



## Use of mid infrared spectroscopy to analyze the ripening of Brazilian bananas

Leandro da Conceição LUIZ<sup>1,2\*</sup> , Cláudia Aline NASCIMENTO<sup>1</sup>, Maria José Valenzuela BELL<sup>1</sup>,  
Rafaela Tavares BATISTA<sup>3</sup>, Seshadri MERUVA<sup>1</sup>, Virgílio ANJOS<sup>1</sup>

### Abstract

Banana is one of the most consumed fruits in the world due to its flavor and nutritional value. The knowledge of the ripening stage of bananas is essential for its commercialization, since, after harvesting it can take days in transportation until reaching their destination. The use of spectroscopic techniques in the infrared region has been widely used in the food industry. In this study, a nondestructive analytical method was developed to monitor the maturation of silver bananas, dwarf bananas, gold bananas and bread bananas using Fourier Transform Mid Infrared spectroscopy. To accompany the ripening of the bananas, 3 regions in each banana were chosen (bottom, middle and top) and from these, 3 measures were taken around the banana perimeter. Beyond the ripening process of the bananas, functional groups are identified as well as the beginning of the stage previous to the mature fruit, characterized by the emission of ethylene and CO<sub>2</sub>. Principal components analysis allowed the identification of the process irrespective of the banana type which suggest the automation possibility of the process.

**Keywords:** infrared; maturation; FT-IR; optical absorption; fruit.

**Practical Application:** Use of FT-MIR to accompany the ripening of bananas. Spectral identification of banana varieties.

### 1 Introduction

Fruits are complementary foods of great importance in daily nutrition. Sources of vitamins, fibers and minerals, the fruits are essential in all age groups and consumed by different species. Banana is one of the most consumed fruits in the world due to its flavor and nutritional value (Du et al., 2016). Banana fruit has flesh not only rich in starch which changes into sugars on ripening but is also a good source of resistant starch. Banana is known to be rich in carbohydrates, dietary fibers, vitamins (B5, B6, choline and C), minerals (magnesium, phosphorus, potassium, sodium and zinc), and many health-promoting bioactive phytochemicals (antioxidant properties, reducer of the risk of cardiovascular diseases (CVD) and cancer, potential hepatoprotective effects, cholesterol reducer, precursors of vitamin A, antimicrobial, anti-inflammatory, antiallergic, among others) (Sidhu & Zafar, 2018). Brazil is the fourth largest banana producer in the world with approximately 7 million tons in 2017, representing 6% of the world production (Sá et al., 2019). According to preliminary results from Food and Agriculture Organization of the United Nations (2020), the biggest exporters of bananas are Ecuador, Philippines, Guatemala, Colombia and Costa Rica, while the largest importers are the European Union, United States, Russia and Japan. Brazil does not occupy a prominent position in international banana trade even if one considers South American countries, such as: Ecuador, Colombia, Peru and Bolivia (Voora et al., 2020; Workman, 2020).

The most planted banana varieties in Brazil are: Silver, Pacovan, Silver Dwarf, Mysore, Plantain, D'Angola, Dwarf, Big Dwarf, Grand Naine, Gold, Gray Fig, Red Fig, Green Caru and Purple Caru (Sousa et al., 2017). The knowledge of banana ripening stage is essential for its trade, since, after harvesting it can take days in transport until reaching its destination.

The use of spectroscopic techniques in the infrared region has been widely used in the food industry. In 2015, Wang et al. used visible/near infrared spectroscopy to evaluate the ripeness of melons (Wang et al., 2015). In 2016, Guo et al., evaluated chemical components and properties of jujube fruit using near infrared spectroscopy and chemometrics (Guo et al., 2016). In 2019, Skolik et al., used infrared spectroscopy and chemometrics to determine developmental and ripening stages of whole tomato fruit (Skolik et al., 2019). In the same year, Bureau et al., showed the contributions of Fourier-transform mid infrared spectroscopy to the study of fruits and fresh vegetables (Bureau et al., 2019). A year later, in 2020, Manimaran et al., studied the natural cellulosic fibers extracted from Nendran Banana Peduncle plants. To measure the physical-chemical, mechanical and thermal properties of the bananas, they used, among other techniques, Fourier-Transform infrared spectroscopy (FT-IR). The FT-IR results demonstrated the existence of different chemical compositions and their corresponding functional groups (Manimaran et al., 2020). Recently, in 2021, Long Zhang et al. (2021), evaluated the effect of sample presentation (tissue type) and maturity (ripe

