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Ripening Stage-Dependent Variation in Anthocyanin and Vitamin C Content of *Flacourtia inermis*: Evidence from an Underutilized Tropical Fruit in Lombok, Indonesia

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Abstract

Flacourtia inermis (lobi-lobi) is an underutilized Southeast Asian fruit with reported phytochemical potential, yet quantitative evidence describing how ripening influences accumulation of key antioxidant compounds, particularly anthocyanin and vitamin C, remains limited. This study aimed to quantify anthocyanin and vitamin C content across three ripening stages of *F. inermis* (unripe, half-ripe, fully ripe) and to identify the maturity stage associated with optimal bioactive compound concentration. An experimental laboratory design with a post-test-only approach was applied. Fruits were collected from Narmada, West Lombok, freeze-dried, and extracted via cold maceration using 70% ethanol. Anthocyanin was determined using the differential pH method (expressed as cyanidin-3-glucoside equivalents), while vitamin C was determined by iodometric titration. Data were analysed using one-way ANOVA with Tukey's HSD or Kruskal-Wallis with Mann-Whitney post-hoc tests, depending on normality. Anthocyanin content increased progressively with maturity, from 3.05 ± 0.13 mg/L (unripe) to 10.66 ± 0.13 mg/L (half-ripe) and 14.08 ± 0.21 mg/L (fully ripe), with all pairwise differences significant ($p < 0.001$). Vitamin C exhibited a non-linear pattern, peaking at the half-ripe stage (1501.87 ± 32.52 mg/L) before declining sharply in fully ripe fruit (469.33 ± 16.26 mg/L; $p = 0.027$). Ripening stage significantly and differentially governs anthocyanin and vitamin C accumulation in *F. inermis*. As no single stage maximizes both compounds simultaneously, the half-ripe stage represents the optimal harvest point for balanced bioactive value, supporting the development of lobi-lobi as a functional tropical fruit ingredient.

I. Introduction

Fruits are widely recognized as important sources of bioactive phytochemicals that contribute to nutritional quality and may support health maintenance. Tropical fruits are considered promising sources of natural antioxidant compounds due to their diverse secondary metabolites (Sarkar *et al.*, 2023). Dietary intake of anthocyanin-rich fruits has been associated with reduced risk of non-communicable diseases due to their bioactive properties, supporting the importance of exploring underutilized tropical fruits as potential functional food sources (Adriani *et al.*, 2023). Indonesia possesses extensive genetic resources of edible tropical fruits, with more than 400 species available for consumption; however, many remain poorly inventoried and lack comprehensive scientific documentation (Kumoro, Alhanif and Wardhani, 2020; Mustaqim and Raihandhany, 2020). Among these underutilized fruits is lobi-lobi (*Flacourtia inermis*), a Southeast Asian species reported to exhibit promising phytochemical potential and antioxidant activity (Astari, Yulianti and Ferdinal, 2024).

Previous studies have demonstrated that *F. inermis* contains various phytochemicals, including flavonoids, phenolics, anthocyanins, alkaloids, and terpenoids, indicating its potential as a natural source of bioactive compounds (Astari, Yulianti and Ferdinal, 2024). Morphological and phytochemical characterization across six maturity stages further revealed the presence of 34 chemical compounds, with phenolics and anthocyanins identified as abundant metabolites in the fruit (Umam, Poerwanto and Matra, 2023). These findings suggest that metabolite composition in lobi-lobi may vary during fruit development and ripening.

Comparative antioxidant analyses using DPPH and spectrophotometric methods have also confirmed notable antioxidant activity in lobi-lobi fruit extracts (Yasin, Zam and Rakhman, 2022). Additionally, anthocyanin content has been quantified in ethanol extracts, supporting the fruit's potential as a natural antioxidant source (Yasin, Zam and Rakhman, 2022). Despite these findings, information regarding the specific phytochemical composition of lobi-lobi remains limited, and the fruit is still relatively unfamiliar to the broader Indonesian population (Astari, Yulianti and Ferdinal, 2024). Although morphological, phytochemical, and antioxidant evaluations have been reported, systematic evidence describing how fruit ripening influences the quantitative accumulation of key bioactive compounds, particularly anthocyanins and vitamin C, remains scarce. However, most existing studies emphasize metabolite identification or general antioxidant activity rather than maturity-based quantitative comparisons.

