Laboratory Standard Operating Procedure

Harmonised SOP for NIRS Measurement on Intact Cassava Roots and Yam Tubers using NIRS FOSS

High-Throughput Phenotyping Protocols (HTPP), WP3

Ibadan, Nigeria, 08/07/2020

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Ethics: The activities, which led to the production of this document, 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 (HTPP)

SOP: Intact Cassava Roots and Yam Tubers Measurement using Near Infrared Reflectance Spectrophotometer

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CONTENTS

Table of Contents

Table of Figures

ABSTRACT

The standard operating procedure for the analysis of intact fresh yam tubers and cassava roots is described in this document. The procedures expressed require minimal sample preparation and spectra data collection using near infrared spectrophotometer (NIRS). In breeding programs, breeders often cultivate many genotypes which require rapid screening as part of the characterization process for the genotypes. However, most conventional methods used for the analysis of yam tubers and cassava roots involve considerably number of processes from peeling and chopping to drying in the oven for about 72 hours; these processes are time consuming and do not qualify as high throughput methods of analysis. To this end, a standard near infrared spectrophotometric procedure for the rapid analysis of yam and cassava samples using the intact tubers/roots is described in this SOP.

Key Words: cassava roots, yam tubers, near infrared spectroscopy, oven-drying, spectra data, high throughput, quality traits, WinISI, ISIscan

1 SCOPE APPLICATION

Fresh cassava rootcontains approximately 60 - 70%moisture, while the pulp accounts for about 10-15% of the root (Thongkratok *et al*., 2010). On the other hand, yam has been reported to contain crude protein ranges from 4% to 14%, starch from 70% to 80% and vitamin C content ranges from 40 to 120 mg/g/edible portion on a dry weight basis (Baah *et al*., 2009). Most processing procedure requires oven drying to reduce the moisture content and improves the storability of the intermediate or final products. Quality traits phenotyping of cassava/yam involves oven-drying of samples before biochemical analysis and this tends to increase the duration for laboratory analysis. However, modern breeding programs require rapid phenotyping methods for samples analysis. Therefore, the feasibility of using intact fresh roots/tubers for NIRS analysis has been examined, and a sampling and sample preparation protocolhas been developed.

2 REFERENCES

Baah, F.D.; Busie, M.D.; Asiedu, R.; Ibok, O (2009). Physicochemical and pasting characterization ofwater yam (Dioscorea spp.) and relationship with eating quality of pounded yam. J. Food Agric. Environ.7: 107–111.

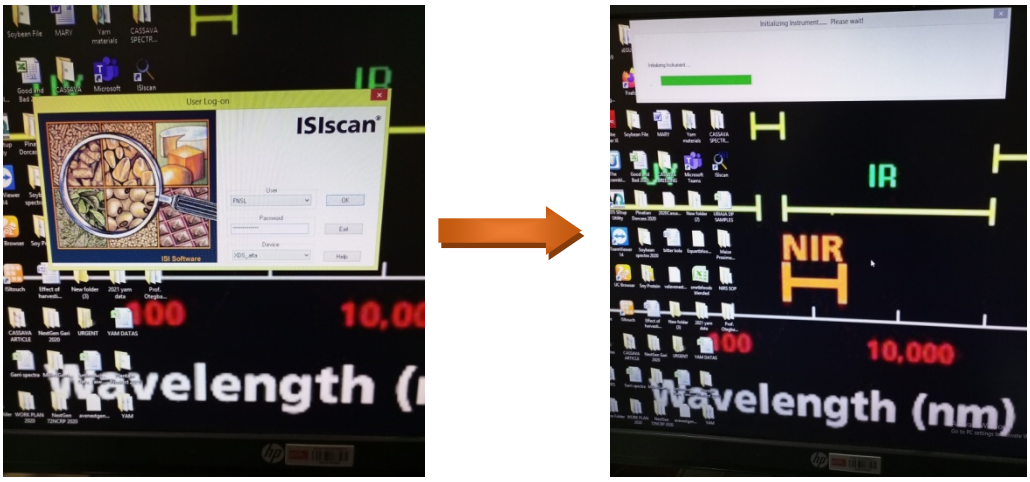
Thongkratok R, Khempaka S and Molee W (2010). Protein Enrichment of Cassava Pulp Using Microorganisms Fermentation Techniques for Use as an Alternative Animal Feedstuff. J Anim Vet Adv 9: 2859-2862.

3 INSTRUMENTATION

3.1 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.
- NIRS scan was conducted on intact cassava/yam root/tuber by placing the smooth face (pulp part) of each section (proximal, middle and the distal) on the sample analysis compartment of the NIRS machine.
- The chamber is closed, and scanning of the cross-section of the root/tuber was conducted twice.

