Response surface optimization of ultrasound-assisted protein extraction from *Spirulina platensis*: investigation of the effect of extraction conditions on techno-functional properties of protein concentrates

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Abstract In the present study, optimum extraction conditions of proteins from *Spirulina platensis* were determined by using response surface methodology. Box–Behnken design of experiments was performed to investigate the effects of temperature (25, 35, 45 °C), pH (7, 8, 9) and time (60, 90, 120 min) on water absorption capacity (WAC), oil absorption capacity (OAC), emulsifying activity (EA), emulsifying stability (ES), foaming capacity (FC) and foaming stability (FS) of the protein concentrates (PC). WAC of the PC varied with time while OAC changed with temperature and pH. The PC had high values of OAC (10.22 g of oil/g of protein) and WAC (5.12 g of water/g of protein) under extraction conditions of pH of 8 temperature of 45 °C and time of 60 min. On the contrary, there were no significant changes in emulsifying properties of PC under different extraction conditions ($p > 0.05$), while foaming stability of the PC varied with the interactive effect of temperature and time. The optimum extraction conditions were 43.87 °C for temperature, 7.16 for pH and 60 min for time. Under these conditions, WAC, OAC, FC, FS, EA and ES values were obtained as 4.41 g of water/g, 10.13 g of oil/g, 300%, 83.94%, 42.99% and 92.50%, respectively. The results obtained through this investigation indicated that protein concentrates from *Spirulina*

platensis have higher functional properties than other algae. Therefore, *Spirulina platensis* proteins can be thought as an inexpensive source of protein for food industry due to its extraordinary functionality.

Keywords *Spirulina platensis* · Microalgae · Protein · Functionality · Response surface methodology (RSM)

Introduction

Microalgae are microscopic single-celled organisms with ability of photosynthesis. They contain numerous valuable bioactive compounds such as proteins, carbohydrates, lipids, carotenoids or vitamins (Priyadarshani and Rath 2012). They are considered as one of the most interesting and unconventional sources of proteins with potential beneficial effects on human health and its functional properties. Therefore, they can be incorporated to enhance the nutritional content of conventional foods such as meat, bakery products, candy bars, gums and beverages (Grossmann et al. 2018). Moreover, they are added to foods as foaming, emulsifying, water and oil binding, and viscosity-modifying agents (Kumar et al. 2014). Román et al. (2002) pointed out that phycobiliproteins extracted from *Synechococcus* spp. and *Porphyridium cruentum* have been used as natural colorants in foods such as chewing gums and dairy products (Román et al. 2002). In addition, various health products based from algae such as tablets, capsules, powder or extracted bioactive ingredients including beta-carotene and phycocyanin have been marketed recently (Samarakoon and Jeon 2012).

Spirulina platensis, which is classified as blue-green microalgae (Cyanobacterium) is known as a plentiful source of protein. Besides having a considerably high

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protein content (up to 70% in dry weight), proteins extracted from *Spirulina platensis* can be used in order to improve functional properties of food products (Benelhadj et al. 2016). There are only few studies regarding to the functional properties of *Spirulina* protein, whereas nutritional properties of *Spirulina* proteins were well studied (Benelhadj et al. 2016; Devi and Venkataraman 1984; Nirmala et al. 1992). To the best of our knowledge, there are no studies about the combination effect of extraction temperature, time and pH on functional properties of *Spirulina* proteins. In study of Benelhadj et al. (2016), only the effect of pH was investigated on functional properties of *Spirulina platensis* protein isolate such as emulsion, foaming and film forming. Therefore, in the present study, we aimed to investigate the effects of temperature, pH and time on functional properties of *Spirulina platensis* proteins. The aims of this study were (i) optimization of ultrasound assisted extraction of proteins from *Spirulina platensis* by using RSM (ii) investigation of the effect of extraction conditions on functional properties of protein concentrates extracted from microalgae.

Materials and methods

Material

Spirulina platensis powder (SPP) was supplied from a local manufacturer in Turkey (Akuatik Fisheries and Cosmetics Products Ltd., Adana, Turkey). All chemicals used in analysis were reagent grade.

