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Degradation Kinetics and Antioxidant Capacity Study of Suji Leaves Extract (*Dracaena angustifolia*)

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ABSTRACT

Synthetic colorants, prized for their diverse colors, homogeneity, and cost-effectiveness, are highly stable under various environmental conditions. Despite these advantages, health concerns have fueled a growing interest in natural alternatives. This study employs Dracaena angustifolia, a pigment-rich plant that produces green extracts suitable for food. The study aimed to determine the stability of Suji leaf extracts through degradation kinetics analysis and assess the physicochemical properties over four weeks. Optimal conditions for colorant stability were identified based on the concept of degradation kinetic concept, which measures absorbance at different pH levels and temperatures. The physicochemical properties studied over four-weekend the antioxidant capacity, total phenolic, color in color, and chlo, prophyll content for both Suji leaves exleaf (Dracaena angustifolia) and synthetic powder colourantcolorantse four weeks, an inverse relationship was observed between the DPPH scavenging effect and total phenolic content. A decrease in total phenolic content and an increase in IC50 values in the Suji leaf extract suggested a potential weakening of its antioxidant capacity. Additionally, the Suji leaves extract exhibited significantly higher chlorophyll content but lower color intensity and stability than synthetic powder, positively correlating with total phenolic content. The degradation kinetics study revealed that Suji leaf extract was the most stable at pH 7.0 and 4°C. In contrast, the synthetic powder colorants showed optimal stability at pH 4.5 and 40°C. These findings and in-depth research underscore the potential of Suji leaf extract as a stable and natural colourant, particularly in the food industry, replacing synthetic powder colorants.

INTRODUCTION

Synthetic colorants are favored in the food industry due to their diverse range of colors, uniformity, brightness, and cost-effectiveness compared to natural alternatives. They offer enhanced stability across

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different environmental conditions such as temperature, pH levels, light exposure, and oxygen concentrations [1]. However, natural food colorants offer a healthier alternative to synthetic options, presenting potential health benefits due to their antioxidant content [2]. Chlorophyll, a natural pigment responsible for the green color in plants, is increasingly sought after as a natural colorant due to rising awareness of its benefits. Natural pigments like chlorophyll offer potential health advantages, including antioxidant and anti-inflammatory properties, which may help combat chronic diseases such as cancer [3]. This research assesses Suji leaf extracts as potential food colorants by analyzing their stability under different temperature and pH conditions typical in food storage. Understanding their stability is vital for evaluating their suitability and guiding breeding programs.

EXPERIMENTAL

Plant materials and synthetic colourant

Suji leaves from *Dracaena angustifolia* were sourced from a farm, and the synthetic powder, Apple Green Food Colour Powder (Kijang Brand), was obtained from a local supermarket due to its high chlorophyll concentration and diverse pigment composition.

Extraction of suji leaves extract

Suji leaves were cut into small pieces and wash them thoroughly. The extraction was conducted by blending the leaves with water at a 1:1 ratio by weight and volume until a uniform consistency was achieved. The mixture underwent filtration, and the filtrate was stored in ambient bottles at -20 °C for analysis. Subsequently, the filtrate was concentrated using a rotary evaporator to remove excess water and solvent. The concentrated Suji leaf extract was freeze-dried and stored at -18 °C for further analysis [1,3,4].

Physicochemical Analysis Properties (1-Week Interval For 4 Weeks)

Spectrophotometric determination of total phenolics

Total phenolic content was assessed using Folin's reagent via colorimetric analysis. 50 µL of the colorant solution or water (as a blank) was mixed with 3700 µL of water and 250 µL of Folin-Ciocalteu reagent (12111-13-6). After 3 minutes, 1 mL of a 20 % Na_2CO_3 (497-19-8) solution was added, and the mixture was incubated at 37 °C in darkness. Absorbance was measured at 765 nm after 60 minutes using a GENESYS 20 visible spectrophotometer. Gallic acid (149-91-7) served as the reference compound for calibration, and total phenolic content (%) was calculated relative to initial conditions. Analytical-grade standards and reagents were obtained from Sigma Aldrich [5].

