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Antioxidant activity optimisation of *Spirulina platensis* C-phycocyanin obtained by freeze-thaw, microwave-assisted and ultrasound-assisted extraction methods

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RESEARCH ARTICLE

Abstract

In vitro antioxidant activities of C-phycocyanin extracts obtained from *Spirulina platensis* by freeze-thaw, microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE) methods were evaluated according to 1,1-diphenyl-2-picryl-hydrazyl radical scavenging capacity. Full factorial design was used to optimise the extraction time and medium pH using multiple linear regression analysis. The third-order polynomial models with high R^2 values (0.925, 0.946 and 0.858) were sufficiently fitted to the experimental antiradical activities of C-phycocyanin extracted by the three extraction methods. The optimised results showed that the lowest IC_{50} value for C-phycocyanin extracted by freeze-thaw extraction method (IC_{50} = 0.031 mg/ml) was obtained after 4 h of extraction at pH=8.00. While these values for MAE (IC_{50} = 0.021 mg/ml) and UAE (IC_{50} = 0.036 mg/ml) methods were 22.5 s at pH=8.00, and 6.71 min at pH=7.57, respectively. The models adequacy was confirmed by extracting the C-phycocyanin under the optimum values suggested by the models. Results showed that MAE could be a better method for C-phycocyanin extraction from *S. platanssis* in terms of antioxidant activity.

Keywords: algae, functional components, microwave-assisted extraction, phycocyanin, radical scavenging capacity, ultrasonic

1. Introduction

Spirulina platensis, the blue-green planktonic algae, is classified under the cyanobacteria group. Currently, *S. platensis* is gaining more attention owing to its various nutritional and medicinal characteristics. The nutritional value of this high-quality health-food derives from the high content of its protein (~70%), vitamins, minerals, polyunsaturated fatty acids, zeaxanthin and mycoxanthophyll (Li *et al.*, 2003; Simpore *et al.*, 2006). C-phycocyanin is a blue pigment found in all cyanobacteria (Gantar and Svircev, 2008). Existence of such natural antioxidants can inhibit several major human diseases including different kinds of cancers (Belay *et al.*, 2002). Bobbili *et al.* (2003) and Reddy *et al.* (2003) have particularly described the induction of apoptosis as a mechanism of C-phycocyanin activity. C-phycocyanin can potentially be used in different food preparations to provide a unique natural blue colour and when necessary to postpone the peroxidation of unsaturated fatty acids in various food formulas. To obtain C-phycocyanin from its algal sources, a proper extraction method needs to be sought. The laboratory involved with the current study has also been practicing several methods including supercritical fluid extraction, microwave-assisted extraction assisted (MAE), ultrasound-assisted extraction (UAE) and regular solvent extraction to obtain numerous functional components from their natural sources (Bashi *et al.*, 2012; Kazazi and Rezaei, 2010; Rezaei *et al.*, 2013; Rezvanpanah *et al.*, 2008; Temelli *et al.*, 2013). MAE was mainly used for the extraction of essential oils and water soluble compounds (Golmakani *et al.*, 2008a,b; Mazidi *et al.*, 2012; Rezaei *et al.*, 2013). Considering the extraction of C-phycocyanin from *S. platensis*, several methods such as conventional

water extraction, sonication, freezing and thawing, homogenisation, buffer extraction, acid extraction have been reported in literature (Sivasankari *et al.,* 2014). One other extraction method that is time-incentive is MAE, which has not been reported for the extraction of C-phycocyanin. Moreover, the extraction process of this component from *S. platensis* needs to be optimised by certain mathematical models to obtain the highest antioxidant activity. Compared to the single-factor method, a full-factorial design can be more efficiently applied for the optimisation of parameters with all numbers of experimental trials. To the best of authors' knowledge, no specific study has so far been reported on the optimisation of radical scavenging capacities of C-phycocyanin extracted from *S. platensis* using different extraction methods. Therefore, the objective of the present study was to optimise the antioxidant properties of C-phycocyanin obtained using a freeze-thaw (FT) extraction method, MAE and UAE as pre-treatments before a freeze-thaw cycle applying a full factorial design considering the extraction time and medium pH as main parameters.