Experimental design and statistical analysis

Based on the results of our previous study, extraction temperature (25, 35 and 45 °C), pH (7, 8 and 9) and extraction time (60, 90 and 120 min) factors were selected as independent variables for optimization of extraction parameters by using RSM with a BBD. Experimental design consisted of 17 experimental runs divided into twelve factorial points and five replicates at central point. Extraction parameters were normalized as coded variables. Variables were coded according to Eq. (1).

$$X = \frac{x_i - x_0}{\Delta x} \quad (1)$$

where X is the coded value, x_i is the corresponding actual value, x_0 is the actual value in the center of domain, and Δx is the increment of x_i corresponding to a variation of 1 unit of X .

The response functions (Y) were water absorption capacity, oil absorption capacity, emulsifying activity, emulsifying stability, foaming capacity and foaming

stability. The response variables were fitted to second-order polynomial model (Eq. 2) to obtain the regression coefficients (β). This model describe between the responses and the independent variables.

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

Regression coefficients of the polynomial model are defined as β_0 for constant term, β_i for linear effects, β_{ii} for quadratic effects and β_{ij} for interaction effects. ε is the random error term that represents the variability of the response Y . Analysis of variance (ANOVA) was used in order to evaluate model adequacy and determine regression coefficients and statistical significance. Statistical analysis was performed using Design Expert 7.1 software (Stat-Ease, Inc., MN, USA). The results were statistically tested at the significance level of $p = 0.05$. The adequacy of the model was determined using model analysis, the coefficient of determination (R^2), correlation of variation (CV%) and lack of fit testing. A mathematical model was established to describe the influence of single process parameter and/or interaction of multiple parameters on each investigated response. 3D contour plot response surface was generated with the same software and drawn by using the function of two factors, keeping the others constant.

Ultrasound assisted extraction of protein concentrates from SPP

Proteins of SPP were extracted according to the methods of Benelhadj et al. (2016) and Stone et al. (2015). Briefly, 1 g of the defatted samples were mixed with 15 ml of distilled water on a magnetic stirrer at room temperature for 60 min. Then, pH of samples was adjusted by adding 0.1 M HCl and 0.1 M NaOH at given pH values in Table 1. Ultrasound was applied to the samples by using an ultrasound device (VWR Ultrasonic cleaner, VWR International, USA) at $+4\text{ °C} \pm 1\text{ °C}$ for 60 min. Samples were kept in a shaking water bath (Classic C76, New Brunswick Scientific, USA) at 135 rpm at given extraction time and temperature in Table 1. After the centrifugation step at 1250 g, 4 °C for 30 min, supernatant was taken and pH was adjusted to 3.0 which is isoelectric point of *Spirulina platensis* (Benelhadj et al. 2016). Afterwards, samples were again centrifuged at 1250 g, 4 °C for 30 min. After centrifugation process, supernatant was discarded and sediments were freeze-dried. Freeze-dried *Spirulina platensis* protein concentrates (SPPCs) were stored at 4 °C until the analysis.

Table 1 Box–Behnken experimental design with natural and coded extraction conditions and experimentally obtained values of all investigated responses

