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Effect of Drying Method and Sugar Type (Sucrose, Glucose, and Fructose) on the Concentration and Stability of Phycocyanin During Storage

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ABSTRACT

Phycocyanin is a major water-soluble pigment derived from Spirulina, valued for its strong antioxidant activity and potential as a natural food colorant. However, its stability is influenced by factors such as temperature, light intensity, and pH. This study investigated the impact of different drying methods (freeze-drying, oven-drying, and spray-drying) and different sugar concentrations (glucose, fructose, and sucrose) on the stability of phycocyanin extracted from Spirulina platensis. Additionally, the effect of storage duration (up to 120 days in 15-day intervals) on pigment stability was assessed. Results showed that Freeze-drying resulted in significantly higher phycocyanin concentration and stability than oven and spray drying ($p<0.05$). Among the treatments, freeze-drying with sucrose yielded the highest pigment retention ($p< 0.05$). Furthermore, sugar-treated samples demonstrated greater pigment preservation than sugar-free samples, with sucrose outperforming glucose and fructose in all drying methods tested. Overall, the stability and concentration of phycocyanin decreased over time during storage. The degradation kinetics followed a second-order model, with thermal degradation constants decreasing as pH and fructose concentration increased but increasing with temperature. Moderate fructose levels extended the pigment half-life, whereas excessive amounts reduced it. These findings suggest that freeze-drying combined with sucrose addition is the most effective strategy for enhancing phycocyanin stability during storage.

What is "already known":	• Drying method may affect the thermal stability of phycocyanin pigment • Drying methods could be freeze-drying, spray-drying, and oven-drying • Content of Sugar may affect pigment stability.
What this article adds:	• Degradation rate constant and $t_1/_2$ of the extracted pigment was measured • Phycocyanin stability changed with and without addition of glucose, fructose, and sucrose. • Freeze-drying with sucrose yielded the highest pigment retention

1. Introduction

In recent years, algae have gained increasing commercial importance owing to their diverse applications in the food, cosmetics, and medicine industries (1). Among the various bioactive compounds derived from microalgae, pigments hold particular commercial value because of their wide range of uses and ease of extraction. *Spirulina platensis*, a species of cyanobacteria, has been recognized by the World Health Organization (WHO) as one of the most nutritious organisms on earth. One of its major pigments, phycocyanin, is notable for its high protein, vitamin, mineral, and essential fatty acid content, making it a valuable dietary supplement (2). *Spirulina platensis*, a cyanobacterium, is one of the richest and most readily available natural sources of phycocyanin. Phycocyanin is the major pigment of phycobiliproteins, hence the common name of this algae is “blue-green algae.” This water-soluble pigment is extensively used as a color additive in food and cosmetics and as a fluorescent reagent (3). The primary and most important application of phycocyanin is as a food colorant, with established use in jellies, ice creams, chewing gums, candies, and more (4). They also serve as emulsifiers, thickening agents, gelling agents, prebiotics, and natural color agents in food products, and have applications in cosmetics (4). Moreover, their strong fluorescence properties make them valuable for cell histochemistry, fluorescence microscopy, flow cytometry, immunofluorescence assays, and other labeling techniques (4, 5).

Phycocyanin can be considered a good substitute for artificial additives in the food industry, as it increases food quality. Due to its proteinaceous nature, phycocyanin is sensitive to microbial and heat degradation and structural breakdown (3). When exposed to temperatures above 75°C, the protein structure of phycocyanin denatures, and if irreversible, this significantly reduces its stability. Despite its functional potential, the industrial application of phycocyanin remains limited because of challenges in extraction efficiency and post-extraction stability. Its degradation depends on the integrity of its protein structure, which is affected by environmental conditions, including light, pH, temperature, and protein concentration (6, 7). Stabilizers, such as sucrose, glucose, and fructose, can enhance pigment stability across various pH and temperature conditions. Sugars influence not only stability, but also activation energy, color intensity, and antioxidant activity. These effects are mainly due to glycosidic interactions between sugars and proteins, which may lead to polymerization and protect phycocyanin from thermal degradation (8).

