Laboratory Standard Operating Procedure

SOP for Hyperspectral Imaging Analysis of Fresh RTB Crops

Montpellier, France, June 2020

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Ethics: The activities, which led to the production of this manual, were assessed and approved by the CIRAD Ethics Committee (H2020 ethics self-assessment procedure). When relevant, samples were prepared according to good hygiene and manufacturing practices. When external participants were involved in an activity, they were priorly informed about the objective of the activity and explained that their participation was entirely voluntary, that they could stop the interview at any point and that their responses would be anonymous and securely stored by the research team for research purposes. Written consent (signature) was systematically sought from sensory panelists and from consumers participating in activities.

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RTBfoods

WP3: High-throughput phenotyping protocols

SOP: Hyperspectral imaging analysis of fresh RTB crops

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1 SCOPE AND APPLICATION

In the last years, hyperspectral imaging (HSI), was used extensively in food quality for the characterization of biochemical constituents, physical parameters and internal and external defects of several foods such as vegetables (potato, tomato, maize, spinach….) **(Amjad et al., 2018; Del Fiore et al., 2010; Diezma et al., 2013; Susič et al., 2018).** In fruits such as apple, strawberry, grape, mango, citrus, banana….etc. **(ElMasry et al., 2007; Fernandes et al., 2011; Li et al., 2016; Mehl et al., 2004; Rajkumar et al., 2012; Vélez Rivera et al., 2014)** and lamb, chicken, beef and pork meat **(ElMasry et al., 2011; Feng and Sun, 2013; Kamruzzaman et al., 2012).**
In RTBfoods project, the main objective is to develop high throughput phenotyping methods which will allow breeders to make a fast and inexpensive post-harvest selection of root, tuber and banana. So far, no research work has been done for the characterization of fresh yam and cassava using HSI. Therefore, the purpose of this SOP is to use HSI analysis to detect the longitudinal distribution of the main component (water) of fresh tuber.
This SOP describes the use of HSI to detect the longitudinal distribution of water in fresh root and tuber from sample preparation to multivariate analysis applied to hyperspectral images. In this protocol fresh yam is taken as an example.

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3 PRINCIPLE

Hyperspectral imaging (HSI) integrates the main advantages of Near Infrared Spectroscopy which is useful to determine the product quality through the measurement of their optical properties and computer vision which is able to measure the external features of products to attain spatial information from an object. HSI system produces a stack of hundreds of images of the same object at different spectral wavelength band. Hence, each pixel in a hyperspectral image contains the spectrum of that specific position, which is a fingerprint useful to characterize the composition of that particular pixel **(Baiano, 2017)**. The major advantage of HSI is the time savings, not only for sample preparation but also for database registration. With conventional NIR techniques, one measure gives one average spectrum. Thousands of spectra can be obtained with HSI, providing a complete picture of the distribution of chemical compounds at the pixel level and the possibility of simultaneously getting the spectral and spatial description of the sample **(Dale et al., 2013)**. HSI analysis involves several steps. First, white and dark images of the sample are acquired, and then the hyperspectral image is corrected with a white and a dark reference **(ElMasry et al., 2007)**. The images pre-processing (image correction, thresholding, segmentation) and multivariate analysis can be performed by using Matlab R2018b (The Mathworks Inc., Natick, MA, USA) along with PLS_Toolbox and MIA_Toolbox.

4 APPARATUS

Ceramic (or coated metal) knife

White reference

HSI image is corrected by a white reference image, which is the Teflon whiteboard with 99% reflectance.

Rectangular inox plate

Will be used to cut, deposit and transport the samples.

Oven for drying process at 105 °C

Hyperspectral Imaging SPECIM FX 17 (900-1700 nm)

5 PROCEDURE

5.1 Sample preparation

Sample of fresh yam tubers used in this study was purchased in the market at Montpellier (France). The maximum dimensions of samples must be 40 cm of length and 20 cm of diameter. Sample preparation may differ between product and product profile. As an example, we describe here the sample preparation for fresh yam tuber characterization.

1. Choose one healthy and non-defect tuber with a representative size (maximum length 40 cm and 20 cm of diameter). Please note that this dimension must not exceed the dimension of the scanner (i.e. 40 cm × 20 cm).
2. After peeling, washing and paper wiping, the tuber is divided longitudinally into two pieces by using a ceramic knife (fig.1).

Figure 1: fresh whole peeled yam tuber divided longitudinally into two sections.

