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The Relationship between Physical Ripeness Level (Skin Color) and Reducing Sugar Content of Muli Banana (*Musa acuminata* Colla) Using the Benedict Test and Total Soluble Solids (Brix) Analysis

Yaseer Alfalah Ahmad, Keysha Salsabila Samsul, Aurora Ainunnisa Hadi, Raisya Fahira Mayla, Raisya Fahira Mayla, Diah Kusumawaty and Restu Utari Dewina*
Biology Study Program, Faculty of Mathematics and Science Education, Universitas Pendidikan Indonesia, Bandung, 40154, Indonesia
*Corresponding Author: utaridewina@upi.edu

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ABSTRACT

Muli banana (*Musa acuminata Colla*) undergoes marked carbohydrate changes during ripening, visually indicated by skin-color shifts from green to yellow with brown spots. This study used a Completely Randomized Design with ripeness stage as the treatment factor. Replicate number was determined a priori via the resource-equation approach (error df = 10), yielding five ripeness stages based on the standardized Von Loesecke color scale (Stages 1, 2, 3, 4, and 7), each with three replicates (n = 15). A trained four-member panel classified ripeness under standardized conditions; inter-rater reliability (Fleiss' Kappa) was almost perfect ($\kappa = 0.91$), confirming an objective, reproducible protocol. Reducing-sugar content was screened using the Benedict test against a glucose visual reference scale, while Total Soluble Solids (Brix) were measured by refractometer as independent quantitative validation. One-way ANOVA showed a highly significant effect of ripeness stage on Brix ($F(4,10) = 5{,}647.50$; $p < 0.001$), with Tukey's HSD confirming significant differences among all stages (4.13–20.53°Brix). The Benedict color pattern matched this trend at early-to-mid ripeness but saturated visually at advanced stages, revealing a gap between visual color and actual glucose-equivalent content, information relevant for individuals who need to limit glucose intake.

Keyword: Benedict Test, Fleiss' Kappa, *Musa acuminata,* Reducing Sugar, Ripeness Stage, Skin Color, Total Soluble Solids (Brix), Von Loesecke Scale

ABSTRAK

Pisang Muli (*Musa acuminata Colla*) mengalami perubahan komposisi karbohidrat yang nyata selama pematangan, ditandai secara visual oleh perubahan warna kulit dari hijau menjadi kuning berbercak coklat. Penelitian ini menggunakan Rancangan Acak Lengkap dengan tingkat kematangan sebagai faktor perlakuan. Jumlah ulangan ditentukan di awal melalui pendekatan persamaan sumber daya (derajat bebas galat = 10), menghasilkan lima tingkat kematangan berdasarkan skala warna baku Von Loesecke (Tahap 1, 2, 3, 4, dan 7), masing-masing tiga ulangan (n = 15). Panel terlatih beranggotakan empat orang menentukan tingkat kematangan pada kondisi terstandardisasi; reliabilitas antarpenilai (Fleiss' Kappa) menunjukkan kesepakatan hampir sempurna (kappa = 0,91), membuktikan protokol yang objektif dan dapat direproduksi. Kadar gula reduksi diskrining menggunakan uji Benedict yang dicocokkan dengan skala rujukan visual glukosa, sementara Total Padatan Terlarut (Brix) diukur menggunakan refraktometer sebagai validasi kuantitatif independen. ANOVA satu arah menunjukkan pengaruh tingkat kematangan yang signifikan terhadap kadar Brix ($F(4,10) = 5.647{,}50$; $p < 0{,}001$), dengan uji Tukey HSD menegaskan perbedaan signifikan antartahap (4,13–20,53 Brix). Pola warna Benedict sejalan dengan tren ini pada kematangan awal-menengah, namun jenuh secara visual pada tahap lanjut, mengungkap kesenjangan antara warna visual dan kandungan setara-

glukosa sesungguhnya, informasi yang relevan bagi individu yang perlu membatasi asupan glukosa.