Ecologically, *F. inermis* is distributed across forests in Maluku and various other regions of Indonesia, including the *F. inermis var. rindjanica* reported as endemic to Lombok (West Nusa Tenggara) and Sumba (East Nusa Tenggara) (Mustaqim and Raihandhany, 2020), underscoring the ecological and ethnopharmacological relevance of this region for further investigation.

Although previous studies have described the phytochemical composition and antioxidant activity of *F. inermis*, quantitative evidence explaining how ripening influences the accumulation of key antioxidant-related compounds remains limited. A clearer understanding of these maturity-associated changes is important for supporting evidence-based utilization of underutilized tropical fruits and for identifying optimal harvest stages that preserve nutritional value. Therefore, this study aimed to quantify anthocyanin and vitamin C content across distinct ripening stages of *F. inermis* and to determine the maturity stage associated with the highest concentration of these bioactive compounds.

II. Research Method

This study employed an experimental laboratory design with a post-test-only approach to evaluate differences in anthocyanin and vitamin C content of lobi-lobi (*F. inermis*) fruit at different ripening stages.

II.1 Sample Collection and Ripening Classification

Fresh lobi-lobi fruits were collected from Narmada District, West Lombok, Indonesia. Fruit maturity was determined based on rind color variation following previously described morphological criteria (Umam, Poerwanto and Matra, 2023). Fruits were categorized into three stages: unripe (green rind with firm texture), half-ripe (reddish-green rind indicating the onset of pigmentation), and fully ripe (dark red to purplish rind with softer texture). Visual assessment was performed immediately after harvest to minimize post-harvest physiological changes. Only fruits with uniform physical characteristics and free from visible defects were selected for analysis. Although *F. inermis var. rindjanica* has been reported as endemic to the Lombok region (Mustaqim and Raihandhany, 2020), fruit identification in this study was confirmed only at the species level based on morphological characteristics; intraspecific or varietal classification was not verified through molecular or phylogenetic analysis.

Figure 1. Visual appearance of *F. inermis* (lobi-lobi) fruit at three ripening stages: unripe (green rind, left), half-ripe (reddish-green rind, center), and fully ripe (dark red to purplish rind, right), prior to sample preparation

II.2 Preparation of Fruit Powder

The collected fruits were washed, cleaned, and cut into small pieces prior to drying. Samples were freeze-dried (lyophilized) for 24-72 hours using a freeze dryer (Christ Alpha 1-2 LDplus®, Germany) to remove moisture while preserving phytochemical stability. The dried samples were then ground using a laboratory blender to obtain a fine fruit powder (simplisia), which was subsequently used for extraction.

Figure 2. Workflow of lobi-lobi (*Flacourtia inermis*) fruit powder preparation prior to phytochemical analysis

II.3 Materials

Ethanol 70% (Merck®, Germany) was used as the extraction solvent. Reagents for anthocyanin analysis included potassium chloride (KCl), hydrochloric acid (HCl), sodium acetate trihydrate ($CH_3COONa \cdot 3H_2O$), and sodium hydroxide (NaOH). Vitamin C determination utilized iodine solution, potassium iodide (KI), potassium iodate (KIO_3), sodium thiosulfate ($Na_2S_2O_3$), starch indicator, and sulfuric acid (H_2SO_4). All chemicals were of analytical grade.

II.4 Instrumentation

Samples were freeze-dried using a freeze dryer (Christ Alpha 1-2 LDplus®, Germany) and weighed with an analytical balance (Ohaus®, USA). Absorbance measurements were conducted using a UV-Visible spectrophotometer (Thermo Scientific®, USA), and pH values were measured with a digital pH meter (Hanna Instruments®, USA).

II.5 Extraction Procedure

Powdered samples (simplisia) from each ripening stage (60 g per stage) were extracted by cold maceration using 70% ethanol as solvent at a sample-to-solvent ratio of 1:10 (w/v), with a total of 600 mL ethanol used per sample. Cold maceration was selected to prevent thermal degradation of heat-sensitive compounds, including anthocyanins and vitamin C. Maceration was conducted at room temperature for 3 x 24 hours (72 hours total), with manual stirring performed every 24 hours to enhance solute diffusion. Following maceration, the extracts were filtered through filter paper to separate the filtrate from the residue, and the collected filtrate was used for subsequent quantitative analysis (Ibrahim *et al.*, 2023; Hidayati *et al.*, 2024).

