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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 one of the major water-soluble pigments 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. 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 level extended the pigment half-life, whereas excessive amount reduced it. Results suggest combined freeze-drying with sucrose addition as the most effective strategy to enhance pigment 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 : [14]:

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

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 ºC 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:

$dA/dt = -Dc \cdot t$ **Equation 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 $t = 0 \rightarrow A = A_o$ at $t = t_o \rightarrow A = A$

$Dc \cdot t = \ln(A/A_o)$ **Equation 3**

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

$t(1/2) = \ln(2)/Dc$ **Equation 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 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 $\alpha = 1.7$. 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].

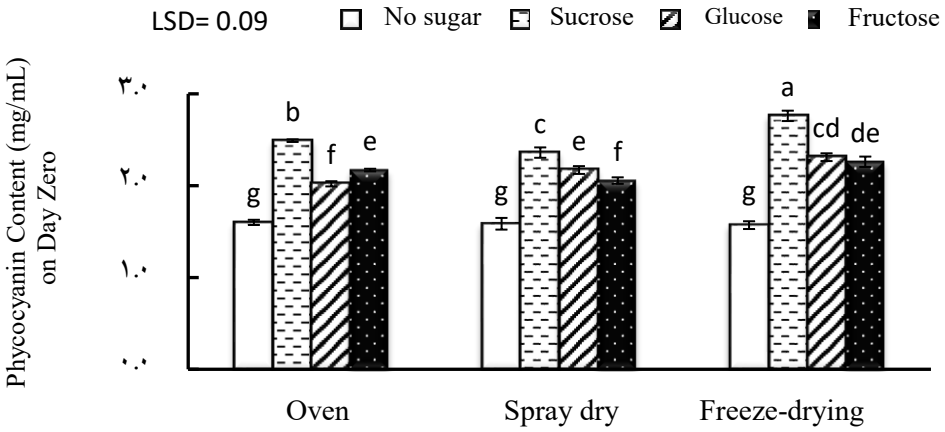


Figure 1. Comparison of the mean interaction effect of drying method and sugar type on phycocyanin content on day zero. Values in each column with at least one similar letter are not significantly different based on LSD test ($P < 0.05$).

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

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

Drying Method	Sugar Type	Time (day)			
		15	30	45	120
Oven-drying	No sugar	1.52 ± 0.03^{jk}	1.39 ± 0.04^{kl}	1.05 ± 0.06^{no}	0.50 ± 0.007^{s}
	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}	$1.73 \pm 0.11^{f-i}$	$1.75 \pm 0.21^{e-i}$	0.83 ± 0.04^{pqr}
	Fructose	1.86 ± 0.05^{ef}	$1.67 \pm 0.16^{g-j}$	$1.65 \pm 0.16^{g-j}$	0.71 ± 0.08^{r}
Spray-drying	No sugar	1.40 ± 0.13^{kl}	1.29 ± 0.06^{lm}	0.92 ± 0.08^{opq}	0.49 ± 0.05^{s}
	Sucrose	2.20 ± 0.02d	2.19 ± 0.09d	1.91 ± 0.06ef	0.88 ± 0.03o-r
	Glucose	1.81 ± 0.15^{efg}	1.80 ± 0.08^{efg}	1.57 ± 0.11^{ij}	0.85 ± 0.01^{pqr}
	Fructose	$1.68 \pm 0.22^{g-j}$	1.61 ± 0.12^{hij}	1.53 ± 0.05^{jk}	0.74 ± 0.01^{qr}
Freeze-drying	No sugar	1.28 ± 0.03^{lm}	1.20 ± 0.08^{mn}	0.90 ± 0.06^{opq}	0.52 ± 0.007^{s}
	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}	$1.76 \pm 0.09^{e-h}$	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, $p < 0.05$).

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-

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

According to the analysis of variance in Table 4, the independent effects of drying method, type of sugar, and their interaction on day 0 were statistically significant at the 1% level. Based on the mean comparison in Table 4, the concentration of phycocyanin on day 0 in the freeze-dried treatment was significantly higher than in oven-dried and spray-dried treatments ($p < 0.05$). Also, the phycocyanin concentration (2.5 mg/L) in the treatment with sucrose was significantly higher than those without sugar, with glucose, or with fructose ($p < 0.05$) (Table5).