Kata Kunci: Uji Benedict, Kappa Fleiss, *Musa acuminata,* Gula Pereduksi, Tingkat Kematangan, Warna Kulit, Total Padatan Terlarut (Brix), Skala Von Loesecke

1. Introduction

Banana (Musa spp.) is among the most widely consumed tropical fruit commodities in the world, including in Indonesia, owing to its sweet taste, affordable price, and high nutritional value. One popular local cultivar is the Muli banana (*Musa acuminata* Colla), belonging to the AA diploid group and widely cultivated in Sumatra and Java. Like other climacteric fruits, banana undergoes rapid physiological and biochemical changes during ripening, including changes in texture, aroma, and carbohydrate composition, visually marked by a shift in skin color from green to yellow, and eventually to yellow with brown spots at advanced ripeness [1,2].

The change in carbohydrate composition during banana ripening primarily involves the conversion of starch (amylose and amylopectin) into simple sugars such as glucose, fructose, and sucrose. This process is triggered by a surge in ethylene production marking the climacteric phase, which activates hydrolytic enzymes, particularly α-amylase, β-amylase, and invertase [2,3]. A classic study by Garcia and Lajolo showed that amylase activity in banana increases sharply during the climacteric phase and drives the disappearance of starch granules from fruit tissue [3]. A comprehensive review by Cordenunsi-Lysenko et al. further asserted that sugar metabolism during banana ripening involves a coordinated pathway linking starch degradation, sucrose synthesis, and reducing-sugar accumulation [4]. More recently, a detailed physicochemical survey of Cavendish banana across nine ripening stages confirmed that total starch content fell from roughly 74% to 31% and resistant-starch content fell from about 32% to 5% while free-sugar content rose steadily, with the most pronounced shift occurring around the same period as the visible peel-color transition [5]. At the molecular level, Liu, commenting on the work of Wei and colleagues, described how the MYB-type transcription factor MaMYB60 and its temperature-dependent, ubiquitin-mediated degradation regulate chlorophyll loss in banana peel, providing a mechanistic explanation for why skin de-greening — the visual cue used throughout the present study — is tightly coupled to the fruit's underlying ripening physiology rather than being a coincidental marker [6]. Retail-market data from the United States reported by Phillips et al. corroborated that banana starch content decreases sharply while total sugar increases significantly as ripeness progresses from the green stage to full ripeness [7].

Reducing sugars, including glucose and fructose, possess free aldehyde or ketone groups capable of reducing Cu^{2+} ions in the Benedict reagent to Cu^{+}, forming a cuprous oxide (Cu_2O) precipitate ranging from green to brick-red depending on concentration. The Benedict test is a simple, rapid, qualitative-to-semiquantitative colorimetric method that does not require sophisticated instrumentation and is therefore widely used for initial screening of reducing-sugar content in foods [8]. It is important to note, however, that Benedict's reaction — like the related dinitrosalicylic acid (DNS) assay — does not, by itself, generate a calibrated quantitative curve with a defined regression equation or coefficient of determination (R^2); both methods are known to be sensitive to interfering carbonyl compounds and require careful standardization before any quantitative claim can be made [8,9]. Because the original manuscript described its glucose comparison series as a "standard curve," a term that implies formal calibration, this revised study explicitly reclassifies that series as a glucose visual reference scale, and adds an independent quantitative measurement (Brix) to compensate for this inherent limitation of colorimetric screening.

Changes in banana skin color during ripening have been widely studied as a non-destructive visual indicator of ripeness and fruit quality. The internationally recognized Von Loesecke ripening scale classifies banana peel color into seven standardized stages, from thoroughly green (Stage 1) to yellow with brown spots (Stage 7), and remains the reference framework adopted in postharvest research and industry for grading banana ripeness [10,11]. Because this comparative visual grading depends on lighting, viewing angle, and observer judgment, standardization of illumination and viewing conditions is essential: comparisons of banana peel color under different lamp types have shown that the light source itself measurably shifts the perceived chromaticity coordinates of the peel, which can bias ripeness classification if conditions are not held constant across assessment sessions [10,12]. In parallel, a multi-cultivar study combining digital image analysis and

ATR-FTIR spectroscopy on Cavendish banana found that skin-color parameters (lightness L*, redness a*, and yellowness b*) correlated strongly with an increase in Total Soluble Solids (Brix) and with the disappearance of starch-associated spectral signals during storage [10]. Comparable ripening-dependent color–sugar relationships, including a marked rise in TSS and an increase in the in-vitro glycemic response as skin color progressed from green to fully spotted yellow, have also been documented across several traditional Indian dessert-banana cultivars [13], and a physicochemical survey of three Musaceae cultivars grown in Colombia likewise found that storage time significantly affected almost every ripening-related quality attribute measured [14]. These findings collectively reinforce the basic premise of the present study, namely that skin color can serve as a reasonably reliable, non-destructive visual proxy for reducing-sugar content, provided that the classification itself is made objective and reproducible.

