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Spectroscopic Techniques for Non-Destructive Ripeness Monitoring of Banana (Musa Acuminata CV.): A Vis-NIR Approach

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Abstract

This manuscript examines the application of Visible Near-Infrared (Vis-NIR) spectroscopy using the reflection method to predict banana ripeness. Over 10 days, from day 1 (initial shelf life) to day 10, forty uniformly unripe bananas (Musa Acuminata cv.) were monitored using both non-destructive and destructive methods to assess changes in their properties during ripening. Vis-NIR reflectance measurement served as a non-destructive method, while soluble solids content (SSC), pH, and firmness measurements, using a commercial refractometer, pH meter, and a sclerometer fruit hardness tester, were destructive methods. Results revealed changes in reflectance across the wavelength spectrum as the bananas ripened, especially near 980 nm, a region associated with variations in water content resulting from moisture loss during ripening. High correlations were found between the reflectance spectra and SSC, pH, firmness, and shelf days (r^2 = 0.8780, 0.8477, 0.9206, and 0.9720, respectively). In conclusion, Vis-NIR spectroscopy reflectance is an effective method for assessing fruit quality and predicting ripeness, promoting non-destructive methodologies in the agricultural and food industries.

1. Introduction

Consuming foods rich in essential vitamins and minerals is crucial for our well-being, as they provide protection against illnesses and reduce the risk of various health issues. Fruits mature through multiple stages, each characterized by specific changes in texture, flavor, and nutrient content. Unripe fruits often have underdeveloped flavors, such as sourness or bitterness, a hard texture, and a green exterior. When fruits are fully ripened, their nutritional value is at its peak, making them ideal for consumption. These fruits are suitable for fresh eating due to their sweetness and juiciness. Conversely, overripe fruits tend to have a softer texture, may exhibit bruising, and can have an overly sweet taste.

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Determining fruit ripeness is crucial in the agricultural industry, as it impacts both quality and market value [1]. A review on precision agriculture techniques highlights the growing use of non-destructive sensing and machine learning, identifying sugar content, acidity, and firmness as key maturity indicators [34]. However, traditional methods for assessing ripeness, such as tactile examination, visual inspection, and smell tests [2], are subjective, labor-intensive, and impractical for large-scale production. These manual approaches are prone to errors and inefficient, often leading to food wastage. This wastage contradicts the goals of Sustainable Development (SDG) 12, which aims to promote sustainable consumption by ensuring responsible resource use and minimizing environmental impact.

In addition to these traditional approaches, destructive analytical methods remain widely used for assessing fruit ripeness. These techniques involve measuring key physical and chemical properties such as firmness, pH, and Soluble Solid Content (SSC). Firmness, a key indicator of ripeness, is typically measured using a penetrometer, either handheld or automated [6], or by squeezing or pressing the fruit's outer layer. SSC, which indicates the fruit's sweetness, is commonly measured using a refractometer or through conventional physicochemical analysis [7]. Similarly, the acidity or alkalinity of fruit is usually assessed using pH meters after extracting the juice [8]. Although reliable, these destructive methods require extensive sample preparation and cause irreversible damage to the fruit, making it impossible to monitor changes in the same sample over time.

To address these challenges, there is growing interest in non-destructive methods, such as Visible Near-Infrared (Vis-NIR) Spectroscopy, which has been developed to assess fruit quality and ripeness objectively and consistently [3]. Hence, this study employs Vis-NIR Spectroscopy to determine banana (Musa acuminata cv.) ripeness and establish a predictive model based on the relationship between spectral characteristics and conventional destructive measurements, such as soluble solids content (SSC), pH, and firmness measurements.

Banana (Musa acuminata cv.) is a widely known and popular fruit in Malaysia [4], valued for its nutrient content and benefits for human consumption. Bananas are particularly noted for their ability to accelerate the production of serotonin in the brain, thereby enhancing mood and promoting a sense of well-being [5]. Typically, bananas are harvested when they are unripe, hard, and green, and then allowed to ripen off the tree.

Several non-destructive approaches have been proposed, including image processing, capacitive sensing, and spectroscopy. For instance, one study employed image processing techniques with Fuzzy Logic-Based classification to determine the ripeness of bananas [9]. This method employed color segmentation and the intensity of the mean color, based on the RGB color model, to assess ripeness. At the same time, fuzzy logic was used to classify the bananas as either ripe or unripe. Additionally, a convolutional neural network (CNN) classifier was implemented in image processing [10], achieving an accuracy of 98.97% for maturity detection in another study. However, these methods are primarily limited to surface characteristics such as color and texture and cannot directly assess internal chemical changes associated with ripening.