Table 4. Mean Comparison of Drying Methods on Phycocyanin Content at Day 0

Drying Method	Phycocyanin Content (mg/L)

Oven drying	2.07 ± 0.34[b]
Spray drying	2.04 ± 0.31[b]
Freeze drying	2.23 ± 0.46[a]

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

Table 5. Mean Comparison of Sugar Types on Phycocyanin Content at Day 0

Sugar Type	Phycocyanin Content (mg/L)
No sugar	1.59 ± 0.04[c]
Sucrose	2.54 ± 0.19[a]
Glucose	2.17 ± 0.13[b]
Fructose	2.16 ± 0.10[b]

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

3.3. Effect of Drying Method and Sugar Type on Phycocyanin Stability Over Time

The phycocyanin content significantly decreased from day 15, 30, 45, 120 ($p < 0.05$). to 1.91 ± 0.42, 1.80 ± 0.41, 1.62 ± 0.46, 0.79 ± 0.21. The lowest and highest concentrations were observed on days 120 and 15, respectively. The results of Table 6 show that in all drying methods, whether with or without sugar as a stabilizer, the phycocyanin content decreased with increasing storage time ($p < 0.05$). During the storage period (120 days), the phycocyanin content in freeze-dried samples with sucrose was significantly higher than in other treatments ($p < 0.05$). Moreover, 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 6. Interaction Effect of Drying Method, Sugar Type, and Storage Time on Phycocyanin (mg/mL)

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

- 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 stability in oven, spray and freeze drying method was achieved to 1.52 ± 0.55, 1.43 ± 0.52, and 1.64 ± 0.66, respectively. So, 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-dried phycocyanin ($p < 0.05$).

The phycocyanin stability in the sample without sugar, sucrose, glucose and fructose were measured as 1.04 ± 0.37, 1.98 ± 0.59, 1.63 ± 0.49, 1.47 ± 0.45, respectively. The sample treated with sucrose was significantly higher than in the samples treated with glucose and fructose ($p < 0.05$). Furthermore, the phycocyanin stability in the samples treated with sucrose, glucose, and fructose was significantly ($p < 0.05$) higher than in the sample treated without sugar."

According to Figure 2), it is observed that the phycocyanin stability in the samples freeze-dried with sucrose is significantly higher than in other treatments ($p < 0.05$). In all drying methods, the addition of sugar resulted in increased phycocyanin stability compared to the treatments dried without sugar ($p < 0.05$).

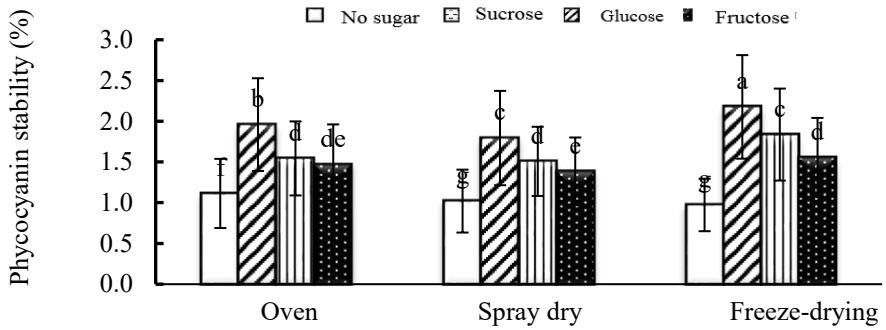


Figure 2. Comparison of the mean interaction effect of drying method and sugar type on phycocyanin stability during the storage period. Numbers in each column that share at least one common letter are not significantly different according to the LSD test ($p < 0.05$).

According to Figure 3, phycocyanin stability decreases with increasing storage time. Moreover, the highest phycocyanin content during the storage period was observed in the sucrose treatment, which was significantly higher than in the other treatments ($p < 0.05$).

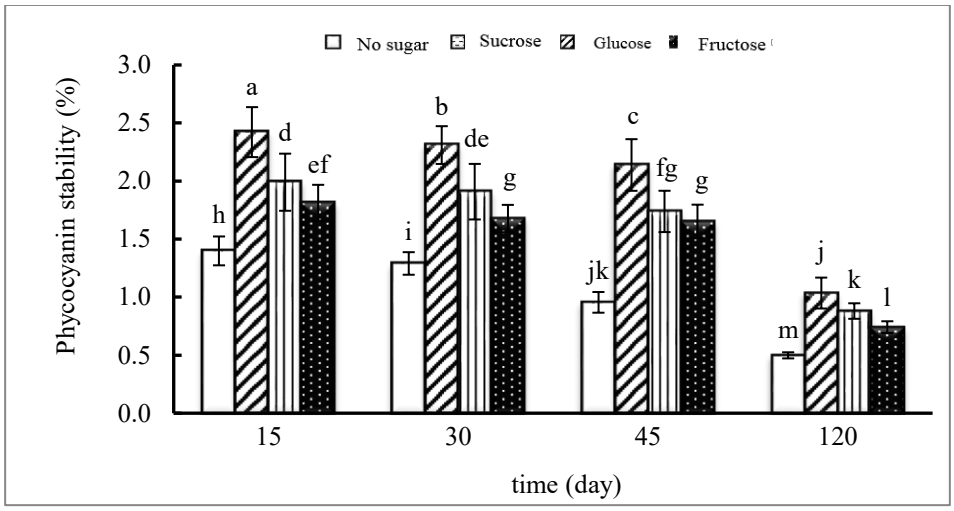


Figure 3. Comparison of the mean interaction effect of sugar type and storage time on phycocyanin stability during the storage period. Numbers in each column that share at least one common letter are not significantly different according to the LSD test ($p < 0.05$).

