# NON-DESTRUCTIVE ESTIMATION OF MATURITY LEVEL OF 'CRYSTAL' GUAVA FRUIT BY MEANS OF FLUORESCENCE SPECTROSCOPY
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# *PENDUGAAN TINGKAT KEMATANGAN BUAH JAMBU KRISTAL SECARA NON DESTRUKTIF DENGAN SPEKTROSKOPI FLUORESENSI*

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## ABSTRACT
*This research aims to explore the potential use of fluorescence spectroscopy combined with chemometrics for predicting the maturity level of crystal guava fruits non-destructively. The physicochemical properties including total soluble solids (TSS), water content, firmness, and pH were obtained from laboratory tests and used as a reference in developing the predictive model. The fluorescence emission spectra under excitation of 365 nm UV LED were used as predictors. The fluorescence spectra were acquired and recorded using a miniaturized UV-Vis spectrophotometer with an effective 340 – 800 nm range. In total, 240 sets of data from crystal guava fruit samples with varying ages of 90-106 DAA (days after anthesis) were used for calibration and validation. A multivariate analysis using Partial Least Squared Regression (PLSR) was used to develop the predictive models. Several data preprocessing methods were applied to enhance the information contained in the spectral data to find the best predictive model. Analysis results showed that the developed model could accurately predict water content with $r_{cal}$=0.94; SEC=0.08%, $r_{val}$=0.84; SEP=0.08%; RPD=2.59, followed by TSS with $r_{cal}$ =0.91; SEC = 0.47%Brix, $r_{val}$ =0.79; SEP=0.48%Brix; RPD=2.13. Although with lower accuracy, it also could predict firmness with $r_{cal}$ =0.86; SEC=0.43 kgf, $r_{val}$ =0.74; SEP=0.43 kgf; RPD=1.82 and pH with $r_{cal}$ =0.81; SEC=0.10, $r_{val}$ =0.79; SEP=0.09; RPD=1.76. The results indicate that fluorescence spectroscopy could be used as an alternative method for non-destructive estimation of physicochemical properties as indicators of the maturity level of crystal guava fruits.*

## ABSTRAK
*Penelitian ini bertujuan untuk mengeksplorasi potensi penggunaan spektroskopi fluoresensi dikombinasikan dengan kemometri untuk memprediksi tingkat kematangan buah jambu kristal secara non-destruktif. Sifat fisikokimia meliputi total padatan terlarut (TPT), kadar air, kekerasan, dan pH diperoleh dari uji laboratorium dan digunakan sebagai acuan dalam pengembangan model prediktif. Spektrum emisi fluoresensi di bawah eksitasi LED UV 365 nm digunakan sebagai prediktor. Spektrum fluoresensi diperoleh dan direkam menggunakan spektrofotometer UV-Vis mini dengan rentang efektif 340 – 800 nm. Total sebanyak 240 set data sampel buah jambu kristal dengan variasi umur 90-106 HSA (hari setelah bunga mekar) digunakan untuk kalibrasi dan validasi. Analisis multivariat menggunakan Partial Least Squared Regression (PLSR) digunakan untuk mengembangkan model prediktif. Beberapa metode pra-pemrosesan data diterapkan untuk menyempurnakan informasi yang terkandung dalam data spektral untuk menemukan model prediksi terbaik. Hasil analisis menunjukkan bahwa model yang dikembangkan mampu memprediksi kadar air secara akurat dengan rcal=0,94; SEC=0,08%, rval=0,84; SEP=0,08%; RPD=2,59, diikuti TPT dengan rcal =0,91; SEC = 0,47%Brix, rval =0,79; SEP=0,48%Brix; RPD=2.13. Meskipun dengan akurasi yang lebih rendah, namun juga dapat memprediksi kekerasan dengan rcal =0,86; SEC=0,43 kgf, rval =0,74; SEP=0,43 kgf; RPD=1,82 dan pH dengan rcal =0,81; SEC=0,10, rval =0,79; SEP=0,09; RPD=1,76. Hasil penelitian menunjukkan bahwa spektroskopi fluoresensi dapat digunakan sebagai metode alternatif untuk estimasi sifat fisikokimia secara non-destruktif sebagai indikator tingkat kematangan buah jambu kristal.*

## INTRODUCTION
Guava (*Psidium guajava* L.) is well known for its abundant nutritional and functional ingredients, such as various vitamins, minerals, phenolics, and dietary fibers *(Jamieson et al.,2021; Sahu et al., 2020)*. It is also known as a good source of ascorbic acid, and even its ascorbic acid content is almost three times higher than oranges *(Hassimoto et al., 2005; Jawaheer et al., 2003)*.