Another method for predicting fruit ripeness is the use of a Capacitive Sensing System [11]. According to a study, this method utilizes the correlation between an avocado's capacitance value and its properties, as measured by destructive methods such as Brix, pH, and moisture content, to predict ripeness. The study found that the optimal frequency for assessing avocados is 1 MHz, due to its highest regression coefficient (r^2 value of 0.71). In another study [12], the capacitance sensing technique was applied to measure the dielectric properties of Sapota Sapodilla fruit. It identified 10 MHz as the optimal frequency for determining SSC and moisture content, with linear equations exhibiting promising regression, yielding r^2 values of 0.9329 and 0.6116, respectively. Similarly, papaya fruit exhibited high correlations between fruit properties (SSC and moisture content) and dielectric properties, with regression, r2 values of 0.9434 and 0.9177, respectively [13]. The Limau Madu fruit also showed a strong relationship between SSC and dielectric properties at 10 MHz, with a regression R-squared (r2) value of 0.8718 [14]. Nevertheless, capacitive sensing requires electrical contact and is sensitive to environmental factors such as temperature and humidity, which may affect consistency.

Compared to these techniques, Vis–NIR spectroscopy offers a distinct advantage because it directly measures light absorption and reflection related to changes in water, sugar, and pigment levels, key biochemical indicators of ripening. Vis-NIR Spectroscopy offers three optical approaches for analyzing spectral data, namely transmittance, absorbance, and reflectance [15]. This method utilizes light within specific wavelength λ ranges, typically between 350-1100 nm, 780-2500 nm, or 350-2500 nm.

In the transmittance method, the spectrometer's detector is positioned opposite the light source to measure the light passing through the sample under test. However, this approach requires higher light intensity, which may lead to fruit damage due to overheating. Additionally, thick fruit skin can impede light penetration. For instance, in a study [16] focusing on pomelo fruit, an optimal light intensity of 600W was determined for detecting internal quality attributes such as Total Soluble Solids (TSS) content and acidity value. Chemometric methods, particularly partial least squares regression, were employed to correlate wavelengths with actual fruit parameters, while deep learning techniques were applied to enhance classification accuracy. Although the accuracy achieved was not exceedingly high, with r^2 values of 0.88 and 0.72 for TTS content in the calibration and validation sets, respectively, and 0.98 and 0.03 for acidity, the data remain acceptable for non-destructive quality grading in post-harvest fruit assessment.

Int. Journal of Integrated Engineering Vol. 18 No. 7 (2026) p. 14-26

In the absorbance method, Vis-NIR Spectroscopy measures the amount of light absorbed by the sample at specific wavelengths, and in a study focused on postharvest tomatoes [17], absorbance mode combined with chemometric methods such as Partial Least Squares (PLS) and Least Squares-Support Vector (LS-SV) proved effective for the rapid determination of SSC, pH, and firmness. The LS-SV models demonstrated superior performance compared to PLS models, achieving a correlation coefficient (r^2) of 0.8676, a root mean square error of prediction (RMSEP) of 0.1192, and a bias of 0.0610 in the validation set.

On the other hand, in the reflectance method, Vis-NIR Spectroscopy measures the amount of light reflected by the sample. However, this approach may present challenges for fruits with dark skin or uneven texture, potentially leading to inaccuracies in reflectance spectra measurements [16], [18].

For instance, in a study on Humilis fruit [19], Vis-NIR spectroscopy coupled with wavelength selection algorithms was employed to predict soluble solids content (SSC). The prediction model, utilizing linear (PLSR) and non-linear (LS-SVM) models of spectral information and SSC, achieved promising results. The calibration set demonstrated an r^2 of 0.8995, with a prediction set r^2 of 0.8579, root mean square error of calibration (RMSEC) of 0.8897, RMSEP of 0.9059, and regression point displacement (RPD) of 1.8766, indicating a strong correlation in predicting CH SSC. According to [20], specific wavelengths correspond to absorption bands related to SSC (sugar content). Specifically, around λ = 980 nm, the second O-H overtone is prominent, while water absorption occurs around 973 nm, 1324 nm, and 1581 nm due to the third O-H overtone. These findings were observed using a reflectance method.

Additionally, a wavelength of 980 nm was found to exhibit a strong linear relationship with SSC and sucrose content in apples, with a coefficient of determination (r^2) of 0.9002 [21]. Moreover, significant peaks in the Visible area between wavelengths, λ of 840 nm and 980 nm, are attributed to water stretching vibrations [22]. It was noted that water interactions with solutes, such as sugar, influence reflectance spectra.