3.4. Effect of Temperature, pH, and Fructose Concentration on Thermal Degradation Kinetics of Phycocyanin

The values of the independent variables — fructose concentration (A), pH (B), and temperature (C) — along with the resulting degradation rate constant (Dc) and half-life (t1/2) are presented in Table 6. Based on the results, the highest degradation rate occurred in test 14 (Dc = 2.6872 h^{-1}) and the lowest in test 4 (Dc = 0.1989 h^{-1}). As shown in Table 8, the longest half-life was in test 7 (t1/2 = 1.01 h) and the shortest in test 10 (t1/2 = 0.37 h).

Table 6. Values of Independent (Temperature, pH, Fructose %) and Dependent Variables (Dc, t1/2)

Test	Fructose (%)	pH	Temperature (°C)	Dc (h^{-1})	t1/2 (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

3.4.1. Effect of Temperature, pH, and Fructose Concentration on the Thermal Degradation Rate (Dc) of Phycocyanin

In the analysis of variance table 7, the effects of three variables—temperature, pH, and fructose concentration—on the thermal degradation rate of phycocyanin pigment are shown. As can be seen, the proposed model was significant ($p < 0.05$), and the lack of fit was not significant ($P = 0.0591$). The coefficient of determination (R^2) is 0.8521, which indicates that the closer this value is to 1, the better the model fits the observed and predicted results, indicating the level of deviation of the data from the linear regression model. Thus, it can be concluded that the obtained results are consistent with the predicted values and the selected numerical range is reliable. The adjusted R^2 is 0.7839, reflecting the distribution of statistical data within the sample. According to the analysis of variance table, the independent linear effects of fructose and temperature are significant. Moreover, the quadratic effect of temperature and the interaction effects between fructose concentration and pH, as well as fructose concentration and temperature, are significant ($p < 0.05$).

Table 7. Final Analysis of Variance Results of the Effect of Three Variables (Temperature, pH, Fructose Concentration) on Thermal Degradation Rate (Dc)

Source of Variation	Degrees of Freedom	Sum of Squares	F	P
Model	6	1.93	12.49	< 0.0001
Fructose (A)	1	0.2749	10.67	0.0061
pH (B)	1	0.0607	2.36	0.1488
Temperature (C)	1	0.3163	12.28	0.0039
AB	1	0.2046	7.95	0.0145
AC	1	0.2925	11.36	0.0050
C^2	1	0.7808	30.31	0.0001
Residual Error	13	0.3348		
Lack of Fit	8	0.2934	4.42	0.0591
Pure Error	5	0.0414		
Total	19	2.26		

R^2: 0.8521
C.V.%: 8.89
Adjusted R^2: 0.7839
Predicted R^2: 0.5475

For a better evaluation of the thermal degradation kinetics, a second-order polynomial model, as given in Equation 1), was applied in coded form to all the data.

Equation 1) Dc = 1.65 - (0.1419A) - (0.0666B) - (0.1522C) - (0.1599AB) + (0.1912AC) + (0.2307C^2)

As shown in Equation 1), fructose concentration, temperature, and pH linearly have significant negative effects on the thermal degradation rate of phycocyanin pigment. In other words, increasing fructose concentration and pH leads to a decrease in Dc. The quadratic effect of temperature and the interaction of temperature and fructose concentration have positive effects on Dc. The interaction of fructose concentration and pH has a negative effect on Dc ($p < 0.05$).

Figure 4 shows that with increasing temperature, the thermal degradation rate of phycocyanin decreases until it reaches a minimum value (74°C), and beyond this point, with further temperature increase, the thermal degradation rate of phycocyanin increases.