Color Intensity (CI)

The measurements were carried out using a GENESYS 20 visible spectrophotometer (4001/4, Thermo Scientific, America North (USA). The changes in visual color were assessed with a Minolta CR-400 Chroma Meter (Konica Minolta Sensing, Inc., Japan) by measuring Hunter lightness (L*), redness/greenness (a*), and blueness/yellowness (b*) values [1].

2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The antioxidant activity of colorant solutions was assessed using the DPPH assay following [6] with modifications. A DPPH (1898-66-4) solution was prepared by dissolving 40 mg of DPPH in 1 L of methanol. Colorant solutions (50 µL) were mixed with the DPPH solution (1000 µL) and incubated for 1 hour in darkness. Absorbance was measured at 515 nm using a GENESYS 20 spectrophotometer. Trolox

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was used for the standard curve, and solvent blanks were included. Measurements were conducted at least three times.

Chlorophyll content

Chlorophyll content was determined according to [7]. Samples (0.2 g) were dissolved in 5 mL of distilled water and mixed with isooctane: isopropanol solution (3:1, v/v). After centrifugation, supernatants were collected, evaporated, and dissolved in acetone. Absorbances at 646 and 664 nm were measured to calculate Chlorophyll a and b using the equations:

$$\text{Chlorophyll a } (\mu g/mL) = 11.24\ A_{662} - 2.04\ A_{646} \quad (1)$$

$$\text{Chlorophyll b } (\mu g/mL) = 20.13\ A_{646} - 4.19\ A_{662} \quad (2)$$

Total chlorophyll content, expressed as µg/g dry powder, was determined by adding the contents of chlorophyll a and b.

Storage stability study of suji leaves extract (1-week interval for 4 weeks)

Degradation kinetics study at different pH and temperature

The impact of pH on the stability of Suji leaves chlorophyll extracts was investigated at pH 2.5, 4.5, and 7.0. The chlorophyll extracts were diluted in various pH buffers following the method outlined by [8], as detailed in Table 1.

Table 1. Buffer solutions used for attaining different pH values in the Suji leaf extracts

pH	Buffer solution
2.5	25 mM KCl (7447-40-7)
4.5	400 mM CH_3OONa (127-09-3)
7.0	50 mM Tris-HCl (1185-53-1)

Source: Reyes & Cisneros-Zevallos (2007)

Analytical-grade chemicals from Sigma Aldrich (Atlanta, USA) were utilized. The extract/buffer ratio for pH levels (2.5, 4.5, and 7.0) was adjusted to achieve an absorbance of approximately 0.7, serving as the standard. The final pH was adjusted using 37 % HCl (7647-01-0). Aqueous solutions of natural and synthetic colorants were similarly prepared and equilibrated at room temperature for 1 hour before baseline measurements. They were then incubated in the dark at 25 °C for 28 days, with weekly sampling. A parallel experiment evaluated temperature effects (4 °C, 25 °C, and 40 °C) following the same procedure. Each experiment comprised 15 jars per colorant type and treatment, totaling 45 jars. At each sampling time, three jars were analyzed, resulting in five sampling moments for each experiment.

Statistical Analysis

All measurements were performed three times (n=3). The total and mean scores per attribute were calculated. The value was analyzed statistically for product differences in each attribute using ANOVA at a 5% level.

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RESULTS AND DISCUSSIONS

Spectrophotometric determination of total phenolics

Table 2 displays significant differences in total phenolic content between Suji leaf extract and synthetic powder colorant. Suji leaf extract exhibited higher phenolic content due to its plant origin and diverse bioactive compounds. Both colorants showed decreasing phenolic levels over time, with Suji leaf extract displaying slower degradation attributed to freeze-drying, which minimizes oxidation. Interestingly, synthetic colorants contained phenolic compounds likely from dextrose anhydrous ("see Appendix"), contributing to total phenolic content, albeit lower than Suji leaves extract [3,9,10].