5.2 Hyperspectral imaging system

The hyperspectral imaging system used is a laboratory-based pushbroom imaging equipment. The main components include (fig.2) : (1) a spectrograph with PGP optical structure (ImSpector, N17E, SPECIM, Finland), (2) a 12-bit CCD camera (V-light, Lowel Light Inc, USA), (3) 150-W tungsten halogen lamps (Fibre-Lite DC950 Illuminator, Dolan Jenner Industries Inc., Boxborough, MA, USA) and (5) a translation LabScanner with dimension (L × l) of 40 × 20 cm by a step motor. The harmonious work of the integral system is assured by using (5) control software LumoScanner (SPECIM, Finland).

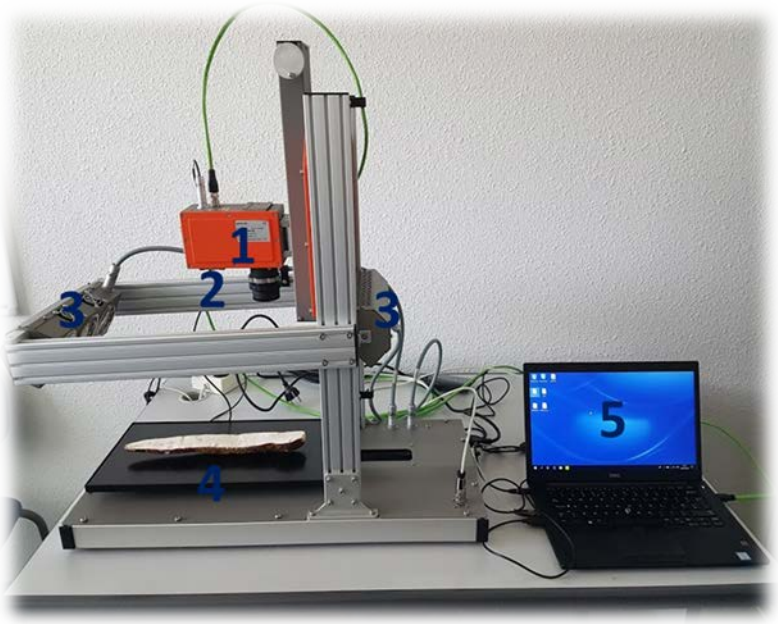

Figure 2: FX17 SPECIM hyperspectral imaging camera system

The assembly disperses the incoming line of light into the spectral and spatial matrices and then projects them onto the CCD. The optics, spectrograph and the camera, has high sensitivity from 900 to 1700 nm with a spectral resolution of 8 nm, and the exposure time is adjusted at 10 ms throughout the whole test. The distance between the lens and the surface of the imaged yam tuber is fixed at 22 cm, and the scanning speed is at 9.5 mm/s. After finishing the scans on a tuber, a three-dimensional (x,y,z) spatial (x,y) and spectral (z) data space are constructed. Images are binned during acquisition in spatial direction to provide images with spatial dimension (x×y) of (832×640) pixels with 224 spectral (900-1700 nm).

5.3 Image acquisition

The aim of this SOP is to visualize water distribution of fresh yam during oven drying at 105 °C. For this purpose, an image is acquired in two replicats, immediately after sample preparation on both

longitidunal slices and then images are taken during oven drying at 105 °C. these images are acquired after 1, 2, 3, 20 hours of oven drying (two reptitions). Between two acquisition times the sample should be put back into the oven.
The system described above (SPECIM FX17) is used to acquire hyperspectral images of fresh pieces of yam. It employs push-broom imaging and acquires one line of the image at a time. Each line of the image has a fixed number of pixels of 640, and the number of lines depends on the length of the object. The samples of fresh yam and white reference sample (Teflon) are placed into the black translation stage with a size of 40×20 cm (fig.3). Once the white and sample positions are fixed using the LumoScanner acquisition software, the recording of the HSI image is started. The dark image (with 0% reflectance) is recorded first by turning off the lighting source with the lens of the camera completely closed, and then the white reference image (Teflon whiteboard with 99% reflectance) is recorded, in the end, the hyperspectral image of the target sample is recorded.