The stability of phycocyanin depends on light, pH, and temperature (9). The most stable pH of phycocyanin is 4.5-5.5 (10, 11). Storage stability of phycocyanin at temperatures (−18 °C, 4 °C, and 10 °C) and pH levels (4.5, 5.5, and 7) showed that phycocyanin content decreased during the shelf-life (12). However, storage at 30, 55, and 65 °C led to rapid degradation, with pigment absorbance dropping to zero within 2–3 d at the highest temperatures (13). Maximum stability was observed at −18 °C, particularly at pH 4.5 (12). Phycocyanin stability at 40 °C declined over time, but when glucose was added as a stabilizer, the pigment retention exceeded 95% (14). However, the addition of 20–40% glucose or sucrose at pH 7 improved retention to 62–70%, extending the half-life from 19 to 30–44 min (15, 16). The effect of sugar on phycocyanin thermal degradation between 25 and 80 °C indicated that sugar concentration played a more crucial role in pigment protection than sugar type. Fructose was identified as the most effective stabilizer due to its high solubility (17). Phycocyanin without sugar degraded significantly, whereas glucose addition increased the activation energy for degradation up to fourfold, likely due to sugar-induced protein polymerization (18). Sodium chloride was ineffective in stabilizing phycocyanin under these conditions, but sugar-based stabilizers are more effective. Sodium chloride exhibited stabilizing effects in a concentration-dependent manner (9, 15, 19).

This study investigated the effects of drying methods (spray drying, oven drying, and freeze drying) and the presence of exogenous sugars (fructose, glucose, and sucrose) on the stability of blue phycocyanin pigment extracted from *Spirulina platensis* during drying and phycocyanin powder storage. This study was designed to investigate the effect of temperature, pH, and on fructose concentrations on the stability of phycocyanin in simulated food conditions. Modeling of the effect of temperature (50–98°C), pH (4–7), and fructose concentrations (0–50%) on thermal degradation constant (Dc) and half-life (t½) of phycocyanin was carried out by the response surface method (RSM). By controlling pigment degradation in thermal processes, similar conditions are predicted and the desired pigment content in food is calculated.

2. Materials and Methods

The study of phycocyanin pigment stability was conducted in two stages:

Stage 1: In this phase, thermal degradation constant (Dc) and half-life (t1/2) of the extracted blue pigment were measured under different concentrations of fructose (0–50%), pH levels (4–7), and temperatures (50–98°C), using response surface methodology (RSM) (Table 1). The independent variables included temperature, pH, and fructose concentration, while the dependent variables were the degradation constant (Dc) and pigment half-life (t1/2). A total of 17 experimental treatments were evaluated based on RSM design with α=1.7 and three replications at the central point.

Stage 2: Phycocyanin stability was evaluated after drying the pigment using three methods: freeze-drying, spray-drying, and oven-drying, both in the presence and absence of sugars (glucose, fructose, and sucrose). The experimental treatments were outlined in Table 2.

2.1. Cultivation of *Spirulina platensis*

Dry biomass of microalgae *S. platensis* (APP1) was provided by Microalgae Culture Collection of Tarbiat Modares University, Tehran, Iran. To preserve phycocyanin, dry biomass was stored at 4 °C in dark

2.2. Pigment Extraction and Drying

Dry biomass of *S. platensis* was soaked in solvent (0.1 M phosphate buffer, pH 6.8) at a ratio of 1:50 solid-to-liquid for 60 min at 27 °C ±2. After centrifugation at 6000 rpm for 10 min at 25 °C, supernatant was used to assess phycocyanin concentration. Absorbance of the crude extract was measured at 615 nm and 652 nm using a spectrophotometer, and phycocyanin concentration was calculated using Eq.1 (20):

$$\text{Phycocyanin (mg/mL)} = [OD_{615} - 0.474\,(OD_{652})] / 5.34 \qquad \text{Eq. 1}$$

Where OD615 and OD652 represent optical densities at respective wavelengths.