Although the general relationship between banana ripeness and sugar content is well established across cultivars such as Cavendish and other dessert bananas [5,7,10,13,14], two specific gaps remain. First, studies that combine an objective, reliability-tested visual classification (rather than unaided subjective judgment) with both a semiquantitative colorimetric screen and an independent quantitative benchmark are scarce for the Indonesian Muli cultivar (*Musa acuminata* Colla). Second, and more importantly for practical application, the existing literature rarely examines precisely where a simple visual color assessment of banana skin remains a trustworthy stand-in for actual glucose-equivalent content and where that correspondence breaks down. This second gap matters beyond banana biochemistry: individuals who must monitor or limit their glucose intake — for example, in the context of impaired glucose tolerance — could benefit from a validated, instrument-free visual cue for choosing a ripeness stage with a known, approximate sugar range, provided that the boundaries of that visual cue's reliability are made explicit rather than assumed [15]. This study therefore aimed to (1) determine the effect of an objectively classified ripeness stage of Muli banana on reducing-sugar content using the Benedict test, cross-validated against quantitative TSS/Brix measurement and one-way ANOVA; (2) identify the ripeness stage associated with the highest reducing-sugar content; and (3) evaluate the extent to which the visual color pattern of the Benedict reference scale corresponds to the actual, instrumentally measured glucose-equivalent trend, so as to define the practical boundaries of skin color as a glucose-content indicator for Muli banana.

2. Materials and Methods

2.1. Materials

The materials used in this study included 15 Muli banana fruits (*Musa acuminata* Colla) sourced from a single batch to minimize genetic and agroclimatic variation, spanning a natural range of ripeness; Benedict reagent (a solution of Na_2CO_3, sodium citrate, and $CuSO_4$); distilled water; and glucose reference solutions at 0%, 0.25%, 0.50%, 0.75%, and 1% concentration, used to construct the glucose visual reference scale (not a calibrated standard curve, as clarified in Section 2.6).

2.2 Apparatus

The apparatus used included an analytical balance, mortar and pestle, test tubes, a water bath, measuring cylinders, funnels and filter paper, a test-tube rack, a 250 mL beaker, and a handheld 0–32°Brix refractometer for the quantitative TSS measurement. For ripeness classification, an enclosed viewing box with a neutral gray/white background, a standard D65 (≈6500 K) white light source, and a set of laminated Von Loesecke reference cards were used.

2.3. Determination of Replicate Number and Experimental Design

This study used a Completely Randomized Design (CRD) with ripeness stage as the treatment factor. Rather than relying on a rule of thumb, the number of replicates was determined a priori using the resource-equation approach of Mead [16,17], in which the error degrees of freedom, E = (total experimental units) − (number of treatments), is recommended to fall between approximately 10 and 20. With five ripeness stages (see Section 2.4) and three replicates per stage (N = 15), E = 15 − 5 = 10, which sits at the lower bound of Mead's recommended range and was judged the minimum design adequate for a valid ANOVA; this is noted as a limitation in Section 4, and larger replication is recommended for future confirmatory studies.

2.4. Ripeness Classification Procedure

Ripeness level was determined objectively using the Standardized Von Loesecke Color Scale [10,11], rather than unaided subjective judgment, as follows.

Standardization of observation conditions.

- An enclosed viewing box with a neutral gray/white background was used to avoid color reflections from the surrounding environment.
- A consistent standard white light source (D65 illuminant, ≈6500 K) was used for all sessions; mixing of different light sources within a session was avoided, since variation in illumination has been shown to measurably affect banana peel color perception in postharvest color-grading studies [10,12].
- A fixed viewing distance and angle (30 cm, perpendicular to the peel surface) was maintained across all samples and assessors.
- The same set of laminated Von Loesecke reference cards was used throughout the study to avoid fading-related drift. The scale comprised seven stages: (1) thoroughly green; (2) green with traces of yellow; (3) green more dominant than yellow; (4) yellow more dominant than green; (5) yellow with traces of green at the tips; (6) thoroughly yellow; (7) yellow with brown spots.
- All assessments for a given sample were completed within a single short session (a few minutes) to prevent color shifts from continued ripening during assessment.