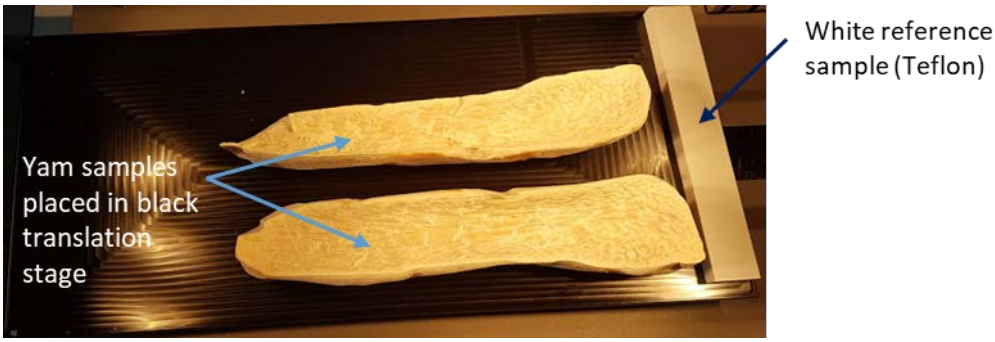

Figure 3: Black translation stage and a white reference sample (Teflon).

5.4 Image correction

When the hyperspectral images are acquired. They firstly corrected with a white and a dark reference. The dark reference was used to remove the effect of the dark current of the thermally sensitive CCD detectors. The corrected image (R) is estimated using Eq. (1):

$$R = \frac{\mathrm{R0} - \mathrm{D}}{W - D}$$

Where R0 is the recorded hyperspectral image, D the dark image and W is the white reference image. The corrected images R will be the basis for the subsequent image analysis to extract information about the spectral properties of fresh yam sample for optimizing surface characteristics identification, selection of effective wavelengths and texture analysis purposes **(ElMasry et al., 2007)**.

5.5 Selection of the region of interest (ROI)

The spectral response of fresh yam samples could be used to characterize and identify the sample uniquely. To collect the spectral response of each sample, a binary mask is first created to produce an image containing only the fresh yam in the image, avoiding any interference from the background. Here, an image at 1325 nm wavelength is taken for this task because the tuber appeared opaque compared with the background and can be segmented easily by simple thresholding at the level of 0.3176. All active pixels in the segmented image are used as a mask to identify all pixels belonging to the yam sample (ROI) and set the others to zero background (fig.4). At each pixel of the region of interest (ROI), the relative reflectance is recorded at each wavelength from 900 to 1700 nm. Each segmented image contains more than 300000 pixels.

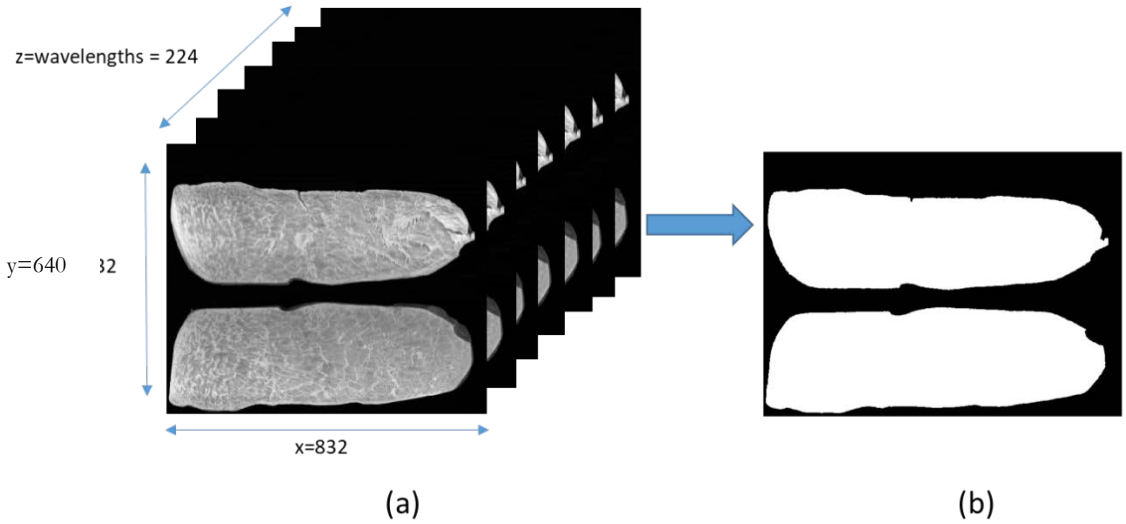

Figure 4: Hyperspectral images of fresh pieces of yam samples. (a) Corrected hyperspectral image, (b) sample mask resulting from thresholding the 1325 nm image at a value of 32 %.