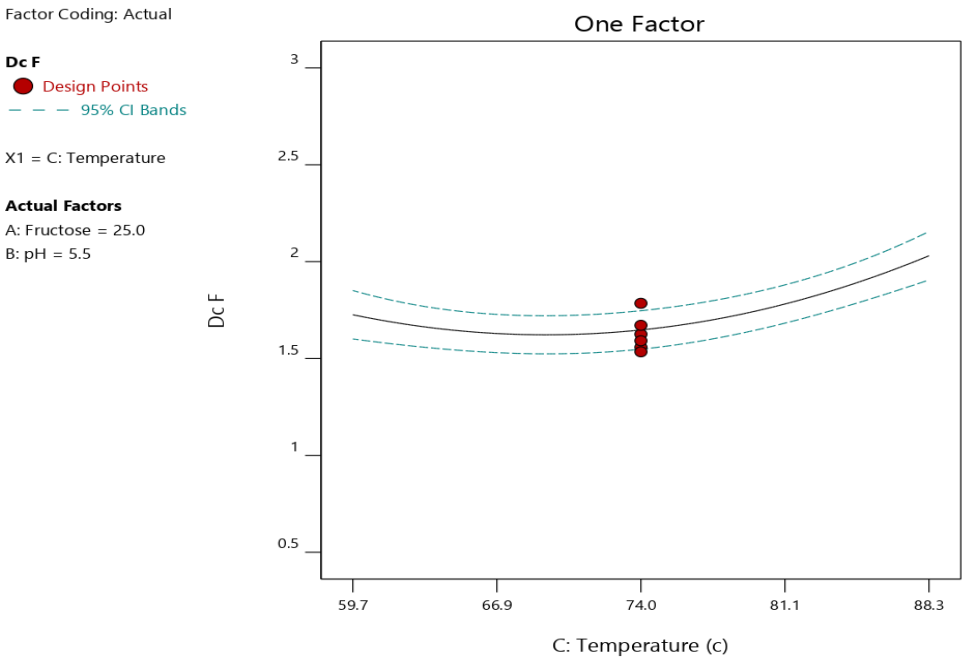


Figure 4. Independent effect of temperature on the thermal degradation rate of phycocyanin.

As observed in Figure 5, with an increase in fructose concentration, the thermal degradation rate of the blue pigment phycocyanin decreases. At higher pH levels (4.6), increasing fructose concentration leads to a further decrease in the thermal degradation rate of phycocyanin. Moreover, the highest thermal degradation rate of phycocyanin is observed at lower fructose concentrations (10%) and lower temperatures

(59.7°C). In the fructose concentration range of 30–40%, with an increase in temperature up to 74°C, the thermal degradation rate of phycocyanin first decreases, and from 74 to 88°C, the thermal degradation rate of phycocyanin increases.