Samples were spread uniformly on shallow trays at 1 cm thickness and dried using the following three methods: (i) oven-drying; (ii) freeze-drying and (iii) spray-drying.

The oven-drying process was conducted at 65 °C for 11 hin an oven (Memmert GmbH + Co.KG, Schwabach, Germany) equipped with an air circulator.

In freeze-drying (FD), the pressure was reduced to 10 Mbar. The temperature in the drying chamber was -76 to -80 °C, the samples were kept in the drying chamber for 22-24 h. The final moisture content of the dried sample was 7% (wet basis).

In spray-drying (SPD), aqueous was dried through an industrial plant spray dryer (Maham Neyshabour Inc., Khorasan, Iran). Drying conditions were defined as follows: feed temperature at 25 °C, inlet temperature of slurry at 170 °C, outlet temperature of dry phycocyanin at 90 °C, atomisation airflow rate of 400 l/h and liquid feed pump rate of 25 m3/h. Spray drying duration was approximately 60 min. The final moisture content of the dried sample was 1-2% (wet basis)

2.3. Kinetic Calculations (Reaction Dynamics)

Effects of temperature (50–98°C), fructose concentrations (0–50%), and pH (4.3–7.7) on Dc and t (½) of phycocyanin extracted from *A. platensis* were assessed using a water bath. Phycocyanin solutions were heated for 30 min in a water bath (50–98°C), and samples were collected after 5 min. Degradation constant obtained in the first-or der kinetic model was expressed according to Equation (2). Regression lines were obtained by plotting changes in the degradation of phycocyanin logarithmically as a function of heat treatment time (19). Dc as the heat degradation constant is response variable calculated by Eq. 2:

$$dA/dt = -Dc \cdot t \qquad \text{Eq. (2)}$$

In this equation, A represents the amount of phycocyanin pigment, t is the time in hours, and Dc is the heat-induced color degradation constant, with units of 1/h. The Equation 2 follows a logarithmic plot, and to convert it into a linear graph, boundary conditions for each parameter must be used in Eq. 3. Thus:

at $t = 0 \rightarrow A = A_0$ at $t = t_0 \rightarrow A = A$

$$Dc \cdot t = \ln(A/A_0) \qquad \text{Eq. (3)}$$

Where, A_0 is the calculated initial phycocyanin amount. The half-life or t1/2 is derived from parameter Dc:

$$t(1/2) = \ln(2)/Dc \qquad \text{Eq. (4)}$$

2.4. Statistical Design and Analysis of the Experimental Plan

To investigate the drying method (oven, freeze-drying, and spray-drying) the presence of exogenous sugars (fructose, glucose, and sucrose) on the stability of the

Table 1. Degradation constant (Dc) and half-life (t½) of phycocyanin produced by Spirulina platensis for various heat treatments, pH, and fructose concentrations.

	Independent variables			Responses	
Treatment	Fructose (A) (%)	pH (B)	Temperature (C) (°C)	Dc (h^{-1})	t½ (h)
1	1.10	6.4	7.59	2.1531	0.4644
2	9.39	6.4	7.59	1.5784	0.5502
3	1.10	4.6	7.59	2.0489	0.4881
4	9.39	4.6	7.59	0.9896	0.4672
5	1.10	6.4	3.88	1.8174	0.6336
6	9.39	6.4	3.88	2.1627	0.4624
7	1.10	4.6	3.88	2.1403	1.0105
8	9.39	4.6	3.88	1.6907	0.5915
9	0.00	5.5	0.74	1.8705	0.4830
10	0.50	5.5	0.74	1.7521	0.3721
11	0.25	4.0	0.74	1.7078	0.5855
12	0.25	7.0	0.74	1.6673	0.5998
13	0.25	5.5	0.50	2.0704	0.5533
14	0.25	5.5	0.98	2.6872	0.5707
15	0.25	5.5	0.74	1.5583	0.6296
16	0.25	5.5	0.74	1.6254	0.6152
17	0.25	5.5	0.74	1.7850	0.5602
18	0.25	5.5	0.74	1.6716	0.5982
19	0.25	5.5	0.74	1.5919	0.6282
20	0.25	5.5	0.74	1.5346	0.6516