5.6 Multivariate analysis of hyperspectral images

5.6.1 Unfolding the hyperspectral (hypercube) image (x, y, z) into a 2D matrix (z,x ×y)

To explore hyperspectral images by multivariate analysis methods, the hypercube is unfolded into a two-dimensional (2-D) matrix (z, x×y) so that each single-band image became a column vector and each spectrum is pixel (fig.5). Where (x,y) are spatial dimensions, and z is a spectral dimension. Each plane of this hypercube is an image acquired at one wavelength, and each pixel is a spectrum over the spectral range (900-1700 nm).

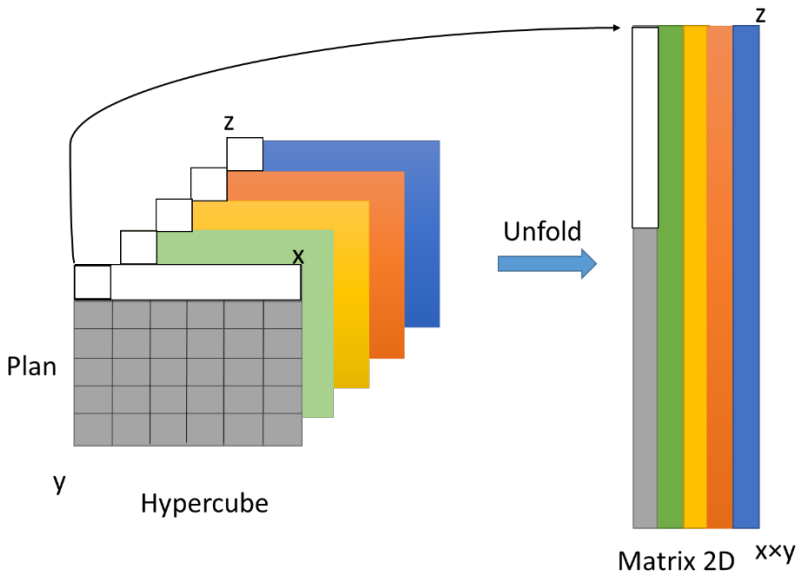

Figure 5: schema of the unfolding of the hyperspectral image (hypercube) (x y z) to a 2D spectral matrix (z, x×y)

5.6.2 Principal component analysis of hyperspectral data

Principal components analysis (PCA) is a conventional multivariate analysis technique for dimensionality reduction and variable selection in spectral data. Typically, PCA finds fewer independent components instead of the original variables through orthogonal transformation. In PCA, spectral data in the matrix X are decomposed into a loading matrix (P) and a score matrix (T). Where X is the N × K spectral data matrix, T is the N × A matrix of score vectors, P' is the K × A matrix of loading vectors, N is the number of examined samples, K is the number of variables (wavelengths), and A is the number of principal components (PCs) (fig.6). The scores of PCA represent the weighted sums of the original variables without significant loss of useful information, and the loadings of PCA (weighting coefficients) can be used to identify important variables that are responsible for the specific features appeared in the corresponding scores.

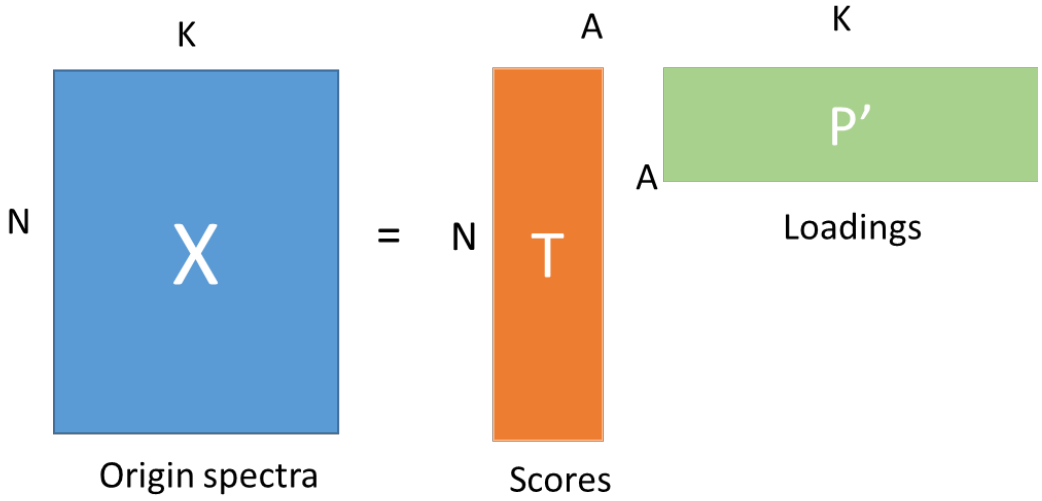

Figure 6: Shema of the principle of reduction of origin spectral data by principal component analysis, X is origin spectra, T is scores matrix, and P' is loading matrix.