There are various varieties of guava fruits, and one of them is crystal guava. This variety was first introduced to Indonesia through the Taiwan Technical Mission in Indonesia under the International Cooperation and Development Fund (ICDF) program and recently has been cultivated in many areas in Indonesia, especially in Java and Sumatra Islands. Crystal guava is known for its superior characteristics, making it one of important and desirable fruits. Some of these include fresh sweet fruit flavors, crisp fruit texture, fewer seeds (less than 3 % parts of fruit or even seedless), relatively large size, thick and hard skins which allow them to last longer than other guava varieties, good storage ability, fast growth rate, and high yielding *(Susanto et al., 2019; Herdiat et al., 2019)*.

Crystal guava fruit (*Psidium guajava* L.) is a non-climacteric fruit because it has a stable respiration pattern, or in other words, it cannot ripen after being picked. This condition requires farmers to harvest crystal guava fruit exactly when it is fully ripe so that it is in accordance with consumer desires. Usually, farmers estimate the level of fruit maturity based on their experience, however, they potentially harvest fruits that are not optimally ripe. According to *Patel et al. (2015)*, the time needed for guava fruit from flower anthesis until ready for harvest ranges from 110 to 120 days, depending on the cultivar. A better predictor of maturity level is to perform laboratory tests but it will take time to get the result and make the measurement not real-time. Therefore, a fast and accurate way is needed to estimate the ripeness level of the guava fruit.

A comprehensive review of advances in the non-destructive assessment of fruit ripeness toward defining optimal time of harvest and yield prediction has been undertaken in *Li et al. (2018)*, from which it was learned that a variety of non-destructive techniques have been introduced to estimate the ripeness or maturity including colorimetry, visible imaging, visible and near-infrared (Vis-NIR) spectroscopy, hyperspectral imaging, multispectral imaging, fluorescence imaging, acoustic impulse technique, acoustical vibration technique, Computed Tomography (CT) scan, Magnetic Resonance Imaging (MRI), and the electronic nose technique, but not all of them are applicable for in situ assessment. In a more specific topic, there are various studies on non-destructive methods for estimating the maturity level and/or physicochemical of guava fruits. A machine vision system was introduced to estimate the ripeness level of guava (green, ripe, overripe, and spoiled) *(Kanade and Shaligram, 2015)*. Using color information (RGB, CIE1931 standard-based tristimulus value, chromaticity coordinates, and average) combined with Principal Component Analysis (PCA) and Artificial Neural Network (ANN) the system could classify the ripening stages with 100% accuracy. The thermal imaging method has been used by *Widodo et al. (2021)*. The result indicated that fruit temperature had some correlation with the physical characteristics (firmness) and chemical characteristics (TSS, free acids, sucrose, and starch) of crystal guava fruit. Resonant frequency and elasticity index (EI) to estimate fruit firmness have been reported to be inversely correlated to its maturity *(Mayorga-Martínez et al., 2016)*. Visible/near-infrared (Vis-NIR) spectroscopy was applied to detect the sugar content and hardness of pearl guava *(Hsieh and Lee, 2005)*. Using three different calibration models, the proposed method could estimate the sugar content and hardness with a validation accuracy of 94%. Near-infrared spectroscopy (NIRS) with an effective range of 1000-2500 nm could be used to predict total soluble solids and moisture content with good accuracy, while they were less viable to predict the flesh hardness of the guava fruit *(Ahmad et al., 2018)*. Computer vision combined with Convolutional Neural Networks (CNN) with LeNet architecture was successfully applied to identify the maturity of guava fruit with 100% accuracy *(Sunarya et al., 2019)*.

Another non-destructive method that has also potential to be used for estimating the maturity level and physicochemical properties of guava is fluorescence spectroscopy. Fluorescence is a light emission phenomenon that arises after the absorption of light at a certain wavelength by fluorescence molecules or a substructure known as a fluorophore. Fluorophore absorbs energy in the form of light with a certain wavelength and releases energy in the form of light emission with longer wavelengths *(Lakowicz, 2006)*. As indicated by *Krishnamoorthy (2018)*, the power of fluorescence spectroscopy in comparison to other types of spectroscopies is as follows: (i) high sensitivity and selectivity, in which a single molecule can be tracked from its fluorescence; (ii) high level of environmental sensitivity of a fluorescent molecule used as a probe and (iii) feasibility of monitoring wide timescale (femtoseconds to seconds) of dynamic processes.

