Harmonized SOP for NIRS Measurement on Blended Cassava and Yam using NIRS FOSS

## High-Throughput Phenotyping Protocols (HTPP), WP3

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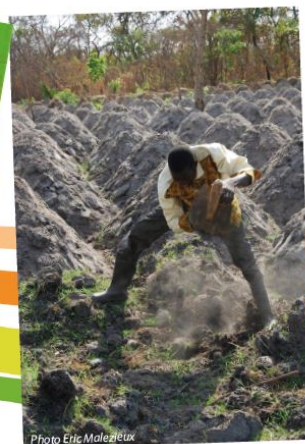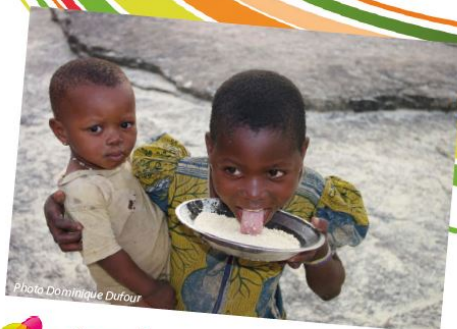
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WP3: High-Throughput Phenotyping Protocols (HTPP)

SOP: Analysis of Blended Cassava and Yam using Near Infrared Reflectance Spectrophotometer

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ABSTRACT

The standard operating procedure for the rapid analysis of blended fresh yam tubers and cassava roots is described in this document. Breeders in their breeding programs often generate hundreds of genotypes which requires rapid screening techniques to characterize them for priority quality traits. Conventional approaches for quality analysis are not cost-effective and time-consuming. Therefore, this standard operating procedure has described near infrared spectroscopy as a rapid method for screening blended cassava and yam tubers. The procedure involves minimal sample preparation using the blended fresh roots and tuber; thus, a process of oven drying was eliminated.

Key Words: near infrared spectroscopy, blended cassava roots, blended yam tubers, chopped cassava, chopped yam, oven-drying, wavelength, ISIscan

1 SCOPE APPLICATION

Conventional methods of sampling and sample preparations for fresh cassava and yam involve bulking the harvested crops and sub-sampling a laboratory size/lot that represents the entire field plot. This is followed by washing and cutting to reduce the size by chopping with a stainless-steel knife and then dried in the Oven for about 72 hrs, then milling to form a fine flour. The drying and milling process contributes to a more extended analysis time in quality traits phenotyping of cassava and yam. These time-consuming procedures pose challenges to breeders where a rapid decision must be made for selection in subsequent breeding stages. However, analysis of cassava and yam using their blended sample presentation form will significantly reduce analysis time and labor cost. This protocol is developed to analyze fresh cassava and yam without converting to dry flour using the Near-Infrared Reflectance Spectrometer.

2 INSTRUMENTATION

2.1 Operating mode to start the instrument

- Put on the instrument by pushing the start button on the right-hand side of the instrument.

- Allow it to run for about 3 minutes while the analysis chamber is cleaned with soft tissue.

- Launch the ISIscan software on a PC desktop. This switches on the instrument's lamp.
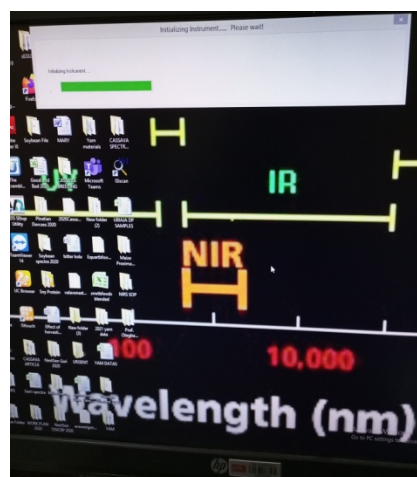
- Leave for 30 minutes for the lamp to warm up before spectra data collection.

- The blended (cassava or yam) mash was transferred quantitatively into the moving coarse cup of the NIRS machine for spectra data collection.

- The chamber is closed, and scanning of the cross-section of the root/tuber was conducted twice.

- After each scan, the sample compartment was cleaned with wet soft tissue.

2.2 Instrument configuration

- The performance test was conducted for wavelength accuracy, and the noise level was conducted before spectra data collections
- Triplicate spectra data were collected for each sample
- Spectra data collection takes 60 secs per scan.

