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Preliminary Purification of C-Phycocyanin through the Foam Fractionation

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ABSTRACT

Objective*: C-Phycocyanin (C-PC) is a blue-colored protein antioxidant produced in the microalgae Spirulina platensis (S. platensis) that is used in the food, cosmetic and pharmaceutical industries. The purification methods are often time-consuming and expensive. In this study, Foam fractionation (FF) was used as a simple, rapid, cost-effective, and environmentally friendly method for the purification of C-PC.*

Methods*: The C-PC pigment solution was extracted from dry S. plantensis biomass in phosphate buffer. The FF method for the purification of C-PC was optimized using the response surface methodology (RSM). A UV-Vis spectrophotometer was used to determine the identifying absorbance peaks of the C-PC solutions.*

Results*: RSM obtained an optimal setting at the pH = 6, aeration rate of 3.5 vvm, and operation time of 21 min for achieving the highest purification fold (PF), purity index (PI), and C-PC recovery percentage (R) of about 1.56 ± 0.02, 0.59 ± 0.02, and 45.59 ± 0.72% respectively.*

Conclusion: *In the FF method there are no additional chemicals, also it is fast and has low operating costs, which make it an attractive method.*

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1. Introduction

C-phycocyanin (C-PC) is a blue-colored protein with antioxidant properties that has widespread applications in the food, cosmetic, and pharmaceutical industries [1]. Cyanobacteria biomass such as *Spirulina platensis* (*S. platensis*) is the main source of C-PC. C-PC as a phycobiliprotein plays a crucial role in capturing light energy during photosynthesis [2]. The C-PC extracted from *S. platensis* contains high levels of undesirable proteins and thus purification steps must be

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applied to obtain a high purity of C-PC. The purity index (*PI*) of C-PC is generally evaluated by the absorbance ratio of A_{620}/A_{280}, wherein a purity of 0.7 is considered as the food grade, 3.9 as the reactive grade, and greater than 4.0 as the analytical grade [3]. The more detailed classification of C-PC is as follows: the purity of 0.5-1.5 for food dye; 1.50-2.50 for cosmetic dye, 2.5-3.5 for biomarkers, bigger than 4 for biomedical grade [4].

Methods such as ammonium sulfate precipitation, filtration, and chromatography are typically used for C-PC purification; these are often time-consuming and expensive [5-7]. Therefore, the development of cost-effective, rapid, and environmentally friendly methods is attractive.

Foam fractionation (FF) due to the difference in the surface activity of molecules with air bubbles could be efficient for the separation and purification purposes especially for proteins [8]. The main part of the FF equipment usually consists of an aerated column in which individual bubbles of a gas like air are introduced into the feed solution after diffusing through a porous body. The rising bubbles adsorb the material on their surfaces when passing in the column and finally the accumulation of the bubbles in the top of the bulk liquid forming a foam layer at the head of the column. The strong amphipathic nature of proteins, resulting from their mixture of polar and non-polar groups, causes them to be preferentially adsorbed at the gas–liquid interface [9]. Important features of FF including low equipment and low operating costs, make it an an attractive method for purifying and recovering proteins from complex mixtures [10]. Generally, factors affecting protein separation in the FF process include pH, concentration, ionic strength, or column operating parameters like gas flow rate, foam column height, and bubble size [9].

Keshavarzi et al. investigated the protein enrichment of bovine serum albumin (BSA) through a foam fractionation system, which required quantities, such as foam stability, interface coverage or bubble size distribution, to be measured in the experiment and fed into the model [11]. Also, a previous study in a FF system for the purification of C-PC from the crude extract obtained from *S.platensis* powder yielded a recovery efficiency of 48.8% and a purification fold (*PF*) of 1.472 without needing any surfactants [12]. Other researchers tried to enhance the recovery of bioactive components using a new high-efficiency FF of anthocyanin from perilla leaves using the surfactant-free active Al_2O_3 nanoparticle (ANP) as collector and frother, which was modified with adipic acid (AA). ANP-AA was able to form a stable foam layer and under optimal conditions, 95.68% of anthocyanin was recovered [13]. Krause et al. finding that diverse natural foam-stabilizing proteins like F-Tags could able to recovery of β-lactamase, penicillin G acylase, and formate dehydrogenase through FF. They suggested the establishment of surfactant-free FF for enzyme workup is a promising method [14].