Table 2. Total phenolic content (mg/GAE/g) of Suji leaves extract and synthetic powder colorant (among samples and weeks)

Sample	Total Phenolic Content (mg/GAE/g)				
	Week 0	Week 1	Week 2	Week 3	Week 4
Suji leaves Extract	34.824 ± 1.913^{aA}	29.882 ± 0.998^{aB}	24.412 ± 1.580^{aC}	14.883 ± 2.579^{aD}	8.294 ± 0.583^{aE}
Synthetic Powder	30.765 ± 2.413^{aA}	18.353 ± 0.666^{bB}	9.765 ± 0.166^{bC}	5.883 ± 0.166^{bD}	3.647 ± 0.167^{bE}

Data are expressed as the mean value of replication (n) ± SD (standard deviation). Means with small letters in the same column differed significantly (Duncan's test, $p<0.05$). Means with different capital letters within a row were significantly differences (Duncan's test, $p<0.05$)

Color intensity (CI)

The experiment revealed significant differences in the properties of Suji leaf extract and synthetic powder colorants over the observation period, as seen in Table 3. For lightness (L*), Suji leaf extract showed higher values initially, likely due to its complex pigment composition, while synthetic powder colorant exhibited darker tones [9]. The redness/greenness (a*) values indicated a shift from green to red for the Suji leaves extract and a reduction in green intensity for the synthetic powder colorant. The observed shift in a* values from negative (greenish tones) to positive (reddish tones) for Suji leaves extract is due to chlorophyll being sensitive to oxidation, and over time, exposure to air and light can lead to degradation [1]. The b* values reflected transitions from yellow to blue tones, with synthetic powder initially displaying more intense yellow hues. However, both colorants eventually shifted toward blue tones.

Table 3. Colour measurement analysis of Suji leaves extract and synthetic powder colorant (among samples and weeks).

	L*				
Sample	Week 0	Week 1	Week 2	Week 3	Week 4
Suji leaves Extract	56.47 ± 0.45^{aA}	55.26 ± 0.25^{aB}	53.83 ± 0.86^{aC}	49.76 ± 0.25^{aD}	46.36 ± 0.12^{aE}
Synthetic Powder	54.40 ± 0.25^{bB}	54.33 ± 0.16^{aB}	53.34 ± 0.08^{aA}	48.53 ± 0.08^{bC}	48.14 ± 0.01^{aD}
	a*				
Sample	Week 0	Week 1	Week 2	Week 3	Week 4
Suji leaves Extract	-1.06 ± 0.13^{aD}	-0.59 ± 0.05^{aC}	-0.38 ± 0.06^{aB}	0.05 ± 0.01^{aA}	0.15 ± 0.08^{aA}
Synthetic Powder	-2.46 ± 0.08^{bE}	-2.16 ± 0.16^{bD}	-1.50 ± 0.01^{aC}	-0.77 ± 0.01^{bB}	-0.34 ± 0.02^{aA}
	b*				
Sample	Week 0	Week 1	Week 2	Week 3	Week 4
Suji leaves Extract	1.75 ± 0.01^{bB}	1.96 ± 0.06^{bC}	2.85 ± 0.10^{bA}	0.76 ± 0.01^{bD}	0.89 ± 0.01^{bD}
Synthetic Powder	3.27 ± 0.04^{aB}	3.49 ± 0.26^{aB}	4.68 ± 0.01^{aA}	1.78 ± 0.06^{aC}	1.22 ± 0.01^{aD}

Data are expressed as the mean value of replication (n) ± SD (standard deviation). Means with small letters in the same column differed significantly (Duncan's test, $p<0.05$). Means with different capital letters within a row significantly differed (Duncan's test, $p<0.05$).

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2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

Table 4 presents the antioxidant activities of all colorant samples, measured in terms of Inhibition Concentration at 50% (IC_{50}). Significant differences were observed between Suji leaf extract and synthetic powder colorants. Suji leaf extract exhibited higher IC_{50} values than ascorbic acid, indicating a weaker ability to scavenge DPPH radicals. Moreover, Suji leaf extract showed increasing IC_{50} values over time, suggesting a decline in antioxidant potency, possibly due to decreased phenolic content, with quercetin contributing to stability. Conversely, synthetic powder colorants showed no measurable IC_{50} values, likely due to their unique interaction with DPPH radicals and the absence of glycosidic bonds found in natural colorants. Glycosidic bonds, crucial for enhancing antioxidant potential, are limited in synthetic colorants, affecting their efficacy in neutralizing free radicals [10-12].

Table 4. Inhibition concentration at 50% (IC_{50}) for ascorbic acid, a mixture of BHA and BHT, and Suji leaves extract.

