RTBfoods

WP3: High-throughput phenotyping protocols (HTPP)

SOP: Analysis of blended cassava and yam using Near Infrared Reflectance Spectrophotometer

Written by: ALAMU Emmanuel Oladeji
ADESOKAN Michael

For information on this SOP please contact:

- MAZIYA-DIXON, Busie; b.maziya-dixon@cgiar.org.
- ALAMU, Emmanuel Oladeji; oalamu@cgiar.org
- ADESOKAN, Michael ; m.adesokan@cgiar.org

This document has been approved by:

Partner	Name of the person who approved	Date DD/MM/YY

Table of contents

1 SCOPE AND APPLICATION

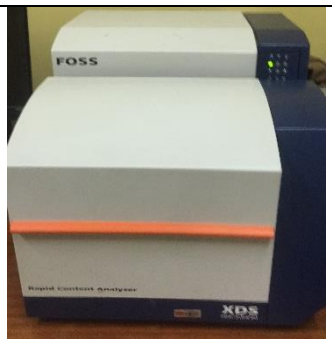
Conventional methods of sampling and sample preparations for fresh cassava and yam involve bulking the harvested crops and sub-sampling a laboratory size/lot which is a representative of the entire field plot. This is followed by washing and cutting to reduce size through chopping with a stainless-steel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

2 REAGENTS

No reagent uses

3 APPARATUS

Material	**Image**
Stainless steel knife. The stainless-steel knife is basically carbon steel with added chromium to resist corrosion and other elements which increase performance levels.	

Electric power Blending machine This is used to for the wet blending of fresh cassava and yam crops. It has a stainless-steel cups and blade.	
Near Infrared Reflectance Spectrophotometer (NIRS) XDS Rapid Content Analyzer Serial No: 3013-0857 Wavelength range of 400-2495 nm	

4 PROCEDURE

Matured and healthy fresh cassava roots of varying sizes i.e. big, medium, and small were selected randomly for each genotype from the pool of cassava roots from at least 5 plant, to obtain a representative of the field plots. The fresh roots were washed and dried with a paper towel to remove dirt. The intact root was cut longitudinally into four sections (See Figure 1). The two adjacent sections were pulled and cut to reduce the size, then the samples were placed in the blended using the Warren Blender until smooth texture is obtained. The blended mash was transferred quantitatively into the coarse cup of the NIRS machine for spectra data collection. Triplicate scan was conducted for each sample.