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<sup>1</sup> Universidade Federal de Juiz de Fora – UFJF, Juiz de Fora, MG, Brasil

<sup>2</sup> Faculdade Bezerra de Araújo – FABA, Rio de Janeiro, RJ, Brasil

<sup>3</sup> Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro – IFRJ, Eng. Paulo de Frontin, RJ, Brasil

\*Corresponding author: [livroleandro@gmail.com](mailto:livroleandro@gmail.com)

and unripe) on the classification of banana (*Musa Cavendish*) samples sourced from two different geographical regions and analyzed using mid infrared (MIR) spectroscopy. Their results demonstrated that MIR spectroscopy might be used to classify the origin of the banana samples at different degrees of ripeness. Still in 2021, Pereira et. al., used X-ray fluorescence to characterize the elemental concentrations of two types of banana, gold and silver. Their results showed that elements of both types are more concentrated in the skin than in the pulp (Pereira et al., 2021). In fact, the usage of infrared spectroscopy as an analytical method to determine authenticity and adulteration in food has increased exponentially (Kamal & Karoui, 2015; Pereira et al., 2020; Luiz et al., 2020; Teixeira et al., 2020; Khan et al., 2021; Silva et al., 2021). Although the studies are being conducted for decades infrared devices in food industries are still scarce. The reason remain in the complexity and temporal dynamic of foods. The basic premise behind the application of spectroscopic techniques for evaluation of food quality depends on the generation of “fingerprints”. In general these are related to vibrational or roto-vibrational modes of solids or molecules that constitute the samples. Eventually electron degrees of freedom may take part in the optical process (Luiz et al., 2018; Luiz, 2019; Sala, 2008).

Due to the complex and subtle response of food systems, multivariate statistics are necessary. They may be supervised or unsupervised. Example of the latter is the Principal Components Analysis (PCA). This mathematical algorithmic, which will be used in this work, allows to reduce the dimensionality of a set of variables in a new set of variables, called principal components. This still maintain the integrity of the data that characterize the studied samples although losing some information from the primary data set (Luiz et al., 2018; Jolliffe & Cadima, 2016; Sant’Ana et al., 2019). Mathematically, PCA is based on an eigenvector (principal component) decomposition of the covariance matrix of the variables in a data set. In PCA analysis, the score matrix provides information about the samples distribution and grouping, and particularly for this study, the loading matrix gives information about the most relevant mid infrared bands used.

The aim of this study was to use the non-destructive FT-MIR technique to determine the day when the ripening process of silver bananas, dwarf bananas, gold bananas and bread bananas start and accompany its evolution in time through the spectral dynamics resulting from the change of characteristic functional groups. The PCA results regardless of the variety of the bananas shows a similar behavior when at the beginning of the ripening process. This suggest the possibility of automation of the maturation process in order to assist in decision making concerning the commercialization of the fruit.

## 2 Materials and methods

The samples were stored, prepared and analyzed in the laboratories of Group of Engineering and Spectroscopy of Materials (GE2M), located in the Physics Department of the Federal University of Juiz de Fora, Brazil.

### 2.1 Sampling and preparation

Bananas samples used were harvested manually from a farm in the city of Guarani, Minas Gerais, Brazil. They are: silver bananas, dwarf bananas, gold bananas and bread bananas. After harvesting, the bananas were immediately taken to the laboratory, where they were cleaned with distilled water in order to standardize the samples ripening process. The measurements were carried out the next day. The bananas were kept stored, in an environment protected from light, with a temperature of around 21 °C, during all measurement days.

### 2.2 Experimental methodology

To accompany the ripening of bananas, it was decided to define 3 regions in each banana (bottom, middle and top) and in each region, 3 measures were taken around the banana perimeter. In this way, the measurements were standardized. The samples were exposed to infrared light only in the selected regions. Figure 1A illustrates the light incidence zone used to maintain a standard in each sample. Two bananas of each type were chosen, so that one banana served as a control sample. The control sample was a banana from the same bunch, but with the stem (on top) wrapped in aluminum foil. Each sample was placed in the ATR (Attenuated Total Reflection) compartment of the FT-MIR spectrometer, and it was lightly pressed so that no light enters between the ATR crystal and the surface of the sample.