UV-induced fluorescence spectroscopy that utilized UV lamps for excitation is one of the most used in various studies. Previous studies had shown that UV light at certain wavelengths could produce fluorescence emissions which contain information about the physicochemical changes of the fruit. *Li et al. (2011)* reported that the excitation of UV light onto navel orange fruit at a wavelength of 365 nm was able to produce emissions identified at wavelengths 385–414 nm. Fluorescence spectroscopy could be developed for non-destructive measurement of the fruit maturity index of satsuma mandarin (*Citrus unshiu* Marc) *(Muharfiza et al., 2017)*.

Other research also reported that excitation at a wavelength of 365 nm resulted in a better fluorescence spectrum in several varieties of citrus in Indonesia *(Siregar et al., 2018)*. A study on the ability to predict fruit maturity of four yellow peach 'August Flame', 'O'Henry', 'Redhaven', and 'September Sun' cultivars using a prototype non-destructive fluorescence spectrometer has been undertaken *(Scalisi et al., 2020)*. Collected spectra were analyzed to predict, among others, flesh firmness (FF) and soluble solids content (SSC). It was found that except for 'September Sun', a good prediction of FF and SSC was observed. Fruit maturity classes were reliably predicted with a high likelihood (F1-score = 0.85) when samples from the four cultivars were pooled together.

*Das et al. (2016)* utilized the smartphone spectrometer to rapidly evaluate the ripeness of different varieties of apples using chlorophyll (ChlF) emissions when excited using UV light. It was observed that there is a satisfactory correlation between the ripeness that was measured by a penetrometer and by ChlF emission using the proposed device. This study aims to explore the potential use of UV-induced spectroscopy in determining the maturity level of crystal guava fruit. There is no such study reported yet previously.

## MATERIALS AND METHODS

### Sample material

Sample materials used in this research were crystal guava fruits harvested directly from the farmer at Cidahu, Sukabumi, Indonesia. In order to get a representative variation of the ripening stage, the samples were harvested at four age categories, starting from 90 DAA, 95 DAA, 100 DAA, and 106 DAA. For this purpose, all crystal guava samples were marked using a colored string during anthesis. Guava crystals were collected and labeled with the identity of the sample, then they were transported to the laboratory for measurement on the same day. Prior to in-lab measurement, the crystal guavas were cleaned of impurities using running water and then dried using a cloth. Next, the measurement points for the fluorescence spectrum and destructive test are marked on the crystal guava fruit in three main parts, top, middle, and bottom. In total, 240 samples were collected and then divided into two groups of datasets for calibration and validation during predictive model development.

### Fluorescence spectra and physicochemical parameter measurements

Fluorescence spectra were acquired using a simple Do It Yourself (DIY) instrument which is similar to that used by *Das et al. (2016*). A UV LED with an effective wavelength of 365 nm was used for excitation and a UV-Vis mini-spectrometer C1266280MA (Hamamatsu, Japan) was used to capture the emission spectra. For each sample, fruit spectra measurement was done at three different points, and the spectra obtained were the average value of five scans. The process is then followed by destructive measurements including total soluble solids (TSS), firmness, pH, and water content. The measurement of total soluble solids (TSS) was carried out using a digital pocket refractometer (Atago, Japan), firmness level was measured using a rheometer (Sun Rheometer CR 500-DX, USA), pH was measured using a handheld pH meter (Extech, USA) and water content of crystal guava was measured by the oven method following AOAC standard *(AOAC, 2018)*.

### Data analysis

A multivariate analysis using Partial Least Squares Regression (PLSR) was selected to develop the predictive model. In many cases, PLSR can provide better prediction results compared to other linear regression approaches such as Multiple Linear Regression (MLR) and Principal Component Regression (PCR). PLSR algorithm considered and modeled both the predictor and response simultaneously to determine the latent variables in the predictor data that will best predict the response *(Kusumiyati et al., 2021)*. From a total of 240 sets of data, it was then divided into two groups, about 2/3 of the data was used for calibration (n=159) and 1/3 for validation (n=81). The obtained raw fluorescence spectrum data as well as the pre-processed data were used in the model development. Data pretreatment was usually needed to reduce or eliminate noise in the fluorescence spectra data and enhance the information in the spectra data. Data pretreatment was carried out by using six methods, namely, Standard Normal Variate (SNV), Multiplicative Scatter Correction (MSC), Smoothing Savitzky-Golay (SSG), baseline correction, normalization, and Detrending (DT). The data analysis was done using Microsoft Excel and The Unscrambler X 10.4 software (CAMO, Norway).