2.3 Instrumental tests

To check the instrument's performance, a diagnostic test was run to ascertain the wavelength accuracy and noise level.
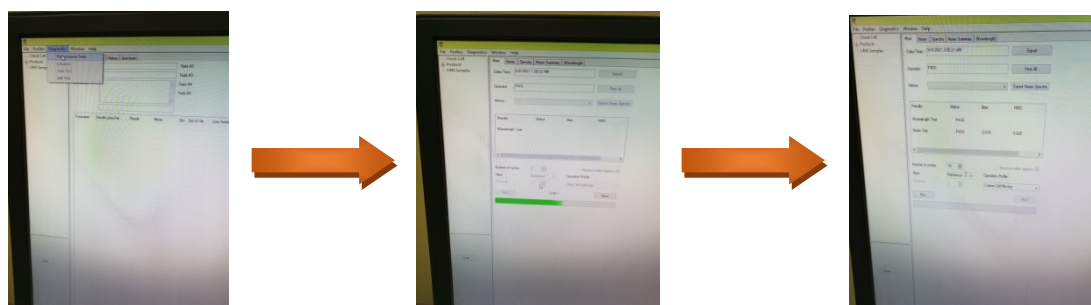
3 PROCEDURE

3.1 Sample preparation/presentation

Matured and healthy fresh cassava/yam roots/tubers of varying sizes, i.e. big, medium, and small, were selected randomly for each genotype from the pool of roots/tubers from at least five plants to obtain a representative of the field plots. The fresh roots/tubers were washed and dried with a paper towel to remove dirt. The root/tuber was cut longitudinally into four sections (See Figure 1).The two adjacent sections were pulled and cut to reduce the size, and then the samples were blended using the Warren Blender until a smooth texture is obtained. The blended mash was transferred quantitatively into the coarse cup of the NIRS machine for spectra data collection. A triplicate scan was conducted for each sample.

3.2 Protocol of spectral measurement and sample codification

- The appropriate code was used for the samples. The code used per sample does not exceed 12 characters to prevent loss of samples codes when exporting spectra data..

3.3 Procedures for spectra storage

- Spectra data were exported to the WinISI software stored in .nir format on the computer's hard drive.

Fresh cassava roots

Stage 1: Randomization of fresh healthy roots for selection

Stage 2: Selection of Big, Medium, and Small root per genotype

Stage 3: Cut roots longitudinally and combining two adjacent sides

Stage 4: Blending of Cassava roots

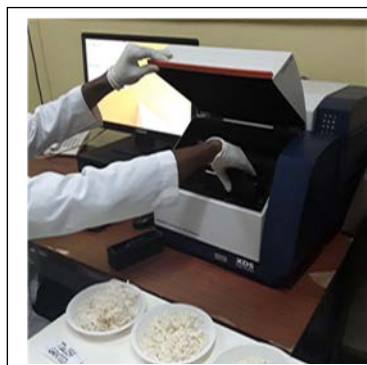**Stage 5:** Spectra data collection using NIRS machine

Figure 1 Work Flow of BlendedCassava/Yam Roots/Tubers Measurement using NIRS

4 LIMITS FOR SPECTRA REPEATABILITY

4.1 Method of calculation and formulae

The repeatability test must be carried out by taking at least ten readings on the blended sample. Absorbances values repeatability is an indication of the stability and performance of the Near Infrared Spectrophotometer. The mean (x) and standard deviation of the absorbances for the average spectra are estimated for each wavelength, and the root mean square error (RMS) was calculated using the equation below :

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

Where:

$\overline{X}_j$ = average of absorbance of wavelength j

p = number of wavelengths (j variate from 1 to p).

X_{ij} = an absorbance value of spectra i for wavelength j.