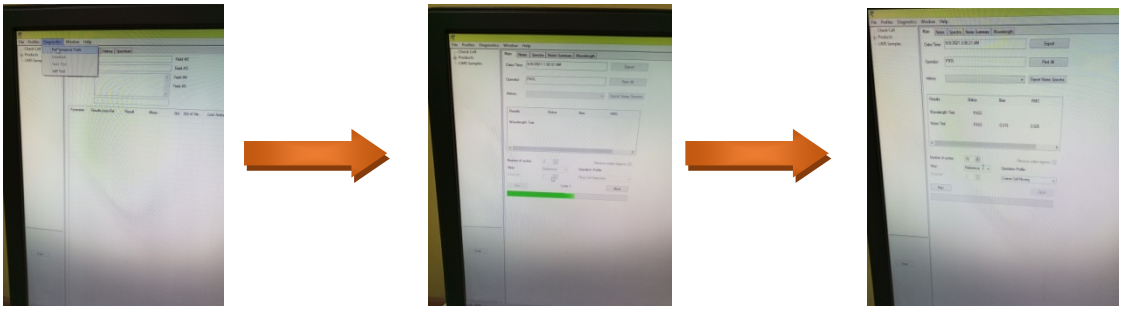
- After each scan, the sample compartment was cleaned with wet soft tissue.

3.2 Instrument configuration

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

3.3 Instrumental tests

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



4 PROCEDURE

4.1 Sample preparation/presentation

Matured, healthy and fresh roots/tubers of varying sizes, i.e. big, medium and small were selected 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 intact roots/tubers were cut axially into proximal, middle and distal sections. The dimension of each section is subjective depending on the length and shape of the root/tuber. However, the stalk on the proximal end is cut off before dissecting into three parts. The surface of each section was cut smoothly with a stainless-steel knife, and then tagged with a labelled ribbon then placed in a clean whirl pack bag for analysis. NIRS scan was conducted by placing the smooth face (pulp part) of each section (proximal, middle and the distal) on the sample compartment of the NIRS machine. The chamber is closed, and scanning of the cross-section of the roots/tubers was conducted twice. After each scan, the sample compartment was cleaned with wet soft tissue.

4.2 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.