The results of the calibration and validation model of the maturity level of guava crystals were evaluated based on several parameters, namely, *R* (correlation coefficient), SEC (standard error of calibration), SEP (standard error of prediction), RPD (ratio of the standard error of prediction to deviation), and consistency.

A good model should have an *R* value close to 1, SEC and SEP value close to 0. An RPD between 1.5 and 2 means that the model can discriminate low from high values of the response variable; a value between 2 and 2.5 indicates that coarse quantitative predictions are possible, and a value between 2.5 and 3 or above corresponds to good and excellent prediction accuracy, respectively *(Nicolai et al., 2007)*. The consistency value is a ratio or value that shows the closeness between SEC and SEP. This value should be in the range of 80-110% for the model with good predictability to avoid overfitting or underfitting during model development (*Cantor et al., 2011*).

## RESULTS AND DISCUSSION

### Physicochemical properties of crystal guava samples

The physicochemical properties of crystal guava fruit obtained from destructive measurements showed different TSS, water content, firmness, and pH values at each harvest age (Table 1). The data showed that the TSS values fluctuate at each harvest age. It seems to be uncommon because generally there is a positive linear correlation between TSS and fruit age. The more the fruit was aged, the TSS value would increase. However, Bashir et al. *(2003)* indicated that when the fruit has reached a certain level of maturity, the fruit will experience a decrease in TSS because the carbohydrates and sucrose in the fruit are used as an energy source. The increase in TSS was caused by the degradation of starch into simple sugars while the decrease in TSS was due to the sugar being used as a respiration substrate to produce energy. The growing conditions such as soil, climate, or other factors that were not measured in this research could also influence the TSS. The value of water content did not change significantly but did increase with harvest age. The firmness level of the fruit appeared to decrease with the increasing harvest age. According to Zainal et al. (2012), the softening of the fruit was caused by the breakdown of insoluble protopectin into soluble pectin or starch or by fat hydrolysis. In fruits, there is a cell wall consisting of cellulose, hemicellulose, pectin, and lignin. Fruit softening is caused by the degradation of hemicellulose and protopectin. The pH value obtained showed a change that is not linear with the age of the fruit, although it has an increasing trend. The increase in pH value with age was caused by acid hydrolysis that occurs during the fruit ripening process (*Rachmayati et al., 2017*). The riper the fruit, the pH value will increase because the total sugar in the fruit also increases. During the ripening process, the fruit becomes sweeter after the organic acids are converted into sugar. Acid hydrolyzed into simple sugars causes the H+ ions in the fruit to decrease so that the pH value becomes higher as the fruit ripens. The obtained destructive measurement data was then used as reference data in developing the predictive model.

**Table 1**

**Physicochemical properties of crystal guava with different harvest age**

| Harvest Age | Number of Samples | TSS (ºBrix) | Water Content (%) | Firmness (kgf) | pH |
|---|---|---|---|---|---|
| 90 | 60 | 5.971 ±0.729 | 87.78 ± 0.013 | 3.29± 1.07 | 3.97± 0.18 |
| 95 | 60 | 7.022± 1.191 | 88.53 ± 0.029 | 3.32± 0.49 | 3.88± 0.17 |
| 100 | 60 | 5.725± 0.710 | 89.56 ± 0.017 | 2.75± 0.50 | 3.98± 0.11 |
| 106 | 60 | 6.143± 0.933 | 90.10 ± 0.011 | 2.30± 1.08 | 4.04± 0.11 |

### Fluorescence Emission Spectra

The fluorescence emission spectrum of all samples under excitation of UV-LED at 365 nm is shown in Figure 1. There were two peaks observed at around 550 nm and 690 nm. Although it was not obvious, a small peak was also observed at the range of 730 nm. The first peak was likely emission from flavonoid substances such as poly-methoxylated flavone (PMF) substances *(Muharfiza, 2017; Siregar, 2018)*. Guava fruit contains high phenolic and flavonoid compounds and hence traditionally is used for curing several diseases *(Gutiérrez, 2018).* This has been confirmed by the study of Sanches et al. (2005) as well as Gull et al. *(2012)* which also stated that the UV (270, 331, 392 nm) spectra data were typical of flavonoids. The second and third peaks were likely emissions from chlorophyll substances (*Muharfiza, 2017; Kusumiyati et al., 2019)*.