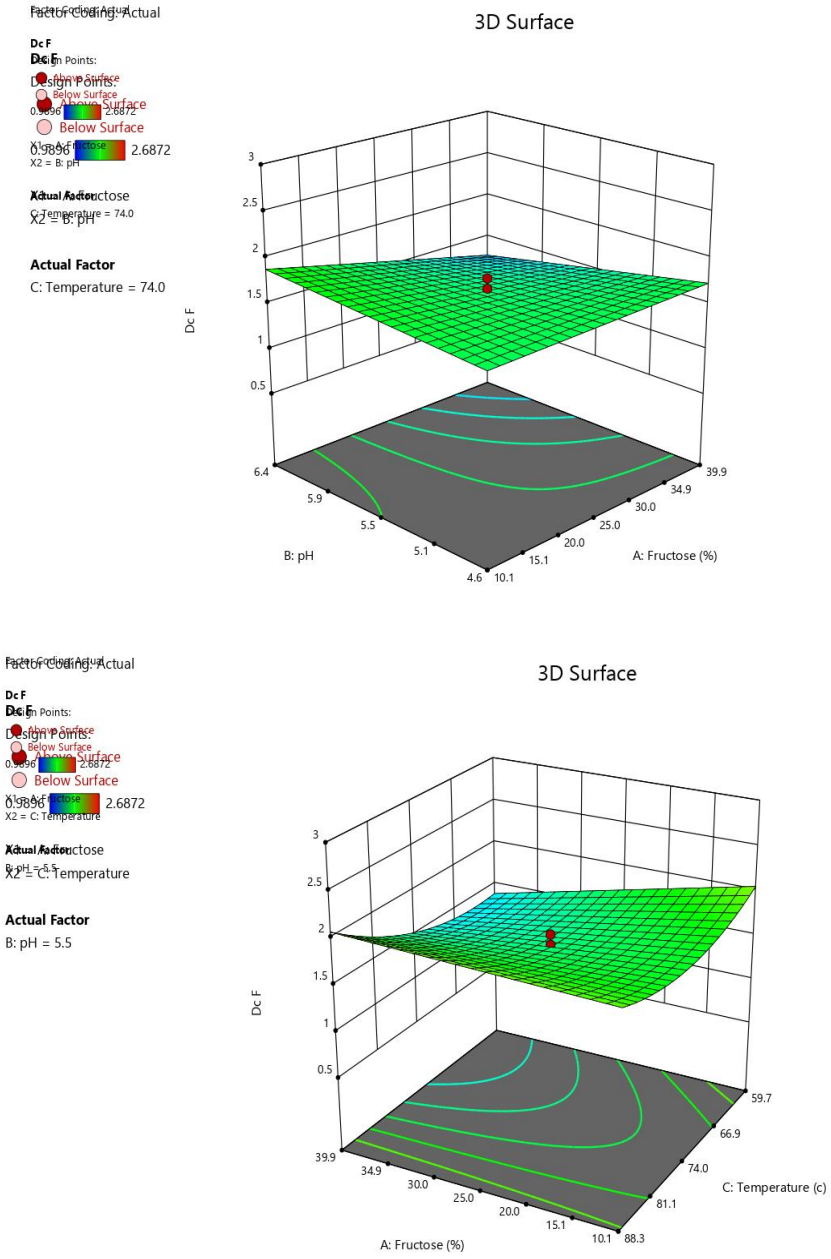


Figure 5. Two-dimensional plot of the interaction effect of pH and fructose concentration (a), and temperature and fructose concentration (b) on the thermal degradation rate of phycocyanin.

3.4.2. Effect of Temperature, pH, and Fructose Concentration on the Half-Life of Phycocyanin Stability

Phycocyanin half-life, reflecting pigment stability under processing stress, is defined as the time required for the pigment concentration to decline to half of its initial level, following first-order degradation kinetics. The analysis of variance table 8 shows the effect of temperature, pH, and fructose concentration on the half-life of phycocyanin. The results indicate that the proposed model is significant ($p < 0.05$), and the lack of fit is not significant ($P = 0.0209$). The R^2 is 0.8112, the adjusted R^2 is 0.7010, and the coefficient of variation (C.V.) is 11.99%. The linear effects of temperature and fructose concentration, as well as the

interaction effects between fructose concentration and temperature, and between temperature and pH, are significant. The quadratic effect of fructose concentration is also significant ($p < 0.05$).

Table 8. Final Analysis of Variance Results of the Effect of Three Variables (Temperature, pH, Fructose Concentration) on the Half-Life of Phycocyanin

Source of Variation	Degrees of Freedom	Sum of Squares	F	P
Model	7	0.2457	7.36	0.0015
Fructose (A)	1	0.0371	7.78	0.0164
pH (B)	1	0.0162	3.40	0.0900
Temperature (C)	1	0.0420	8.81	0.0117
AB	1	0.0157	3.30	0.0945
AC	1	0.0537	11.26	0.0057
BC	1	0.0400	8.38	0.0134
A^2	1	0.0411	8.62	0.0125
Residual Error	12	0.0572		
Lack of Fit	7	0.0522	7.45	0.0209
Pure Error	5	0.0050		
Total	19	0.3029		

R^2: 0.8112
C.V.%: 11.99
Adjusted R^2: 0.7010
Predicted R^2: 0.1236

Equation 2) h1/2 = +0.6119 + (0.0521A) + (0.0345B) + (0.0555C) - (0.0443AB) - (0.0819AC) + (0.0707BC) - (0.0529A^2)

According to Equation 2), fructose concentration has a significant negative effect, while temperature has a linear positive effect on the half-life of phycocyanin pigment. Fructose concentration has a quadratic positive effect, and the interaction between pH and temperature has a negative effect on the half-life of phycocyanin pigment. Furthermore, the interaction between pH and temperature has a positive effect on the half-life of phycocyanin ($p < 0.05$). Figure 6shows that with increasing fructose concentration, the half-life of phycocyanin pigment increases until it reaches a maximum point (25%), and beyond that point, further increase in fructose concentration negatively affects the half-life of phycocyanin pigment, causing it to decrease.