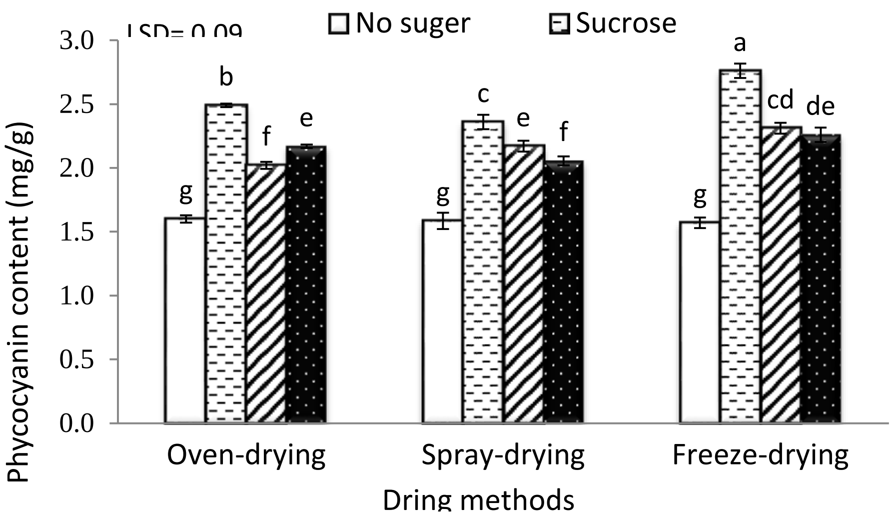

Figure 1. Effects of different drying methods and exogenous sugars (fructose, glucose, and sucrose) on phycocyanin (mg/g).

Values in each column with at least one similar letter are not significantly different based on LSD test ($P < 0.05$).

Table 2. Effects of different drying methods and exogenous sugars (fructose, glucose, and sucrose) on phycocyanin (mg/g) during storge time at 4 °C

		Time (day)			
Drying Method	Sugar Type	15	30	45	120
	No sugar	1.52 ± 0.03^{jk}	1.39 ± 0.04^{kl}	1.05 ± 0.06^{no}	0.50 ± 0.007^{s}
Oven-drying	Sucrose	2.40 ± 0.03^{bc}	2.27 ± 0.06^{cd}	2.12 ± 0.05^{d}	1.06 ± 0.05^{no}
	Glucose	1.87 ± 0.06^{ef}	1.73 ± 0.11^{f-i}	1.75 ± 0.21^{e-i}	0.83 ± 0.04^{pqr}
	Fructose	1.86 ± 0.05^{ef}	1.67 ± 0.16^{g-j}	1.65 ± 0.16^{g-j}	0.71 ± 0.08^{r}
	No sugar	1.40 ± 0.13^{kl}	1.29 ± 0.06^{lm}	0.92 ± 0.08^{opq}	0.49 ± 0.05^{s}
Spray-drying	Sucrose	2.20 ± 0.02d	2.19 ± 0.09d	1.91 ± 0.06ef	0.88 ± 0.030-r
	Glucose	1.81 ± 0.15^{efg}	1.80 ± 0.08^{efg}	1.57 ± 0.11^{ij}	0.85 ± 0.01^{pqr}
	Fructose	1.68 ± 0.22^{g-j}	1.61 ± 0.12^{hij}	1.53 ± 0.05^{jk}	0.74 ± 0.01^{qr}
	No sugar	1.28 ± 0.03^{lm}	1.20 ± 0.08^{mn}	0.90 ± 0.06^{opq}	0.52 ± 0.007^{s}
Freeze-drying	Sucrose	2.67 ± 0.06^{a}	2.48 ± 0.18^{b}	2.39 ± 0.09^{bc}	1.17 ± 0.01^{mn}
	Glucose	2.29 ± 0.04^{cd}	2.20 ± 0.08^{d}	1.90 ± 0.05^{ef}	0.96 ± 0.02^{op}
	Fructose	1.90 ± 0.10^{ef}	1.76 ± 0.09^{e-h}	1.79 ± 0.09^{efg}	0.78 ± 0.03^{qr}

- Values represent the mean ± standard deviation (n = 3).
- Values with at least one letter in common are not significantly different (LSD test, $p < 0.05$).