4.3 Procedures for spectra storage

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

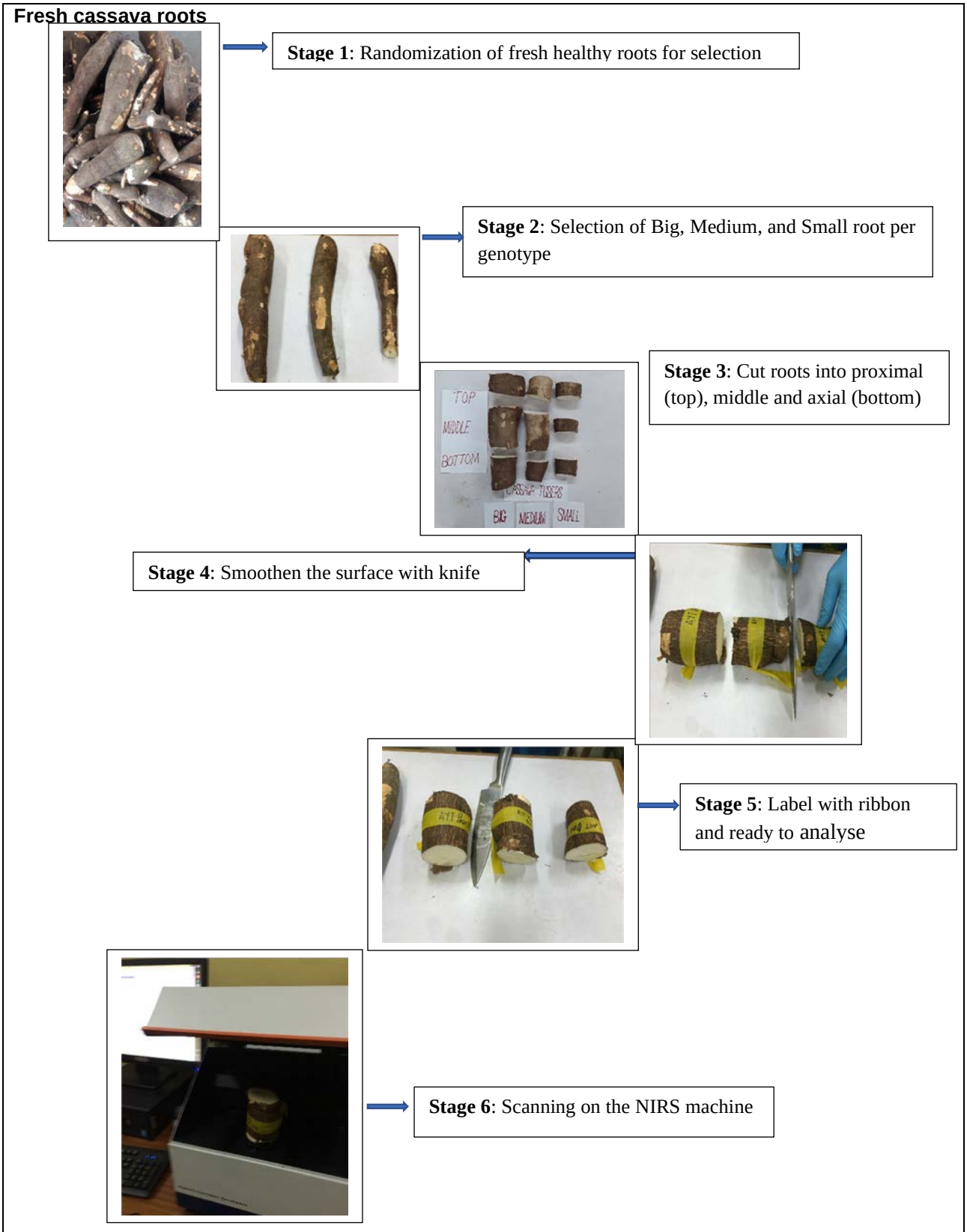


Figure 1 Work Flow of Cassava/Yam Intact Roots/Tubers Measurement using NIRS

5 LIMITS FOR SPECTRA REPEATABILITY

5.1 Method of calculation and formulae

The repeatability test must be carried out by taking at least ten readings on the intact root/tuber (the measurements must be taking from the same part and point). Then, the average of 10 spectra are obtained and compared between them (Figure 2). The mean (x) and standard deviation (s) 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.

5.2 Results and discussions

The RMS values ranged from 578.73to 9899.90µabs with a mean value of 4123 µabs for the ten mean spectra from 400 nm to 2400 nm acquired on intact fresh cassava or yam. However, the RMS values ranged between 1858.45 and 8703.16µabs with a mean value of 4759µabs for the ten spectra from 400nm to 2400nm collected for blended fresh yam. According to these two measurements per parts are recommended, and a RMS value of less than 5000µabsis acceptable between the two replicates. It is essential to point out that the mean values of fresh yam and cassava were very close. Thus, the SOP could be used for both crops.

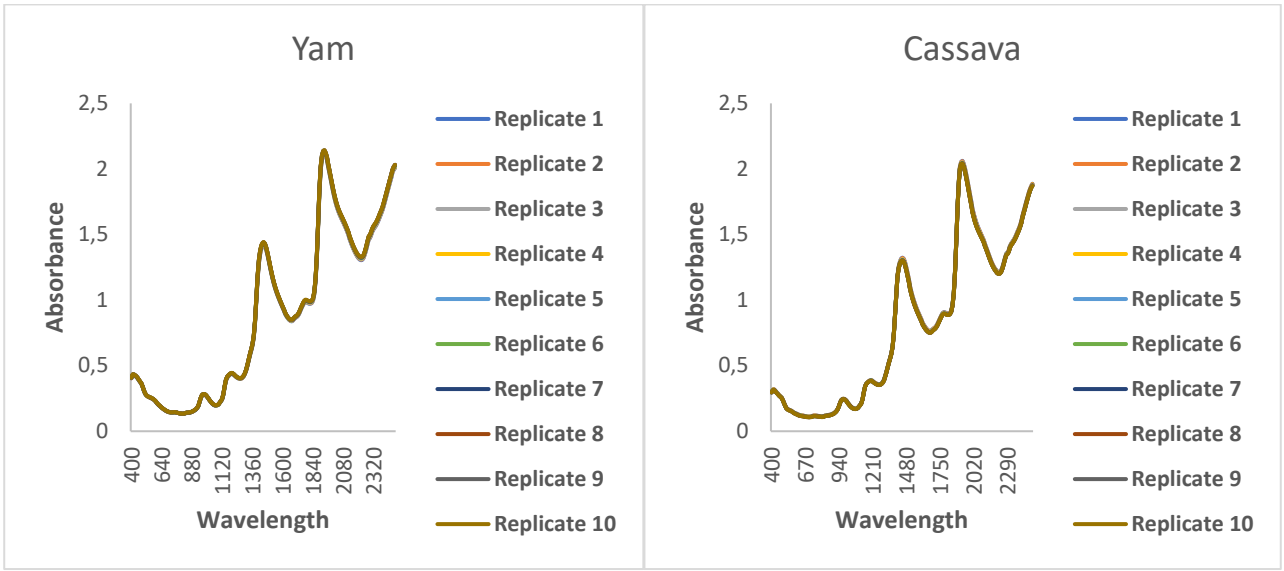


Figure 2 Average Spectra of intact fresh yam and cassava roots (10 replications)

6 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.
- The surface of the roots/tubers to be scanned must be smooth to avoid light scattering
- Sample compartment of the NIRS machine must be wiped with mild solvent after each reading.

7 APPENDICES

7.1 Annex 1: Revision Record

Date	Responsible person	Description of change
11/06/2020	Karima Meghar	Correction
07/07/2020	Alamu Emmanuel	Reviewing, editing and adding more input
08/07/2020	Karima Meghar	Reviewing and validation
09/07/2020	Fabrice Davrieux	Reviewing
21/07/2020	Alamu Emmanuel	Reviewing, editing and adding more input
22/07/2020	Fabrice Davrieux	Validation

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