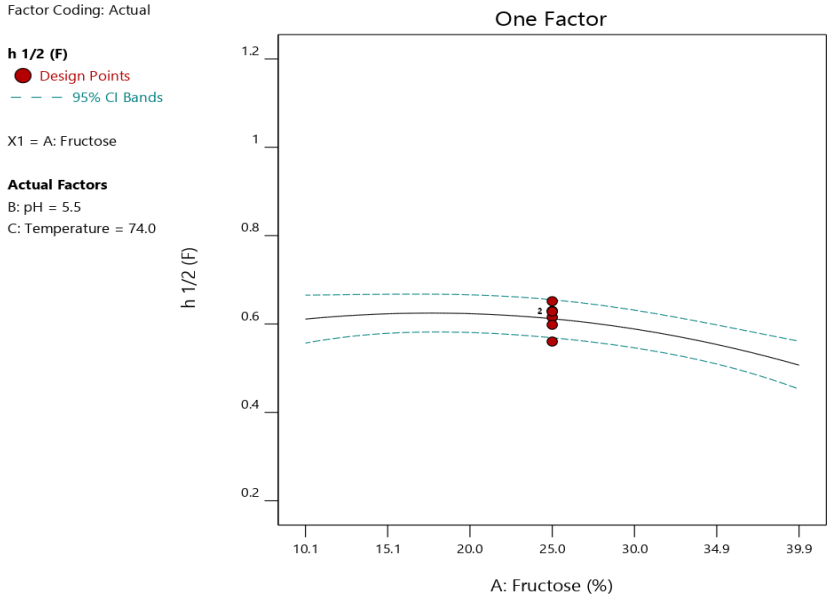
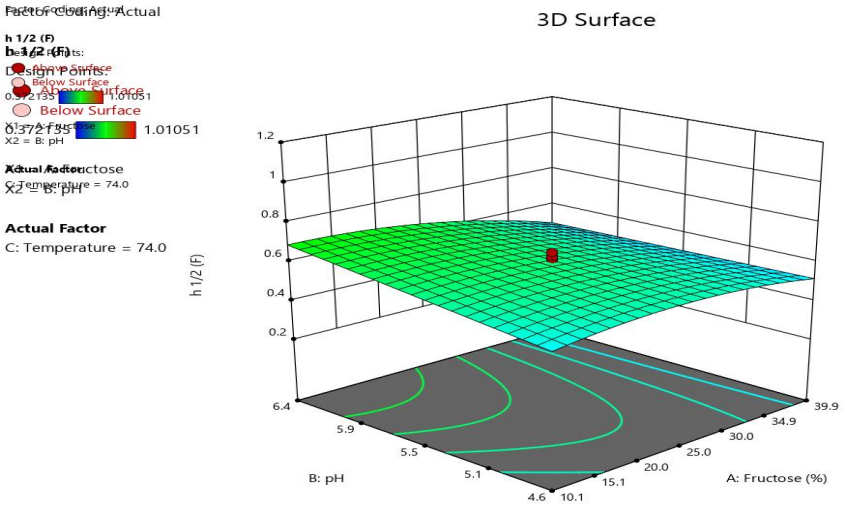
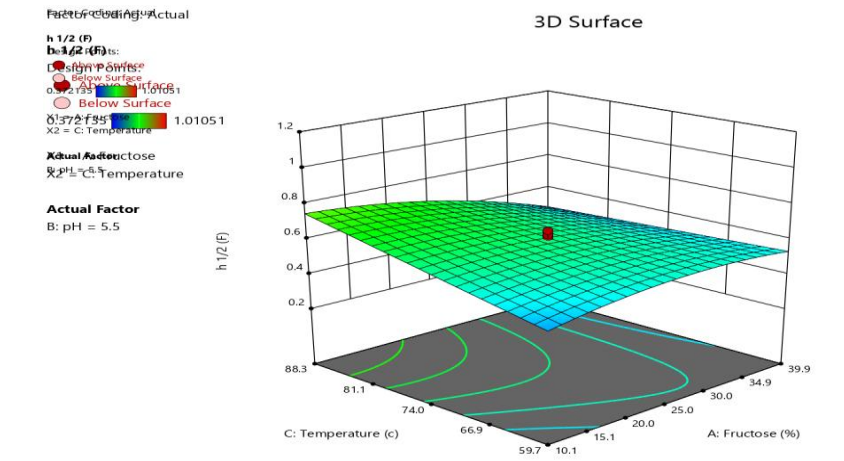


Figure 6. Independent effect of fructose concentration on the half-life of phycocyanin pigment.

According to Figure 7 (a), an increase in pH leads to an increase in the half-life of phycocyanin pigment. As observed, a simultaneous increase of both fructose concentration and pH reduces the half-life of the phycocyanin pigment. Moreover, at high fructose concentrations (39.9%), an increase in pH has little

effect on the half-life of the pigment. The highest half-life of phycocyanin pigment corresponds to lower fructose concentrations (10%) and higher pH levels (4.6). It should be noted that at low fructose concentrations (10%), an increase in temperature positively affects the half-life of phycocyanin pigment. Furthermore, an increase in both temperature and fructose concentration simultaneously reduces the half-life of phycocyanin pigment (Figure 7 b). Figure 7 (c) shows that a simultaneous increase in temperature and pH increases the half-life of phycocyanin pigment. Additionally, the lowest half-life is observed at pH = 4.6 and temperature 59.7°C.