Phycocyanin pigment during drying and powder storge, a factorial test in the form of a completely randomized design with a 95% confidence level and SAS software was used.

In this study, temperature (50–98°C), pH (4–7), and fructose concentrations (0–50%) were analyzed as independent variables using central composite design (CCD) of response surface methodology (RSM) and the Expert Design Software v.7.0.0. Levels of real variables in CCD are shown in Table 1. Effects of significant independent variables were assessed in terms of DC (Y1) and t½ (Y2) of phycocyanin. The research was designed by applying RSM with α = 1.7. Data were analyzed by the Design 7.0.0 Expert software at 95% confidence level (95% CI)

3. Results and Discussion

3.1. Effect of Drying Method and Sugar Type (Sucrose, Glucose, and Fructose) on Phycocyanin Content

Phycocyanin is a water-soluble phycobiliprotein (~28–30 kDa) with linear tetrapyrrole chromophores covalently attached via thioether bonds. While relatively light-resistant, it is heat-sensitive, remaining stable only up to ~47 °C, with degradation and chromophore detachment accelerating near ~60 °C, particularly during thermal processing such as spray- or oven-drying (16, 21). The results of this study showed that the phycocyanin content in the freeze-dried powder was higher than that in the spray-dried and oven-dried treatments. The highest level of phycocyanin was observed in the freeze-drying method in the presence of sucrose significantly ($p < 0.05$). It should be noted that applying the freeze-drying method compared oven and spray drying methods in terms of maintaining the amount and stability of phycocyanin over is due to the fact that this drying method allows the removal of water at low temperatures without causing thermal degradation and breakdown of phycocyanin (22).

In addition, freeze-drying causes minimal changes in the protein structure of phycocyanin. However, with a relative increase in temperature (in the oven), the protein structure of this pigment is affected, and as a result, its amount and stability are reduced (16). The use of sucrose during the drying process with all three methods of freeze-drying, spray-drying, and oven-drying was more effective than glucose and fructose in significantly increasing the concentration of phycocyanin pigment ($p < 0.05$).

The phycocyanin content significantly decreased from 15 to 120 days ($p < 0.05$). The lowest and highest concentrations were observed on days 120 and 15, respectively. The results of Table 2 show that in all drying methods, whether with or without sugar as a stabilizer, the phycocyanin content decreased with increasing storage time ($p < 0.05$) (Table 2). During the storage period (120 days), the phycocyanin content in freeze-dried samples with sucrose was significantly higher than in other treatments ($p < 0.05$). The rate of change or the rate of decline in phycocyanin content up to day 45 in freeze-dried samples with all three sugars (sucrose, glucose, and fructose) was lower compared to other treatments ($p < 0.05$) (Table 2). The reduction of phycocyanin during the 120-day storage period in the oven-dried and spray-dried treatments was significantly greater than in the freeze.

Furthermore, the phycocyanin stability in the samples treated with sucrose, glucose, and fructose was significantly higher than in the sample treated without exogenous sugar. ($p < 0.05$)

3.2. Effect of Drying Method and Type of Sugar (Sucrose, Glucose, and Fructose) on Phycocyanin Content

Table 1 shows the effects of the independent variables of fructose concentration (A), pH (B), and temperature (C) on the thermal degradation constant (Dc) and half-life ($t½$) of phycocyanin. Based on the results, the highest degradation rate occurred in treatment 14 (Dc = 2.6872 h^{-1}) and the lowest in treatment 4 (Dc = 0.1989 h^{-1}). As shown in Table 1, the longest half-life was in test 7 ($t½$= 1.01 h), and the shortest was in test 10 ($t½$= 0.37 h).