2. Materials and methods

Cultivation of microalgae

S. platensis microalgae was obtained from the Department of Food Science, Engineering and Technology, University of Tehran (Tehran, Iran) and cultivated in a modified Zarrouck medium, each litre of which contained 2.5 g $NaNO_3$, 1 g K_2SO_4, 1 g NaCl, 0.2 g $MgSO_4.7H_2O$, 0.04 g $CaCl_2$, 0.01 g $FeSO_4.7H_2O$, 0.08 g Na_2EDTA, 0.5 g K_2HPO_4, 10.8 g $NaHCO_3$, 7.6 g Na_2CO_3, 1 ml trace solution A_5 and 1 ml trace solution B_6. One litre of trace solution A_5 contained 2.86 g H_3BO_3, 1.81 g $MnCl_2.4H_2O$, 0.222 g $ZnSO_4.7H_2O$, 0.079 g CuSO4.5H_2O and 0.015 g MoO_3. One litre of trace solution B_6 contained 0.023 g NH_4VO_3, 0.096 g $KCr(SO_4)_2.12H_2O$, 0.045 g $NiSO_4.6H_2O$, 0.018 g $Na_2O_4.2H_2O$ and 0.048 g $Ti(SO_4)_2$+$TiSO_4$ (Vernerey *et al.*, 2001). All the used chemicals were purchased from Merck Chemical Co. (Darmstadt, Germany). The cells growth was under a continuous white light illumination (at 4,000 Lux) for 15 days at 25 °C, when they reached the exponential phase. At the end of cultivation, the biomass was recovered by filtration under vacuum and dried at 40 °C for 48 h, grinded and sieved (Silveira *et al.*, 2007).

Extraction of C-phycocyanin

C-phycocyanin was extracted using three extraction methods. The freeze-thaw extraction of C-phycocyanin was carried out by mixing the different buffers (1.0 M each) and spirulina powders with a solvent to sample ratio of 0.08 g/ml and 0.02% sodium azide as preservative and went through 4 cycles of freezing at -18 °C and thawing at 4 °C for 4 cycles (each cycle for 1 h). The buffers (1.0 M) were prepared at pH levels of 5.0 (acetate buffer), 6.0 and 7.0 (phosphate buffer), and 8.0 (Tris-Cl buffer) (Silveira *et al.*, 2007). For MAE, a microwave oven (model MC175, frequency 2,450 MHz; AEG, Nuremberg, Germany) was used to extract C-phycocyanin from the algal powders. Samples were irradiated at 90 W for 0-50 s (with 5 s intervals) (selected based on a pre-evaluation process) and then used for one FT cycle. For conducting UAE, a 4-L ultrasound system (model DSA100-SK1; Universal Ultrasonic Cleaner, Guangdong, China) followed by one FT cycle was used to extract C-phycocyanin from *S. platensis* powders for 1-9 min at 1-min intervals. Samples were then cooled in order to avoid local overheating. Preliminary experiments were carried out to determine the effects of various operating conditions in UAE. Frequency and power applied for this process were 40 kHz and 100 W, respectively. After the extraction, the mixtures were centrifuged at 17,000×*g* for 45 min and then the clear blue supernatants were pooled and stored at 4 °C until analysed for the antioxidant activity of C-phycocyanin solution.

DPPH radical-scavenging capacity

Antioxidant activities of the obtained extracts were measured in terms of radical scavenging ability using the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH•; Sigma Aldrich Co., St. Louis, MO, USA) as previously described by Brand-Williams *et al.* (1995). At first, three different concentrations of each extract were prepared. DPPH• was dissolved in methanol at a concentration of 25 mg/ml and stored at 4 °C under dry and dark conditions and 0.1 ml of each concentration was then mixed with 3.9 ml of DPPH• solution. The control sample was prepared with the same volume of methanol (0.1 ml) instead of extract solutions. All samples were placed in the dark for 30 min and their absorbances were then recorded using a UV-visible spectrophotometer (UV-160A; Shimadzu, Kyoto, Japan) at 515 nm. The DPPH• concentration in the reaction medium was determined by linear regression according to the equation $Å_{515} = 0.269\ [DPPH•] + 0.0024$. The percentage of remaining DPPH• was calculated as follows:

$$\%[DPPH•]_{rem} = \frac{[DPPH•]_{test}}{[DPPH•]_{control}} \times 100 \quad (1)$$

where $[DPPH•]_{control}$ is the initial concentration of DPPH• and $[DPPH•]_{test}$ is that of DPPH• after the extract solution was added, measured after 30 min of incubation in dark. $\%[DPPH•]_{rem}$ was then plotted against the extract concentration to determine IC_{50} values. This value is defined as the amount of extract necessary to decrease the initial DPPH• concentration by 50%. A lower IC_{50} value indicates a higher antiradical activity.