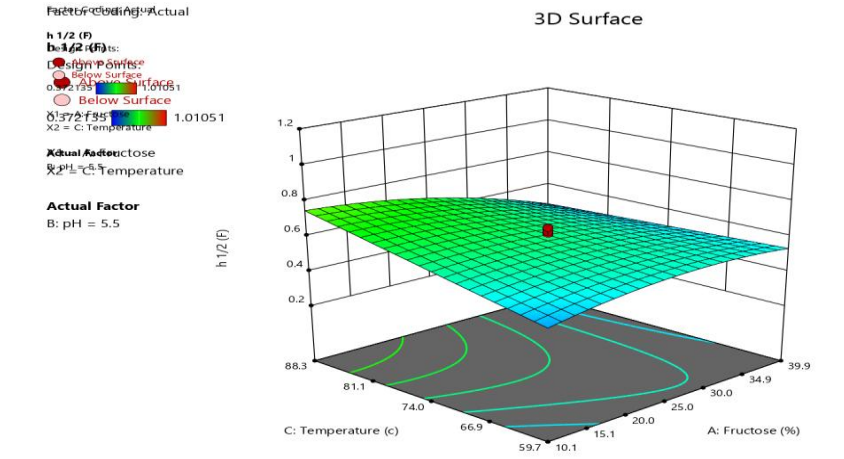


Figure 7. Two-dimensional plot of the interaction effect of pH and fructose concentration (a), temperature and fructose concentration (b), and temperature and pH (c) on the half-life of phycocyanin pigment.

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

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 (Chaiklahan et al., 2012; Adjali et al., 2021).The results of this study showed that the stability of phycocyanin in freeze-dried treatments g different treatments, the freeze-dried treatment with sucrose had the highest concentration and stability of phycocyanin (Figure 4-1). Oven-drying significantly reduces phycocyanin concentration due to heat-induced alterations in its secondary, tertiary, and quaternary structures leading to protein denaturation—resulting in much greater pigment loss compared to other drying methods (Mróz et al., 2024). It is noteworthy that the advantage of freeze-drying in preserving the amount and stability of phycocyanin, compared to oven or spray drying, is due to the removal of water at low temperatures without causing thermal degradation or breakdown of phycocyanin [15]. Additionally, freeze-drying causes the least alteration in the protein structure of phycocyanin. Conversely, the relative temperature increase (in an oven) affects the protein structure of this pigment, reducing its amount and stability [5]. Numerous studies have investigated the effects of various processing methods on phycocyanin pigment, with results indicating that the processing method, temperature, time, and pH significantly influence the concentration and stability of phycocyanin.

In a study conducted by Doke (2005), it was found that the phycocyanin content of Spirulina biomass dried under shade at 25°C with air circulation was higher than that of sun-dried and oven-dried (50°C) samples. Antelo et al. (2008) also reported that increasing the temperature from 50 to 65°C increased the denaturation rate of phycobiliprotein phycocyanin. Similarly, the findings of Güroy et al, (2017) confirmed that the phycocyanin content of freeze-dried samples was higher than that of oven-dried samples (80°C), with a 35.4% reduction in phycocyanin content in the oven-dried method.

According to the results of the present study, the concentration and stability of phycocyanin were higher in treatments with sugar than in those without sugar. The findings suggest that using sucrose during the drying process with all three methods—freeze, spray, and oven drying—was more effective in improving the concentration and stability of phycocyanin pigment compared to glucose and fructose (Tables 4-2 and 4-6, and Figure 4-1). The lower stability of phycocyanin treated with fructose, compared to glucose, can be explained by the fact that glucose readily binds to the protein structure of phycocyanin via glycosidic bonds and acts as a stabilizer, protecting the protein structure of phycocyanin against heat, especially at high temperatures. In contrast, fructose has a more stable and resistant structure and binds less readily to protein, forming sugar-protein complexes with more difficulty. Some researchers believe that at 60°C, glucose easily forms N-linked glycosidic bonds with phycocyanin protein, while fructose starts to break down, and sucrose binds less readily to proteins because it is a non-reducing sugar, and its O-linked glycosidic bond between glucose and fructose makes it less reactive. At higher temperatures (80°C), the O-linked glycosidic bond in sucrose starts to break down into glucose and fructose, which then form N-linked glycosidic bonds with proteins, thereby enhancing stability [16].

Previous studies have also shown that various sugars, including sucrose, glucose, fructose, and even sugar alcohols, can act as protein stabilizers and reduce the rate of thermal degradation of phycocyanin during heat processing. In this regard, Antelo et al. (2008) reported that sugar alcohols such as sorbitol can prevent thermal degradation of phycocyanin across a wide pH range (5–7) and temperatures (50–65°C) [14]. Mishra et al. (2008) found that both calcium chloride and sucrose were effective in maintaining the stability of phycocyanin at low temperatures. Miyawaki et al. (2016) reported that trehalose improved the

thermal stability of phycocyanin. Hadiyanto et al. (2018) stated that the phycocyanin concentration combined with glucose during thermal processing was higher than that combined with fructose and sucrose. Additionally, the concentration of phycocyanin combined with sucrose was higher than with fructose when used as a stabilizer.

