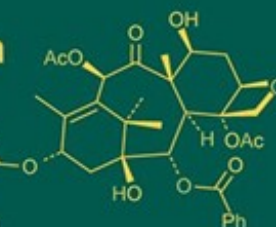
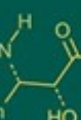
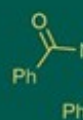


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Environmental stability and dairy-food application of Spirulina-derived phycocyanin: Color retention, microbiological quality and economic feasibility

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ABSTRACT

Spirulina-derived C-phycocyanin is an attractive natural blue colourant, but its industrial use is constrained by sensitivity to light, acidity and heat. This study evaluated the stability of an extracted phycocyanin preparation under dark, LED-daylight and UV-A exposure; at pH 5.0-7.0; and at 30-75 °C. Its performance was subsequently examined in whipping cream and flavoured milk. Pigment retention was monitored spectrophotometrically at 620 nm for 7 days, while product colour was followed through CIELAB coordinates for 15 days. Optimized products containing 100 mg pigment (T_2) were selected sensorially and their microbiological quality was assessed during 28 days of refrigeration. Dark storage retained 94.6% of initial absorbance, compared with 66.7% under LED daylight and 26.9% under UV-A. After 7 days, pH 6.0 provided the greatest retention (93.5%), followed by pH 5.5 (91.4%), pH 7.0 (88.0%) and pH 5.0 (78.0%). Retention was 96.8% at 30 °C and 93.5% at 45 °C, but only 57.7% at 60 °C and 37.0% at 75 °C. In whipping cream and flavoured milk, chroma decreased by 13.8% and 17.5%, respectively, over 15 days, while hue remained in the blue to blue-purple region. T_2 achieved overall acceptability scores of 8.4 and 8.3, respectively. Coliforms were not detected in either product through 28 days, although total plate and yeast/mould counts rose during storage. Correcting the arithmetic of the thesis cost sheet gave an estimated batch cost of ₹192.55; at the reported approximate 6-g yield, this was ₹32.09/g. The findings support light-protective packaging, mildly acidic systems near pH 5.5-6.0 and low-temperature processing for practical food use of phycocyanin.

Keywords: C-phycocyanin, Natural blue colourant, *Spirulina*, Pigment stability, Flavoured milk, Whipping cream, Clean label

1. Introduction

Consumer preference for recognisable, naturally derived ingredients has intensified interest in replacing synthetic food colours with pigments from plants, microorganisms and algae. Blue remains a technically difficult colour because relatively few natural sources provide an intense hue with acceptable food compatibility. C-phycocyanin, the principal blue phycobiliprotein of *Arthrospira* (commercially known as *Spirulina*), combines colouring capacity with reported antioxidant functionality and is therefore promising for clean-label foods.

Unlike many low-molecular-weight pigments, phycocyanin is a protein-chromophore complex. Its colour is consequently sensitive to unfolding, aggregation and chromophore disruption. Temperature, pH, illumination, oxygen and food-matrix interactions can accelerate these changes. Maximum stability has commonly been reported around pH 5.5-6.0, below approximately 45 °C and in the absence of light (Chaiklahan *et al.*, 2012; Adjali *et al.*, 2022) [2, 1]. This narrow stability window is a central barrier to wider use in thermally processed and brightly displayed foods.

Model-food trials are necessary because stability in a buffered pigment solution does not necessarily predict behaviour in a complex food. Dairy matrices contain proteins, fat, lactose and minerals that may either protect the pigment or alter its optical expression. Previous work has demonstrated that food-grade phycocyanin can retain useful colour in frozen dairy systems and beverage matrices, although performance depends on formulation and storage conditions (de Amarante *et al.*, 2020; Garcia *et al.*, 2021) [3, 4].

The present paper addresses the third and fourth objectives of a broader pigment-development study. It evaluates the stability of extracted phycocyanin against light, pH and temperature; examines colour retention and acceptability in whipping cream and flavoured milk; monitors microbiological quality during refrigerated storage; and assesses preliminary production economics. This downstream focus avoids duplication of cultivation and extraction optimization and directly addresses feasibility as a food colourant.