Experimental design and statistical analysis

After conducting the preliminary study, effects of two main parameters of the extraction time (X_1), and buffer pH (X_2) on the antioxidant activities (Y) of C-phycocyanin extracts obtained by FT, MAE and UAE methods were studied using Design-Expert software (trial version 7.1.6; Stat-Ease Inc., Minneapolis, MN, USA) according to a full factorial design. Each variable was selected based on preliminary literature review and primary experimental trials (Table 1). Responses were then analysed to provide information about main effects and interactions. The response functions (Y) were related to the coded variables (X_i, i = 1, 2) by a third-order polynomial using Equation 2:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{111}X_1^3 + b_{222}X_2^3 + b_{112}X_1^2X_2 + b_{122}X_1X_2^2 \quad (2)$$

The coefficients of the polynomial are represented by b_0 (constant term), b_1 and b_2 (linear effects), b_{11} and b_{22} (quadratic effects) and b_{12} (interaction effects), b_{111} and b_{222} (cubic effects) and b_{112} and b_{122} (interaction effects).

The significance of the equation parameters for each response variable was also assessed by *F*-ratio at a probability (*P*) of 0.05. The adequacy and fitness of mathematical models were tested by the analysis of variance (ANOVA). Adequacy levels for the regression models were evaluated by determining R^2, adjusted R^2, coefficient of variation (CV), and adequate precision (ADP).

3. Results and discussion

Model fitting

Model validation and verification are possibly the most important steps in the model building sequence. Most of the time, this evaluation is carried out by R^2 statistical analysis, lack of fit (assessing the correctness of the functional part of the model) and graphical residual analysis. As analysis showed no indication of significant (P<0.05) lack of fit was observed for the final reduced MAE and UAE models so ensuring satisfactory fitness of the reduced cubic models to the significant (P<0.0001) independent variable effects (Tables 2 and 3). In contrast, in FT experiments significant model term may occur due to the noise (Table 4). All the remaining parameters were significant at P<0.05. The statistical analysis of the results generated the following polynomial equations for antioxidant activities (shown by IC_{50} value) of C-phycocyanin extracted by MAE (Y_1, Equation 3), UAE (Y_2, Equation 4) and FT (Y_3, Equation 5) methods, respectively:

$$Y_1 = 0.052 - 9.773E\text{-}003X_1 + 0.018X_2 - 9.982E\text{-}003X_1^2 + 0.014X_2^2 + 3.847E\text{-}003X_1X_2 - 4.741E\text{-}003X_1^3 - 5.927E\text{-}003X_2^3 \quad (3)$$

$$Y_2 = 0.059 - 0.045X_1 - 0.010X_2 + 2.171E\text{-}003X_1^2 + 7.077E\text{-}003X_1X_2 + 8.153E\text{-}003X1X_2^2 + 0.025X_1^3 \quad (4)$$

$$Y_3 = 0.049 - 0.014X_1 - 3.583E\text{-}003X_2 \quad (5)$$

The suitability/fit of the models were also tested using the R^2 and R^2_{adj} for them. High values of R^2 (0.925, 0.946 and

Table 1. Experimental high and low levels of independent variables for the antioxidant activity in the current study to be used in the full factorial design.[1]

Variables	Symbol	-1	+1
Buffer pH	X_1	5	8
Extraction time	X_2 (MAE, s)	15	50
Extraction time	X_2 (UAE, min)	1	9
Extraction time	X_2 (freeze-thaw, h)	2	4

[1] MAE = microwave-assisted extraction; UAE = ultrasound-assisted extraction.

Table 2. Estimated regression coefficients for the cubic polynomial model and ANOVA for the experimental results in the optimisation of antioxidant activity of C-phycocyanin (expressed as IC_{50}) obtained by microwave-assisted extraction.[1]

Source	DF	Coefficient	Sum of squares	*P*-value
Model	7	0.052	0.023	<0.0001
Linear				
X_1	1	-9.773E-003	4.019E-004	<0.0001
X_2	1	0.018	1.805E-003	<0.0001
Quadratic				
X_{11}	1	-9.982E-003	1.889E-003	<0.0001
X_{22}	1	0.014	2.470E-003	<0.0001
Interaction				
X_{12}	1	3.847E-003	3.383E-004	0.0001
Cubic				
X_{111}	1	-4.741E-003	8.526E-005	0.0449
X_{222}	1	-5.927E-003	1.362E-004	0.0118
Residual	88		1.813E-003	
Lack-of-fit	24		1.299E-003	0.1011
Pure error	64		5.148E-004	
Core Total	95		0.024	
R^2		0.9257		
Adj-R^2		0.9198		
CV		8.72		
ADP		43.490		

[1] X_1 = pH; X_2 = Time; X_{11} = pH^2; X_{12} = pH×Time; X_{111} = pH^3; X_{222} = Time; Adj-R = adjusted R; CV = coefficient of variation; ADP = adequate precision.