The results of the final analysis of the effects of heat treatment, pH, and fructose content on the thermal degradation constants (Dc) and half-life ($t½$) of phycocyanin produced by Spirulina platensis are presented in Table 2. Both models were significant ($p < 0.05$), and the lack of fit was not significant ($p = 0.059$, $p = 0.121$). The distribution of statistical data in the samples of Dc and $t½$ of phycocyanin were 8.22 and 11.9, respectively. The value of the regression coefficient, R2, was 0.9060 and showed significant relationships between experimental Dc and predicted and experimental $t½$ and predicted values according to Equations 5 and 6:

$$Dc = 1.65-(0.1419A)-(0.0666B)-(0.1522C)-(0.1599AB) + (0.1912AC) + (0.2307C^2) \quad \text{Eq. (5)}$$

$$t½ = +0.6119 + (0.0521A) + (0.0345B) + (0.0555C) - (0.0443AB) - (0.0819AC) + (0.0707BC) - (0.0529A^2) \quad \text{Eq (6)}$$

where Dc was the degradation constant (h^{-1}) and t½was the half- life value, A was the variable fructose concentration, B was the variable pH, and C was temperature.

4. Conclusion

The results of this study showed that freeze-drying of phycocyanin and the use of sucrose as a stabilizer were superior in increasing the concentration and improving the stability of phycocyanin during 120 days of storage compared to oven drying and spray drying. Regardless of the drying method or sugar type, phycocyanin stability decreased over the 120-day storage period. Based on the findings of this study, a second-order model with R^2 values was suitable for describing and predicting the thermal kinetics (thermal degradation constant and half-life) of this pigment. Increasing pH and fructose concentration reduced the thermal Dc, whereas increasing the temperature increased it.

Table 3. Analysis of variance the effect of heat treatments, pH, and fructose content on thermal degradation constants (Dc) and half- life value (t½) of phycocyanin produced by *Spirulina platensis*.

Source	Thermal degradation constant (Dc)			Half-life (t½)		
	df	SS	P-value	df	SS	P-value
Model	6	1.93	< 0.0001	7	0.2457	0.0015
Fructose content (A)	1	0.2749	0.0061	1	0.0371	0.0164
pH (B)	1	0.0607	0.1488	1	0.0162	0.0900
Temperature (C)	1	0.3163	0.0039	1	0.0420	0.0117
AB	1	0.2046	0.0145	1	0.0157	0.0945
AC	1	0.2925	0.0050	1	0.0537	0.0057
BC	-	-	-	1	0.0400	0.0134
A^2	-	-	-	1	0.0411	0.0125
C^2	1	0.7808	0.0001	-	-	-
Residual Error	13	0.3348		12	0.0572	
Lack of Fit	8	0.2934	0.0591	7	0.522	0.121
Pure Error	5	0.0414		5	0.0050	
Total	19	2.26		19	0.3029	
R^2	0.8521			0.8112		
C.V (%)	8.89			11.99		
Adjusted R^2	0.7839			0.7010		