Previous research has also investigated the use of Vis–NIR spectroscopy in assessing banana ripeness, showcasing its potential for non-destructive quality evaluation. In one study, [30] utilized Vis–NIR reflectance spectroscopy to estimate the sugar and chlorophyll content in bananas at various ripening stages. The findings revealed strong correlations, with coefficients of determination (r^2) exceeding 0.90, indicating that spectral features, particularly within the visible range, are susceptible to pigment breakdown and sugar accumulation during ripening. Similarly, [31] employed Near-Infrared (NIR) spectroscopy combined with chemometric analysis to differentiate between naturally ripened and artificially ripened bananas. Their model achieved high calibration accuracy ($R^2CV > 0.74$), confirming the technique's reliability in distinguishing ripening mechanisms based on internal chemical variations. Additionally, [32] designed a versatile Vis–NIR wireless sensing system for real-time monitoring of banana ripening. This system effectively tracked reflectance spectral changes associated with moisture loss and sugar development throughout ripening, validating the feasibility of in-field, non-destructive analysis.

Despite these advances, most earlier studies on bananas have primarily focused on classification or qualitative assessment rather than quantitative modeling across the continuous ripening stages. Consequently, this research expands on previous work by employing Vis–NIR reflectance spectroscopy over a 10-day ripening period to develop predictive calibration models correlating spectral features, particularly near λ = 980 nm, with SSC, pH, and firmness. This method promotes a more comprehensive understanding of how biochemical alterations are reflected in spectral responses, enhancing the precision and practicality of non-destructive ripeness evaluation in banana (Musa acuminata cv.).

In this study, Vis-NIR Spectroscopy, using the reflectance approach, aims to identify specific wavelengths, λ, that strongly correlate with banana fruit attributes and their associated parameters. This focused approach is crucial for developing predictive models or calibration equations to assess banana ripeness (Musa acuminata cv.) accurately. Based on these considerations, the reflectance Vis-NIR method was chosen as the most effective, non-destructive, and dependable technique for monitoring banana ripeness, enabling accurate spectral measurements without harming the fruit while providing reliable data on water, sugar, and pigment transformations during ripening.

Compared to traditional and existing approaches, the proposed method demonstrates clear advantages in both accuracy and practicality. Conventional techniques such as visual inspection, tactile assessment, and smell tests are inherently subjective and unsuitable for large-scale applications, often leading to inconsistent grading and increased food wastage. Similarly, destructive analytical methods although reliable in measuring parameters such as SSC, pH, and firmness require sample preparation and permanently damage the fruit, making continuous monitoring impossible. In contrast, the proposed Vis–NIR reflectance spectroscopy method offers a non-destructive, rapid, and objective alternative. By focusing on spectral responses, particularly at the wavelength of 980 nm, this method enables direct correlation with key biochemical changes such as sugar accumulation and moisture variation, allowing repeated measurements on the same fruit across different ripening stages.

Furthermore, when compared to other non-destructive techniques such as image processing and capacitive sensing, the proposed method provides a more comprehensive assessment of fruit ripeness. Image-based approaches, including RGB analysis and deep learning models, primarily rely on external features like color and

texture, limiting their ability to capture internal chemical transformations. Capacitive sensing, while capable of reflecting internal properties, requires physical contact and is sensitive to environmental conditions such as temperature and humidity, which may affect measurement stability. In contrast, the Vis–NIR reflectance approach directly measures light interaction with internal constituents, offering higher reliability and deeper insight into fruit physiology. Additionally, unlike many previous studies that focus mainly on classification, this study advances the field by developing quantitative predictive models using polynomial regression to estimate SSC, pH, firmness, and even shelf life. This not only improves prediction accuracy but also enhances the method's applicability for real-time, non-destructive monitoring in agricultural and postharvest management systems.

Nomenclature	
Vis-NIR	Visible-Near Infrared
SSC	Soluble Solid Content
R^2	Coefficient of determination (correlation measure)
°Brix	Degree Brix, a unit for sugar content
°C	Degrees Celsius (used for temperature)
nm	Nanometres (wavelength units)
kg/cm^2	Kilograms per square centimeter (firmness unit)

2. Methodology

This research employed a non-destructive Vis-NIR spectroscopy reflectance technique and destructive commercial SSC, pH, and firmness testers to determine banana properties at various ripening stages over a 10-day shelf life period.

The bananas were collected from a local market in Seksyen 7, Shah Alam, Selangor. All forty bananas were green and firm, indicating the same level of unripe maturity. In the laboratory, the banana samples were cleaned, numbered, and visually inspected for size and color. They were then stored at room temperature (24-28°C) before experimentation.