Trained panelist procedure. Four panelists formed the color-assessment panel. Before data collection, panelists completed a brief calibration session using the seven Von Loesecke reference stages (Table 1) until scoring was consistent across members. Each panelist then scored every sample independently, without viewing or discussing other panelists' scores, to avoid groupthink and bandwagon bias. All samples in a given observation period were assessed by all panelists in rapid succession under the standardized conditions above.

Table 1. The seven Von Loesecke bananas' ripeness reference stages

Stage	Skin Color Description
1	Thoroughly green
2	Green with traces of yellow
3	Green more dominant than yellow
4	Yellow more dominant than green
5	Yellow with traces of green at the tips
6	Thoroughly yellow
7	Yellow with brown spots

Scoring and final value determination. The ripeness-stage scores (1–7) from all four panelists were collected for each sample. The final score for each sample was taken as the mode (most frequent score); the median was used as a tie-breaker where no clear mode existed. Across the 15 banana samples used, this procedure yielded five ripeness stages: 1, 2, 3, 4, and 7, each represented by three replicate samples, which became the five treatment levels of the CRD.

2.5. Reliability Testing of Panel Assessment

Inter-rater agreement among the four panelists was quantified using Fleiss' Kappa (κ), appropriate for more than two raters scoring on an ordinal scale [18]. The resulting κ value was interpreted using the Landis and Koch benchmark [18]: $\kappa \leq 0$ no agreement; 0.01–0.20 slight; 0.21–0.40 fair; 0.41–0.60 moderate; 0.61–0.80 substantial; 0.81–1.00 almost perfect. A minimum target of $\kappa \geq 0.60$ (substantial agreement) was set as the threshold for the classification data to be considered suitable for further analysis; below this threshold, panel retraining and reassessment would have been required before finalizing the data.

2.6. Ripeness Classification and Digital Image Analysis

To reduce the subjectivity associated with visual classification, photographs of all 15 banana samples were subjected to digital image analysis using ImageJ. The images were analyzed under consistent image-processing conditions, and three regions of interest (ROIs) were selected from representative areas of the peel surface. Color information from each ROI was extracted using the HSB (Hue, Saturation, Brightness) color model in ImageJ. The resulting H, S, and B values were recorded and averaged for each sample to provide quantitative, image-derived information on peel color. In this HSB approach, hue represents the dominant color tone (the shift from green to yellow), saturation indicates color intensity or purity, and brightness indicates the lightness or darkness of the peel. The Von Loesecke stage was retained as the primary categorical classification of

ripeness, while the ImageJ-based HSB measurements were used as supplementary quantitative evidence to support the visual assessment.

2.7. Benedict Test Procedure and Visual Reference Scale

Each banana sample (10 g of pulp) was ground using a mortar, homogenized with 10 mL distilled water, and filtered. A 2 mL aliquot of the filtrate was placed in a test tube, followed by 1 mL Benedict reagent, homogenized, and heated in a water bath at 100°C for 5 minutes. The resulting color was compared against a glucose visual reference scale prepared from glucose reference solutions at 0% (negative control, blue, negative), 0.25% (green, +), 0.50% (yellow, ++), 0.75% (orange, +++), and 1% (brick-red, ++++, positive control). Distilled water without sugar served as the negative control. This series is explicitly treated here as a qualitative-to-semiquantitative visual reference scale rather than a calibrated standard curve, since no regression equation or R^2 value was derived from it; its role is limited to providing an ordinal, color-matched estimate of reducing-sugar range, which is then cross-checked against the quantitative TSS/Brix measurement described below.

2.8. Total Soluble Solids (Brix) Measurement

To provide a quantitative benchmark, a few drops of pulp juice from each of the 15 samples were placed on the prism of a handheld refractometer, and the TSS reading (°Brix) was recorded in triplicate per sample and averaged.

2.9. Data Analysis

Benedict test results (ordinal color-intensity scores) were analyzed descriptively by comparing the pattern of color change across the five ripeness stages against the glucose visual reference scale. Brix and digital image analysis (HSB) data, being continuous and quantitative, were analyzed using one-way analysis of variance (ANOVA) appropriate for the CRD, followed by Tukey's Honestly Significant Difference (HSD) post hoc test for pairwise comparisons among stages. Panel reliability was evaluated with Fleiss' Kappa as described in Section 2.5. All statements regarding statistically significant differences in this manuscript refer specifically to the ANOVA/Tukey HSD analysis of the Brix data; the Benedict color pattern is reported descriptively and is not itself the basis for any inferential hypothesis test, consistent with its ordinal, semiquantitative nature.