Run	Independent variables						Responses						
	pH		Temperature (°C)		Time (min)		WAC (g of water/g of protein dw)	OAC (g of oil/g of protein dw)	EA (%)	ES (%)	FC (%)	FS (%)	
1	0	8	0	35	0	90	4.34	9.78	44.18	100	350	88.88	
2	1	9	0	35	1	120	3.87	7.72	45.98	95	400	85	
3	0	8	0	35	0	90	3.90	10.22	40	100	350	88.88	
4	1	9	− 1	25	0	90	4.48	6.54	44.32	100	300	87.5	
5	− 1	7	0	35	− 1	60	4.60	9.84	42.22	100	250	85.71	
6	0	8	− 1	25	1	120	4.67	9.56	42.20	94.74	325	88.23	
7	0	8	0	35	0	90	4.12	9.69	40.48	94.12	350	88.88	
8	− 1	7	0	35	1	120	4.25	7.37	42.22	100	300	87.5	
9	0	8	1	45	1	120	3.35	9.93	44.32	97.44	300	87.5	
10	0	8	− 1	25	− 1	60	4.03	10.14	43.68	100	350	88.88	
11	1	9	0	35	− 1	60	4.26	7.06	40.43	100	325	88.23	
12	0	8	1	45	− 1	60	5.12	10.22	43.68	94.74	325	82.35	
13	− 1	7	− 1	25	0	90	4.51	6.66	40.48	100	325	88.23	
14	0	8	0	35	0	90	3.62	9.89	43.96	95	350	88.88	
15	− 1	7	1	45	0	90	4.61	8.96	44.32	100	300	87.5	
16	1	9	1	45	0	90	4.51	7.71	44.44	95	250	85.71	
17	0	8	0	35	0	90	3.71	10.15	42.86	83.33	350	88.88	

Water absorption capacity (WAC)

WAC (g of H₂O/g of protein concentrate) was determined according to the method of Kumar et al. (2014). Freeze-dried protein concentrate (0.1 g) and distilled water (10 ml) were transferred into a pre-weighed centrifuge tube. The mixture was subjected to high speeds using a vortex mixer for 30 s. It was kept at room temperature for 30 min, and centrifuged at 3000 g for 20 min. The supernatant was removed and the centrifuge tube containing sediment was weighed. The water absorption capacity was determined by using Eq. (3).

$$WAC (gH_2O) = \frac{W_2 - W_1}{W_0} \times 100 \quad (3)$$

where W_0 is the weight of protein concentrate (g), W_1 is the weight of the tube plus the protein concentrate (g) and W_2 is the weight of the tube plus the sediment (g).

Oil absorption capacity (OAC)

OAC (g of oil/g of protein concentrate) was determined by using the method of Kumar et al. (2014). In order to determine the oil absorption capacity, 1.0 g of protein concentrate was taken in a pre-weighed centrifuge tube and thoroughly mixed with 5 ml of sunflower oil. This mixture was then centrifuged at 3000 g for 20 min, the supernatant

was decanted and the tubes were weighed. The oil absorption capacity was determined by using Eq. (4).

$$OAC (gOil) = \frac{O_2 - O_1}{O_0} \times 100 \quad (4)$$

Where O_0 is the weight of protein concentrate (g), O_1 is the weight of the tube plus protein concentrate (g) and O_2 is the weight of the tube plus the sediment (g).

Emulsifying activity (EA) and emulsifying stability (ES)

EA and ES were determined according to the method of Tan et al. (2014). Briefly, 0.1 g of the samples were mixed with 10 ml of distilled water in a beaker. Then, protein concentrate suspension was homogenized by using a high-shear disperser (Ultra-Turrax IKA® T18, Janke & Kunkel, Staufen, Germany) for 2 min at room temperature. After the homogenization, 10 ml of olive oil was added into beaker and again homogenized by using the high-shear disperser for 2 min at room temperature. Slurry was put into falcon tube and centrifuged at 1200 g for 5 min. The height of emulsion layer was recorded and the emulsion activity was determined by using Eq. (5).

$$EA(\%) = \frac{\text{Height of emulsified layer}}{\text{Height of contents of tube}} \times 100 \quad (5)$$

In order to determine the ES value, the samples were heated at 80 °C for 30 min, then centrifuged at 1200 g for 5 min. The ES value was determined by using Eq. (6).

$$ES = \frac{\text{Height of remaining emulsified layer}}{\text{Height of original emulsified layer}} \times 100 \quad (6)$$

Foaming capacity (FC) and foaming stability (FS)

FC and FS of the samples were determined according to the method of Jarpa-parra et al. (2014). Briefly, 0.02 mg of the protein concentrate was mixed with 20 ml of distilled water and volume of the mixture was recorded as volume before homogenization. Then, it was homogenized by using a high-shear disperser (Ultra-Turrax IKA® T18, Janke & Kunkel, Staufen, Germany) for 2 min and volume of the mixture was recorded. The FC value was obtained by using Eq. (7).