2. Materials and Methods

2.1 Pigment and experimental design

Food-grade phycocyanin previously extracted and purified from cultivated *Spirulina* biomass was used. Stability was followed by absorbance at 620 nm, with higher absorbance representing greater retention of the characteristic blue pigment. Unless otherwise stated, values represent mean $\pm$ standard deviation of three determinations.

2.2 Light, pH and temperature stability

For light stability, identical pigment portions were stored in darkness, under LED daylight and under UV-A exposure. Absorbance was recorded daily for 7 days. The pH trial used pigment systems adjusted to pH 5.0, 5.5, 6.0 and 7.0 and monitored from day 0 to day 7. Thermal stability was evaluated at 30, 45, 60 and 75 °C over the same period. Retention at the final observation was calculated as final absorbance divided by initial absorbance $\times$ 100.

2.3 Preparation of model dairy foods

Whipping cream was prepared from 66.90 mL milk, 30.00 g unsalted butter, 2.50 g powdered sugar and 0.50 g vanilla flavour, with phycocyanin added according to treatment. Milk was heated to 40–45 °C, butter was melted into it, remaining ingredients were mixed, and the blend was homogenized for 2–3 min. After cooling at 4 °C for 2–4 h, it was whipped at high speed for approximately 20 min and refrigerated. Flavoured milk contained 91.80 mL milk, 8.00 g sugar, 0.10 g vanilla flavour and treatment-specific phycocyanin. Milk and sugar were heated at 70–80 °C for 5–10 min; flavour and pigment were added after cooling. Treatment T₂ contained 100 mg phycocyanin and was selected for detailed evaluation.

2.4 Sensory and colour measurements

Control and pigment treatments (0, 50, 100 and 150 mg) were assessed by a semi-trained panel of academic staff and students using a nine-point hedonic scale for appearance, colour, taste, flavour, texture and overall acceptability. The thesis did not report panel size; therefore, no unsubstantiated sample size is assigned here. Colour was measured with a Hunter 45/0-L MiniScan XE Plus after black-and-white-tile calibration. L^* , a^* and b^* were recorded and chroma (C^*) and hue angle (h°) were calculated. Colour of optimized products was monitored on days 0, 3, 7, 10 and 15.

2.5 Microbiological evaluation

Refrigerated products were analysed on days 0, 7, 14, 21 and 28. Serial dilutions were prepared in sterile 0.85–0.90% saline. Total plate count was determined on nutrient agar after incubation at 37 °C for 48

h. Yeasts and moulds were assessed on potato dextrose agar. Coliforms were recorded as detected or not detected. Results were expressed as CFU/g for whipping cream and CFU/mL for flavoured milk.

2.6 Economic assessment and statistical analysis

Water, cultivation medium and inoculum were costed at recorded experimental prices. Processing cost was defined as 20% of raw-material cost. Because the original cost table contained an arithmetic inconsistency, both the recorded inputs and a transparent recalculation are reported. Experimental treatment data were analysed in a completely randomized design; standard error and critical difference at the 5% level were reported where available.

3. Results and Discussion

3.1 Effect of light

Table 1. Phycocyanin absorbance at 620 nm during exposure to different light conditions

Day	Dark	LED daylight	UV-A
1	0.93 $\pm$ 0.12	0.93 $\pm$ 0.20	0.93 $\pm$ 0.16
2	0.93 $\pm$ 0.13	0.90 $\pm$ 0.18	0.84 $\pm$ 0.13
3	0.92 $\pm$ 0.12	0.86 $\pm$ 0.15	0.72 $\pm$ 0.11
4	0.92 $\pm$ 0.10	0.81 $\pm$ 0.13	0.59 $\pm$ 0.08
5	0.91 $\pm$ 0.09	0.75 $\pm$ 0.09	0.46 $\pm$ 0.06
6	0.90 $\pm$ 0.09	0.69 $\pm$ 0.08	0.35 $\pm$ 0.05
7	0.88 $\pm$ 0.08	0.62 $\pm$ 0.07	0.25 $\pm$ 0.04

Values are mean $\pm$ SD ($n = 3$).