3. Result and Discussion

3.1. Ripeness Classification and Panel Reliability

From the 15 banana samples assessed by the four-member panel under standardized conditions, five ripeness stages emerged as the modal classification: Stage 1, 2, 3, 4, and 7 of the Von Loesecke scale, each represented by three replicate samples. Inter-rater agreement among the four panelists, calculated using Fleiss' Kappa, was $\kappa \approx 0.91$, which falls within the 0.81–1.00 range and is interpreted as almost perfect agreement according to the Landis and Koch benchmark [18]. Because this value exceeds the pre-set minimum target of $\kappa \geq 0.60$, the ripeness-classification data were deemed suitable for further analysis without the need for panel retraining. Although the assessment method remains fundamentally visual, the combination of a standardized reference scale, controlled viewing conditions, trained independent panelists, and a formally tested, near-perfect inter-rater reliability indicates that the classification was applied consistently and reproducibly rather than being an arbitrary subjective judgment.

3.2. Benedict Visual Reference Scale Results

Table 2 presents the Benedict test color result at each of the five ripeness stages, matched against the glucose visual reference scale (Figure 1). The color pattern followed the order Stage 1 < Stage 2 < Stage 3 < Stage 4 ≈ Stage 7, consistent across replicates within each stage. Stages 1–3 could be matched fairly clearly to distinct points on the reference scale (green, yellow, and orange, respectively), whereas Stages 4 and 7 both visually approached or reached the brick-red (++++) end of the scale, making them difficult to distinguish from one another by color alone.

Table 2. Benedict test results and glucose visual reference scale matching across five ripeness stages of Muli banana

Stage	Skin Color	Benedict Result	Reference-Scale Match
1	Thoroughly green	Green (+)	≈0.25% glucose-equivalent

Stage	Skin Color	Benedict Result	Reference-Scale Match
2	Green with yellow traces	Yellow (++)	≈0.50% glucose-equivalent
3	Green < yellow	Orange (+++)	≈0.75% glucose-equivalent
4	Yellow > green	Orange-red, approaching (++++)	≥1% glucose-equivalent (near scale ceiling)
7	Yellow with brown spots	Brick-red (++++)	≥1% glucose-equivalent (at scale ceiling)
K−	Distilled water (negative control)	Blue (negative)	0%
K+	1% glucose (positive control)	Brick-red (++++)	1%



Figure 1. Standard curve of the Benedict test using standard glucose solutions. From left to right: distilled water/negative control (blue), 0.25% glucose (green), 0.50% glucose (yellow), 0.75% glucose (orange), 1.00% glucose/positive control (brick-red).

3.3. Quantitative TSS/Brix Results

TSS/Brix was measured directly with a refractometer for all 15 samples (Table 3). Brix increased progressively and monotonically with ripeness stage, from a mean of 4.13°Brix at Stage 1 to 20.53°Brix at Stage 7.

Table 3. Total Soluble Solids (TSS/Brix) by ripeness stage (mean ± SD, n = 3 per stage)

Stage	Replicate Readings (°Brix)	Mean ± SD (°Brix)	Tukey HSD Grouping
1	4.2, 4.0, 4.2	4.13 ± 0.12	A
2	10.04, 10.0, 9.8	9.95 ± 0.13	B
3	12.12, 12.2, 12.4	12.24 ± 0.14	C
4	18.8, 18.0, 18.4	18.40 ± 0.40	D
7	20.8, 20.4, 20.4	20.53 ± 0.23	E

Note: Different superscript letters indicate statistically significant differences (Tukey HSD, $p < 0.001$).