According to this study, the amount and stability of phycocyanin decreased over the 120-day storage period (Table 4-3). This decrease can be explained by the protein nature of phycocyanin, which likely undergoes structural changes during storage, leading to reduced stability (Doke et al., 2005). In a study by Chaiklan et al. (2012), it was found that phycocyanin concentration at 4°C decreased after 120 days, with a greater decrease at pH 5 than at pH 6 or 7.

3.6. Effect of Temperature, pH, and Fructose Concentration on the Thermal Degradation Kinetics of Phycocyanin

The results of the thermal degradation kinetics study of phycocyanin showed that with increasing pH (up to 4.6) and fructose concentration (up to 39%), the thermal degradation rate of phycocyanin decreased, while an increase in temperature, especially at higher levels, increased this parameter (Equation 4-1, Figures 4-2 and 4-3). Increasing fructose concentration up to moderate levels (25%) increased the half-life of phycocyanin pigment, but further increasing the fructose concentration reduced its half-life (Figure 4-4). Since phycocyanin is protein-based, it is highly sensitive to heat and high temperatures. Accordingly, in this study, the thermal degradation rate of phycocyanin increased with rising temperatures. At high temperatures (thermal processing), the breaking of inter- and intra-chain bonds affects the spatial structure of the protein, leading to protein denaturation, which accelerates phycocyanin degradation and reduces its stability [5] .

Duangsee et al. (2009) reported that phycocyanin remains more stable at low temperatures and pH values of 4.5–5, but rapidly denatures and precipitates when the temperature increases to 45–48°C. Other studies have also shown that at high temperatures (45–75°C), the protein structure of phycocyanin denatures, reducing its stability. Therefore, the use of various stabilizers such as sucrose, glucose, and sodium chloride can enhance the stability of phycocyanin under different temperature and pH conditions [5]. In this study, using appropriate fructose concentrations reduced the thermal degradation rate of phycocyanin under various temperature and pH levels.

The positive effect of fructose on the thermal stability of phycocyanin can be explained by the fact that adding sugar to phycocyanin during thermal processing increases the activation energy by up to four times, and at higher sugar concentrations, the sugar can form more bonds with the protein, thus improving pigment stability and reducing its thermal degradation [16]. Many researchers have also noted that when Spirulina solution is formulated with monosaccharides, organic acids, and salts, it significantly reduces the negative effect of heat on phycocyanin (Faietaet al., 2020). The mechanism of thermal stability enhancement of proteins such as phycocyanin by monosaccharides involves binding to the protein molecule and controlling the orientation of secondary, tertiary, and quaternary structures, preventing protein inactivation under various temperature and pH levels [17].

Sharma et al. (2021) reported that adding sugars such as glucose, fructose, sucrose, lactose, and galactose to phycobiliproteins extracted from Oscillatoria (a type of cyanobacterium) prevents further protein breakdown during heat processing by reducing entropy and increasing enthalpy due to the formation of glycosidic bonds. Martelli et al. (2014) found that adding sugars or honey reduced the thermal degradation rate of phycocyanin compared to the control group, with the highest thermal stability achieved using fructose, sucrose, and glucose, respectively.

It is worth noting that another factor affecting the degradation of the protein structure of phycocyanin under different temperature conditions is pH, with acidic, neutral, and alkaline pH levels having varying effects. In this regard, Safari et al. (2017) concluded that the stability of phycocyanin was higher at low temperatures compared to high temperatures, and the stability was greater at pH 4.5 than at pH 5.5 or 7.

4. Conclusion

The results of this study showed that freeze-drying 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, spray drying, and the use of glucose or fructose. Regardless of the drying method and type of sugar, the stability of phycocyanin decreased over the 120-day storage period. Based on the findings of this research, a second-order model with R^2 values above 0.80 was suitable for describing and predicting the thermal kinetics (thermal degradation rate and half-life) of this pigment. Increasing pH and fructose concentration reduced the thermal degradation rate, while increasing temperature increased it. Using appropriate fructose concentrations (25%) reduced the half-life of the pigment. Other innovative non-thermal methods for extracting phycocyanin is recommended. Also, investigating the effect of type and concentration of sugars on the antioxidant activity and color of phycocyanin could be future trend. Stabilizing agents such as sugars in the extraction and processing industry of phycocyanin pigment could be applied.

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 adheried 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.

5.6. Funding

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5.7. Using Artificial Intelligent chatbots

None.

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