### 2.3 Spectral measurements

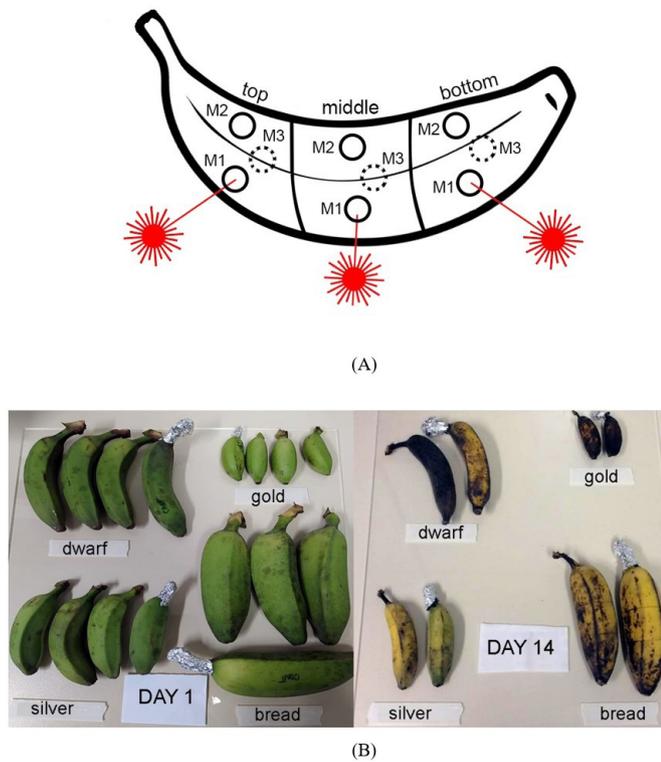
Measurements of the samples were carried out with the Vertex 70 FT-MIR Analyzer from Bruker operating in the absorbance mode with ATR accessory. The OPUS software version 5.5 was used for data acquisition. Background signals were first acquired. After properly positioning the sample, spectral measurements were performed on each sample region. The spectra were acquired in the range of 4000 to 400  $\text{cm}^{-1}$  wavenumbers with a resolution of 4  $\text{cm}^{-1}$ , an average of 32 scans, temperature of  $20 \pm 0.5$  °C and humidity of  $43 \pm 0.5\%$ . Each measurement was performed in triplicate and were taken over 14 consecutive days at 2 pm.

### 2.4 Statistical analysis

Absorbance spectra were constructed and analyzed using OriginPro software, version 2019b. The principal component analysis was conducted with the software The Unscrambler® version 9.2.

## 3 Results and discussion

Figure 1B shows the different types of bananas used in the measurements, on the first day and on the fourteenth day, the last day. Although the main objective of the work was to accompany the ripening of bananas, the measurements persisted until all bananas were with black peels. The banana control, with the top coated with aluminum foil, which was used as an attempt to delay ripening did not present defined trend.

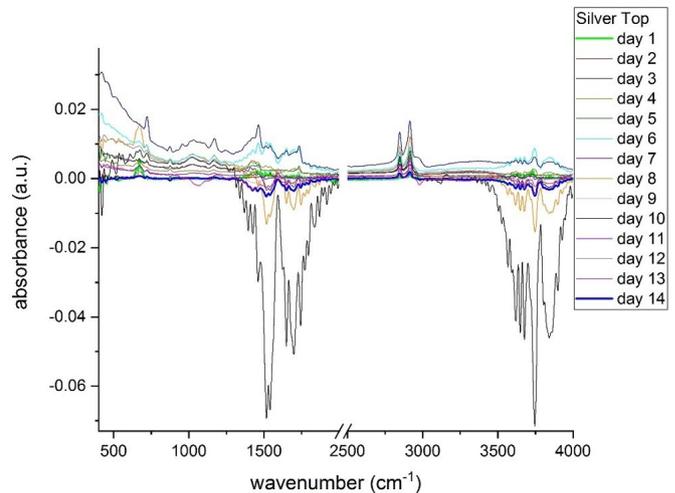


**Figure 1.** (A) Schematic diagram of the infrared light incidence zone around the banana; (B) Bananas samples on the first and last day of measurement. M1 = measure 1, M2 = measure 2 and M3 = measure 3.