Before any measurements, a paper towel was used to remove additional dirt and moisture from the skin surface of the samples. The experiment spanned 10 days, during which a total of 40 bananas were evaluated. Each day, four bananas of similar size, weight, and maturity were randomly selected for analysis. Non-destructive measurements were performed first on each sample, followed by destructive tests on the same fruits. This cross-sectional approach allowed independent evaluation of ripening stages from day 1 to day 10. Figure 1 shows the progress of banana shelf days.



Fig. 1 *The progress of banana shelf days*

The reflectance spectrum was measured using an AvaSpec-2046L Visible-Near Infrared Spectrometer (manufactured by Avantes) equipped with a R400-7-SR reflectance probe (manufactured by Ocean Insight) and a LS-1 tungsten halogen light source (manufactured by Ocean Optic). In this measurement, the spectrometer operated within the wavelength range of 430-950 nm to avoid noise present outside this region, due to limitations in equipment specifications. Measurements were conducted in a lightproof box to eliminate interference from ambient light and shadows. The interior surfaces of the box were covered with dark cloth to prevent reflections [24]. The halogen light source emitted light through the reflection probe onto the sample, and the built-in sensor within the probe detected and collected the reflectance data, which was then processed by the spectrometer.

The distance between the reflectance probe and the sample was consistently maintained at 1.2 cm. Increasing this distance would result in the light source covering a larger area of the skin surface, potentially leading to inaccurate readings. Conversely, a shorter distance would limit the area of reflectance measurement on the skin surface [25]. To determine the diameter of the reflectance area on the fruit surface, trigonometric principles were applied. The angle of the emitted light from the probe was $\theta = 12.5°$. Given the distance between the probe and the sample as 1.2 cm, the radius of the circle formed by the emitted light (opposite side) was calculated as $x = 0.266$ cm. Consequently, the diameter of the emitted light was found to be $2x = 0.532$ cm, as shown in the Figure. 2.

Int. Journal of Integrated Engineering Vol. 18 No. 7 (2026) p. 14-26

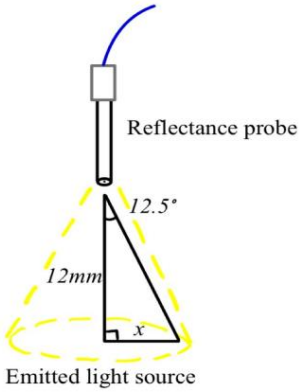


Fig. 2 *Diameter of collected reflectance data*

The probe was positioned at a 90° angle using a retort stand, as shown in the Figure. 3. Calibrations were performed using a white reference, and a dark reference was recorded in AvaSoft Software. The integration time for the reflectance measurements was set to 500 ms, with a 5-wavelength averaging to ensure smoother output. Application Origin software was also used to further smooth any noise in the wavelength data, as illustrated in Figure 4.

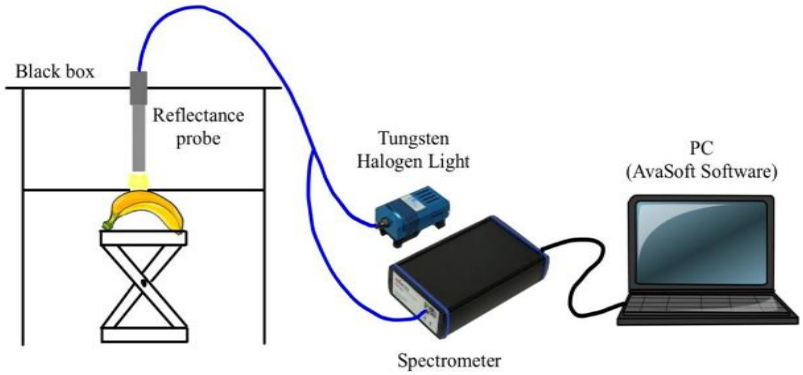


Fig. 3 *Schematic of the reflectance measurement set-up*

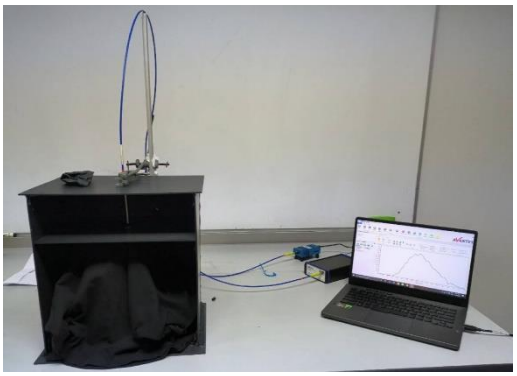


Fig. 4 *Actual set-up for reflectance measurement*

Reflectance spectra data were obtained by measuring the outer skin of the samples. Each sample was scanned four times at four marked locations around the banana, resulting in an average of four scans per fruit. Measurements were taken daily for ten days, from the unripe to the overripe stages. Each day, four bananas were measured, covering the full spectrum of ripeness from day 1 to day 10. All destructive measurements were performed four times per sample and averaged to ensure a thorough assessment from day 1 to day 10.