Flavonoids are a class of polyphenolic compounds that are widely present in nature and have grown to be an important part of the human diet *(Mutha et al., 2021).*

TSS is the function of several factors of which total sugars and organic acids constitute the major part with minor contributions from other small components (phenolics, amino acids, vitamins, and minerals) (*Bexiga et al., 2017*). TSS also correlates with the pH value because changes in the value of TSS are followed by changes in the amount of sugar and other compounds that will affect the pH value. Therefore, it is hypothesized that the estimation of the parameter value of the maturity level of the guava fruit could be done indirectly by utilizing the spectrum information showing the levels of flavonoids and chlorophyll. The spectrum intensity data of 450-800 nm was chosen to be used to develop the predictive model of physicochemical properties (i.e., TSS, water content, firmness, and pH) as indicators of the maturity level of crystal guava fruits.

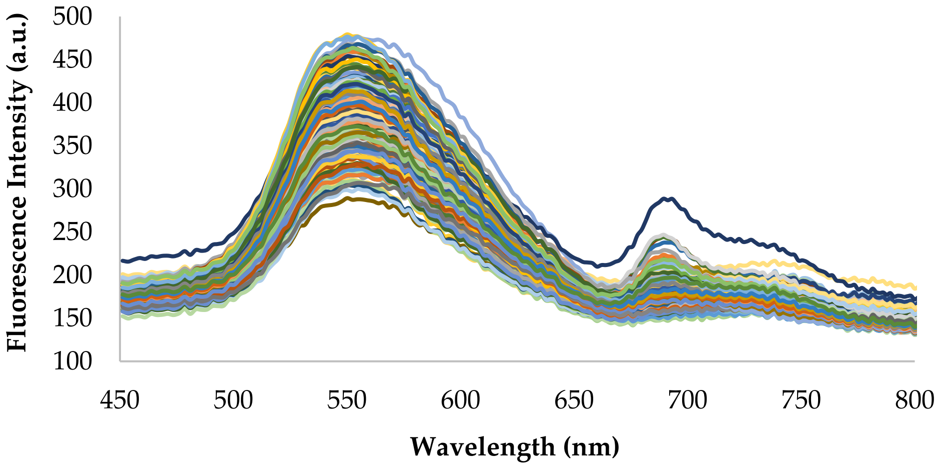


**Fig. 1 - The fluorescence emission spectrum of crystal guava**

**Prediction of physicochemical properties of crystal guava fruits**

This study used fluorescence spectroscopy combined with chemometrics to non-destructively predict some of the physicochemical properties as indicators of crystal guava fruit maturity. These physicochemical properties can be used to determine the maturity level of the fruit more accurately as an alternative to conventional methods which are only based on the age or external appearance of the fruit. Table 2 shows the calibration and validation results of crystal guava's physicochemical properties prediction using the PLSR method with original/raw and several pre-processed data.

The results indicate that each parameter requires a different pre-processing method to produce the best model. This study's normalization treatment provides a better prediction for TSS and pH, baseline correction for water content, and MSC for firmness. The best developed model could accurately predict water content with $r_{cal}$=0.94; SEC=0.08%, $r_{val}$=0.84; SEP=0.08%; RPD=2.59; consistency=99.05%, followed by TSS with $r_{cal}$=0.91; SEC = 0.47%Brix, $r_{val}$=0.79; SEP=0.48%Brix; RPD=2.13; consistency=97.67%. Although with lower accuracy, it also could predict firmness with $r_{cal}$=0.86; SEC=0.43 kgf, $r_{val}$=0.74; SEP=0.43 kgf; RPD=1.82; consistency=100.47% and pH with $r_{cal}$=0.81; SEC=0.10, $r_{val}$=0.79; SEP=0.09; RPD=1.76; consistency=109.65%.

Based on the RPD value, the pH and firmness parameters were above 1.5, indicating that the estimation model for these parameters was feasible, while the moisture content and TSS parameters were at a value of 2-3 indicating that the prediction model for these parameters was good for rough screening. Furthermore, the consistency value of each parameter was between 80% - 110%, indicating that the model was neither under-fitted nor over-fitted. Scatter plots between reference and predicted values for all parameters are shown in Figure 2. The data distribution for the calibration and validation stages seems acceptable and represents the entire data population.