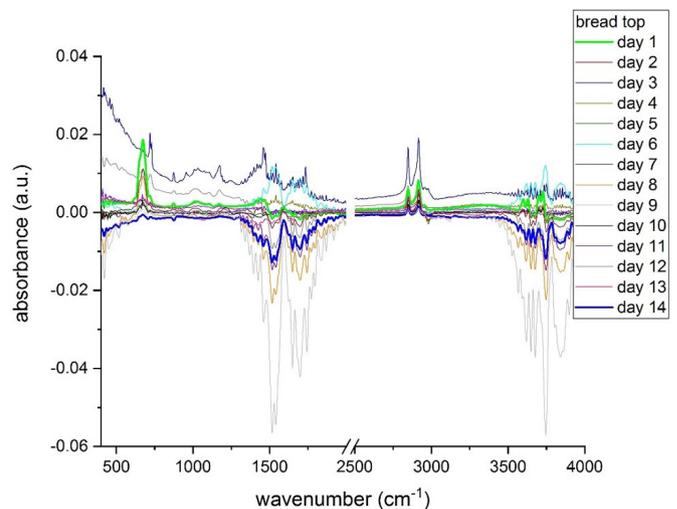
### 3.1 Vibrational spectra description

Figures 2, 3, 4 and 5 show the vibrational spectra obtained by FT-MIR in ATR mode in the range of 4000 to 400  $\text{cm}^{-1}$  for each type of banana during the 14 days of analysis. From Figure 1B, it is apparent that the ripening occurs from bottom to the top of the fruits. However, checking the spectra of the three analyzed regions, it was observed that there was no significant difference in the ripening process between them. Therefore, the top region was the one chosen to be presented. The spectra of bananas used as controls were not shown. It is important to note that the band between 2300  $\text{cm}^{-1}$  and 2400  $\text{cm}^{-1}$  attributed to the  $\text{CO}_2$ , referred to the ATR crystal, was taken in the Figures 2, 3, 4 and 5 (Ohlin et al., 2013).

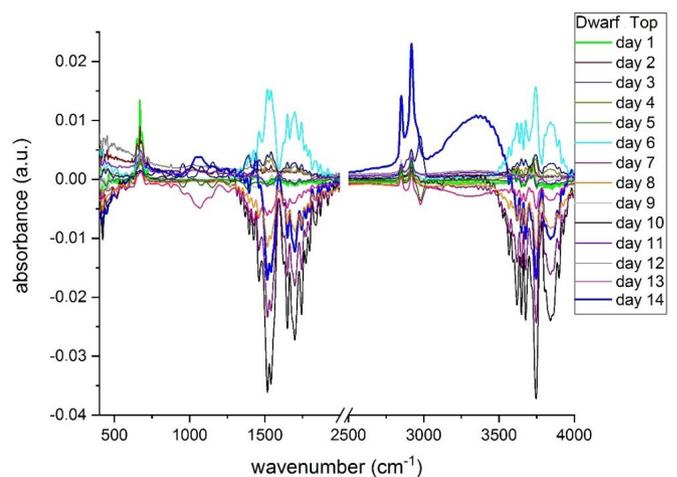
The profiles of the spectra obtained during the 14 days are similar for the 4 types of bananas (Figures 2, 3, 4 and 5). Around 1500  $\text{cm}^{-1}$  and 3750  $\text{cm}^{-1}$  an interesting phenomenon occurred for all types of bananas. The bands present negative absorption from days 6 until day 8 depending on the type of banana. These days corresponds those where the bananas show visually the first signs of maturation. We claim that this negative absorption is due to the emission of some gas in the banana ripening process. The assignments regarding the observed bands are as follow: symmetrical ester C-O-P stretch (1025  $\text{cm}^{-1}$ ), phospholipids (1020  $\text{cm}^{-1}$  to 1282  $\text{cm}^{-1}$ ), asymmetrical C-O stretch of C=O-O-C (1170  $\text{cm}^{-1}$ ), asymmetrical phosphate diester PO2 (1245  $\text{cm}^{-1}$ ), lipids C-H stretch (1400  $\text{cm}^{-1}$  to 1477  $\text{cm}^{-1}$ ), amide I (1650  $\text{cm}^{-1}$ ), amide II (1540  $\text{cm}^{-1}$ ), H-OH stretching (1650  $\text{cm}^{-1}$ ), lipids C=O (1750