Table 3. Estimated regression coefficients for the quadratic polynomial model and ANOVA for the experimental results in the optimisation of antioxidant activity of C-phycocyanin (expressed as IC_{50}) obtained by ultrasound-assisted extraction.[1]

Source	DF	Coefficient	Sum of squares	*P*-value
Model	6	0.059	0.032	<0.0001
Linear				
X_1	1	-0.045	8.806E-003	<0.0001
X_2	1	-0.010	4.548E-003	<0.0001
Quadratic				
X_{11}	1	2.171E-003	1.005E-004	0.0203
Interaction				
X_{12}	1	7.077E-003	1.252E-003	<0.0001
Cubic				
X_{111}	1	0.025	2.562E-003	<0.0001
X_{122}	1	8.153E-003	5.332E-004	<0.0001
Residual	101		1.825E-003	
Lack-of-fit	29		6.388E-004	0.1610
Pure error	72		1.186E-003	
Core Total	107		0.034	
R^2		0.9460		
Adj-R^2		0.9428		
CV		7.07		
ADP		46.986		

[1] X_1 = pH; X_2 = Time; X_{11} = pH^2; X_{12} = pH×Time; X_{111} = pH^3; X_{222} = Time; Adj-R = adjusted R; CV = coefficient of variation; ADP = adequate precision.

Table 4. Estimated regression coefficients for the linear model and ANOVA for the experimental results in the optimisation of antioxidant activity of C-phycocyanin (expressed as IC_{50}) obtained by freeze-thaw method.[1]

Source	DF	Coefficient	Sum of squares	*P*-value
Model	2	0.049	2.966E-003	<0.0001
Linear				
X_1	1	-0.014	2.658E-003	<0.0001
X_2	1	-3.583E-003	3.082E-004	<0.0001
Residual	21		4.551E-004	
Lack-of-fit	5		3.629E-004	0.0801
Pure error	16		9.227E-005	
Core Total	23		3.422E-003	
R^2		0.8670		
Adj-R^2		0.8543		
CV		9.59		
ADP		21.511		

[1] X_1 = pH; X_2 = Time; X_{11} = pH^2; X_{12} = pH×Time; X_{111} = pH^3; X_{222} = Time; Adj-R = adjusted R; CV = coefficient of variation; ADP = adequate precision.

0.867) and R^2_{adj} (0.919, 0.942 and 0.854) indicate that the fitted models can be used for the prediction with reasonable precision. The CV values of 8.72, 7.07 and 9.59% indicate that the experiments performed are more precise and highly reliable. The ADP measures the signal-to-noise ratio, with a ratio greater than 4 being desirable. The ADP values of 43.490, 46.986 and 21.511 indicated that the quadratic models could be used to navigate the design space. The validity of the polynomial models has also been established by comparing their experimental and predicted values (Figure 1) and drawing their normal probability plot of the studentized residuals to check for normality of residuals (Figure 2). The results adequately confirmed the models validity.

Optimisation of the microwave-assisted extraction

The ANOVA results (Table 2) revealed that the antioxidant activity of C-phycocyanin obtained by the MAE method was significantly affected by the linear terms (P<0.0001 for both X_1 and X_2), quadratic terms (P<0.0001 for both X_{11} and X_{22}), cubic terms (P=0.0449 for X_{111}, P=0.0118 for X_{222}) and the interaction term (P<0.0001) among all variables. Based on sum of squares and coefficients in Equation 3, positive and linear effects of irradiation time had the largest effect on the IC_{50} value (Table 2). Figure 3A shows that the IC_{50} value decreased until 25 s then increased by increasing irradiation time in all studied pH levels. Figure 4A shows that IC_{50} values could decrease with an increase in the buffer pH. Figure 5A suggests that an increase in the IC_{50} value or a decrease in the antioxidant activity of C-phycocyanin could be obtained by increasing the extraction time from 25 to 50 s. The lowest IC_{50} level (0.021 mg/ml, optimum point suggested by the model) during MAE process was obtained at 22.45 s and pH=8.00, respectively (Table 5).