Figure 3 shows the plot of the regression coefficient of Factor-1 of PLSR for all parameters. From the regression coefficient profile, the variables that contributed most to the predictive model for TSS were similar to firmness, although with the opposite trend. Meanwhile, the variables and trends for water content tend to be similar to pH. In the TSS and firmness estimation, the flavonoid content appears to be more dominant, while for the water content and pH estimation both the flavonoids and chlorophyll have a comparable contribution.

Figure 3 also indicates that in the 500-650 nm range, there is likely more than one fluorescence component, although this needs further investigation.

Table 2

**The results of physicochemical prediction of crystal guava fruit using the PLSR**

| Pre-processing | PLS Factor | Calibration | | Validation | | RPD | Consistency (%) |
|---|---|---|---|---|---|---|---|
| | | $r_{cal}$ | SEC | $r_{val}$ | SEP | | |
| **Total Soluble Solid -TSS (%Brix)** | | | | | | | |
| Original/raw | 13 | 0.90 | 0.50 | 0.78 | 0.51 | 2.03 | 98.77 |
| SNV | 12 | 0.90 | 0.48 | 0.77 | 0.50 | 2.07 | 97.47 |
| MSC | 12 | 0.90 | 0.49 | 0.75 | 0.52 | 2.00 | 94.87 |
| SSG 1 | 20 | 0.85 | 0.59 | 0.62 | 0.61 | 1.69 | 97.26 |
| SSG 2 | 20 | 0.87 | 0.57 | 0.70 | 0.55 | 1.87 | 103.39 |
| *Baseline* | 13 | 0.90 | 0.48 | 0.78 | 0.50 | 2.08 | 97.48 |
| ***Peak Normalize*** | **13** | **0.91** | **0.47** | **0.79** | **0.48** | **2.13** | **97.67** |
| *Max Normalize* | 13 | 0.91 | 0.48 | 0.77 | 0.50 | 2.07 | 96.51 |
| DT 1 | 12 | 0.90 | 0.50 | 0.76 | 0.51 | 2.02 | 98.80 |
| DT 2 | 12 | 0.90 | 0.49 | 0.75 | 0.53 | 1.94 | 92.14 |
| **Firmness (kgf)** | | | | | | | |
| Original/raw | 12 | 0.86 | 0.43 | 0.72 | 0.45 | 1.74 | 95.54 |
| SNV | 10 | 0.86 | 0.43 | 0.78 | 0.44 | 1.79 | 98.18 |
| MSC | 10 | 0.86 | 0.43 | 0.77 | 0.44 | 1.77 | 96.89 |
| SSG 1 | 20 | 0.80 | 0.51 | 0.67 | 0.49 | 1.59 | 102.67 |
| SSG 2 | 20 | 0.83 | 0.48 | 0.78 | 0.46 | 1.70 | 103.03 |
| ***Baseline*** | **10** | **0.86** | **0.43** | **0.74** | **0.43** | **1.82** | **100.47** |
| *Peak Normalize* | 10 | 0.80 | 0.50 | 0.66 | 0.48 | 1.64 | 104.87 |
| *Max Normalize* | 11 | 0.85 | 0.45 | 0.72 | 0.44 | 1.76 | 100.71 |
| DT 1 | 4 | 0.85 | 0.45 | 0.70 | 0.45 | 1.74 | 98.51 |
| DT 2 | 10 | 0.86 | 0.43 | 0.74 | 0.43 | 1.82 | 100.47 |
| **Water Content (%)** | | | | | | | |
| Original/raw | 17 | 0.93 | 0.08 | 0.84 | 0.08 | 2.61 | 103.85 |
| SNV | 16 | 0.93 | 0.08 | 0.84 | 0.08 | 2.56 | 102.07 |
| **MSC** | **16** | **0.94** | **0.08** | **0.84** | **0.08** | **2.59** | **99.05** |
| SSG 1 | 20 | 0.79 | 0.15 | 0.62 | 0.12 | 1.82 | 125.33 |
| SSG 2 | 20 | 0.81 | 0.14 | 0.62 | 0.12 | 1.81 | 117.80 |
| *Baseline* | 16 | 0.93 | 0.09 | 0.82 | 0.09 | 2.48 | 103.20 |
| *Peak Normalize* | 16 | 0.93 | 0.09 | 0.82 | 0.09 | 2.47 | 102.31 |
| *Max Normalize* | 16 | 0.93 | 0.09 | 0.81 | 0.09 | 2.39 | 98.80 |
| DT 1 | 15 | 0.93 | 0.09 | 0.82 | 0.09 | 2.41 | 98.14 |
| DT 2 | 14 | 0.92 | 0.09 | 0.82 | 0.09 | 2.41 | 102.71 |
| **pH** | | | | | | | |
| Original/raw | 13 | 0.79 | 0.10 | 0.80 | 0.09 | 1.78 | 98.77 |
| SNV | 11 | 0.79 | 0.10 | 0.75 | 0.10 | 1.63 | 105.19 |
| MSC | 11 | 0.78 | 0.10 | 0.79 | 0.09 | 1.73 | 117.59 |
| SSG 1 | 20 | 0.67 | 0.12 | 0.67 | 0.10 | 1.44 | 116.39 |
| SSG 2 | 20 | 0.72 | 0.11 | 0.74 | 0.10 | 1.53 | 117.30 |
| *Baseline* | 12 | 0.77 | 0.11 | 0.78 | 0.09 | 1.72 | 118.77 |
| *Peak Normalize* | 12 | 0.79 | 0.10 | 0.74 | 0.10 | 1.56 | 104.11 |
| ***Max Normalize*** | **13** | **0.81** | **0.10** | **0.79** | **0.09** | **1.76** | **109.65** |
| DT 1 | 11 | 0.78 | 0.10 | 0.78 | 0.09 | 1.69 | 113.44 |
| DT 2 | 11 | 0.78 | 0.10 | 0.81 | 0.10 | 1.59 | 104.26 |