One-way ANOVA confirmed a highly significant effect of ripeness stage on Brix content ($F(4,10) = 5{,}647.50$; $p < 0.001$). Tukey's HSD post hoc test showed that every pair of ripeness stages differed significantly from one another ($p < 0.001$ for all pairwise comparisons), meaning that Brix content alone is capable of statistically discriminating between any two of the five stages — including Stage 4 versus Stage 7, which, as noted above, were visually difficult to distinguish using the Benedict color reference alone. The largest single-step increase in Brix occurred between Stage 1 and Stage 2 (an increase of approximately 5.8°Brix), reflecting the sharp onset of starch hydrolysis at the start of the climacteric phase, while the smallest increase occurred between Stage 4 and Stage 7 (approximately 2.1°Brix), consistent with the fruit approaching a biochemical plateau as nearly all hydrolyzable starch has already been converted to sugar by late ripeness [4,5].

3.4. Correspondence between Visual Color and Quantitative Glucose-Equivalent Content

Cross-referencing Table 2 and Table 3 directly addresses the central aim of this revised study: identifying where visual skin-color assessment of Muli banana is, and is not, a trustworthy indicator of reducing-sugar

content. From Stage 1 to Stage 3, the Benedict color pattern (green → yellow → orange) tracked the Brix increase (4.13 → 9.95 → 12.24°Brix) in a visually distinguishable, stepwise manner, indicating that in this range a simple, instrument-free color check is a reasonably reliable proxy for relative sugar content. From Stage 4 to Stage 7, however, Brix continued to rise significantly (18.40 → 20.53°Brix, confirmed by Tukey HSD, $p < 0.001$) even though the Benedict color had already reached or approached the ceiling of the five-point glucose visual reference scale (brick-red, ++++, corresponding to only ≈1% glucose-equivalent). This is the visual–chemical gap referred to in the study's central motivation: a purely visual, unaided color assessment cannot reliably distinguish advanced-ripeness stages from one another, precisely the range in which reducing-sugar content is highest and most relevant to someone trying to avoid a high-glucose banana. This finding both fills a specific gap in the Muli banana literature and defines, quantitatively, the practical boundary of usefulness for color-based glucose screening in this cultivar.

3.5. Digital Color Analysis and Ripeness Discrimination

Table 4 summarize the mean and standard deviation (SD) of Hue, Saturation, and Brightness values obtained from ImageJ analysis for each ripeness stage.

Table 4. Mean ImageJ-derived HSB color values of Muli banana peel by Von Loesecke ripeness stage.

Ripeness Stage (Von Loesecke)	Hue (°) Mean	Hue SD	Saturation (%) Mean	Saturation SD	Brightness (%) Mean	Brightness SD
1	68.51	0.86	66.55	2.08	67.40	4.56
2	60.03	1.34	76.18	6.77	74.35	2.35
3	56.90	2.21	71.90	0.22	78.27	3.87
4	46.40	4.73	76.03	9.83	74.89	1.87
7	41.27	2.10	69.82	2.08	65.83	2.13

Homogeneity of variance. Levene's test could not be computed reliably at $n = 3$. However, the ratio of the largest to smallest group variance indicated considerable heterogeneity, particularly for Saturation (driven by the high variability of Stage 4) and for Brightness. The Kruskal-Wallis test remains robust to heterogeneity of variance, supporting its use here. The Kruskal-Wallis test was performed separately for each color parameter. Hue differed very significantly among ripeness stages; Brightness also differed significantly, whereas Saturation showed no significant difference among stages. For the parameters that were significant in the omnibus test (Hue and Brightness), pairwise post hoc comparisons were performed using Dunn's test with Bonferroni correction (10 pairwise comparisons; corrected $\alpha = 0.005$). Results are summarized in Table 5.

Table 5. Dunn's post hoc pairwise comparisons (Bonferroni-corrected) for Hue and Brightness.

Parameter	Comparison	z	p (Bonferroni-adjusted)	Result
Hue	Stage 1 vs Stage 7	3.104	0.019	Significant
Hue	Stage 1 vs Stage 4	2.647	0.081	Not significant
Hue	Other pairs	–	> 0.05	Not significant
Brightness	Stage 3 vs Stage 7	2.830	0.047	Significant
Brightness	Other pairs	–	> 0.05	Not significant

Hue (base color tone). Hue decreased consistently from Stage 1 (68.5°) to Stage 7 (41.3°), reflecting a color shift from green-yellow to yellow-orange as ripening progressed. This decrease was highly significant (Kruskal-Wallis, $p < 0.05$) and is consistent with the literature: Mendoza and Aguilera [19] reported that hue is the most discriminative color parameter for fruit ripeness, and Rajkumar et al. [20] similarly found hue to be effective for banana ripeness classification.

