol. 38, Issue 4, 2016, pp. 391-398.

[11] Ruangyot T., Jaturonglumlert S., Nitatwichit C., and Varith J., Factors affecting phycocyanin extraction from Spirulina platensis by using freezing and thawing combined with ultrasonic method, Journal of fisheries technology research, Vol. 10, Issue 4, 2016, pp. 78-87.

[12] Jaturonglumlert S., Promya J., and Varith J., Modeling of *Spirulina* growth rate with led illumination and applications, Engng.J.CMU., Vol. 24, Issue 1, 2017, pp. 142-151.

[13] Makris D., Kinetics of ultrasound-assisted flavonoid extraction from agri-food solid wastes using water/glycerol mixtures, Resources, Vol. 5, Issue 7, 2016, pp.1-10.

[14] Minkova K. M., Tchernov A. A., Tchorbadjieva M. I., Fournadjieva S. T., Antova R. E., and Busheva M. C., Purification of C-phycocyanin from Spirulina (Arthrospira) fusiformis, Journal of Biotechnology, Vol. 102, Issue 1, 2003, pp. 55-59.

[15] Yan S.-G., Zhu L.-P., Su H.-N., Zhang X.-Y., Chen X.-L., Zhou B.-C., Single-step chromatography for simultaneous purification of C-phycocyanin and allophycocyanin with high purity and recovery from Spirulina

(Arthrospira) platensis, Journal of Applied Phycology, Vol. 23, Issue 1, 2011, pp. 1-6.

[16] Shaochen Guan, Extracting phycocyanin from spirulina and hydrothermal liquefaction of its residues to produce bio-crude oil," Master, Agricultural and Biological Engineering, Illinois at Urbana-Champaign, Illinois, 2016.

[17] Manirafasha E., Murwanashyaka T., Ndikubwimana T., Yue Q., Zeng X., Lu Y., Ammonium chloride: a novel effective and inexpensive salt solution for phycocyanin extraction from Arthrospira (Spirulina) platensis, Journal of Applied Phycology, Vol. 29, Issue 3, 2017, pp. 1261-1270.

[18] Choi W. and Lee H., Effect of Ultrasonic Extraction on Production and Structural Changes of C-Phycocyanin from Marine Spirulina maxima, International Journal of Molecular Sciences, Vol. 19, Issue 1, 2018, pp. 220-231.

[19] Duangsee R., Phoopat N., and Ningsanond S., Phycocyanin extraction from Spirulina platensis and extract stability under various pH and temperature, Asian Journal of Food and Agro-Industry, Vol. 20, Issue 4, 2009, pp. 819-826.

[20] Vinatoru M., An overview of the ultrasonically assisted extraction of bioactive principles from herbs, Ultrasonics Sonochemistry, Vol. 8, Issue 3, 2001, pp. 303-313.

[21] Tavanandi H. A., Mittal R., Chandrasekhar J., and Raghavarao K. S. M. S., a Simple and efficient method for extraction of C-Phycocyanin from dry biomass of Arthospira platensis, Algal Research, Vol. 31, 2018, pp. 239-251.

[22] Antelo F. S., Costa J. A. V., and Kalil S. J., Thermal degradation kinetics of the phycocyanin from Spirulina platensis, Biochemical Engineering Journal, Vol. 41, Issue 1, 2008, pp. 43-47.