In general, it was found from this study that flavonoid and chlorophyll fluorescence were strongly correlated and could be used to indirectly estimate some physicochemical properties of crystal guava. Furthermore, those physicochemical properties could be used to calculate the maturity index of crystal guava. Such an approach has been applied by *Khumaidi et al. (2022)* to determine the maturity index of intake mango. This method is also considered to be more objective, standardized, and free from bias that may arise due to subjectivity. Overall, based on the performance evaluation of the developed predictive model, the results of this study suggested that fluorescence spectroscopy combined with chemo-metrics could be used as an alternative method for non-destructive estimation of physicochemical properties of crystal guava fruits as indicators of the maturity level of crystal guava fruits.

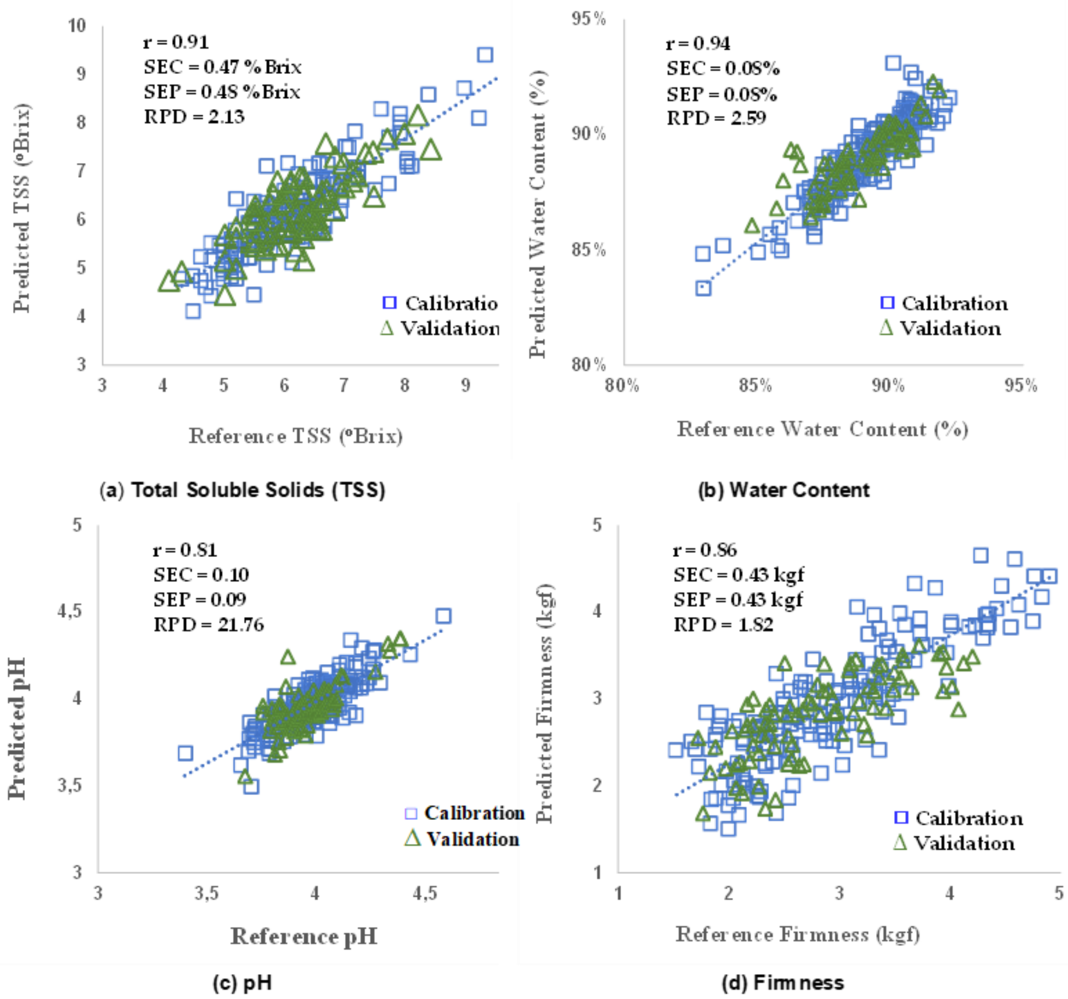


**Fig. 2 - Prediction and reference value of each maturity parameter**

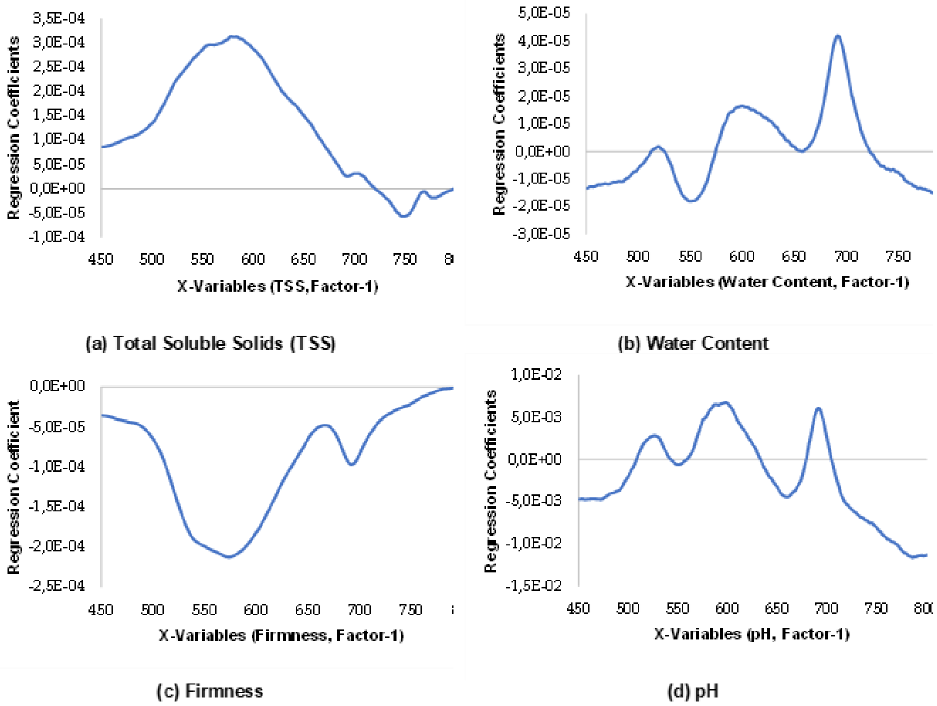


**Fig. 3 - The regression coefficient of Factor-1 PLSR**

## CONCLUSIONS

This study explored an alternative method for non-destructive estimation of the physicochemical properties of crystal guava fruit using fluorescence spectroscopy combined with chemometrics. Using fluorescence emission spectra at 450-800 nm under excitation of a 365 nm UV LED combined with PLSR, the developed models were able to accurately predict water content (r=0.94; SEP=0.08%; RPD=2.59; consistency=99.05%) and TSS (r=0.91; SEP=0.48; RPD=2.13; consistency=97.67%). Although with lower accuracy, it also could predict firmness (r=0.86; SEP=0.43; RPD=1.82; consistency=100.47%) and pH (r=0.81; SEP=0.09; RPD=1.76; consistency=109.65%). The results indicate that fluorescence spectroscopy could be used as an alternative method for non-destructive estimation of physicochemical properties as indicators of the maturity level of crystal guava fruits.

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