All treatments began at an absorbance of 0.93, but their trajectories separated rapidly. Dark storage retained 94.6% of initial absorbance after 7 days, compared with 66.7% under LED daylight and 26.9% under UV-A. UV-A therefore produced a 73.1% loss, whereas darkness limited loss to 5.4%. The result confirms that light protection is not merely desirable but essential for preserving this pigment. Photon-driven disruption of the protein-chromophore complex and photo-oxidation plausibly account for the accelerated decline, particularly under UV-A. Opaque or UV-barrier packaging should therefore be used during storage and retail display.

3.2 Effect of pH

Table 2. Phycocyanin absorbance at 620 nm under different pH conditions

Day	pH 5.0	pH 5.5	pH 6.0	pH 7.0
0	0.91 $\pm$ 0.19	0.93 $\pm$ 0.17	0.93 $\pm$ 0.14	0.92 $\pm$ 0.16
1	0.90 $\pm$ 0.16	0.92 $\pm$ 0.14	0.93 $\pm$ 0.16	0.91 $\pm$ 0.11
2	0.88 $\pm$ 0.15	0.91 $\pm$ 0.16	0.93 $\pm$ 0.11	0.90 $\pm$ 0.12
3	0.85 $\pm$ 0.13	0.90 $\pm$ 0.15	0.92 $\pm$ 0.13	0.89 $\pm$ 0.15
4	0.82 $\pm$ 0.12	0.89 $\pm$ 0.12	0.91 $\pm$ 0.12	0.88 $\pm$ 0.13
5	0.79 $\pm$ 0.09	0.88 $\pm$ 0.10	0.90 $\pm$ 0.10	0.86 $\pm$ 0.12
6	0.75 $\pm$ 0.08	0.87 $\pm$ 0.09	0.88 $\pm$ 0.08	0.84 $\pm$ 0.09
7	0.71 $\pm$ 0.06	0.85 $\pm$ 0.05	0.87 $\pm$ 0.07	0.81 $\pm$ 0.07

Values are mean $\pm$ SD ($n = 3$).

The most favourable condition was pH 6.0, which retained 93.5% of initial absorbance. Retention at pH 5.5 was 91.4%, compared with 88.0% at pH 7.0 and 78.0% at pH 5.0. The ranking—pH 6.0 > 5.5 > 7.0 > 5.0—agrees with the recognized stability maximum around pH

5.5–6.0 (Chaiklahan *et al.*, 2012) [2]. Increasing acidity toward pH 5.0 probably promoted conformational destabilization and aggregation, while the modest loss at pH 7.0 may reflect a less favourable oligomeric state. The pigment is thus better suited to mildly acidic or near-neutral foods than strongly acidic beverages.

3.3 Effect of temperature

Table 3. Phycocyanin absorbance at 620 nm during storage at different temperatures

Day	30 °C	45 °C	60 °C	75 °C
0	0.93 ± 0.12	0.93 ± 0.15	0.78 ± 0.12	0.54 ± 0.18
1	0.93 ± 0.16	0.92 ± 0.12	0.73 ± 0.17	0.48 ± 0.15
2	0.93 ± 0.16	0.92 ± 0.11	0.68 ± 0.15	0.43 ± 0.11
3	0.92 ± 0.13	0.91 ± 0.14	0.64 ± 0.11	0.37 ± 0.10
4	0.92 ± 0.14	0.90 ± 0.10	0.59 ± 0.13	0.32 ± 0.08
5	0.91 ± 0.12	0.89 ± 0.11	0.54 ± 0.07	0.28 ± 0.07
6	0.91 ± 0.11	0.88 ± 0.09	0.49 ± 0.08	0.24 ± 0.04
7	0.90 ± 0.10	0.87 ± 0.08	0.45 ± 0.06	0.20 ± 0.02

Values are mean ± SD ($n = 3$). Initial absorbance differed at higher temperatures, indicating rapid early thermal loss before or at the first recorded measurement.