High activity of C-phycocyanin as a water-soluble and highly fluorescent protein may be due to high degree of conjugation of double bonds, which strongly stabilised and quenched the DPPH radical (Wada *et al.,* 2013). Moreover, some amino acid residues in the apoprotein part of C-phycocyanin are known to exhibit antioxidant activity (Patil and Raghavarao, 2007). The increase in the extraction time increases the reactive site to the effective extraction process, which enhances the concentration and antioxidant activity of C-phycocyanin obtained from *S. platensis* (Tong *et al.*, 2012). It has been demonstrated that *S. platensis* protein extract can effectively inhibit the generation of hydroxyl radical in a concentration-dependent manner (Bermejo *et al.*, 2008). Tong *et al.* (2012) also reported that a 45 min-time can optimally enhance the dissolving power of chlorophylls in *S. platensis* during MAE procedure. Kao *et al.* (1975) showed that the medium pH is a key parameter in controlling C-phycocyanin aggregation and dissociation to form monomers, trimers, hexamers and other oligomers in solution. Chaiklahan *et al.* (2012) found that 82% of

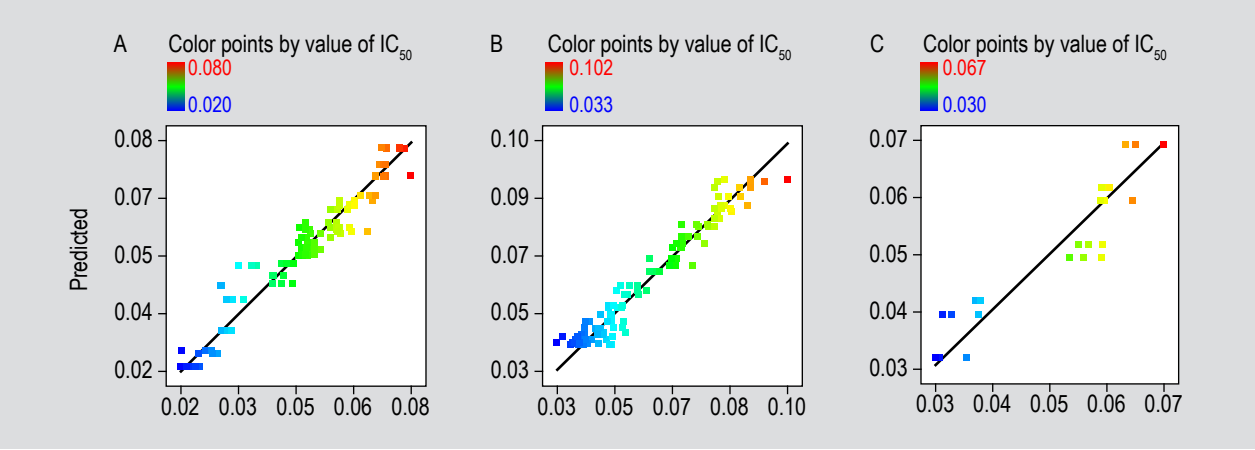

Figure 1. The predicted versus actual antioxidant activities (IC_{50}) of C-phycocyanins extracted by (A) microwave-assisted extraction, (B) ultrasound-assisted extraction, and (C) freeze-thaw methods.