In the present study, following the extraction of C-PC in a phosphate buffer solution, the crude C-PC extract was purified using the FF method. The ultimate objective of this research is to determine the optimal purification conditions for the FF method using Response Surface Methodology (RSM) and to investigate the interactions between operational factors during the purification process. RSM comprises a set of mathematical and statistical techniques used to develop a suitable functional relationship between a desired response and a number of relevant control (input) variables. In general, such a

relationship is unknown, but it can be represented by a polynomial model. Therefore, employing this method provides a better understanding of the influential factors [15]. Previous studies have not investigated the purification of C-PC using a FF from this perspective. In this study, the purification process is carried out within a FF system using simple equipment and without the use of chemicals, factors that are desirable for the industrial-scale application and environmental friendliness.

2. Materials and Methods

2.1. Chemicals

S. platensis (dried powder) and commercial C-PC were purchased from a local supplier in Iran (bioGreens, http://biogreens.ir). Potassium dihydrogen phosphate (KH_2PO_4), dipotassium hydrogen phosphate (K_2HPO_4) were of Merck.

2.2. Preparation of C-PC extract

Various methods such as the use of phosphate buffer, freeze-thaw cycles, ultrasonication, enzymes, and surfactants have been employed to extract C-PC from *S. platensis* pigment solutions. The extraction, using phosphate buffers, requires neither specialized equipment nor expert knowledge, making it a suitable and cost-effective option for industrial-scale applications; furthermore, the method offers good reproducibility [16, 17]. For these reasons, the phosphate buffer extraction method was selected for this study. The extraction process was carried out in a dark flask to prevent the effect of light on the pigment**.** The C-PC extraction was done according to previous research with a slight modification [18]: (0.5, 1 or 1.5) g of dried *S.platensis* powder was mixed with 100 mL of 20 mM phosphate buffer at different pH values of 6, 7, or 8. Other conditions were identical for all samples; only the final purity index was considered, without accounting for PI changes occurring during the extraction process. Phosphate buffers of a specific pH were prepared using appropriate amounts of phosphate salt solutions, potassium dihydrogen phosphate (KH_2PO_4) and dipotassium hydrogen phosphate (K_2HPO_4). The resulting mixture was then stirred at 300 rpm for 4 h. Subsequently, the solution was separated from cell debris via centrifugation (at 6,000 × g for 15 minutes), and the crude supernatant was used for purification in the FF system.

2.3. Foam fractionation set up

A schematic view of the FF system used in the present study is presented in Figure 1. This system includes a glass column with a length of 28 cm and the inner diameter of 7 cm which is equipped with a porous glass disperser at the bottom. Aeration to the FF column was supplied by a controllable air pump and a rotameter measured the air flow transfer to the FF system. The accumulated foam at the top of the column was transferred to a funnel and the foamate (the collapsed foam) was collected in a separate tank.

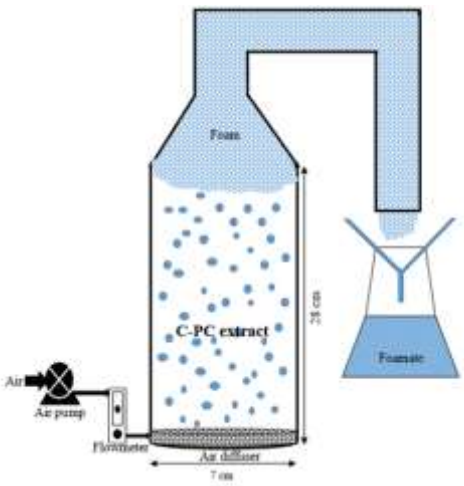

Figure 1. Schematic of the FF system used for the C-PC purification.

2.4. Optimization of the C-PC purification by the FF process

Each experiment was performed at room temperature (25 ± 2 ºC). In each experiment, 100 mL of C-PC crude extract was transferred into the FF system and the process was performed at different pH (6, 7 and 8), operation times (15, 30, and 45 min) and aeration rates (2, 3, and 4 vvm).