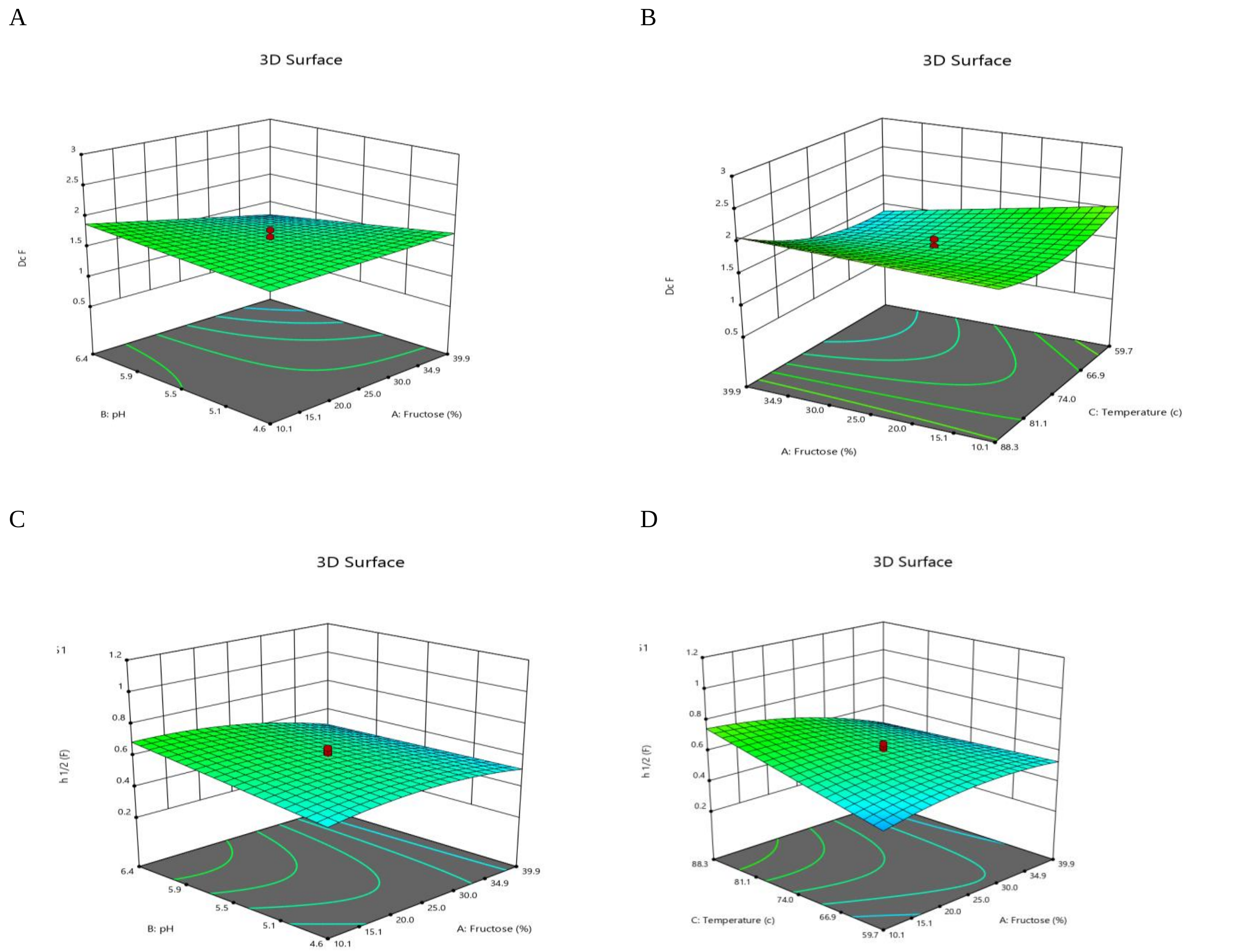

Figure 2. The 3D response surface plot demonstrating effects of: (A) pH and fructose content, fructose content and temperature (B) on the thermal degradation constant (Dc) (h^{-1}); and pH and fructose content (C) fructose content and temperature (D) on half-life (t½) of phycocyanin

Using an appropriate fructose concentration (25%) reduced the half-life of the pigment. Other innovative non-thermal methods for extracting phycocyanin are recommended. In addition, investigating the effects of sugar type and concentration on the antioxidant activity and color of phycocyanin could be a future trend. Stabilizing agents, such as sugars, can be applied in the extraction and processing of phycocyanin pigment.

5. Declarations

5.1. Acknowledgments

None.

5.2. Authors' Contributions

All authors equally contributed to this study.

5.3. Declaration of Interest

The authors of this article declared no conflict of interest.

5.4. Ethical Considerations

All ethical principles were adhered in conducting and writing this article.

5.5. Transparency of Data

In accordance with the principles of transparency and open research, we declare that all data and materials used in this study are available upon request.

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5.7. Using Artificial Intelligent chatbots

None.

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