$$FC(\%) = \frac{\text{Volume after whipping (ml)} - \text{Volume before whipping (ml)}}{\text{Volume before whipping (ml)}} \times 100 \quad (7)$$

After the homogenization step, it was kept at room temperature for 30 min and volume of the mixture was recorded. The FS value was calculated by using Eq (8).

$$FS(\%) = \frac{\text{Volume after standing (ml)} - \text{Volume before whipping (ml)}}{\text{Volume before whipping (ml)}} \times 100 \quad (8)$$

Result and discussion

Model fitting

Each response (WAC, OAC, EA, ES, FC and FS) was evaluated as a function of linear, quadratic and interaction terms of the independent variables including extraction temperature, pH and extraction time using BBD (Table 1). Analysis of variance of the fitted second-order polynomial model and coefficients of determination (R^2) for each dependent variable are shown in Table 2. The R^2 values were 0.87, 0.95, 0.57, 0.37, 0.49 and 0.79 for WAC, OAC, EA%, ES%, FC% and FS%, respectively. According to

particularly high R^2 for the models (> 0.80), except for the EA% (0.57), ES% (0.37) and FC% (0.49), applied polynomial models were in accordance with experimental results. Moreover, low CV ranged from 1.37% to 12.65% for most of the responses (Table 2), suggested good reproducibility of the investigated systems, since CV describes dispersion of the data and small values indicate low variation in the mean value.

A significant lack of fit shows that the models failed to represent the data in the experimental domain at which points were not included in the regression. The lack of fit was not significant for all variables ($p > 0.05$), except for oil absorption capacity ($p = 0.0322$), meaning that all models accurately predicted the related responses (Table 2). The results suggest that the models used in this study were able to identify the extraction conditions for protein extraction from *Spirulina platensis*.

Functional properties of SPPCs

WAC and OAC

WAC of a protein is one of the important parameters among its functional properties. Proteins, which have high water absorption property, help to reduce moisture loss to improve texture and increase the shelf life of food products (Benelhadj et al. 2016). It is a crucial property for viscous food products like soups and dough, since these are supposed to absorb water without dissolution of protein, thereby providing thickening and viscosity (Kumar et al. 2014).

According to the results of our study, extraction time was found as a significant factor ($p = 0.0329$), whereas extraction temperature and pH were found as not significant effects ($p > 0.05$) on WAC. The interaction between extraction temperature and time and quadratic effect of extraction temperature were significant ($p = 0.0017$ and $p = 0.0322$) on WAC. Estimated coefficients of the fitted second-order polynomial model is shown for all response variables in Table 3. The variation of WAC with temperature and time at constant pH (8.0) is presented in Fig. 1. The graph plot revealed that WAC increased under the experimental conditions of extraction temperature of ~ 25–30 °C and time of ~ 105–120 min and extraction temperature of > 40 °C and time of ~ 60–75 min (Fig. 1).

WAC values of SPPCs were ranged from 3.35 g of water/g of protein concentrate to 5.12 g of water/g of protein concentrate, as shown in Table 1. These values were similar to the WAC values of *Spirulina platensis* protein concentrate (3.25 ± 0.07 – 4.29 ± 0.15 g of water/g protein concentrate) in the study conducted by Benelhadj et al. (2016) and also the study reported by Abdel-Aal et al.