To statistically optimize FF for the C-PC purification, the effect of the initial concentration of the extract was first investigated at three concentrations (0.5, 1.0 and 1.5 wv%) at the pH of 7, time of 30 min and aeration rate of 3 vvm and the optimal concentration was selected. Then an experimental design, according to the face center composite design (CCD, α=1) and considering pH (X_1), time (X_2), and aeration rate (X_3) as the influential factors of the system, was selected. Table 1 shows the actual and coded levels of these factors. The CCD considered twenty combinations of these factors as the experimental conditions. All tests were conducted on the fresh extracts prepared from a single biomass source using the same method. To evaluate the impact of these factors and examine their interactions, the F-test (with a confidence level superior to 95% and Design-Expert® software (version 11) were employed. Maximizing the *PF* value and the C-PC recovery percentage (*R*) served as the system responses and the criteria for determining optimal conditions.

2.5. Analytical methods

A UV-Vis spectrophotometer was used to determine the identifying absorbance peaks of the C-PC solutions as 280, 620, and 652 nm. The UV–Vis measurements were performed in triplicate and the error analysis was 0.01%. Then, *PI*, *PF*, the concentration of C-PC (C, mg /mL), *R* (%), and the extraction yield of C-PC (*Y*, mg/g) were calculated as follows [16, 19]:

$$PI = \frac{A_{620}}{A_{280}} \quad (1)$$

$$PF = \frac{PI_p}{PI_c} \quad (2)$$

$$C = \frac{A_{620} - 0.477A_{652}}{5.34} \quad (3)$$

$$R = \frac{C_p \times V_p}{C_c \times V_c} \times 100 \quad (4)$$

$$Y = \frac{C \times V}{m} \quad (5)$$

Where *C* (mg/mL) and *V* (mL) are the concentration and the volume of C-PC. The subscripts of *p* and *c* denote the phase with concentrated C-PC and crude C-PC extract , *Y* is the extraction yield of C-PC in mg per gram of dry biomass, *V* is the buffer volume (mL), and *m* is the dry biomass (g).

Table 1.
Independent variables and their levels tested in the FF system for the C-PC purification.

Independent factor	Symbol	Units	Levels		
			-1	0	+1
pH	X_1	-	6.0	7.0	8.0
Operatiom time	X_2	min	15.0	30.0	45.0
Aeration rate	X_3	vvm	2.0	3.0	4.0

3. Results and Discussion

3.1. Performance of FF in the different initial concentrations of C-PC

To investigate the effects of the initial concentration of the C-PC extract in the FF process, the crude extract C-PC was prepared in three different concentrations of 0.5, 1.0 and 1.5 wv%, and the experiments were done at the pH = 7, and aeration rate of 3 vvm for 30 min. At the end of the experiments, the PF, and the R values of foamate were determined. Figure 2 shows that the increase in the concentration of the C-PC extract from 0.5 to 1.0 wv% enhanced the PF and R values to 1.32 ± 0.01 and 46.26 ± 0.87% respectively. However, the performance of FF decreased at the higher concentration than 1.0 wv% of the protein. The performance of FF decreases by increasing the concentration of the protein. This behavior is probably exhibited due to the limited capacity of the gas/water interface for the adsorption of proteins. Thus, a certain amount of the protein may be adsorbed to the interface under a certain condition. After the bubble surfaces are saturated, further increase in the concentration of the protein results in a decrease in the rate of foam discharge [20].

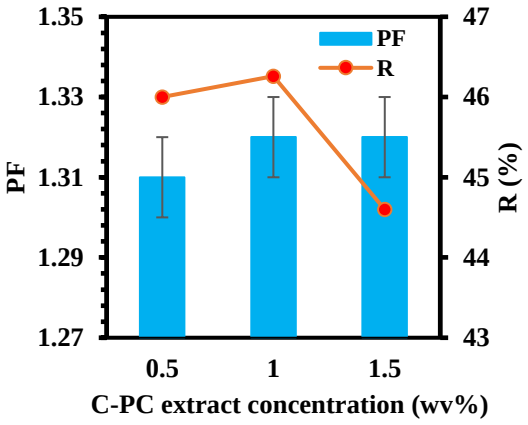

Figure 2. Effects of the initial concentration of the C-PC extract on the performance of the FF process. Each expeiment was performed in triplicate and the mean values ± error were reported.