Temperature produced the largest practical constraint. Final retention relative to the recorded day-0 value was 96.8% at 30 °C and 93.5% at 45 °C, but fell to 57.7% at 60 °C and 37.0% at 75 °C. Moreover, day-0 absorbance was already lower at 60 and 75 °C than at 30 and 45 °C,

suggesting immediate degradation during heat exposure. Chaiklahan *et al.* (2012) [2] identified a critical stability region near 47 °C, while Martelli *et al.* (2014) [5] similarly described the need for stabilization at processing temperatures. Phycocyanin should therefore be added after severe heat treatment whenever possible, or protected through validated stabilizers or encapsulation.

3.4 Colour retention in dairy matrices

Table 4. CIELAB colour of phycocyanin-coloured whipping cream during refrigerated storage

Day	L*	a*	b*	C*	h°
0	82.50 ± 0.37	-6.80 ± 0.14	-18.90 ± 0.19	20.09 ± 0.18	250.21 ± 0.42
3	82.22 ± 0.32	-6.59 ± 0.11	-18.50 ± 0.17	19.64 ± 0.16	250.39 ± 0.35
7	81.87 ± 0.25	-6.24 ± 0.08	-17.84 ± 0.14	18.90 ± 0.13	250.72 ± 0.27
10	81.50 ± 0.23	-6.00 ± 0.05	-17.20 ± 0.12	18.22 ± 0.11	250.77 ± 0.19
15	80.90 ± 0.20	-5.57 ± 0.03	-16.40 ± 0.07	17.32 ± 0.07	251.24 ± 0.12

Values are mean ± SD ($n = 3$).

Table 5. CIELAB colour of phycocyanin-coloured flavoured milk during refrigerated storage

Day	L*	a*	b*	C*	h°
0	87.80 ± 0.49	-4.20 ± 0.10	-15.60 ± 0.30	16.32 ± 0.32	254.93 ± 0.38
3	87.37 ± 0.46	-4.00 ± 0.07	-15.10 ± 0.26	15.62 ± 0.30	255.16 ± 0.40
7	86.87 ± 0.41	-3.70 ± 0.06	-14.37 ± 0.21	14.84 ± 0.27	255.56 ± 0.45
10	86.40 ± 0.39	-3.38 ± 0.03	-13.78 ± 0.16	14.19 ± 0.19	256.23 ± 0.49
15	85.90 ± 0.35	-3.10 ± 0.02	-13.10 ± 0.13	13.46 ± 0.15	256.69 ± 0.52

Values are mean ± SD ($n = 3$).

Whipping cream became slightly darker (L^* decreased 1.60 units) and less saturated (C^* decreased 13.8%) over 15 days. Flavoured milk showed a 1.90-unit decline in L^* and a 17.5% decline in C^* . In both systems, negative b^* values moved toward zero, indicating weakening blue intensity, while hue changed by only 1.03° in cream and 1.76° in milk. Thus, colour vividness diminished gradually without a major shift in hue identity. The cream matrix retained chroma slightly better, possibly because fat and its lower light scattering environment altered pigment protection or optical expression. Comparable successful

dairy applications have been reported in ice cream and model beverages (de Amarante *et al.*, 2020; García *et al.*, 2021) [3, 4].

T₂ (100 mg phycocyanin) provided the best overall acceptability: 8.4 in whipping cream and 8.3 in flavoured milk. It also achieved colour scores of 9.0 and 8.5, respectively. Control overall acceptability was 7.8 for cream and 7.5 for milk. Because the differences exceeded the corresponding critical differences (0.24 and 0.23), the optimized treatments were statistically preferred at the 5% level. The pigment therefore improved appearance without materially reducing taste, flavour or texture.