The firmness of the samples was measured using a GY-03 Sclerometer Farm Hardness tester. An 11 mm diameter probe head was used, capable of measuring firmness in the range of 1-24 kg/cm^2. A small section of the banana's skin was peeled back, and firmness was measured at that exposed area, as shown in Figure 5.



Fig. 5 *Firmness measurement*

Before measuring the Soluble Solids Content (SSC), the ATAGO PAL-1 digital refractometer must be calibrated by zeroing it with distilled water. After calibration, the distilled water is replaced with banana juice, and the juice SSC is measured in units °Brix, as depicted in Figure 6.



Fig. 6 *Measuring SSC using a refractometer*

On the other hand, pH measurements of the banana were then taken using a HGM model digital pH meter, as shown in Figure 7. The pH meter was immersed in the banana juice, displaying values ranging from 1 to 14. Each reading was allowed to stabilize for thirty seconds to ensure accuracy.



Fig. 7 *Measuring pH level using a pH meter*

The second overtone of O-H in sugar occurs at a wavelength, λ, of 980 nm. According to [20], [26], this wavelength, λ, is significant for analyzing reducing sugar content, serving as an indicator for determining banana ripeness based on SSC values from reflectance at 980 nm. Additionally, pH and firmness of the banana samples are analyzed at this wavelength, λ, since these parameters are strongly correlated with SSC [27]. Microsoft Excel will be used to analyze the collected data. The reflectance spectral data will be compared with destructive measurement results, using measured reflectance (%) as the dependent variable and destructive measurement parameters (SSC, pH, firmness) as the independent variables. This analysis will help develop a predictive model for determining the ripeness of bananas.

The relationship between measured reflectance, R (%), and other measured parameters (SSC, pH, firmness, days) is analyzed and developed using a second-order polynomial regression expression:

Int. Journal of Integrated Engineering Vol. 18 No. 7 (2026) p. 14-26

$$y = aR^2 + bR + c \quad (1)$$

Where y can be either SSC or pH or firmness or shelf days (dependent variable), whereas R is the percentage of the reflectance (independent variable) at λ = 980 nm, symbols a, b, and c are fitting coefficients that determine the curvature, slope, and y-intercept of the polynomial curve [28]. The correlation expressions between the reflectance, R at λ = 980 nm, and the SSC, pH, firmness, and days are tabulated in Table 1.

3. Results and Discussion

The measured data on bananas was collected from day 1 to day 10 of the shelf life, covering the unripe to overripe phases. Figs. 8, 9, and 10 illustrate the overall measured SSC, pH, and firmness over the shelf days. The SSC (°Brix) of bananas increases as ripening progresses, consistent with findings from [25] and [29], which also reported an increase in SSC values with maturity. Figure 8 indicates a curvilinear relationship between SSC and days, showing that SSC values rise with the number of days until day 7, after which they begin to decline. This strong curvilinear relationship is supported by a high regression coefficient, r^2=0.9843. Similarly, as SSC increases, the pH value also rises with ripening, as noted in [29]. This is depicted in Figure 9, which also shows a curvilinear relationship between pH and the number of days, resulting in a high regression coefficient, r^2 of 0.9509. The firmness of bananas decreases with increased ripening. Figure 10 illustrates a declining trendline between firmness and days. As the number of days increases, firmness decreases. The regression between firmness and days is also high (r^2 = 0.9528) when using a polynomial trendline.