The optimization of the FF process was carried out using the C-PC extract of the initial concentration of 1.0 wv% (*PI* = 0.37 ± 0.01, *C* = 0.32 ± 0.01 mg/mL and *Y* = 32.08 ± 0.96 mg/g). The C-PC extraction using phosphate buffers is one of the most common methods employed in previous studies. The *PI* obtained via this method varies between 0.2 and 3.5 across different studies, while *Y* ranges from 17 to approximately 200 mg/g [16]. These varying results indicate that the outcome depends not only on the method used but also on the quality of the *S.platensis* biomass employed. The *PI* and *Y* results in this study fall within the intermediate range of previous studies.

3.2. Optimization of FF for the purification of C-PC

Further evaluations on the FF system were performed on pH (X_1), operation time (X_2), and aeration rate (X_3) according to the experiments presented in Table 2 using the central composite design (CCD) for the arrangement of the experiments. The experimental results obtained for *PF*, and the *R* of the foamate from various experiments are presented in Table 2.

The pure error was determined by six replicates of the experiment at the central levels of these factors. The average of the results of *PF* and *R* for these six replicates were 1.31 ± 0.01 and 45.84 ± 0.31 % and the values of the pure error for *PF* and *R* were 0.0004 and 0.5663, respectively.

The values of *PF* and *R* were changed with the variation of the operating parameters. For instance, the lowest value of *PF* was observed in Experiment #14; however the highest value of *R* was obtained at Experiment #13. It indicates that the experimental operational factors can affect the purification performance of C-PC by the FF system.

Table 2.
Eexperimental results obtained for the purification of C-PC by the FF system.

NO	Operational factors			Responses	
	(X_1)	(X_2)	(X_3)	*PF*	R (%)
1	6	45	2	1.36	40.27
2	7	30	3	1.32	45.72
3	8	45	4	1.27	46.50
4	6	15	4	1.51	46.76
5	8	45	2	1.25	40.33
6	7	30	3	1.31	46.25
7	7	30	3	1.33	45.78
8	7	30	3	1.31	45.49
9	6	15	2	1.49	40.18
10	8	15	4	1.22	45.90
11	6	30	3	1.55	45.56
12	7	30	3	1.32	45.49
13	7	30	4	1.28	47.45
14	8	15	2	1.21	40.22
15	7	45	3	1.25	46.20
16	7	30	2	1.21	40.65
17	7	15	3	1.28	45.46
18	8	30	3	1.31	45.12
19	6	45	4	1.41	47.20
20	7	30	3	1.33	46.21

Model evaluation showed that the response behavior provided the best fit with the following quadratic expression:

$$Y_{\mathrm{i}} = \beta_0 + \sum \beta_{\mathrm{i}} X_{\mathrm{i}} + \sum \beta_{\mathrm{ii}} X_{\mathrm{i}}^2 + \sum \sum \beta_{\mathrm{ij}} X_{\mathrm{i}} X_{\mathrm{j}} \quad (5)$$

The significance of the coefficients were analysed using the analysis of variance (ANOVA) at a confidence level of 0.95. The results of ANOVA on the regression models were summarized in Table 3. After removing statistically insignificant coefficients, the following mathematical models were defined to describe the responses in the FF-based C-PC purification process in this study:

$$PF = 1.310 - 0.106\,X_1 - 0.017\,X_2 + 0.017\,X_3 + 0.040\,X_1X_2 + 0.124\,{X_1}^2 - 0.0405\,{X_2}^2 - 0.0605\,{X_3}^2 \quad (6)$$

$$R(\%) = 45.840 - 0.190\,X_1 + 0.198\,X_2 + 3.22\,X_3 - 0.207\,X_1X_3 - 0.557\,{X_1}^2 - 1.850\,{X_3}^2 \quad (7)$$

The models indicate that pH(X_1) has the most effect on *PF* and the operation time (X_2) has the lowest effect, also the aeration rate (X_3) has the utmost effect on *R*, but the pH has the low effect. The effects of pH and ionic strength on the performance of FF are very important, because of the effect on the surface activity of a protein [21]. Increasing the aeration rate can provide more foamate volume and increase the recovery, but it also can provide conditions for other proteins rather than the target protein [22]; so increasing the aeration rate can decrease *PF*.

The accuracy of the models to predict the experimental values of the responses was also illustrated in Figure 3. The comparison of experimental and predicted results indicates that the RSM method has successfully provided a suitable model for predicting outcomes in this study.

Table 3.
ANOVA of the regression models to predict the responses of the FF process.