Sample	IC_{50} (ppm)				
	Week 0	Week 1	Week 2	Week 3	Week 4
Ascorbic Acid	279.795 ± 20.739^{cA}	279.795 ± 20.739^{cA}	279.795 ± 20.739^{cA}	279.795 ± 20.739^{cA}	279.795 ± 20.739^{cA}
BHA and BHT	1063.100 ± 36.770^{aA}	1063.100 ± 36.770^{aA}	1063.100 ± 36.770^{aA}	1063.100 ± 36.770^{aA}	1063.100 ± 36.770^{aA}
Suji leaves Extract	468.190 ± 37.901^{bD}	592.325 ± 18.081^{bC}	599.625 ± 2.949^{bC}	624.925 ± 3.429^{bB}	664.245 ± 1.987^{bA}

Data are expressed as the mean value of replication (n) ± SD (standard deviation). Means with small letters in the same column differed significantly (Duncan's test, $p<0.05$). Means with different capital letters within a row significantly differed (Duncan's test, $p<0.05$).

Chlorophyll content

Table 5 presents the chlorophyll content analysis of the tested colorants, revealing a significant difference between Suji leaf extract and synthetic powder. Suji leaf extract consistently exhibited higher chlorophyll content than synthetic powder colorant over 4 weeks, consistent with its plant-based origin, which contained a higher amount of chlorophyll [13]. The data also indicates a significant decrease in chlorophyll content within Suji leaf extract from Week 0 to Week 4 due to environmental factors' degradation effects [14]. Conversely, synthetic powder colorants showed minimal chlorophyll presence, typical chemically produced colorants devoid of natural chlorophyll components.

Table 5. Chlorophyll content of Suji leaves extract and synthetic powder colorant (among samples and weeks).

Sample	Chlorophyll Content (μg/g)				
	Week 0	Week 1	Week 2	Week 3	Week 4
Suji leaves Extract	18.083 ± 4.774^{aA}	6.807 ± 1.290^{aB}	4.435 ± 1.239^{aB}	3.553 ± 0.296^{aB}	1.002 ± 0.535^{aC}
Synthetic Powder	1.257 ± 0.182^{bA}	0.391 ± 0.045^{bB}	0.074 ± 0.022^{bC}	0.044 ± 0.009^{bC}	-0.928 ± 0.077^{bD}

Data are expressed as the mean value of replication (n) ± SD (standard deviation). Means with small letters in the same column differed significantly (Duncan's test, $p<0.05$). Means with different capital letters within a row significantly differed (Duncan's test, $p<0.05$).

Degradation kinetics study at different pH and temperature

The analysis highlighted the significant influence of storage temperature and pH ($p = 0.05$) on the degradation kinetics of Suji leaf extract and synthetic powder colorants. Notably, Suji leaf extract exhibited remarkable stability at pH 7.0 compared to pH 2.5 and 4.5, while synthetic powder colorant showed optimal

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stability at pH 4.5. Additionally, Suji leaves extract stored at 4 °C demonstrated superior stability attributed to refrigeration inhibiting enzyme and bacterial activity, slowing pigment transformation compared to room temperature (25 °C) and 40 °C [15]. In contrast, synthetic powder colorant showed optimal stability at 40 °C, crucial for applications like baking or food processing requiring heat resistance. Choosing binders and carriers in synthetic powder formulation improves its stability under high temperatures [16]. The stability of synthetic powder colorant is illustrated in Fig. 1.