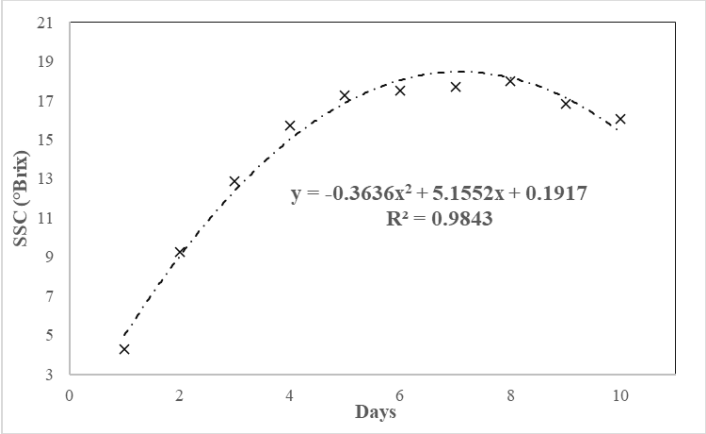


Fig. 8 *Relationship between SSC and shelf days*

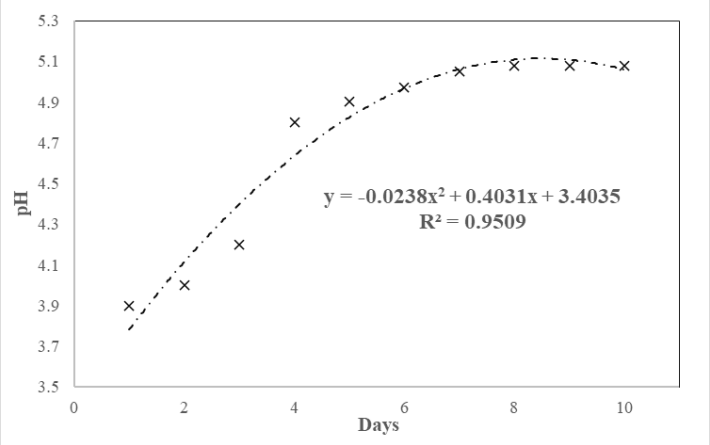


Fig. 9 *Relationship between pH and shelf days*

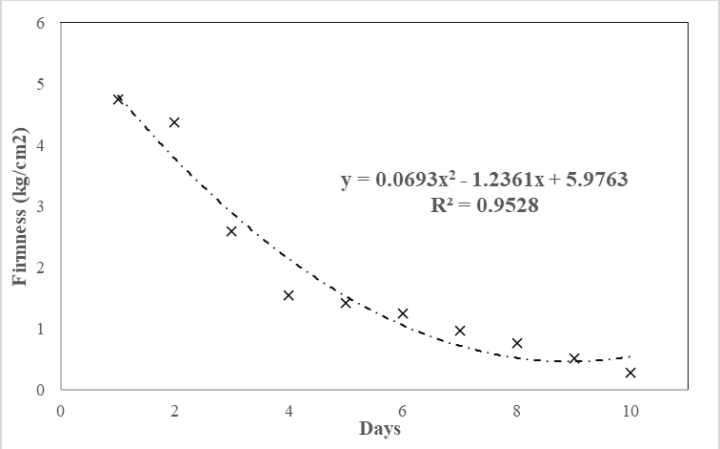


Fig. 10 *Relationship between firmness and shelf days*

Figure 13 illustrates the reflectance spectra curves of bananas during the ripening process. As noted by [29], changes in the banana's reflectance spectra occur due to the transition from unripe to overripe stages. The observed patterns in the graph align with those reported by [25], [29], and [30]. Notably, the graph exhibits a distinct dip between the wavelengths of approximately 680 nm and 980 nm.

At λ = 680 nm, the dip in reflectance, R is due to the absorption of chlorophyll content. As the days progressed, reflectance, R increased until day 5, after which it began to decrease. This pattern reflects the degradation of chlorophyll as the banana's skin colour changes from green to yellow and eventually to dark spots [31].

At a wavelength, λ of 980 nm, there is a noticeable decrease in reflectance, R values over time. As shown in Figure 11, reflectance, R values decrease as the number of days increases, indicating that day 1 bananas have the highest reflectance values while day 10 bananas have the lowest. This relationship between reflectance, R at 980 nm and the number of days is strongly curvilinear, with a regression coefficient of r^2=0.9720. This suggests that reflectance, R at 980 nm can be used to estimate the number of days of ripening.

Figure 12 illustrates the relationship between SSC and reflectance, R which also follows a curvilinear pattern with a regression coefficient of r^2 = 0.878. Lower reflectance, R values correspond to lower shelf days and lower SSC values. As the shelf days of bananas increase, SSC initially increases but then starts to decrease, potentially due to prolonged exposure to ethylene [32].