PF					
Source	**Sum of Squares**	**Df**	**Mean Square**	***F*-value**	***P*-value**
Model	0.1742	7	0.0249	104.5000	< 0.0001
X_1	0.1124	1	0.1124	471.7400	< 0.0001
X_2	0.0029	1	0.0029	12.1300	0.0045
X_3	0.0029	1	0.0029	12.1300	0.0045
X_1X_2	0.0128	1	0.0128	53.7400	< 0.0001
X_{12}	0.0427	1	0.0427	179.0900	< 0.0001
X_{22}	0.0045	1	0.0045	18.9000	0.0010
X_{32}	0.0101	1	0.0101	42.2000	< 0.0001
Residual	0.0029	12	0.0002		
Lack of Fit	0.0025	7	0.0004	4.3900	0.0613
Pure Error	0.0004	5	0.0001		
Cor Total	0.1771	19			
R^2	0.9839		Std.Dev	0.0154	
Adjusted R^2	0.9744		CV (%)	1.1600	
Predicted R^2	0.9425		Adeq.Precision	36.1558	
***R* (%)**					
Source	**Sum of Squares**	**Df**	**Mean Square**	***F*-value**	***P*-value**
Model	129.3200	6	21.5500	334.0600	< 0.0001
X_1	0.3610	1	0.3610	5.6000	0.0342
X_2	0.3920	1	0.3920	6.0800	0.0284
X_3	103.43	1	103.43	1603.0000	< 0.0001
X_1X_3	0.3444	1	0.3444	5.3400	0.0379
X_{12}	0.9946	1	0.9946	15.4100	0.0017
X_{32}	10.9200	1	10.9200	169.2900	< 0.0001
Residual	0.8388	13	0.0645		
Lack of Fit	0.2724	8	0.0341	0.3000	0.9359
Pure Error	0.5663	5	0.1133		
Cor Total	130.16	19			
R^2	0.9936		Std.Dev	0.2540	
Adjusted R^2	0.9906		CV (%)	0.5691	
Predicted R^2	0.9873		Adeq.Precision	48.1986	

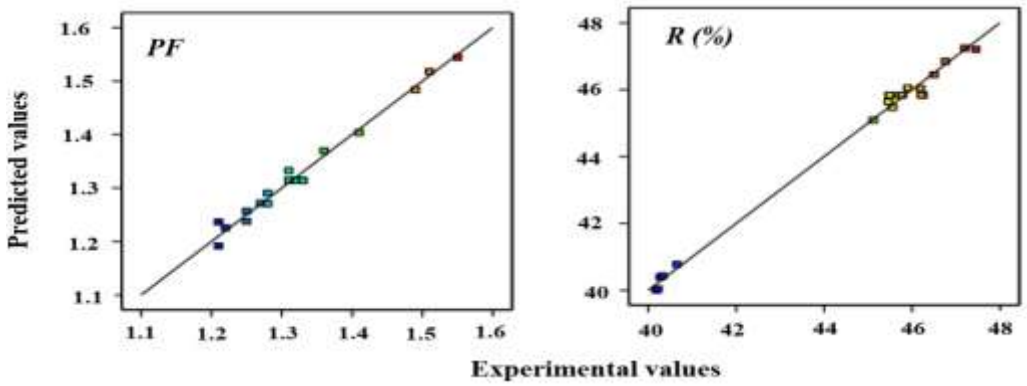

Figure 3. Aaccuracy of the models to predict the experimental values of responses (*PF*, *R*).

The normal probability plot of the residuals for both responses (*PF*, *R*) is shown in Figure 4, indicating that the residual values follow a normal distribution. The coefficient of determination (R^2), which indicates the proportion of the total variability explainable by the models, was 0.9839 for *PF* and and 0.9936 for *R*. The low values for the standard deviation (Std. Dev = 0.154) and the coefficient of variation (CV = 1.16%), along with a favorable adequate precision value of 48.1986, indicate high precision and low experimental error for *PF*. The values for the standard deviation, coefficient of variation, and adequate precision for *R*, were 0.2540, 0.5691%, and 48.1986 respectively, confirming the model's accuracy.