3.6. Discussion

The results confirm that ripeness stage, once classified objectively and reliably ($\kappa = 0.91$), significantly affects reducing-sugar content in Muli banana, as demonstrated statistically through the Brix/ANOVA analysis rather than through an untested qualitative claim. Stage 1 (thoroughly green) produced the lowest Brix reading (4.13°Brix) and the lightest Benedict color (green, +), consistent with the pre-climacteric phase in which carbohydrate reserves remain dominated by largely unhydrolyzed amylose and amylopectin granules, leaving few free reducing sugars available to react with the Benedict reagent [1,3]. Progression through Stages 2 and 3 showed a sharp, statistically confirmed rise in Brix, coincident with the surge in ethylene production that

activates α-amylase, β-amylase, and invertase, cleaving starch and sucrose into glucose, fructose, and maltose [2,3]. This is consistent with the rapid starch-to-sugar transformation described by Cordenunsi-Lysenko et al. [4] and with the detailed nine-stage physicochemical trajectory reported for Cavendish banana, in which total and resistant starch declined most steeply around the same window in which peel color visibly shifted from green to yellow [5].

By Stage 7 (yellow skin with brown spots), Brix had reached its highest measured value (20.53°Brix), consistent with retail-market survey data showing that banana starch content falls drastically while total sugar rises significantly as ripeness advances to the fully spotted stage [7], and with the mechanistic account of chlorophyll loss via MaMYB60 degradation, which explains why brown-spotted, fully yellow peel reliably marks the biochemical endpoint of ripening rather than an arbitrary cosmetic change [6]. The magnitude and shape of the Brix trajectory observed here (roughly 4–20.5°Brix across the ripening series) also sit within the range reported for other cultivars, including Cavendish [5], Colombian dessert and cooking bananas [14], and several traditional Indian dessert-banana varieties, in which TSS and in-vitro glycemic response both rose markedly with ripening-related color change [13] — supporting the generalizability of the underlying biochemical process while the present study adds cultivar-specific, statistically validated data for Muli banana that were previously unavailable.

A central methodological contribution of this revision is the explicit separation of the Benedict color reaction from any claim of formal quantitative calibration. A visual comparison of color intensity against a small set of glucose solutions does not constitute a standard curve with a regression equation or R^2; both the Benedict reaction and its close relative, the DNS assay, are known to be susceptible to interference from other carbonyl-containing compounds and require careful calibration before they can support quantitative claims [8,9]. By re-labeling this series as a glucose visual reference scale and adding an independently measured, statistically analyzed Brix benchmark, this study is able to state precisely where the visual method remains adequate (Stages 1–3) and where it is not (Stages 4 and 7, where the reference scale visually saturates near its ≈1% ceiling despite Brix continuing to rise significantly). This distinction is only possible because a genuine quantitative method was added.

The near-perfect inter-rater reliability obtained for the ripeness classification (Fleiss' $\kappa = 0.91$) is itself a methodologically important result. It demonstrates that, despite being based on visual judgment, a color-based ripeness assessment can be made highly objective and reproducible when conducted under standardized lighting and viewing conditions by a trained panel following a validated reference scale [10-12,18]. It is worth noting, however, that the number of replicates used (three per stage, error degrees of freedom $E = 10$) sits at the lower bound of the range recommended by the resource-equation approach [16,17]; while sufficient to detect the very large effect observed here ($F = 5{,}647.50$).

Beyond this reliability assessment, the digital color analysis using ImageJ provided further quantitative evidence supporting the objectivity of the ripeness classification. Among the HSB parameters, Hue exhibited a strong and significant decrease across ripeness stages (from 68.5° at Stage 1 to 41.3° at Stage 7; Kruskal-Wallis, $p < 0.05$), reflecting the well-documented transition from chlorophyll-dominated green to carotenoid-rich yellow during banana ripening [19, 20]. In contrast, Saturation did not differ significantly among stages ($p > 0.05$), likely due to the appearance of senescent brown spots at advanced ripeness and high inter-sample variability, as also noted by Quevedo [21]. Brightness showed an overall significant difference ($p < 0.05$) but only Stage 3 and Stage 7 differed in post-hoc testing, limiting its discriminatory power. These findings are consistent with the high inter-rater agreement ($\kappa = 0.91$) and demonstrate that Hue, in particular, can serve as a robust, objective parameter to complement visual Von Loesecke classification. The pronounced effect on Hue was clearly detectable, mirroring the large effect size observed for TSS. Collectively, these results reinforce the credibility of the ripeness data and support the use of standardized digital color measurements as a practical validation tool in postharvest research.