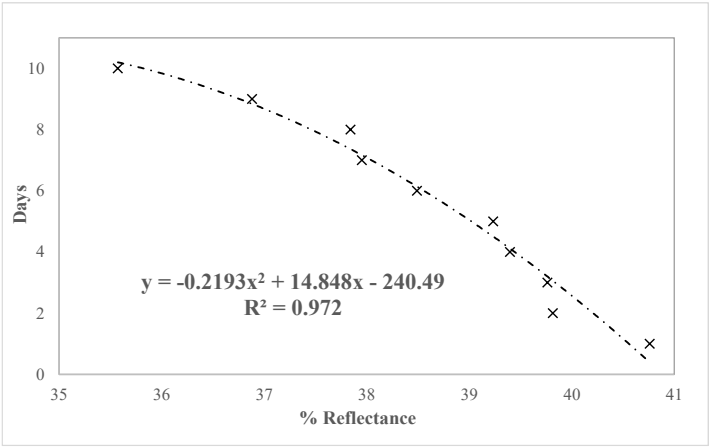


Fig. 11 *Relationship between days and reflectance*

Int. Journal of Integrated Engineering Vol. 18 No. 7 (2026) p. 14-26

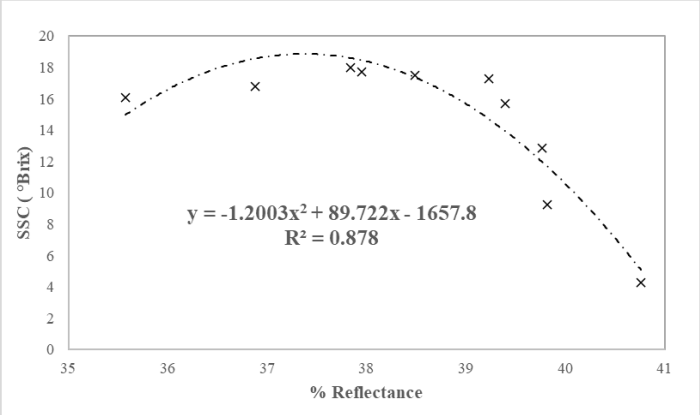


Fig. 12 *Relationship between SSC and reflectance*

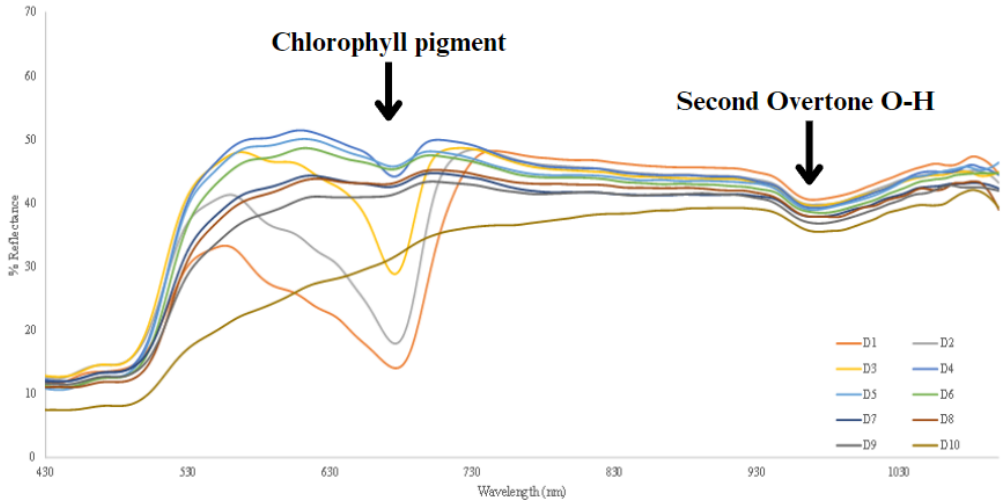


Fig. 13 *The pattern of reflectance spectra of banana from day 1 (D1) until day 10 (D10)*

Moreover, a strong correlation is observed between pH and reflectance, R with a regression coefficient of r^2 = 0.8477. This relationship mirrors the pattern seen in the SSC-reflectance relationship. As depicted in Figure 14, higher reflectance, R corresponds to lower pH values, while decreasing reflectance is associated with increasing pH values, indicating a decrease in acidity. According to [27] and [33], as fruits ripen, their pH values increase, indicating a reduction in acidity.

Furthermore, Figure 15 illustrates the relationship between firmness and reflectance, R displaying a high regression coefficient of r^2 = 0.9206. This suggests that firmness can be characterized by changes in reflectance values. As shown in Figure 15, higher reflectance, R values correspond to higher firmness values. Specifically, at λ = 980 nm, the highest reflectance, R value indicates a lower number of shelf days for the unripe banana. As reflectance, R values increase, firmness decreases, reflecting the ripening process. Table 1 shows the overall equations to determine the banana's properties from measured reflectance, R at wavelength, λ of 980 nm.