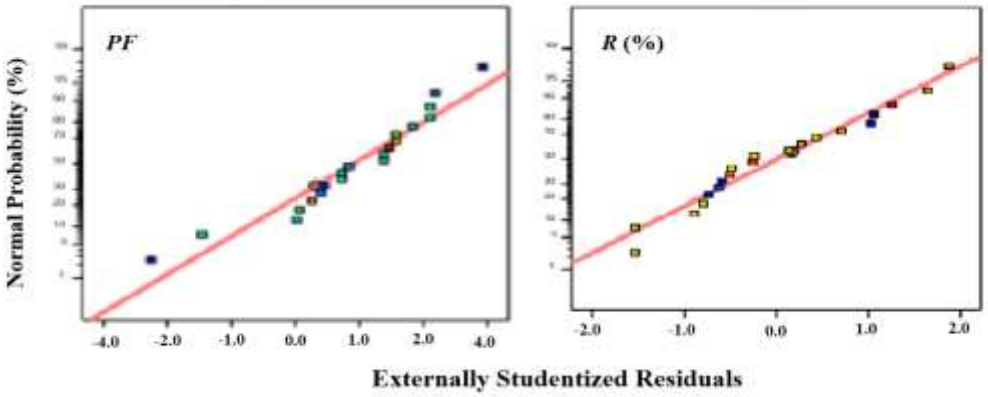

Figure 4. Normal plot of residuals of responses (*PF*, *R*).

To illustrate the interactions between the operational factors in the FF-based purification of C-PC, three-dimensional plots are presented in Figures 5 and 6. Figure 5 shows *PF* as a function of pH (X_1) and time (X_3). The *PF* value decreases by increasing the pH from 6 to 8. The highest *PF* value, 1.55, was obtained at the pH of 6, whereas *PF* decreased to 1.21 at the pH of 8. It has been shown that the surface activity of a protein is maximal at a pH equal to pI. Both of these phenomena are thought to be related to the maximum density of the protein at the interface as a result of minimizing electrostatic repulsion. The isoelectric point (PI) is the pH value at which a protein molecule carries no net surface charge; repulsive forces are minimized, while hydrophobicity and surface activity increase, resulting in maximum protein accumulation at the gas-bubble interface [21]. The PI for C-PC is about 5.8 [23]. For this reason, the highest *PF* is seen at the pH = 6, which is very close to PI. Increasing the time can increase *PF*, and on the other hand, increasing the time will reduce *PF* somewhat. A longer duration can enhance the adsorption of protein molecules onto the bubble surfaces, thereby increasing the loading capacity; however, it may also increase the drainage of entrapped liquid back into the bulk solution, leading to the accumulation of dry foam and a low recovery rate. As time passes and the surface ages, molecules that initially bind to the interface may be replaced by molecules with higher affinity [21].

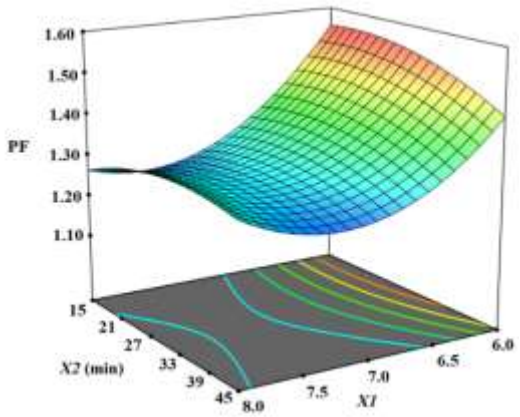

Figure 5. *PF* as a function of pH (X_1) and time (X_2) where the aeration rate (X_3) is set at its central level.

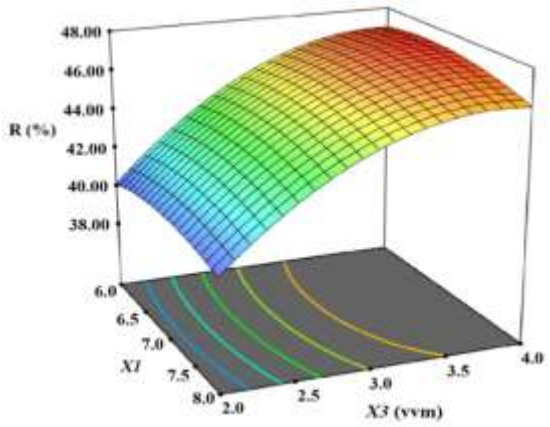

Figure 6. C-PC Recovery (*R*) as a function of pH (X_1) and aeration rate (X_3) where the time (X_2) is set at its central level.