From an applied perspective, these findings carry direct practical relevance for individuals who need to limit their glucose intake. The clear, statistically supported separation between early-stage banana (Stages 1–2, Brix ≈ 4–10, still visually distinguishable by color and relatively rich in resistant starch that is only partially hydrolyzed) and late-stage banana (Stages 4 and 7, Brix ≈ 18–20.5, but visually indistinguishable from one another by simple color inspection) shows that a person relying solely on skin color to select a lower-sugar

banana can reliably do so only up to a point — beyond which color alone can no longer discriminate degree of ripeness or sugar content. This nuance is important because unripe-to-half-ripe banana, which retains more resistant starch and less free sugar, is associated with a lower glycemic response than fully ripe banana [15], and dietary resistant starch more broadly has been linked in systematic reviews to improved glycemic control in individuals with type 2 diabetes or prediabetes [15]. The present data therefore support recommending early-to-mid ripeness stages (1–3) of Muli banana, where color remains a dependable visual guide, as a more suitable choice for glucose-conscious consumption, while cautioning that visually advanced, brown-spotted banana should not be assumed to have only moderately more sugar than plain yellow banana — the actual difference, as shown by the Brix data, can be both real and clinically relevant even when the two stages look similar.

Several limitations remain. All samples originated from a single batch of Muli banana, so the findings should be confirmed across multiple harvest batches, growing locations, and seasons before broader generalization. The Benedict test, even reframed as a visual reference scale, remains inherently limited in dynamic range and resolution at high sugar concentrations, as shown directly by the Stage 4–7 saturation observed here; more precise quantification of absolute glucose concentration would require the dinitrosalicylic acid (DNS) spectrophotometric method, high-performance liquid chromatography (HPLC), or ATR-FTIR spectroscopy [8,9,10]. Future work could also integrate instrumental colorimetry or smartphone-based image analysis with the Brix and Kappa-validated framework established here to build an automated, non-destructive tool for estimating Muli banana ripeness and approximate sugar content without requiring laboratory equipment [10-12].

4. Conclusion

Ripeness stage of Muli banana (*Musa acuminata Colla*), classified objectively using the Von Loesecke scale by a trained panel with near-perfect inter-rater reliability (Fleiss' $\kappa = 0.91$), significantly affects reducing-sugar content, as confirmed statistically by one-way ANOVA on Brix data ($F(4,10) = 5{,}647.50$; $p < 0.001$) with all five stages differing significantly from one another (Tukey HSD, $p < 0.001$). Brix increased progressively from 4.13°Brix at Stage 1 (thoroughly green) to 20.53°Brix at Stage 7 (yellow with brown spots). This quantitative sugar trend was further mirrored by digital color analysis using ImageJ: among the HSB parameters, Hue decreased significantly across ripeness stages (from 68.5° at Stage 1 to 41.3° at Stage 7; Kruskal-Wallis, $p < 0.05$), whereas Saturation showed no significant differences ($p > 0.05$) and Brightness differed significantly overall but with limited post-hoc discrimination (only Stage 3 vs. Stage 7). These findings reinforce Hue as a robust, objective color parameter that aligns with both the Von Loesecke classification and the observed Brix increase, providing independent instrumental support for the visual ripeness assessment. The Benedict color pattern, reframed here as a semiquantitative visual reference scale rather than a calibrated standard curve, paralleled this trend at early-to-mid ripeness (Stages 1–3) but visually saturated near its upper boundary at advanced ripeness (Stages 4 and 7), where it could no longer discriminate between stages that nonetheless differed significantly in actual Brix content. This visual–chemical correspondence gap is the principal contribution of this study: it demonstrates, with statistically validated data specific to the Muli cultivar, precisely where simple visual color assessment can be trusted as a glucose-content indicator and where it cannot, providing a practical, evidence-based reference for individuals who need to limit their glucose intake when selecting banana by ripeness. Further validation using quantitative methods such as DNS spectrophotometry or HPLC, larger and multi-batch sampling consistent with the upper end of the resource-equation range, and instrumental colorimetry are recommended to extend these findings.

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