II.6 Determination of Total Anthocyanin

Total anthocyanin content was determined using the differential pH method adapted from Giusti & Wrolstad (2001). Sample extracts were diluted with 0.025 M potassium chloride buffer (pH 1.0) and 0.4 M sodium acetate buffer (pH 4.5) at a dilution factor of 5. Absorbance was measured at 510 nm and 700 nm using a UV-Visible spectrophotometer (Thermo Scientific®, USA) with a 1 cm path length cell. The absorbance difference (ΔA) was calculated as: $\Delta A = (A_{510}-A_{700})\text{pH } 1.0 - (A_{510}-A_{700})\text{pH } 4.5$. Monomeric anthocyanin content was expressed as cyanidin-3-glucoside equivalents (mg/L) using the formula: Anthocyanin (mg/L) = (ΔA x MW x DF x 1000) / (ε x l), where MW = 449.2 g/mol (molecular weight of cyanidin-3-glucoside), ε = 26,900 $L \cdot mol^{-1} \cdot cm^{-1}$ (molar extinction coefficient), l = 1 cm (path length), and DF = 5 (dilution factor).

II.7 Determination of Vitamin C

Vitamin C content was determined using the iodometric titration method adapted from (Syafitri *et al.*, 2023). The procedure consisted of three sequential stages: preparation of reagents, standardization of iodine solution, and determination of vitamin C in the sample extract.

Preparation of reagents: Iodine solution (0.05 N) was prepared by dissolving 8.3 g potassium iodide (KI) in a small volume of distilled water, followed by addition of 6.34 g iodine (I_2) and 10 mL sulfuric acid (H_2SO_4). The solution was transferred to a 1000 mL volumetric flask, diluted to the mark with distilled water, and stored in a dark bottle to prevent photodegradation. Amylum (starch) indicator (3%) was prepared by dissolving 3 g starch in 100 mL distilled water.

Standardization of iodine solution: The normality of the iodine solution was verified by standardization against As_2O_3 (arsenic trioxide). A total of 150 mg As_2O_3 was dissolved in 20 mL NaOH 2N, diluted with 40 mL distilled water, and acidified with 16 mL HCl 7.3% until a pink endpoint was reached using methyl orange indicator. After adding 2 g $NaHCO_3$ and 50 mL distilled water, the solution was titrated with the prepared iodine solution using 3% amylum as indicator until a persistent blue color was observed. The actual normality of the iodine solution was calculated using the formula: N I_2 = (mg As_2O_3 × valence) / (mL iodine × MW As_2O_3), where 1 mL of 0.1 N iodine is equivalent to 4.946 mg As_2O_3.

Determination of vitamin C in sample extract: A 25 mL aliquot of the sample filtrate was transferred to an Erlenmeyer flask with a dilution factor (fp) of 4. Three drops of 3% amylum indicator were added, and the solution was titrated slowly with 0.05 N standardized iodine solution until a persistent blue color appeared, indicating that all ascorbic acid had been oxidized to dehydroascorbic acid. The titration volume was recorded and performed in triplicate for each ripening stage. Vitamin C concentration was calculated using the formula: Kadar Vit. C (mg/L) = (V titrasi × N I_2 × BM Vit. C × fp) / V sampel, where BM Vit. C = 176 g/mol. Titrations were conducted promptly after extraction to minimize oxidative degradation of ascorbic acid by ambient oxygen.

II.8 Statistical Analysis

All measurements were performed in triplicate, and data were expressed as mean ± standard deviation. Normality was assessed using the Shapiro-Wilk test, and homogeneity of variance was assessed using Levene's test. For variables satisfying both assumptions, one-way analysis of variance (ANOVA) was applied, followed by Tukey's HSD post-hoc test for pairwise comparisons. For variables violating the normality assumption, the non-parametric Kruskal-Wallis test was applied, followed by pairwise Mann-Whitney exact tests. Statistical significance was set at $p < 0.05$.

III. Results and Discussion

This study demonstrates that fruit ripening significantly influences the accumulation of anthocyanin and vitamin C in *F. inermis,* although each compound exhibits a distinct pattern of change. Statistical analysis confirmed significant overall differences among ripening stages for both compounds (ANOVA for anthocyanin, $p < 0.001$; Kruskal-Wallis for vitamin C, $p = 0.027$), indicating that fruit maturity is a critical determinant of phytochemical composition. Detailed statistical outcomes, including post-hoc comparisons, are presented in the corresponding subsections below.