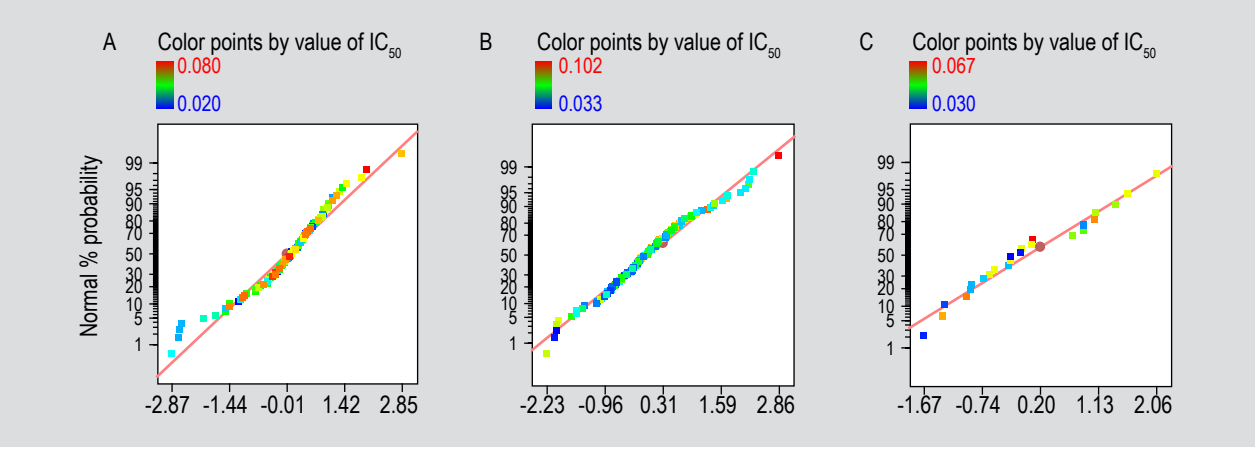

Figure 2. Normal probability plots of the studentized residuals for the antioxidant activities (IC_{50}) of C-phycocyanin extracts obtained by (A) microwave-assisted extraction, (B) ultrasound-assisted extraction, and (C) freeze-thaw methods.

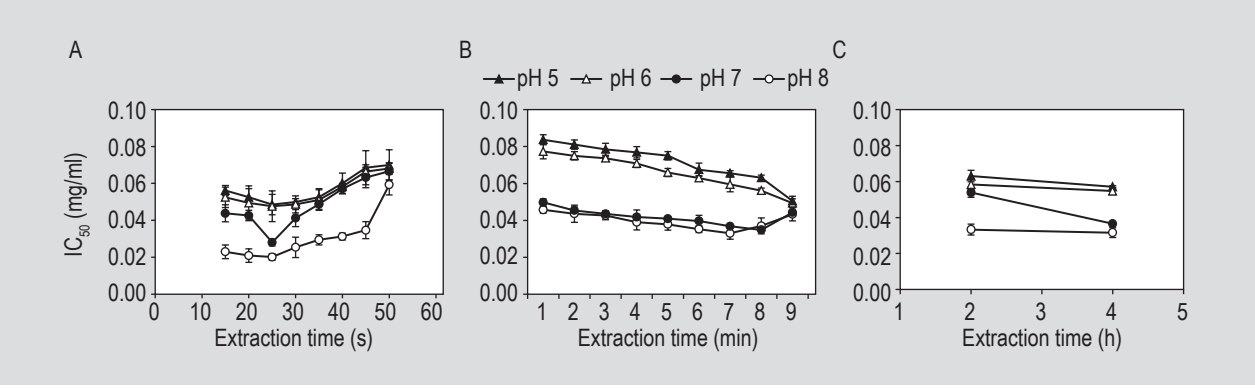

Figure 3. Antioxidant activities of C-phycocyanins obtained by (A) microwave-assisted extraction method, (B) ultrasound-assisted extraction method, and (C) freeze-thaw method as a function of extraction time and medium pH.

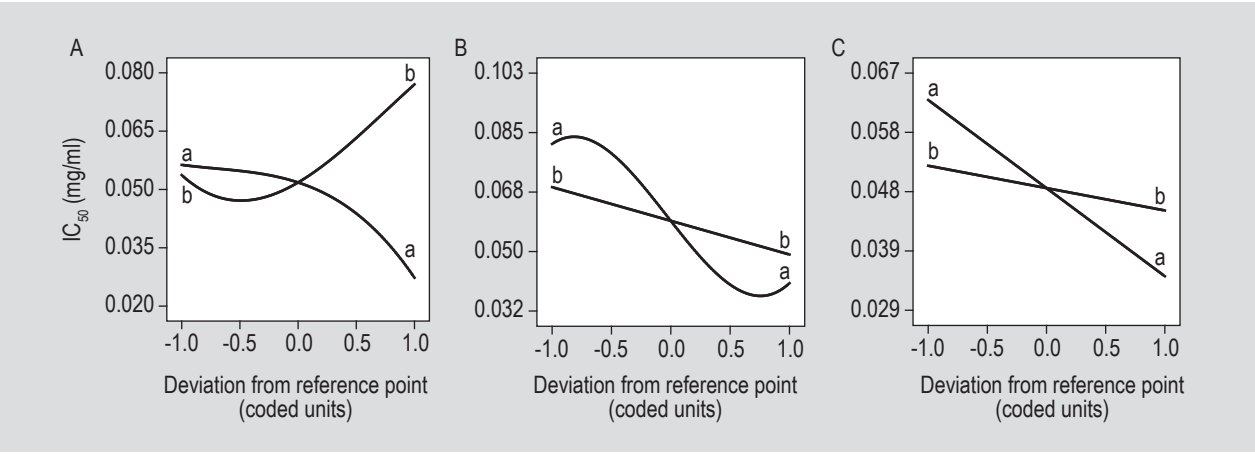

Figure 4. Perturbation graphs showing the effects of independent variables on the antioxidant activities of C-phycocyanin obtained by (A) microwave-assisted extraction method (actual factors: a: pH=6.5; b: time = 32.5 min), (B) ultrasound-assisted extraction method (actual factors: a: pH=6.5; b: time = 5.0 min), and (C) freeze-thaw method (actual factors: a: pH=6.5; b: time = 3 h).