**Figure 2.** Mid infrared spectra of the silver banana sample obtained during 14 days.



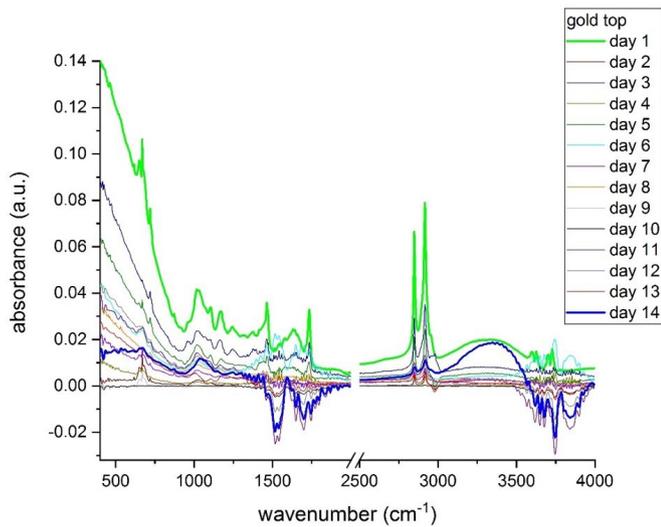
**Figure 3.** Mid infrared spectra of the bread banana sample obtained during 14 days.



**Figure 4.** Mid infrared spectra of the dwarf banana sample obtained during 14 days.

cm<sup>-1</sup>), CH<sub>3</sub>, CH<sub>2</sub> stretch (2900 cm<sup>-1</sup>), OH bend (2900 cm<sup>-1</sup>) (Andrade et al., 2020; Pereira et al., 2020; Meng et al., 2014).

Some factors may contribute to a negative absorbance spectrum, such as: inadequate cleaning of the ATR crystal, Christiansen effect, high humidity, among others. However, all of them were discarded, considering that the equipment undergoes a rigid maintenance operation with periodic check of humidity and temperature. Additionally, the measurement was repeated several times but maintaining the same tendency.



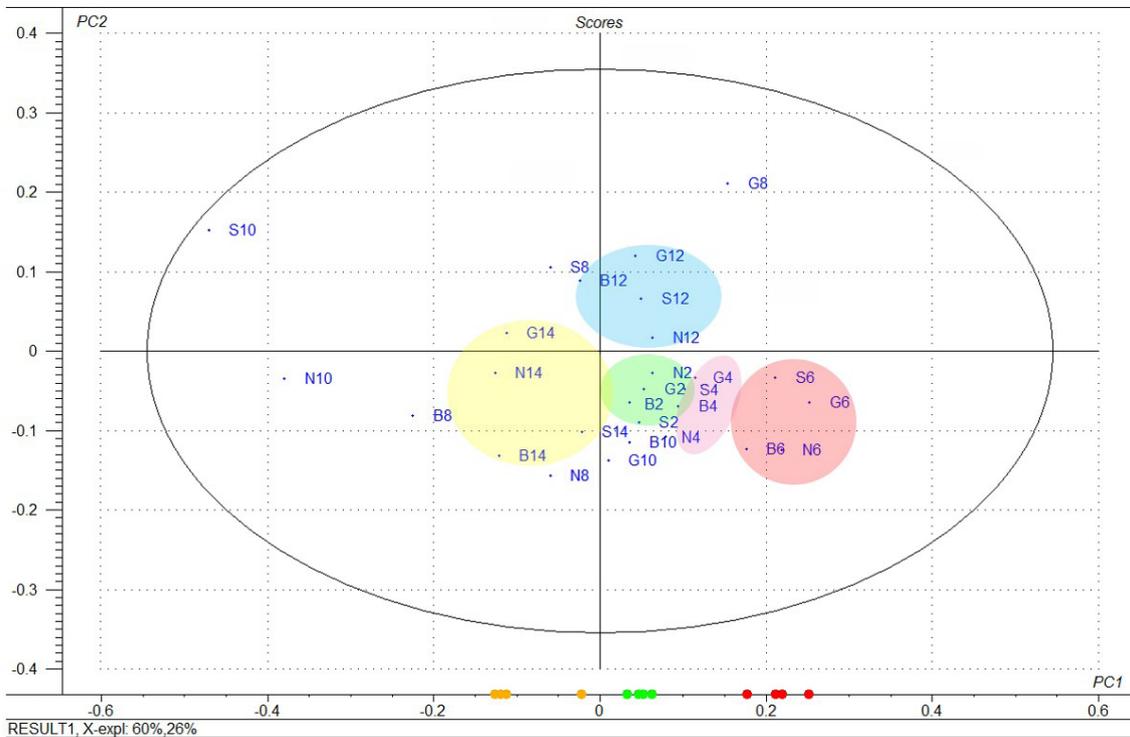
**Figure 5.** Mid infrared spectra of the gold banana sample obtained during 14 days.