4.2 Results and discussions

The root means square error (RMSE) for 10 repetitions of the blended yam samples ranged from 1875 – 32079µabs and an average of 15422µabs. Also, RMSE for fresh blended cassava samples had a range of 5745 to 26899µabs and average values of 12744µabs, respectively. The closeness of the RMSE indicates that this protocol could be used for both blended fresh cassava roots and yam tubers.
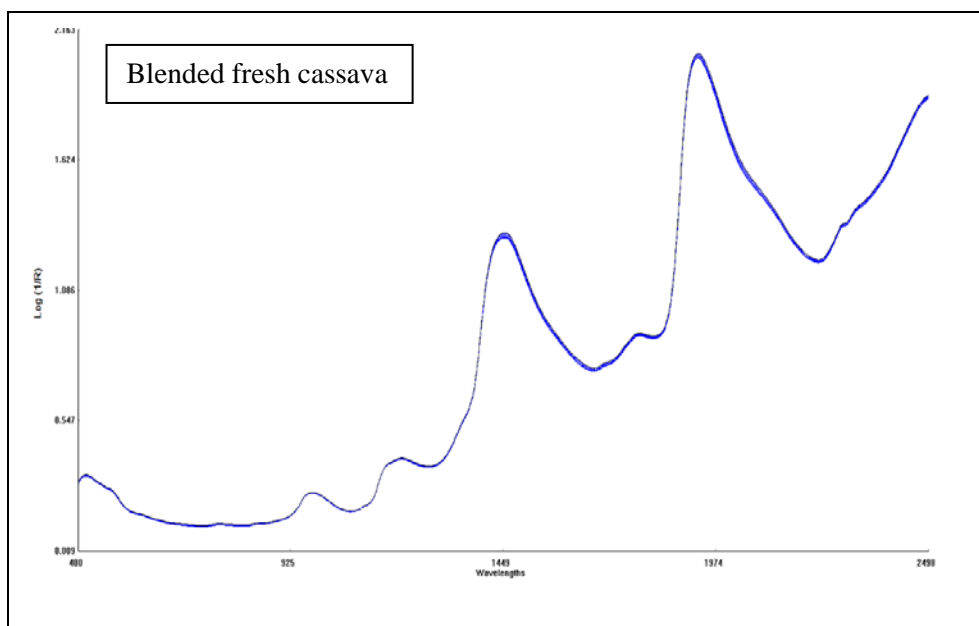
Figure 2 Spectra of 10 replications of blended fresh cassava using the NIRS
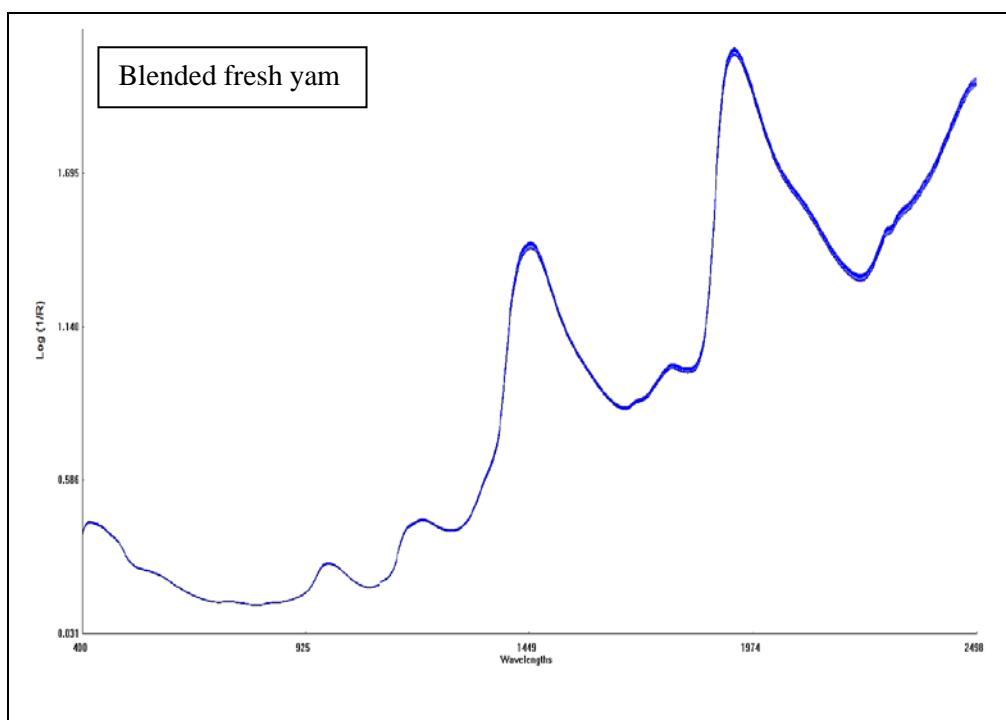
Figure 3 Spectra of 10 replications of blended fresh yam using the NIRS

5   CRITICAL POINTS OR NOTES ON THE PROCEDURE

➢ The Tabletop Near Infrared Spectrophotometer must be switch on 30 minutes before taking spectra reading. Also, the diagnostics test of the equipment must be completed.

➢ There must be precautions to ensure quantitative transfer of the content after blending into the NIRS sample cup.

➢ A minimum of triplicate spectra (different sampling from the sample) must be collected for each sample.

6   REVISION RECORD

Date	Responsible person	Description of change
27/10/2020	Karima Meghar	Reviewing

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