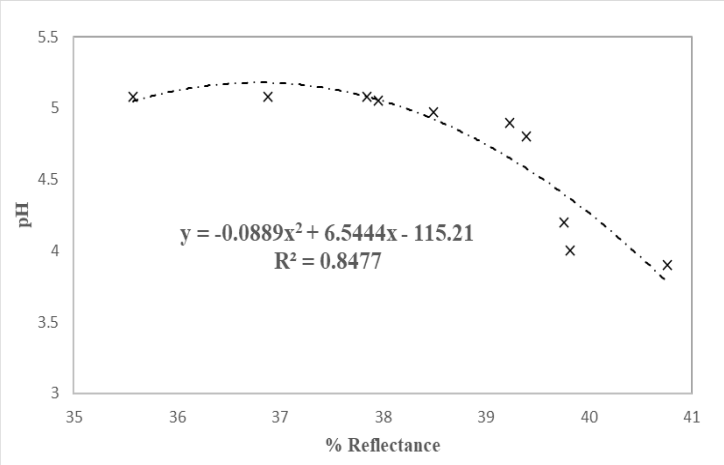


Fig. 14 *Relationship between pH and reflectance*

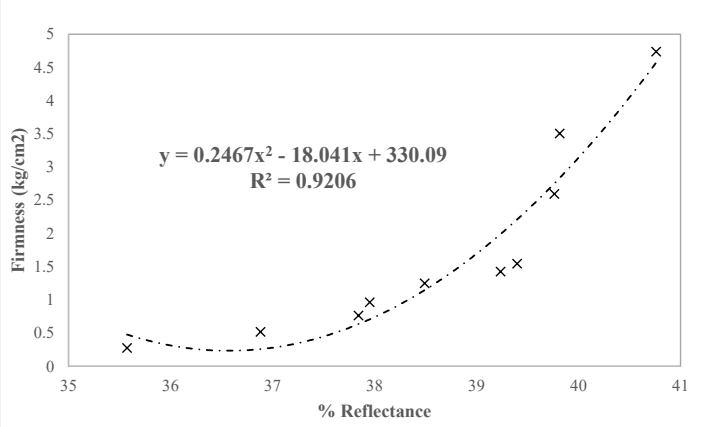


Fig. 15 *Relationship between firmness and reflectance*

Table 1 *Modelling equations to determine SSC, pH, firmness and number of shelf days by using reflectance measurement at 980 nm*

Parameters	Correlation Equations
SSC (°Brix)	$SSC = -1.2003(Reflectance)^2 + 89.222(Reflectance) - 1657.8$
pH	$pH = -0.0889(Reflectance)^2 + 6.5444(Reflectance) - 115.21$
Firmness (kg/cm²)	$\text{Firmness} = 0.2467(Reflectance)^2 - 18.041(Reflectance) + 330.09$
Number of Shelf Days	$Days = -0.2193(Reflectance)^2 + 14.484(Reflectance) - 240.49$

4. Conclusion

The Visible Near-Infrared (Vis-NIR) spectroscopy using the reflectance method proved effective in determining the ripeness of bananas (Musa acuminata cv.) across various stages, from unripe to overripe shelf days. The obtained results revealed high coefficients of determination (R^2) for predicting SSC, pH, firmness, and shelf days based on the 980 nm wavelength, yielding values of 0.8780, 0.8477, 0.9206, and 0.9720, respectively. These findings underscore the robustness of reflectance spectra measurements in predicting banana ripeness. The close association between the 980 nm wavelength and banana properties throughout the ripening process highlights the significance of O–H overtone absorption related to water and sugar interactions as key biochemical indicators.

The strong correlation between reflectance and shelf days (R^2 = 0.9720) demonstrates that the proposed model is not only capable of estimating physicochemical properties but also effective in tracking the temporal progression of ripening. This capability provides a practical advantage over conventional destructive methods, as it allows continuous monitoring without damaging the fruit. The application of second-order polynomial regression further enhanced prediction accuracy, offering a simple yet reliable modelling approach for non-destructive quality evaluation.

Moreover, this study confirms that the reflectance approach within the 430–950 nm range is sufficient to capture meaningful spectral variations associated with chlorophyll degradation (around 680 nm) and moisture–sugar interactions (around 980 nm). This also supports the feasibility of developing cost-effective and portable grading systems.

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of the paper.

Author Contribution

The authors confirm contribution to the paper as follows: ***study conception and design:*** *Timothy Kana anak Intai, Rafidah Rosman;* ***data collection:*** *Timothy Kana anak Intai;* ***analysis and interpretation of results:*** *Timothy Kana anak Intai, Rafidah Rosman, Anees Abdul Aziz;* ***draft manuscript preparation:*** *Rafidah Rosman, Velaiyudam. All authors reviewed the results and approved the final version of the manuscript.*

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