According to Du et al. (2016), the banana is a climacteric fruit that produces a significant explosion of autocatalytic ethylene and respiration (CO<sub>2</sub>) at the beginning of ripening (Du et al., 2016). It is important to know that one of the absorbance bands in the infrared that is attributed to ethylene is, among others, at 1500 cm<sup>-1</sup>. From National Institute of Standards and Technology (2021) the CO<sub>2</sub> band may be found, among others, at 700 cm<sup>-1</sup> and 3700 cm<sup>-1</sup>. Therefore, the ethylene and CO<sub>2</sub> bands superpose the regions which in the sixth (eighth) day occurred the absorbance inversion

According to Boniolo, 2008, some functional groups present in bananas are: Alcohols, phenols and carbonyl (3439 cm<sup>-1</sup>), hydroxyl (1628 cm<sup>-1</sup>), alkanes aliphatics (2847 cm<sup>-1</sup> to 2933 cm<sup>-1</sup>), aldehydes, esters and ketones (1741 cm<sup>-1</sup>) and alcohols, esters saturated, unsaturated and aromatic (1053 cm<sup>-1</sup>) (Boniolo, 2008). These bands are pronounced in the measured spectra.

To complement the information obtained from the absorbance data presented in Figures 2, 3, 4 and 5, a PCA was performed to show the significant differences in the ripening process of bananas over even days. The even days were chosen to clean the PCA score.

In Figure 6, some samples formed a cluster on days 2, 4, 6, 12 and 14, with 3 regions highlighted in PC1. The first region (green) refers to two days after harvesting. The samples remain grouped, close to each other. The same occurs on day 4 when the cluster (purple) moves to the right in PC1. On the sixth day, the cluster (red) significantly distances itself from the origin in relation to PC1. It is on this day that the bananas ripening process began. Between days 8 and 12, the bananas behave



**Figure 6.** Hotelling score plot of statistical analysis (PCA) showing clustering data for bananas samples along 14 days: dwarf banana (N), gold banana (G), silver banana (S), bread banana (B).

differently in the ripening process so as not to form clusters with the 4 types of bananas. On the last day of measurement, in the yellow cluster, it was observed that the dwarf (N), gold (G) and bread (B) bananas are very close together, showing a strong relationship, while the silver (S) banana moved away from the others, being located close to the origin of PC1. This represents that there is some difference of S14 from other bananas on this day, which can be confirmed by looking at Figure 1A, where on the last day, the silver banana seems to be the less mature.

## 4 Conclusions

With the present work it was found that FT-MIR was able to accompany the ripening process of different variety of bananas, to identify characteristic functional groups and the passage from green to intermediate degree of maturation and from this to mature and very mature. Visually the intermediate process is the previous one before the mature banana where the peels becomes yellow. The intermediate process is characterized by the emission of ethylene and CO<sub>2</sub>. This stage begins between the sixth and eighth day when the infrared spectra is inverted, causing a negative absorption. The PCA confirmed the spectral information when, in the sixth day, the PC1 score presented the maximum positive value. This suggest the possibility of automation of the ripening process in order to assist in decision making concerning the commercialization of the fruit.

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