Table 2 Analysis of variance (ANOVA) of the fitted second-order polynomial model

Source	Sum of squares	DF	Mean square	F-Value	<i>p</i> value
Water absorption capacity					
Model	2.48	9	0.28	5.12	0.0213*
<i>Linear</i>					
X_1	0.00125	1	0.00125	0.023	0.8832
X_2	0.076	1	0.076	1.41	0.2736
X_3	0.38	1	0.38	7.02	0.0329*
<i>Quadratic</i>					
X_1X_1	0.38	1	0.38	7.10	0.0322*
X_2X_2	0.29	1	0.29	5.34	0.0541
X_3X_3	0.005609	1	0.005609	0.1	0.7564
<i>Interaction</i>					
X_1X_2	0.0009	1	0.0009	0.017	0.9008
X_1X_3	1.3	1	1.3	24.12	0.0017*
X_2X_3	0.0001	1	0.0001	0.001856	0.9668
Residual	0.38	7	0.054	—	—
Lack of fit	0.069	3	0.023	0.3	0.8261
Pure error	0.31	4	0.077	—	—
Cor total	2.86	16	—	—	—
$R^2 = 0.8681$; C.V. (%) = 5.80					
Source	Sum of squares	DF	Mean square	F-Value	<i>p</i> value
Oil absorption capacity					
Model	25.75	9	2.86	13.98	0.0011*
<i>Linear</i>					
X_1	1.72	1	1.72	8.41	0.0230*
X_2	1.6	1	1.6	7.83	0.0266*
X_3	0.8	1	0.8	3.91	0.0855
<i>Quadratic</i>					
X_1X_1	0.25	1	0.25	1.24	0.3026
X_2X_2	18.53	1	18.53	90.56	< 0.0001
X_3X_3	0.28	1	0.28	1.39	0.2772
<i>Interaction</i>					
X_1X_2	0.28	1	0.28	1.37	0.2797
X_1X_3	0.018	1	0.018	0.089	0.7740
X_2X_3	2.19	1	2.19	10.71	0.0136*
Residual	1.43	7	0.22	—	—
Lack of fit	1.24	3	0.41	8.6	0.0322*
Pure error	0.19	4	0.048	—	—
Cor total	27.18	16	—	—	—
$R^2 = 0.9473$; C.V. (%) = 5.37					
Source	Sum of squares	DF	Mean square	F-Value	<i>p</i> value
Emulsion activity					
Model	28.95	9	3.22	1.01	0.5054
<i>Linear</i>					
X_1	5.81	1	5.81	1.83	0.2182
X_2	4.40	1	4.40	1.38	0.2780
X_3	1.97	1	1.97	0.62	0.4569

Table 2 continued

Source	Sum of squares	DF	Mean square	F-Value	<i>p</i> value
Emulsion activity					
<i>Quadratic</i>					
X_1X_1	4.40	1	4.40	1.38	0.2778
X_2X_2	0.020	1	0.020	0.00644	0.9383
X_3X_3	0.50	1	0.50	0.16	0.7033
<i>Interaction</i>					
X_1X_2	3.46	1	3.46	1.09	0.3315
X_1X_3	0.45	1	0.45	0.14	0.7182
X_2X_3	7.70	1	7.70	2.42	0.1635
Residual	22.24	7	3.18	–	–
Lack of fit	7.00	3	2.33	0.61	0.6420
Pure error	15.24	4	3.81	–	–
Cor total	51.19	16	–	–	–
$R^2 = 0.5665$; C.V. (%) = 4.15					
Source	Sum of squares	DF	Mean square	F-Value	<i>p</i> value
Emulsion stability					
Model	111.41	9	12.38	0.46	0.8602
<i>Linear</i>					
X_1	7.14	1	7.14	0.27	0.6211
X_2	12.50	1	12.50	0.47	0.5161
X_3	7.14	1	7.14	0.27	0.6211
<i>Quadratic</i>					
X_1X_1	5.28	1	5.28	0.20	0.6701
X_2X_2	41.51	1	41.51	1.55	0.2528
X_3X_3	5.28	1	5.28	0.20	0.6701
<i>Interaction</i>					
X_1X_2	6.25	1	6.25	0.23	0.6435
X_1X_3	15.84	1	15.84	0.59	0.4666
X_2X_3	6.25	1	6.25	0.23	0.6435
Residual	187.15	7	26.74	–	–
Lack of fit	1.49	3	0.50	0.011	0.9982
Pure error	185.66	4	46.42	–	–
Cor total	298.56	16	–	–	–
$R^2 = 0.3732$; C.V. (%) = 5.33					
Source	Sum of squares	DF	Mean square	F-Value	<i>p</i> value
Foaming capacity					
Model	11369.49	9	1263.28	0.75	0.6608
<i>Linear</i>					
X_1	1953.12	1	1953.12	1.17	0.3159
X_2	1250.0	1	1250.00	0.75	0.4162
X_3	703.13	1	703.13	0.42	0.5376
<i>Quadratic</i>					
X_1X_1	2631.58	1	2631.58	2.46	0.2502
X_2X_2	4111.84	1	4111.84	2.46	0.1611
X_3X_3	0.000	1	0.000	0.000	1.0000