Figure 6 shows recovery (*R*) as a function of pH (X_1) and aeration rate (X_3). Increasing the aeration rate can provide more foamate volume and increase the recovery, and on the other hand, the recovery rate decreases somewhat with higher aeration rate about 4 vvm. The minimum value of *R* (40.18%) was obtained at an aeration rate of 2 vvm, and the maximum value (47.45%) was obtained at an aeration rate of 4 vvm. At high air flow rates, the concentration of the target protein in the foamate decreases, but the amount of the foamate collected and the recovery percentage increase. On the other hand, when the air volumetric flow rate is low, a greater number of smaller air bubbles are present. This increases the effective surface area and enhances the efficient adsorption of protein molecules at the gas-liquid interface [22]. Larger bubbles form at higher air flows, and larger bubbles can increase the surface area for greater protein adsorption at the gas-liquid interface [24]. However, larger bubbles trap liquid within the foam, leading to its breakdown and a reduction in quality [25].

RSM predicted that the application of FF at the pH of 6, the aeration rate of 3.5 vvm and the time of 21 min gained a simultaneous highest *PF* = 1.55 and *R* = 46.8% for C-PC. The measurements of *PF*, *PI*, and *R* were performed three times under optimal conditions, and the standard deviation of the results was calculated. The examination of the optimal condition yielded a *PF* = 1.56 ± 0.02 and *R* = 45.6 ± 0.72%. The good correlation between the predicted responses and experimental results demonstrated the accuracy of the RSM method in predicting system behavior and optimization. *PI* was 0.59 ± 0.02.

The UV–Vis spectrum of the C-PC purified by FF was compared with those of the crude C-PC extract and a commercial product in Figure 7. A comparison of the results shows that the absorption peak at approximately 260 nm, which is associated with nucleic acid, has shifted to 280 nm (associated with proteins) in both the purified and commercial C-PC samples; furthermore, the absorption peak at 620 nm, which associated with C-PC, showed a significant increase following purification via the FF method [19].

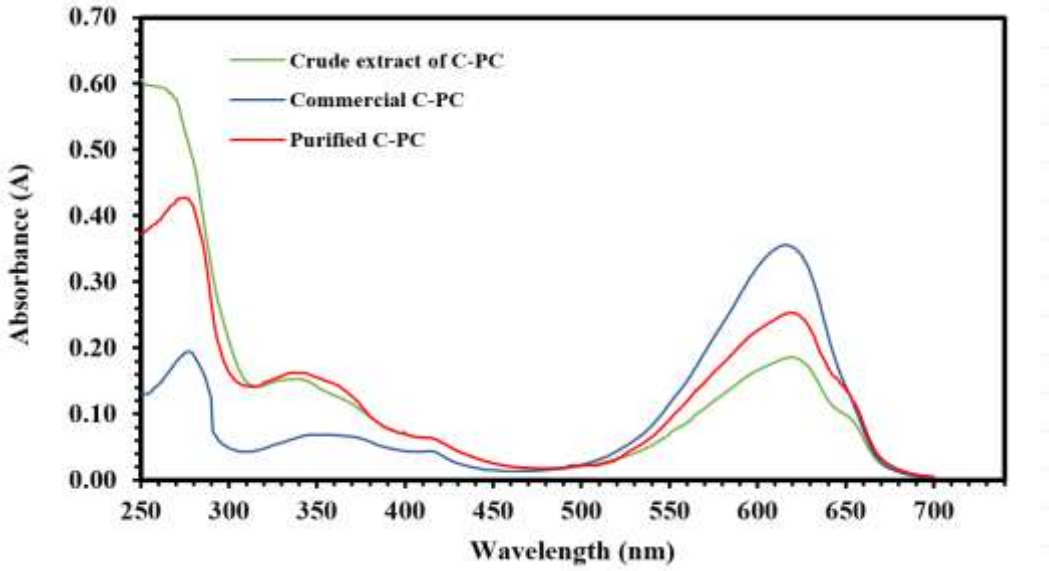

Figure 7. UV-Vis absorption spectra of the crude C-PC extract, the purified C-PC obtained by FF and commercial products.