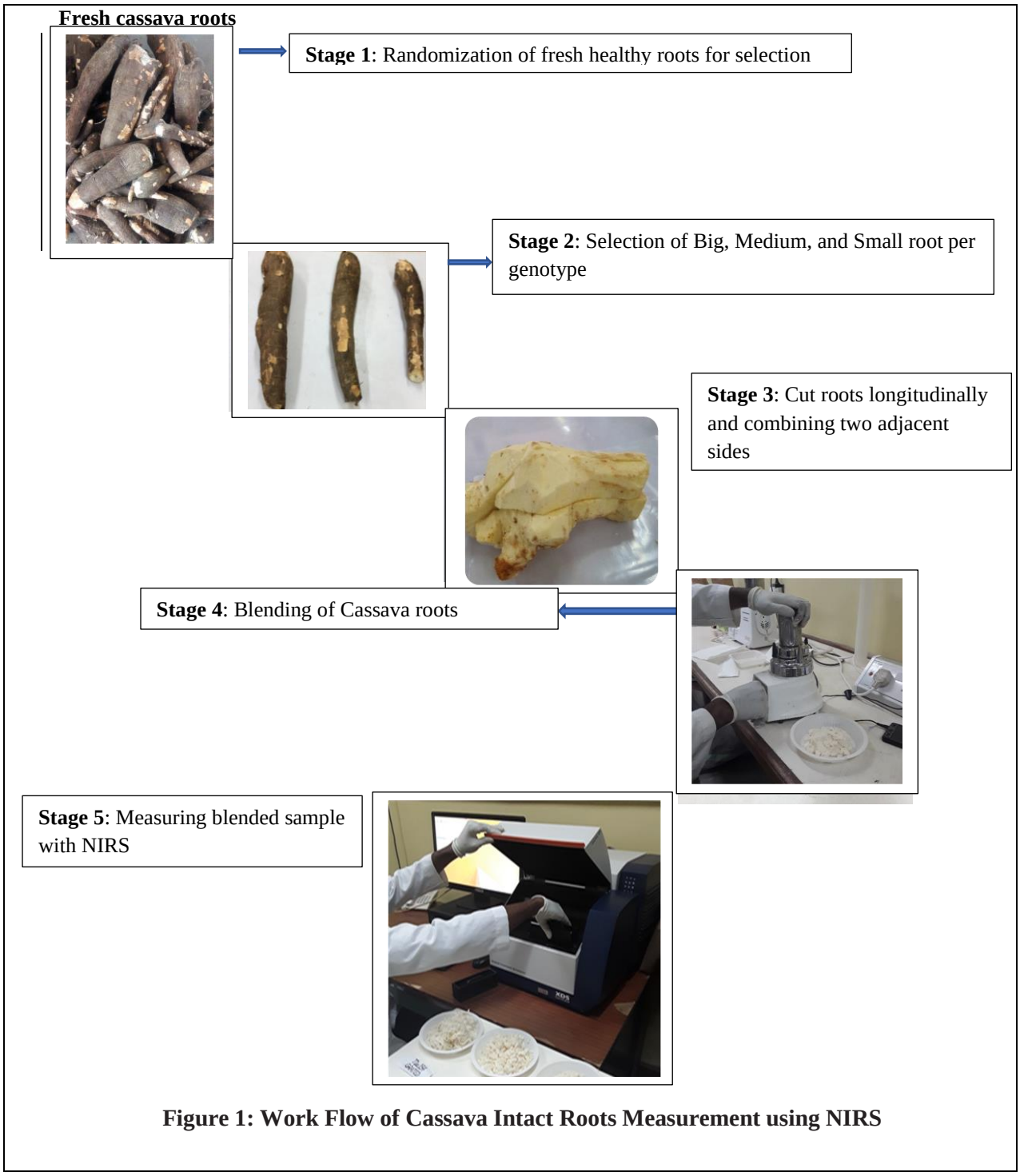


Figure 1: Work Flow of Cassava Intact Roots Measurement using NIRS

5 EXPRESSION OF RESULTS

Results are expressed on fresh weight basis of the unit of measurement e.g. % (w/w)

6 CRITICAL POINTS OR NOTE 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 successfully completed.
- There must be precautions to ensure quantitative transfer of the content after blending into the NIRS sample cup.
- Minimum of triplicate spectra (different sampling from the sample sample) must be collected for each sample

- 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} is an absorbance value of spectra i for wavelength j.

