*Laboratory Standard Operating Procedure*

# NIRS Acquisition on Fresh Cassava Roots using the Benchtop NIRS FOSS DS2500 and Relating Spectra to Root Dry Matter Content by Oven Method

High-Throughput Phenotyping Protocols (HTPP), WP3

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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: NIRS Acquisition on Fresh Cassava Roots using the Benchtop NIRS FOSS DS2500 and Relating Spectra to Root Dry Matter Content by Oven Method

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# ABSTRACT

Developing the right metric for dry matter is vital in defining measurable parameters related to biochemical properties that define root yield and root quality. In this SOP, the procedure for production of definitive spectra that defines the connection between the spectra acquired from grated cassava fresh root and the dry matter content is described. The procedures describe the scanning and spectral acquisition of fresh root cassava spectra using the FOSS DS2500 NIRS equipment. Cassava samples are harvested from labelled fields using appropriate harvesting tools and prepared by peeling and grating using a laboratory grater. The grated material is placed on a sample plate with accompanying barcode and moved to NIRS equipment. This is followed loading the grates into a sample cup and scanning of the grated material filled in the sample cup. The procedure is repeated by loading fresh material from the main sample into the sample cup producing two subsets of scans from one particular accession. Spectral data produced from these scans is downloaded and further processed for use in calibration development while it is also uploaded on cassava base as an additional file. Reference data generation is carried out by approved reference methods in repeatability analyses carried out at NaCRRI. Critical points of consideration include the development and maintenance of correct labelling.

**Key words: Fresh cassava, Reference, spectra, procedure, repeatability.**

# 1 SCOPE AND APPLICATION

This SOP applies to fresh cassava roots that are scanned with prior processing by grating the fresh root after peeling and scanning the grated material using the small sample cup with benchtop NIRS equipment. The scanned grates are after that used to determine dry matter content using the oven at 105°C.

# 2 REFERENCES

Part of this protocol has been informed by work from the following publications

1. Sánchez, T., Ceballos, H., Dufour, D., Ortiz, D., Morante, N., Calle, F., ... & Davrieux, F. (2014). Prediction of carotenoids, cyanide and dry matter contents in fresh cassava root using NIRS and Hunter color techniques. Food chemistry, 151, 444-451.
2. Ikeogu, U.N., Davrieux, F., Dufour, D., Ceballos, H., Egesi, C.N., Jannink, J.L. (2017). Rapid analyses of dry matter content and carotenoids in fresh cassava roots using a portable visible and near infrared spectrometer (Vis/NIRS). PLoSONE 12(12), e0188918. https://doi.org/10.1371/journal.pone.0188918

# 3 DEFINITIONS

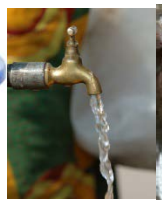
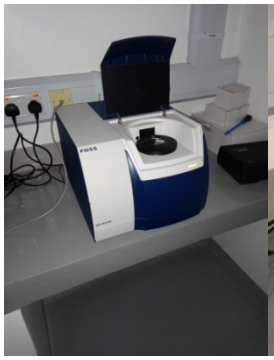
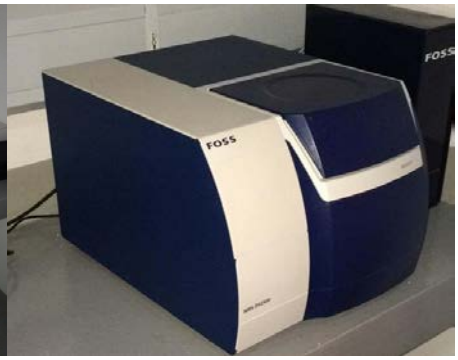
In this SOP, phenotyping refers to a quantitative description of the cassava root anatomical/physical properties concerning biochemical properties of the root.

# 4 PRINCIPLE

In this SOP, the relationship between the spectra acquired from grated cassava fresh root and the dry matter content is investigated. Developing the right metric for dry matter is vital in defining measurable parameters related to biochemical properties that define root yield and root quality.

# 5 APPARATUS

| Process | Equipment/pictorial presentation of process |
|---|---|
| Harvesting equipment |    |

| Process | Equipment/pictorial presentation of process |
|---|---|
| Labelling equipment such as markers or bar code generator and bar code reader |   |
| Washing equipment and water source |    |
| Sample cutting or chopping and grating equipment |    |
| Benchtop NIRS equipment for spectra generation |   |

# 6 PROCEDURE

**Sample preparation**

I. Harvest 4 uniformly sized roots per plot. The root should be at least (20 cm length and 6cm diameter). After harvest, label samples and wash with water in a bucket to remove soil and other debris. Dry the samples by wiping with a towel.