Table 2 continued

Source	Sum of squares	DF	Mean square	F-Value	<i>p</i> value
Foaming capacity					
<i>Interaction</i>					
X_1X_2	156.25	1	156.25	0.093	0.7689
X_1X_3	0.000	1	0.000	0.000	1.0000
X_2X_3	156.25	1	156.25	0.093	0.7689
Residual	11718.75	7	1674.11	–	–
Lack of fit	11718.75	3	3906.25	–	–
Pure error	0.000	4	0.000	–	–
Cor total	23088.24	16	–	–	–
$R^2 = 0.4924$; C.V. (%) = 12.65					
Source	Sum of squares	DF	Mean square	F-Value	<i>p</i> value
Foaming stability					
Model	38.83	9	4.31	2.98	0.0818
<i>Linear</i>					
X_1	7.57	1	7.57	5.23	0.0560
X_2	0.031	1	0.031	0.022	0.8873
X_3	1.17	1	1.17	0.81	0.3983
<i>Quadratic</i>					
X_1X_1	1.08	1	1.08	0.75	0.4153
X_2X_2	1.71	1	1.71	1.18	0.3128
X_3X_3	11.22	1	11.22	7.76	0.0271*
<i>Interaction</i>					
X_1X_2	0.22	1	0.22	0.15	0.7076
X_1X_3	8.41	1	8.41	5.81	0.0467*
X_2X_3	6.30	1	6.30	4.36	0.0753
Residual	10.13	7	1.45	–	–
Lack of fit	10.13	3	3.38	–	–
Pure error	0.000	4	0.000	–	–
Cor total	48.96	16	–	–	–
$R^2 = 0.7932$; C.V. (%) = 1.37					

$$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{33}X_3^2 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3 + \varepsilon$$

Where X_1 : Extraction temperature (°C), X_1 : PH and X_3 : Extraction time (min)

(1986) regarding *Egyptian fenugreek* (3.52 ml water/g of protein). On the other hand, our values were higher than what was reported by Kandasamy et al. (2012) for algae *Kappaphycus alvarezii* (2.22 ± 0.04 g water/g of protein concentrate), *Enteromorpha compressa* (1.53 ± 0.07 g water/g protein concentrate), *Enteromorpha tubulosa* (1.32 ± 0.11 g water/g protein concentrate) and *Enteromorpha linza* (1.22 ± 0.06 g water/g protein concentrate). These differences among the values of WAC could be caused from different extraction methods/parameters applied in these studies.

OAC is a crucial determinant of flavour retention. High oil absorption is necessary for the formulation of foods such as sausage, cake and mayonnaise (Chandi and Sogi 2007). According to the results of our study, extraction

temperature and pH have significant effects ($p < 0.05$), while extraction time had no a significant effect ($p > 0.05$) on OAC of the samples. OAC values of SPPCs changed from 6.54 to 10.22 g oil/g protein, as shown in Table 1. The maximum OAC value was obtained as 10.22 g oil/g protein under the extraction conditions of pH of 8, extraction temperature of 35 °C and time of 90 min and pH value of 8, extraction temperature of 45 °C and time of 60 min. Estimated coefficients of the fitted second-order polynomial model is shown for all response variables in Table 3.