III.1 Anthocyanin Content

Anthocyanin levels increased markedly with fruit maturation, rising from 3.05 ± 0.13 mg/L in unripe fruits to 10.66 ± 0.13 mg/L in half-ripe fruits and reaching the highest concentration in fully ripe fruits (14.08 ± 0.21 mg/L) (Table 1). This progressive increase suggests that ripening plays a central role in promoting anthocyanin accumulation, which is closely associated with the development of red to purplish pigmentation in fruits.

Normality (Shapiro-Wilk, $p > 0.05$ for all groups) and homogeneity of variance (Levene's test, $p = 0.898$) assumptions were satisfied; therefore, one-way ANOVA was applied. Anthocyanin content differed significantly among the three ripening stages, with the magnitude of this difference far exceeding what would be expected by chance (one-way ANOVA: $F(2,6) = 8147.99$, $p < 0.001$), confirming that ripening stage is a strong determinant of anthocyanin accumulation. Tukey's HSD post-hoc test confirmed that all three ripening stages differed significantly from one another ($p < 0.05$ for all pairwise comparisons).

Table 1. Total anthocyanin content of lobi-lobi (*F. inermis*) fruit at different ripening stages

Ripening Stage	Replicate	Measured Value (mg/L)	Mean ± SD (mg/L)
Unripe	1	3.06	3.05 ± 0,13
	2	2.92	
	3	3.17	
Half-ripe	1	10.66	10.62 ± 0,09
	2	10.69	
	3	10.52	
Fully ripe	1	14.11	14.08 ± 0,21
	2	13.86	
	3	14.28	

These results align with previous studies indicating that anthocyanin biosynthesis intensifies during late ripening stages due to the activation of flavonoid pathways (Mattioli *et al.*, 2020). Morphological and phytochemical characterization of lobi-lobi has likewise identified anthocyanins as dominant secondary metabolites in advanced maturity stages (Umam, Poerwanto and Matra, 2023). Collectively, these findings reinforce the role of ripening as a key biological regulator of pigment formation.

The enhanced accumulation observed in this study is consistent with the activation of anthocyanin biosynthesis genes during late ripening, contributing to pigmentation development and increased antioxidant potential. Anthocyanins are water-soluble flavonoid pigments responsible for red, purple, and blue coloration in fruits and vegetables and have been widely investigated for their medicinal properties, including antidiabetic, anti-inflammatory, antimicrobial, and cardioprotective effects (Sapian *et al.*, 2022). The magnitude of increase further supports ripening as a major determinant of pigment-related phytochemicals in tropical fruits.

III.2 Vitamin C Content

In contrast to anthocyanin, vitamin C exhibited a non-linear pattern across ripening stages. The highest concentration was observed in half-ripe fruits (1501.87 ± 32.52 mg/L), followed by a substantial decline in fully ripe fruits (469.33 ± 16.26 mg/L) (Table 3). This pattern suggests that vitamin C accumulation reaches a physiological optimum prior to full ripening.

Normality testing (Shapiro-Wilk) indicated that vitamin C values were normally distributed in the unripe ($p = 1.000$) and half-ripe groups ($p = 0.637$), but not in the fully ripe group ($p < 0.001$); therefore, the non-parametric Kruskal-Wallis test was applied. Vitamin C content differed significantly among ripening stages ($\chi^2 = 7.261$, $df = 2$, $p = 0.027$). Pairwise Mann-Whitney exact tests showed consistent rank separation between half-ripe and unripe fruits, and between half-ripe and fully ripe fruits ($U = 0$ for both comparisons; exact $p = 0.100$), reflecting the minimum attainable significance level for pairwise comparisons with $n = 3$ per group despite complete rank separation between groups.