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

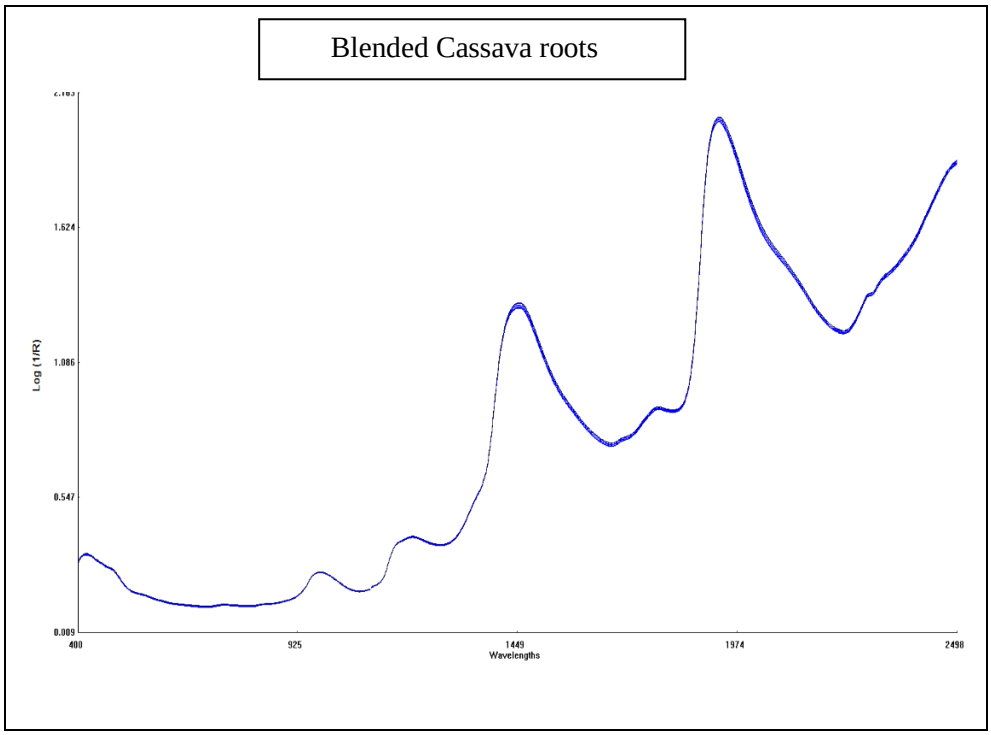


Figure 2: Spectra of 10 replications of blended fresh cassava using the NIRS

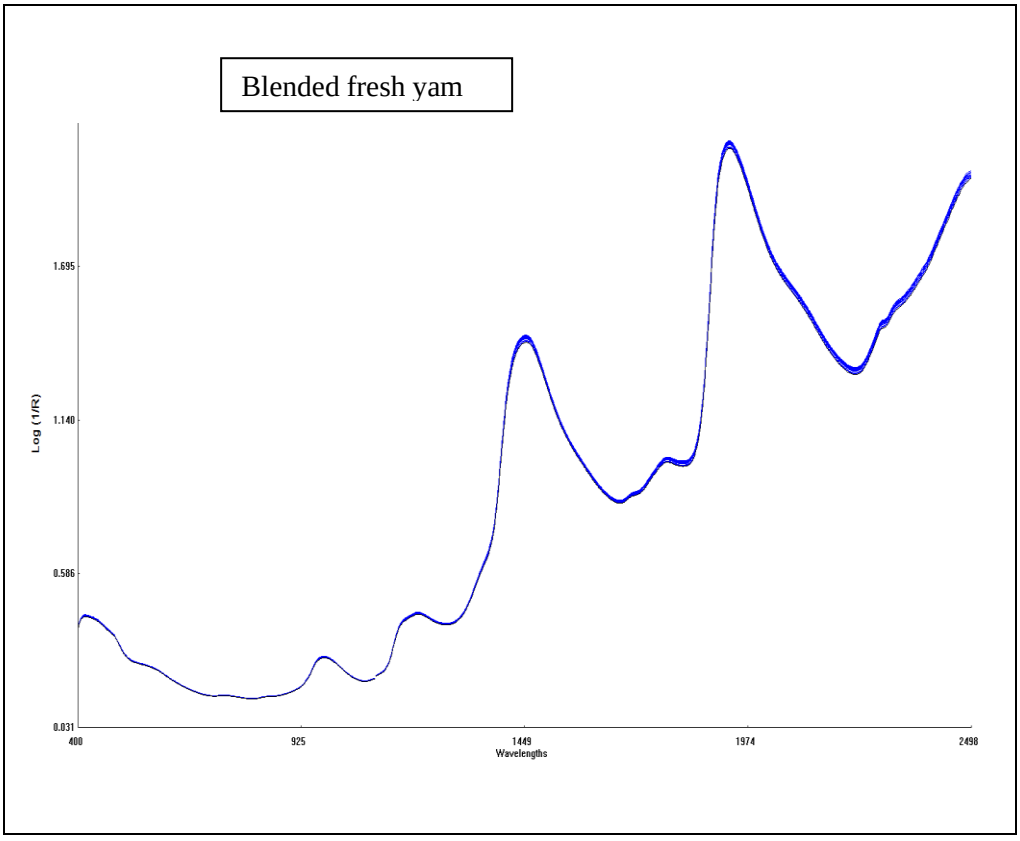


Figure 3: Spectra of 10 replications of blended fresh yam using the NIRS

7 REVISION RECORD

Date	Responsible person	Description of change
27/10/	Karima Meghar	Reviewing
29/10/	Emmanuel Alamu	Reviewing and editing
29/10/	Fabrice Davrieux	Reviewing and validating

8 APPENDIX