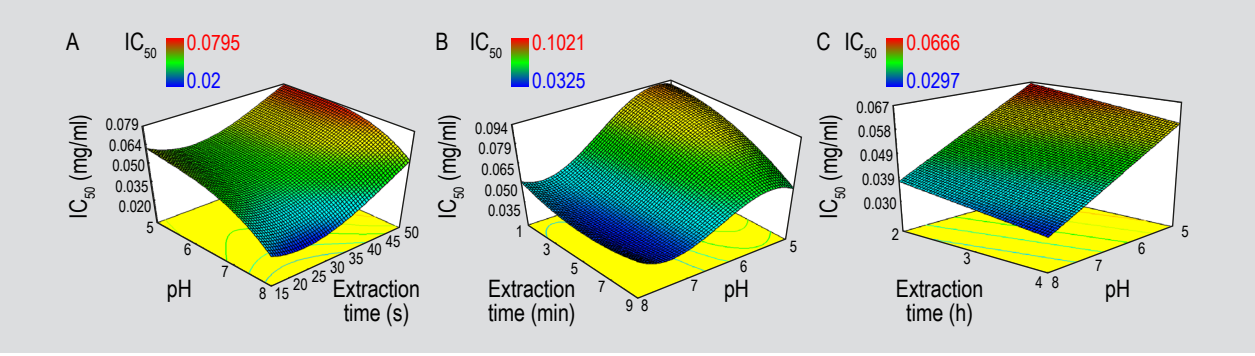

Figure 5. Combined effects of buffer pH and extraction time on the antioxidant activities of C-phycocyanin extracted by (A) microwave-assisted extraction, (B) ultrasound-assisted extraction, and (C) freeze-thaw methods.

Table 5. Optimum conditions of independent variables predicted and experimental IC_{50} values in the three extraction methods applied in the current study.

Extraction method	Optimum independent variables	Optimum response (mg/ml)	
		Predicted	Experimental[1]
MAE	X_1 (8.00) + X_2 (22.45 s)	0.021	0.022±0.002
UAE	X_1 (7.57) + X_2 (6.71 min)	0.036	0.033±0.003
Freeze-thaw	X_1 (8.00) + X_2 (4 h)	0.031	0.031±0.003

[1] Mean ± standard deviation (n=3).

C-phycocyanin at pH=7.0 was trimeric and highly soluble and higher extraction rate of this bioactive component and its antioxidant activity at this pH can be related to its higher solubility and diffusion rate.

Optimisation of the ultrasound-assisted extraction

As shown in Table 3, the linear, cubic and quadratic interaction effects of two independent variables were significant ($P<0.0001$). The variables with the largest effect on IC_{50} value were the linear terms of extraction time and buffer pH, respectively. It was clear that increasing

extraction time at all pH levels of this study decreased the IC_{50} value (Figure 3B). The effects of independent variables on the IC_{50} value as shown in perturbation graph (Figure 4B) and response surface plot (Figure 5B) also revealed that extraction time and buffer pH considerably decreased this factor. IC_{50} value varied between 0.102 and 0.033 mg/ml under different extraction conditions of UAE. The lowest IC_{50} (0.036 mg/ml, optimum point by the model) was observed when extraction time and extraction pH were 6.71 min and 7.57, respectively (Table 5). Duangsee *et al.* (2009) reported that C-phycocyanin is stable in a pH range of 5.0-8.0, however, low levels of this antioxidant component at pH values lower than neutral point can probably be contributed to the conformational changes of tetrapyrrole chromophores due to the unfolding of protein part imposing a particular conformation in the C-phycocyanin structure.