After the projection of each pixel of the image to the PCA, the score value of each pixel is calculated and stored in the scores matrix. Moreover, each score vector was folded back to form a 2-D score image with the same dimensions of the single-band image. Score images were then further explored with image post-processing to obtain classification map, and the resulting classification map is displayed in colours. In the classification image, pixels belong to the same class will appear in the same colour.

6 REPEATABILITY

In order to estimate HSI repeatability and spectra dissimilarities, 10 replicates were acquired from the same sample of fresh yam. Then, for each image, an average spectrum is calculated. 10 average spectra are obtained and compared between them (fig.11). The mean (x) and standard deviation (s) of the absorbances for 10 average spectra are calculated for each wavelength, and the root mean square error (RMS) is then estimated (eq.2) :

$$RMS(i) = \sqrt{\frac{\sum_j^p (X_{ij} - \overline{X}_j)^2}{p}}$$

$\overline{X}_j$ average of absorbance of wavelength j
p number of wavelengths (j variate from 1 to p).
X_{ij} is an absorbance value of spectra i for wavelength j.

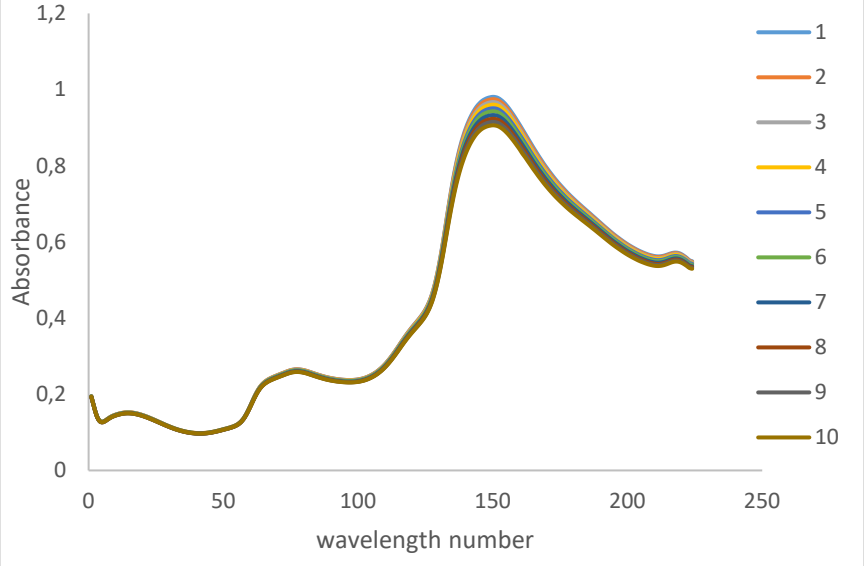

Figure 7: average spectra in absorbance mode of 10 hyperspectral images acquired in repeatability conditions on fresh yam pieces.

The average RMS value is equal to 9113 µabs for the 10 mean spectra from 900 nm to 1700 nm of the 10 hyperspectral images acquired on fresh yam slices. The RMS values range between 990 and 16631 µabs. The high RMS value (on average) could be the result of several factors, including the heterogeneous nature of the sample and rapid evaporation of water at the surface of the fresh tuber, due to the heat, generated by the halogen lamps. This result suggests that at least two images have to be scanned in order to be representative of the heterogeneity of the root. However, acceptable values for each product should be set by trial and error (ISI, 1992) (Alomar et al., 1999).

7 CRITICAL POINT OR NOTE OF THE PROCEDURE

- Acquisition of the images on fresh slices must be carried out immediately after cutting as well as after leaving the oven to avoid oxidation and loss and modification of the main components.
- Measurements must be carried out in a bright room with constant light.

8 PROSPECTS

Use hyperspectral imaging to measure traits of interest of RTBfoods project:
- Oxidation, Color, size, shape, weight, fibers
- Texture, firmness, cooking degree
- Water content, dry matter, carotenoids, protein, starch
- Diseases detection etc.

9 REVISION RECORD

Date (arial 11 bold)	Responsible person	Description of change
27/04/2020	Emmanuel Oladeji ALAMU	Reviewing and Editing
19/05/2020	Fabrice DAVRIEUX	Reviewing and editing
12/06/2020	Karima MEGHAR	Correction and editing
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24/06/2020	Karima MEGHAR	2 nd correction end editing
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