Laboratory Standard Operating Procedure

NIRS Acquisition on Fresh Cassava Roots using the ASD Quality Spec (QST) 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 ASD Quality Spec (QST) and Relating Spectra to Root Dry Matter Content by Oven Method

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ABSTRACT

The Standard Operating Procedures (SOPs) detailed herein are applicable in the acquisition of spectra from largely intact cassava roots. The procedures allow for scanning and spectral acquisition after a scanning surface has been provided on the intact root. Cassava samples are harvested using approved harvesting tools and labelled after which preparation commences by removing the tail ends of the root. This is followed by sectioning of the root into four equal portions and surface trimming of each section immediately before spectral acquisition. Thereafter, labelling and scanning of the trimmed surface using the ASD-QUALITY SPEC produces four scans representative of one particular root. The procedure is repeated for the rest of the roots producing four subsets of scans from one particular accession. Spectral data produced from these scans is downloaded and further processed for use in calibration development and root chemical composition determination. Processed spectral data is also uploaded as an additional file to the cassava base. Reference data generation is carried out by approved reference methods in repeatability analyses carried out at NaCRRI and NARL. Critical points of consideration include the development of a sample flow and spectra acquisition matrix coupled to correct labelling since sample numbers involved are usually many.

Key words: Cassava, spectra, procedure, Reference data, repeatability

1 SCOPE AND APPLICATION

This SOP applies to fresh cassava roots that are scanned without prior processing apart from providing a root surface/root flesh surface amenable for scanning with a portable NIRS equipment. The scanned portion is after that grated and dry matter content determined 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 in relation to biochemical properties of the root.

4 PRINCIPLE

In this SOP, the relationship between the spectra acquired from an intact root.

5 APPARATUS

Process	Equipment/pictorial presentation of the process
Harvesting equipment	  

Process	Equipment/pictorial presentation of the process
Labelling equipment such as markers or bar code generator and bar code reader	 
Washing equipment and water source	  
Sample cutting or chopping equipment	
ASD quality spec equipment for spectra generation	

6 PROCEDURE

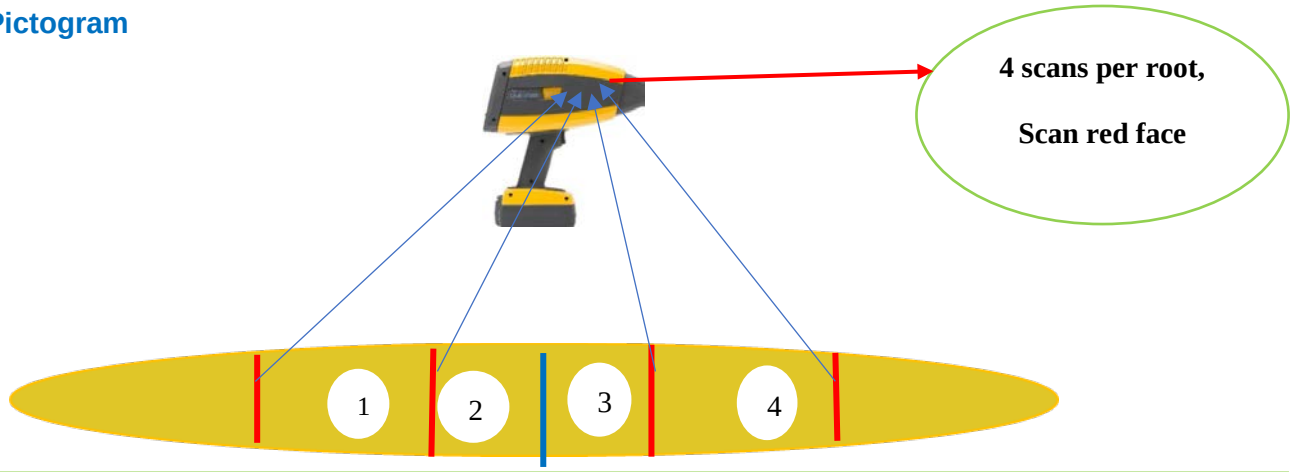
Sample preparation

Harvest 4 relatively uniform-sized roots per plot [about 6 cm diameter and about 30 cm long]. After harvest, samples are labelled and washed with water in a basin to remove soil and other debris. These are then dried by wiping with a towel. From the harvested roots per plot, administer treatments as follows;

i. From each root, the proximal (top 3-5cm) and distal (3-5 cm at the tail end) ends are cut off. The remaining portion is prepared for scanning with the ASD quality spec.
ii. The portion is divided into 4 equal slices using a knife
iii. The slices (4) from above are labelled by the accession name and root number and sent to the ASD quality spec for spectra generation.

iv. Each portion is scanned four times with a scan on each of the opposite root surfaces, making 4 scans per root per accession.
v. This process is repeated for the second root until all the four roots have been scanned.

Pictogram



Notes

1. **Spectra generation**
   - Scan the red face of pieces 1, 3, 4 and 6
2. **Dry matter determination**
   - Grate together pieces 1, 3, 4 and 6 to make 1 composite sample
   - Weigh off 100 g using an analytical weighing scale
   - Dry to constant weight in an oven at 105^0C
   - Take weight of the oven dried sample
   - Express dry weight as a percentage of the fresh weight to get dry matter content (%)

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 the results from all labs to check lab error

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). Data collected on the softness of boiled roots at 30 and 45 minutes of boiling. Results expressed as N/Area and collated to Spectral readings for calibration or validation.

8 CRITICAL POINTS OR NOTE ON THE PROCEDURE

1. Be sure to maintain the same sample id (accession name) for all root sections derived from the same root.
2. Be sure to read the manual supplied with the ASD before use in the field;
3. At least 2 people should operate the ASD. As one person scans, the other will take note of the sample id generated the machine. This is essential to match the spectra with the corresponding field sample and softness data;
4. Use a tablet to enter dry matter readings
5. Have a well-documented and reviewed sample flow before the start of exercise

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