Optimisation of the freeze-thaw extraction method

The results of FT experiments showed that the linear effects of the two studied independent variables on the IC_{50} value were highly significant (P<0.0001) (Table 4). The most significant effect on the antioxidant activity of C-phycocyanin was revealed to be the linear effect of pH followed by the linear term of extraction time (Table 4; Equation 5). For all the pH levels studied (5.0-8.0), increasing the extraction time up to 4 h decreased the IC_{50} value (Figure 3C). An increase in the FT extraction time and the medium pH can decrease the IC_{50} value and increase the antioxidant activity of C-phycocyanin obtained from *S. platensis* (Figure 4C and 5C). The IC_{50} value varied between 0.030 to 0.067 mg/ml in different extraction conditions, the lowest IC_{50} value (0.031 mg/ml; optimum point of the model) was observed when extraction time and extraction pH were 4 h and 8.00, respectively (Table 5). Increasing the extraction time up to 4 h accelerates the penetration of solvent into cells and the release of C-phycocyanin from cells into the solvent resulting in higher antioxidant activity (Siddhuraju and Becker, 2007). More extraction times can lead to the drainage of other components from the cell walls and as a consequence decrease the purity of C-phycocyanin and thus its antioxidant activity. Also, the aggregation of impurities in the surface layers can inhibit C-phycocyanin secretion into the outer layers (Zhang *et al.*, 2015).

Verification of the predictive models

The suitability of the models for predicting optimum response (lowest IC_{50} value) was tested at pH=8.00 and extraction time of 22.5 s (in MAE method), pH=7.57 and extraction time of 6.71 min (in UAE method), and pH=8.00 and extraction time of 4 h (in FT extraction method). Verification experiments performed at the predicted conditions derived from the analysis of full factorial design exhibited that experimental values were reasonably close to the predicted values (P<0.05) confirming the validity and adequacy levels of the predicted models (Table 5). Figure 6 also compared the IC_{50} value of C-phycocyanin obtained by the three studied procedures. Results showed that C-phycocyanin obtained from MAE had the lowest IC_{50} values followed by C-phycocyanin obtained from FT and UAE methods. Although UAE did not require long extraction time, it is usually known that ultrasonication could induce the generation of free radicals within the liquid medium causing the oxidation and degradation of C-phycocyanin (Hemwimon *et al.*, 2007). Degradation of C-phycocyanin in MAE process could also be resulted from the microwave radiation (Pan *et al.*, 2010).

4. Conclusions

In the current study, C-phycocyanin was extracted with selected combinations of extraction time and buffer pH. Decreased polynomial models with high R^2 (0.925, 0.946 and 0.867) values were satisfactorily fitted to the experimental data. Under the optimal conditions, the lowest IC_{50} values for MAE, FT, and UAE processes were 0.021, 0.031, 0.036 mg/ml, respectively, which corresponded well with the values predicted by the constructed models. Although the C-phycocyanin extracted by MAE method under the

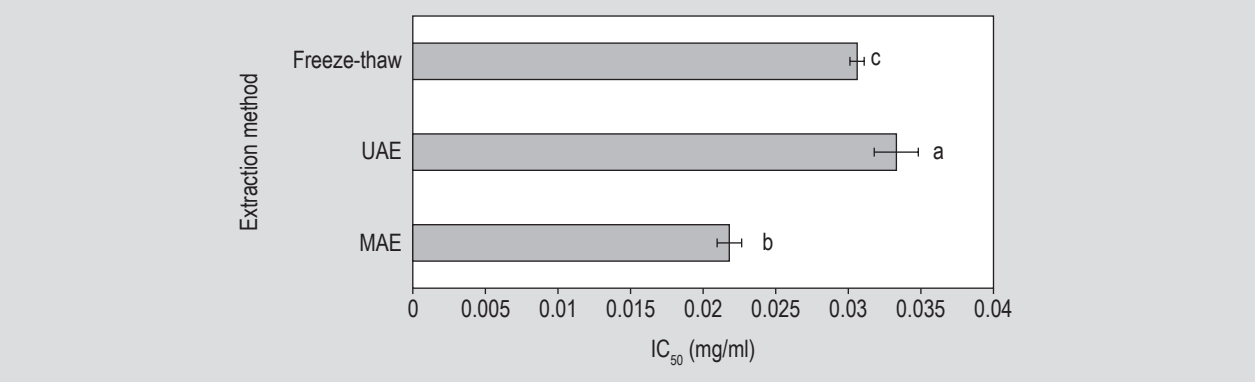

Figure 6. Comparison of the antioxidant activities of C-phycocyanin obtained by microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), and freeze-thaw (FT) methods at their optimal points.

optimal conditions had higher antioxidant activity than those of UAE and FT methods, it is necessary to provide a feasibility programme by considering the short-time extraction and other parameters such high C-phycocyanin concentration and purity ratio in order to select an optimal technique for extracting this functional component from *S. platensis* in an industrial scale.

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