Table 3. Vitamin C content of *F. inermis* fruit across ripening stages

Ripening Stage	Replicate	Measured Value (mg/L)	Mean ± SD (mg/L)
Unripe	1	1126.40	1126.40 ± 56.32
	2	1182.72	
	3	1070.08	
Half-ripe	1	1520.64	1501.87 ± 32.52
	2	1520.64	
	3	1464.32	
Fully ripe	1	469.33	469.33 ± 16.26
	2	450.56	
	3	478.72	

Fluctuations in vitamin C during fruit maturation have been widely reported and are generally attributed to the balance between biosynthesis and oxidative degradation of ascorbic acid (Fenech *et al.*, 2019). The reduction observed in fully ripe fruits may therefore reflect increased metabolic turnover and reduced ascorbate stability during advanced ripening stages.

Given its central role in plant antioxidant defense, ascorbic acid functions as a key regulator of fruit development, ripening, and postharvest physiology. Its accumulation is influenced by transcription factors, protein interactions, phytohormones, and environmental conditions, indicating that maturity-associated changes likely arise from coordinated metabolic regulation rather than simple degradation processes (Zheng *et al.*, 2022).

As a major scavenger of reactive oxygen species, ascorbic acid contributes to cellular protection under oxidative stress, further highlighting its physiological relevance in fruit metabolism. The contrasting trajectories of anthocyanin and vitamin C observed in this study suggest that ripening does not uniformly enhance all antioxidant-related compounds but instead selectively modulates metabolite accumulation.

III.3 Metabolic Implications of Ripening

Fruit ripening is associated with extensive metabolic reprogramming that alters fruit composition and biochemical quality (Parijadi *et al.*, 2018). These coordinated metabolic transitions often involve the activation of flavonoid biosynthetic pathways, promoting anthocyanin accumulation during the later stages of maturation (Shen, Wan and Tan, 2024). Anthocyanins, beyond their role as pigments, are recognized for their strong antioxidant capacity and diverse biological activities, further supporting the functional relevance of pigment enrichment in ripening fruits (Sapian *et al.*, 2022). This mechanistic framework aligns with the progressive increase in anthocyanin observed in the present study.

III.4 Implications for Harvest Strategy and Functional Utilization

The present findings indicate that no single ripening stage simultaneously maximizes all measured phytochemicals. Fully ripe fruits contained the highest anthocyanin levels, whereas half-ripe fruits retained greater vitamin C concentrations. These results emphasize that harvest timing should be strategically determined according to the targeted phytochemical profile. Previous studies have confirmed that *F. inermis* contains diverse phytochemicals with promising biological potential; however, maturity-based quantitative evidence remains limited (Astari, Yulianti and Ferdinal, 2024). Therefore, this study provides quantitative evidence supporting maturity-based optimization of phytochemical value. Collectively, these findings position *F. inermis* as a promising underutilized tropical fruit with measurable phytochemical value influenced by ripening stage, supporting its potential application in functional food development.

Polyphenolic compounds, including anthocyanins, are recognized natural antioxidants capable of scavenging reactive oxygen species, protecting DNA from oxidative damage, and modulating inflammatory signaling pathways. Beyond antioxidant activity, phenolics have also been associated with immune-enhancing and anti-infective properties, further supporting the functional relevance of phytochemical-rich fruits (Liu *et al.*, 2026).

III.5 Study Limitations

This study has two main limitations. First, only anthocyanin and vitamin C content were quantified; total antioxidant capacity and biological activity were not assessed. Second, fruit identification was based on morphological criteria and confirmed only at the species level, so potential infraspecific variation, including the locally reported *F. inermis var. rindjanica* could not be verified without molecular or phylogenetic analysis. Future studies incorporating functional antioxidant assays and DNA barcoding are recommended to address these gaps. Despite these limitations, the present findings provide baseline quantitative data supporting future research on the nutritional and functional properties of this underutilized tropical fruit.

IV. Conclusion

This study demonstrates that ripening stage significantly influences the accumulation of anthocyanin and vitamin C in *Flacourtia inermis*. Anthocyanin content increased progressively from 3.05 ± 0.13 mg/L in unripe fruits to 14.08 ± 0.21 mg/L in fully ripe fruits, while vitamin C peaked at the half-ripe stage (1501.87 ± 32.52 mg/L) before declining in fully ripe fruits (469.33 ± 16.26 mg/L). These findings confirm that phytochemical composition in lobi-lobi is strongly maturity-dependent, and that half-ripe fruits represent an optimal harvest stage for maximizing the combined presence of both bioactive compounds, supporting the potential of *F. inermis* as a functional tropical fruit with measurable phytochemical value influenced by ripening stage.

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