The variation of OAC with extraction time and pH at constant extraction temperature (35 °C) is shown in Fig. 2. The graph plot revealed that OAC increased under the experimental conditions of pH of ~ 7.5 –8.5 °C and time

Table 3 Estimated coefficients of the fitted second-order polynomial model for all response variables

Regression coefficient	Responses					
	WAC	OAC	EA	ES	FC	FS
β_0	3.72	9.41	42.30	94.49	350.00	88.88
<i>Linear</i>						
β_1	− 0.012	0.46	0.85	− 0.95	− 15.62	− 0.97
β_2	− 0.097	− 0.45	0.74	− 1.25	12.50	− 0.063
β_3	− 0.22	− 0.32	0.50	− 0.95	9.38	0.38
<i>Cross product</i>						
β_{12}	− 0.015	− 0.27	− 0.93	− 1.25	− 6.25	0.24
β_{13}	− 0.57	0.068	0.34	1.99	0.00	1.45
β_{23}	− 5.000E−003	0.74	1.39	− 1.25	6.25	− 1.26
<i>Quadratic</i>						
β_{11}	0.3	− 0.25	1.02	1.12	− 25.00	− 0.51
β_{22}	0.26	− 2.1	0.070	3.14	− 31.25	− 0.64
β_{33}	0.037	0.26	0.34	1.12	0.00	− 1.63

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \varepsilon$$

Where X_1 : Extraction temperature (°C), X_2 : PH and X_3 : Extraction time (min)

Fig. 1 3D contour plot response surface for the effect of cross-interaction between extraction temperature and extraction time on water absorption capacity at pH 8.0

Design-Expert® Software

WAC
5.119
3.35

X1 = A: Temperature
X2 = C: Time

Actual Factor
B: pH = 8.00

of ~ 90–120 min (Fig. 2). The maximum OAC values were obtained under the experimental conditions of extraction time of approximately 80 min and pH of ~ 7.5–8.5 (Fig. 2).

The results indicated that SPPCs have higher OAC than the OAC values of other algae. OAC might increase because of the presence of high hydrophobic proteins that exhibit superior binding to hydrocarbon chains of lipids (Turan et al. 2015). In the study of Benelhadj et al. (2016), oil absorption capacity of *Spirulina platensis* protein

isolate was found as 2.53 ± 0.01 g of oil/g of protein isolate. In a study conducted by Kumar et al. (2014), OAC value of protein concentrate of algae *Kappaphycus alvarezii* was found as 1.29 ± 0.20 g oil/g of protein. Similarly, OAC value of algae *Enteromorpha compressa* was determined as 1.34 ± 0.10 g oil/g protein concentrate (Kandasamy et al. 2012). Reason of this difference among OAC values could be the presence of nonpolar residues side chains of proteins (Benelhadj et al. 2016). The other reasons of this difference may be relating with protein

Fig. 2 3D contour plot response surface for the effect of cross-interaction between extraction time and pH on oil absorption capacity at extraction temperature of 35 °C

Design-Expert® Software

OAC
10.22
6.514

X1 = B: pH
X2 = C: Time

Actual Factor
A: Temperature = 35.00

Fig. 3 3D contour plot response surface for the effect of cross-interaction between extraction temperature and extraction time on foaming stability at pH 8.0

Design-Expert® Software

FS
88.88
82.35

X1 = A: Temperature
X2 = C: Time

Actual Factor
B: pH = 8.00

derivative, protein solubility and degree of protein distortion (Aurelia et al. 2009).

Emulsifying activity and emulsion stability

EA and ES of proteins are very important for their utilisation as emulsifying agents (Fernandez-Avila et al. 2016).

EA is expressed as the capability of a protein to form an emulsion by adsorbing oil at the water–oil interface. On the other hand, ES is defined as potential to stabilize emulsion without forming coalescence and flocculation over a period of time (Karaca et al. 2011; Kumar et al. 2014; Tan et al. 2014).