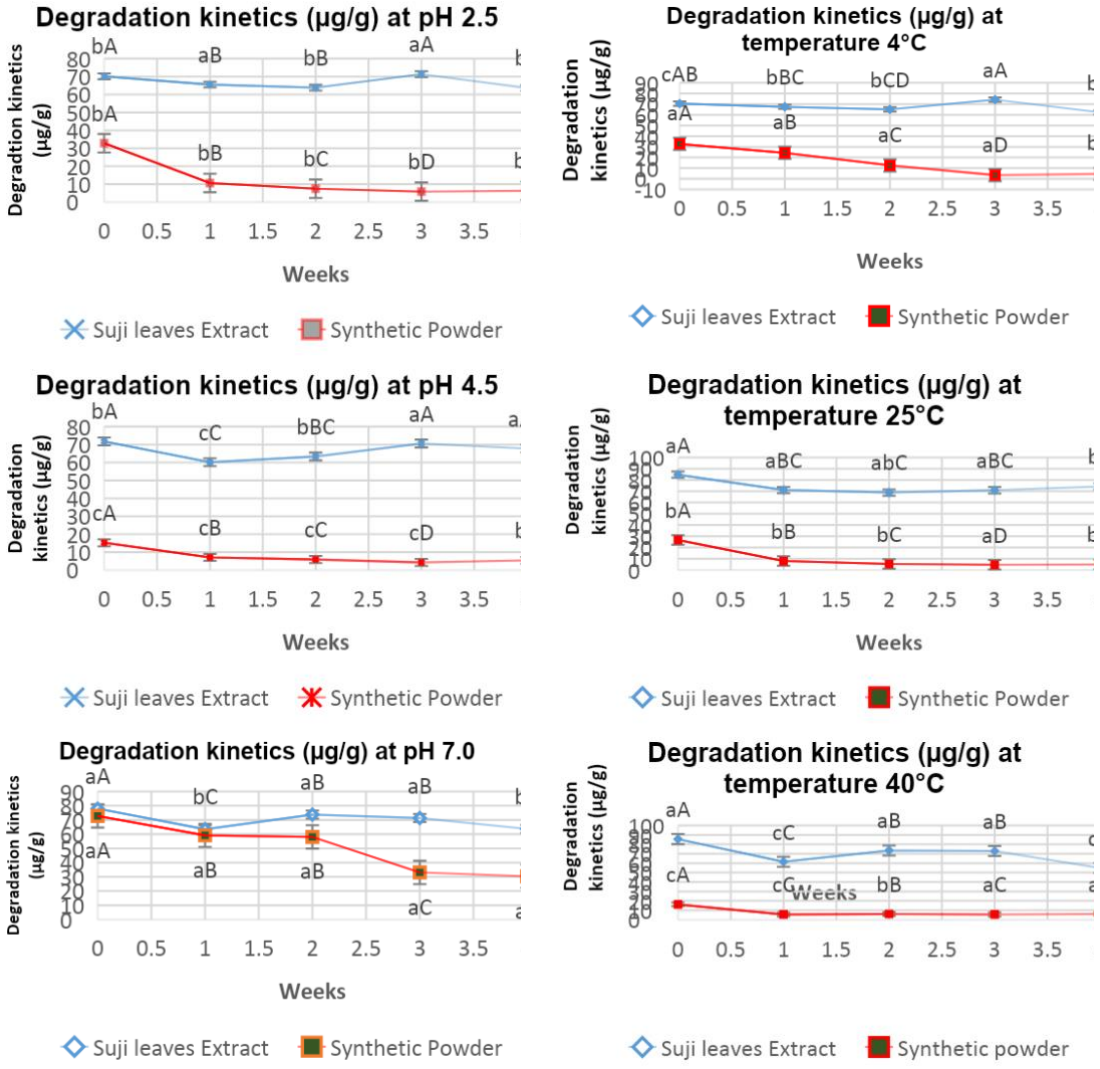

Fig. 1. Time-course variation for degradation kinetics of Suji leaves extract and synthetic powder at different pH and temperature over 4 weeks.

CONCLUSION

This study evaluated Suji leaf extract and synthetic powder stability across different pH levels and temperatures, identifying pH 7.0 as optimal for Suji leaf extract and pH 4.5 for synthetic powder. Storage

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recommendations include 4 °C for Suji leaves extract and 40 °C for synthetic powder. The findings emphasize formulation's importance for stability and provide insights for industrial applications. Also, Suji leaf extract showed higher total phenolic content, antioxidant capacity, and chlorophyll levels but lower stability and color intensity than synthetic powder, offering valuable distinctions for industry decisions.

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CONFLICT OF INTEREST STATEMENT

The authors agree that this research was conducted without any self-benefits or commercial or financial conflicts and declare the absence of conflicting interests with the funders.

AUTHOR'S CONTRIBUTION

Rumaisya Yasmin carried out the research and wrote the article. Wan Saidatul Syida supervised the research progress. Wan Saidatul Syida also anchored the review and revised and approved the article submission.

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APPENDIX

Front cover and list of ingredients of synthetic powder colourant used in the analysis