Antecka et al. used the FF method to purify C-PC from crude extract. The FF process resulted in a recovery efficiency of 48.8% and a *PF* of 1.47 in 40 min [12]. Therefore, the applied system in the present work gained a higher PF value at a shorter operation time. The tested sample containing the 0.5 mM Triton X-100 under optimal conditions, yielded the *PF* = 1.54 ± 0.02 and *R* = 46.4 ± 0.7%. Based on the results, no detergent is required for the purification of the crude extract by the FF system. Apparently, there is enough protein in the mixture to achieve good foaming and form a stable foam [12].

Several methods have been used for the purification of C-PC from microalgae biomass with different values of *PF* and *R*. The comparative economic and operational assessment of some different C-PC purification methods was illustrated in Table 4. Moras et al. employed a combination of ammonium sulfate precipitation, gel filtration, and ion exchange processes to purify C-PC to a high degree of purity (PI = 3.16). At the chromatography step, the *PF* was 2.3 with a recovery of 41.7% [5]. However, the use of multi stages in the purification increased the costs of the product and decreased the overall recovery. Ultra filtration is a fast method with no additional chemicals and high recovery, but operating costs are high [12]. One relatively simple purification method that facilitates the purification process under mild conditions is the aqueous two-phase system (ATPS) [12, 26]. The most studied ATPSs include polyethylene glycol (PEG) with different molecular weights, However, issues related to recovering PEG from the system on a large scale lead to environmental pollution and costly operations [7]. The FF processes are often suggested as an early step in recovering bioproducts of which the protein content is very low [27]. The comparison of these studies shows that the FF method has low recovery, because the protein content in the C-PC extract is high, but this method is fast, there are no additional chemicals and the operational cost is moderate. So, the FF method can be used as an efficient and environmentally friendly method for preliminary purification of C-PC.

Table 4.
Comparative, economic and operational assessment of the C-PC purification methods.

Purification method	Additional chemicals	Processing time	Operating costs	*PF*	*R* (%)	Ref.
Chromatography	Tris-HCl + NaOH	High (> 8 h)	High	2.30	41.70	[5]
Ultrafiltration	None	Moderate	High	1.92	92.00	[12]
	None	Moderate	High	1.37	-	[28]
Aqueous two-phase system	PEG 4000 + Phosphate Salt	30 min centrifugation	High	2.43	81.20	[26]
	PEG 6000 + Phosphate Salt	24 h	High	1.47	97.80	[12]
Foam fractionation	None	40 min	Moderate	1.47	48.80	[12]
	None	21 min	Moderate	1.56	45.60	This study

4. Conclusion

In this study, the FF system was used as a cost-effective and environmentally friendly method for the preliminary purification of C-PC. The highest values of *PF*, *PI* and *R* equaled to 1.56 ± 0.02, 0.59 ± 0.02 and 45.59 ± 0.72% respectively. Adding Triton X-100 as a surfactant did not have a significant effect on the recovery and purification results. The results show that pH is the most effective parameter affecting *PF* and the aeration rate has the utmost effect on the C-PC recovery. A satisfactory result for *PF* was obtained in this study. Therefore, the FF method can be employed as one of the purification steps for C-PC, and a higher *PI* can be achieved by combining it with other purification methods. The C-PC recovery in the FF process, compared to other purification methods, is lower, but in the FF method there are no additional chemicals, also it has low operational costs, which make it an attractive method.

List of Symbols

C	Concentration
Y	Extraction yield
R	Recovery percentage
Y_i	Each response of process
Greek symbols	
β_0	Mean of the response
β_i	Main effects of factors
β_{ij}	Interactions of the factors
β_{ii}	Quadratic effects of each factor
Abbreviations	
FF	Foam fractionation
C-PC	C-Phycocyanin
PF	Purification fold
PI	Purity index

Author Contributions

Shadi Azar: Investigation, Visualization, Validation, Methodology, Formal analysis, Software, and Writing the original draft. Alireza Habbibi: Conceptualization, Supervision, Resources, Methodology, Software, Funding acquisition, and Writing, review & editing. Farshad Rahimpour:

Conceptualization, and Writing, review & editing.

Data Availability Statement

Data will be made available on request.

Ethical considerations

This article does not contain any studies with human participants.

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Conflict of interest

The authors declare no conflict of interest for the review and publication of the manuscript.

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