EA and ES values of SPPCs were shown in Table 1. There were no significant changes in emulsifying activity and emulsion stability of SPPCs under different extraction conditions ($p > 0.05$). Estimated coefficients of the fitted second-order polynomial model is shown for all response variables in Table 3. EA and ES of SPPCs ranged from 40 to 45.98% and from 83.33 to 100%, respectively (Table 1). In our study, EA% of SPPCs were close to the values acquired in the study conducted by Benelhadj et al. (2016). They investigated emulsifying activity of *Spirulina platensis* protein isolate at different pH intervals, and they found the values as $44.1 \pm 0.9\%$ at pH 3, $52.1 \pm 0.4\%$ at pH 2 and $46 \pm 0.8\%$ at pH 4 (Benelhadj et al. 2016). Emulsifying properties of a protein may be affected by protein surface hydrophobicity and solubility (Benelhadj et al. 2016). According to Amita and Ahluwalia (2010), there is an important correlation of emulsification properties and surface hydrophobicity and solubility of proteins and a protein with a high relative hydrophobicity tends to be surface active (Amita and Ahluwalia 2010).

Foaming capacity and foaming stability

FC is defined as the potential of a protein to form a foam, while foaming stability exhibits how well the protein could retain the foam volume over a measured period of time (Benelhadj et al. 2016). FC and FS results of SPPCs are given in Table 1. There were no significant changes in FC% of SPPCs under different extraction conditions ($p > 0.05$), while interactive effect of extraction temperature and time and quadratic effect of extraction time were significant on FS% ($p = 0.0467$ and $p = 0.0271$). Estimated coefficients of the fitted second-order polynomial model is shown for all response variables in Table 3. The variation of FS with extraction temperature and time at constant pH (8.0) is presented in Fig. 3. The graph plot revealed that FS increased while approaching to central point (Fig. 3).

FC% of SPPCs ranged from 250 to 400%, whereas FS ranged from 82.35 to 88.88% (Table 1). FC% of SPPCs are slightly higher than the protein concentrate from *Kappaphycus alvarezii* algae ($53.33 \pm 2.31\%$) (Kumar et al. 2014), the fenugreek protein concentrate (89.5%) (El Nasri and El Tinay 2007) and *Enteromorpha linza* ($33.3 \pm 5.7\%$) (Kandasamy et al. 2012). SPPC has the potential to find application in food products such as cake baking and coffee whiteners, because of its high FC and FS (Omosuli et al. 2011).

Optimization and verification

Optimization procedures were carried out to predict the optimum level of independent variables to obtain

maximum values for WAC, OAC, EA, ES, FC and FS in triplicate. The optimum conditions (extraction temperature: 43.87°C , pH: 7.16 and extraction time: 60 min) provided the highest value of WAC (4.41 g of water/g of protein concentrate), OAC (10.13 g of oil/g of protein concentrate), FC (300%), FS (83.94%), EA (42.99%) and ES (92.50%) in a 'desirability' of 1.00. Adequacy of the models for predicting the optimum response values was tested by *Spirulina platensis* protein extraction in the optimum levels of variables obtained by the RSM optimization (extraction temperature: 43.87°C , pH: 7.16 and extraction time: 60 min). Predicted and mean of experimental values for WAC (4.41 g of water/g of protein concentrate and 4.80 ± 0.15 g of water/g of protein concentrate), OAC (10.13 g of oil/g of protein concentrate and 9.80 ± 0.89 g of oil/g of protein concentrate), FC (300% and 300%), FS (83.94% and 87.5%), EA (42.99% and $43.5 \pm 0.22\%$) and ES (92.50% and 94.28 ± 0.17) were not statistically different at the 5% significance level.

Conclusion

As a conclusion, in this study, the protein concentrates from *Spirulina platensis* in desired functional properties were obtained under extraction temperature of 43.87°C , pH of 7.16, extraction time of 60 min. Under these conditions, WAC, OAC, FC, FS, EA and ES were obtained as 4.41 g of water/g, 10.13 g of oil/g, 300%, 83.94%, 42.99% and 92.50%, respectively. The results showed that *Spirulina platensis* protein concentrates had higher WAC, OAC, FC and FS than other algae and plant proteins. Therefore, *Spirulina platensis*, which is rich in protein, may be used as a potential source of a functional protein in new product formulation and it can be considered as a suitable food ingredient. Hence, it offers an exciting alternative protein source for use in various foods such as meat, bakery, ice cream.

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Compliance with ethical standards

Conflicts of interest The authors declare no conflicts of interest.

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