3.5 Microbiological quality

Table 6. Microbiological quality of optimized dairy products during refrigerated storage

Day	Cream TPC (CFU/g)	Cream Y&M (CFU/g)	Cream coliform	Milk TPC (CFU/mL)	Milk Y&M (CFU/mL)	Milk coliform
0	ND	ND	ND	1.2×10^1	ND	ND
7	ND	ND	ND	2.5×10^1	ND	ND
14	2.0×10^1	ND	ND	8.0×10^1	1.0×10^1	ND
21	5.0×10^1	1.0×10^1	ND	2.2×10^2	2.0×10^1	ND
28	1.2×10^2	1.5×10^1	ND	6.5×10^2	4.0×10^1	ND

TPC, total plate count; Y&M, yeast and mould; ND, not detected at the tested dilution.

Microbial populations increased with storage time but remained numerically low in both products. Cream showed no detectable growth through day 7; TPC reached 1.2×10^2 CFU/g and yeast/mould 1.5×10^1 CFU/g by day 28. Flavoured milk began at 1.2×10^1 CFU/mL and reached 6.5×10^2 CFU/mL, while yeast/mould appeared on day 14 and reached 4.0×10^1 CFU/mL. Coliforms were not detected.

These observations support hygienic processing, but absence of an explicitly stated detection limit and product-specific regulatory comparison prevents a definitive 28-day shelf-life claim. The PDA method was described as streak plating in the thesis; future validation should use a quantitative spread- or pour-plate protocol with replicate variability.

3.6 Preliminary economic feasibility

Table 7. Recorded and corrected cost estimate for phycocyanin production

Item	Rate	Quantity	Recorded cost (₹)	Corrected cost (₹)
Water	₹20/L	1,000 mL	20.00	20.00
Medium	₹5,500/kg	12.23 g	6.66	6.66
<i>Spirulina</i> culture	₹6,690 (basis recorded)	20 mL	133.80	133.80
Raw-material total	-	-	160.46	160.46
Processing (20%)	20% of raw materials	-	30.00	32.09
Batch production cost	-	-	190.46	192.55
Approximate cost/g*	-	~6 g yield	31.74	32.09

*The per-gram calculation assumes the approximately 6-g pigment yield implied by the original cost sheet. Market prices and yield must be revalidated before commercialization.

The source table reported ₹160.46 as raw-material cost but listed processing cost as ₹30.00, although 20% equals ₹32.09. Correct arithmetic gives a batch cost of ₹192.55. Using the approximately 6-g yield implied by the reported ₹31.74/g figure, the corrected production cost is ₹32.09/g. This remains far below the thesis's stated reference retail price of approximately ₹400/g, but the comparison should be treated cautiously: purity grade, moisture basis, labour, utilities, equipment depreciation, packaging, quality control and distribution were not aligned. The result is a promising laboratory cost estimate, not a bankable commercial feasibility analysis.

4. Conclusion

Spirulina-derived phycocyanin was most stable in darkness, at pH 5.5-6.0 and at temperatures no higher than 45 °C. UV-A and temperatures of 60-75 °C caused substantial loss of absorbance. When applied at the optimized 100-mg level, the pigment produced highly acceptable whipping cream and flavoured milk and retained its blue-to-blue-purple hue through 15 days, although chroma declined gradually. Microbial counts increased during 28-day refrigeration, but coliforms remained undetected. The corrected laboratory cost was approximately ₹32.09/g at the yield implied by the thesis records. Practical adoption should use light-barrier packaging, post-heat pigment addition or mild processing, refrigeration and product-specific shelf-life validation.

5. Limitations

The study did not report pigment purity grade, exact UV-A irradiance, LED intensity, dissolved oxygen, packaging type, sensory panel size or detection limits. Repeated-measures significance was not reported

for stability datasets. The microbial yeast/mould procedure requires quantitative validation, and the economic analysis omitted commercial overheads. These omissions should be addressed before journal submission or scale-up.

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Conflict of interest

The authors declare no conflict of interest.

Data availability

The data supporting this study are presented in the article and the underlying thesis records.

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