II. From each root, cut off the proximal (leading 3-5cm), and distal (3-5 cm at the tail end) ends. The remaining portion peeled ready for grating.

III. The peeled roots are aggregated and given the same label matching the accession name using a barcode.
IV. Each of the aggregated samples is sent for grating to produce grates. Immediately after grating, the sample is placed in the small sample cup and scanned using a benchtop NIRS equipment
V. The sample is scanned twice by picking from the same sample two times to produce two average spectra representing one sample (see Figure 1).

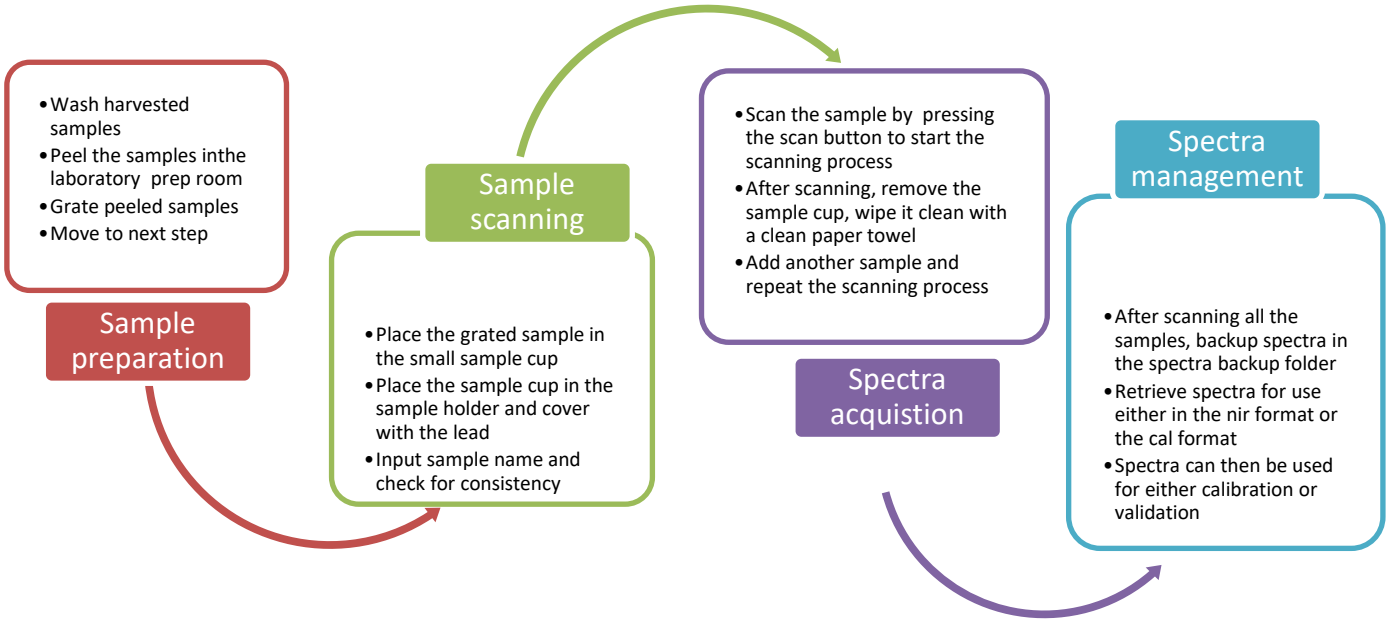



**Figure 1: Description of sample preparation, scanning and spectra acquisition process**

**Repeatability of reference method measurements**

I. Divide the grated sample used for NIRS measurements into 4 (four) equal portions
II. Randomly select two of the four portions and assign them to two technicians in two different labs (In our case NaCRRI and NARL)
III. Undertake the reference method on the above set of subsamples at the same time
IV. Aggregate the data obtained and compare result from all labs to check lab error

Note: For dry matter measurements, the samples are assigned to four different ovens located in four different labs at NaCRRI.

# 7 EXPRESSION OF RESULTS

Percentage dry matter contents of each of the grated portions of the roots determined for each root and each accession as above and used in calibration for DM (Ikeogu et al., 2017).

# 8 CRITICAL POINTS OR NOTE ON THE PROCEDURE

I. Be sure to maintain the same sample id (accession name) for all root sections derived from the same root.
II. Be sure to read the manual supplied with the NIRS FOSS DS2500 before use in acquiring spectra
III. Be sure to use the minimal time between sample preparation (peeling and grating) and sample spectra generation as scanned samples rapidly lose moisture.
IV. Use the Tablet to enter dry matter readings. Where bar code labels are used, scan the bar code with the Tablet to ensure entering of data to the right accession
V. Have a well-documented and reviewed sample flow before the start of exercise

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