Laboratory Standard Operating Procedure

NIRS Measurement on Fresh Ground Cassava

High-Throughput Phenotyping Protocols (HTPP), WP3

Cali, Colombia, December 2020

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This report has been written in the framework of RTBfoods project.

To be cited as:

John BELALCAZAR, Thierry TRAN, Karima MEGHAR, Fabrice DAVRIEUX (2021). *NIRS Measurement on Fresh Ground Cassava. High-Throughput Phenotyping Protocols (HTPP), WP3.* Cali, Colombia: RTBfoods Laboratory Standard Operating Procedure, 9 p. https://doi.org/10.18167/agritrop/00676

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.

Acknowledgments: This work was supported by the RTBfoods project https://rtbfoods.cirad.fr, through a grant OPP1178942: Breeding RTB products for end user preferences (RTBfoods), to the French Agricultural Research Centre for International Development (CIRAD), Montpellier, France, by the Bill & Melinda Gates Foundation (BMGF).

RTBfoods

WP3: High-Throughput Phenotyping Protocols (HTPP)

SOP: NIRS Measurement on Fresh Ground Cassava

Date: 15/12/2020	**Release: 1**

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ABSTRACT

Type of document: Standard Operating Protocol (SOP)

Context: NIRS data collection requires standardized and repeatable sample preparation, to minimize variability between measurements. The present SOP aims to achieve this in the case of fresh cassava roots.

Content: This document describes the protocol in use at CIAT to prepare samples of fresh cassava roots for NIRS analysis in a standardized and repeatable manner.

Objectives: Standardize the preparation of fresh cassava samples for NIRS data collection.

Key Words (10 maximum): Cassava, NIRS, Near Infrared Spectroscopy

1 SCOPE AND APPLICATION

This SOP describes the preparation of fresh mashed cassava root samples for reading by NIRS.

The SOP includes the management of the cassava roots after receipt of the field on the day of harvest, sample preparation and assignment of identification codes for each sample.

2 REFERENCES

Davrieux F., Dufour D., Dardenne P., Belalcazar J., Pizarro M., Luna J., Londoño L., Jaramillo A., Sanchez T., Morante N., Calle F., Becerra Lopez-Lavalle L.A., Ceballos H. (2016). LOCAL regression algorithm improves near infrared spectroscopy predictions when the target constituent evolves in breeding populations. Journal of Near Infrared Spectroscopy 24, 109-117.

Sanchez T., Ceballos H., Dufour D., Ortiz D., Morante N., Calle F., Zum Felde T., Dominguez M., 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.

3 DEFINITIONS

NIRS spectroscopy (Near Infrared Reflectance Spectroscopy) is an analytical technique that is based on the irradiation of samples at different wavelengths. In NIRS these wavelengths are located between the visible and the near infrared. Specific wavelengths of NIRS radiation are absorbed by the sample, producing vibrations of the carbon-hydrogen (CH), oxygen-hydrogen (OH) and nitrogen-hydrogen (NH) bonds, which are the main constituents of the basic structure of organic molecules. The absorbance values over the range of NIRS wavelengths give rise to a characteristic spectrum for each sample that can be considered its fingerprint.

NIRS spectroscopy usually requires chemometrics statistics, and has wide and varied applications in pharmaceutical, chemical, physical and process analysis.

For the development of a NIRS calibration, the information provided by the spectrum of a given sample is related through algorithms with the results from the analyses of said sample by wet means using conventional laboratory methods (a.k.a. reference analyses). When the calibration between NIRS and reference analyses is successful, it becomes possible to skip the conventional analyses and predict the results by NIRS, which saves time and costs of quality traits analyses.

4 PRINCIPLE

When roots are delivered from the field for phenotyping and characterization of quality traits, some biophysical and functional properties need to be analysed immediately (on the same day as the harvest) in order to capture the characteristics of the fresh roots which may change with time, such as dry matter, cooking ability, texture, NIRS, retting ability, post-harvest physiological deterioration (PPD). Other properties do not change after harvest, and can be analysed at a later date, provided the fresh roots material can be stabilized for long-term

storage, typically by dehydration (drying). Such “stable” properties include composition: starch content, fiber, sugars, organic acids, ash, pasting profile (RVA), gelatinization profile (DSC), etc.

This SOP describes how to transform fresh cassava roots into a homogeneous mash, and the protocol for NIRS analysis of the mashed roots.

5 APPARATUS

a. Food processor (rasper or blender)

b. Balance

d. Plastic containers to collect the rasped roots

e. NIRS capsules

f. NIRS spectrophotometer

No reagents are used in this protocol.

6 PROCEDURE

Harvest fresh cassava roots and prepare them as soon as possible for analyses, to minimize biochemical changes that may occur during transportation from the field and storage. For each genotype, select two to three commercial-size roots, and wash and peel them. Homogenize (grind) the roots together with the food processor and collect the resulting mash in a plastic container.

Take from the homogeneous mash enough sample to fill two NIR spectroscopy capsules (figure 1), i.e. ~8g per capsule. Label the capsules accordingly to the sample coding system in place within the laboratory. The coding system must clearly identify the genotype (e.g. Gxx) and the repetition (Rxx) to read by NIRS. Two capsules (i.e. repetitions) per genotype are collected (2 spectra per sample), the variability between capsules is low enough not to affect the relationship between spectrum and chemical information (Davrieux *et al*., 2016). This result reflects a high homogeneity of the mashed samples.*Remark:* Cassava roots from the same genotype (or even the same plant) can have markedly different biophysical properties, even when grown under the same conditions (same field) and harvested on the same day. As mentioned above, it is therefore important to pool at least two or three roots together to prepare the fresh mash, in order to get a representative sample of the genotype. Tests conducted at CIAT on the prediction of dry matter by NIRS indicate that three roots is optimum to reduce variability of the results. Using more than three roots reduces variability further, but the gains are smaller, and may not be worth the additional work (in terms of root preparation). Also, the number of commercial-size roots is usually limited, and it is not always possible to allocate three roots for NIRS analyses.

Figure 1: Sample capsules for NIRS

Measure each NIRS capsule once, using the NIR spectrophotometer available at the laboratory (Figure 2). For the development of this SOP, the NIR spectrophotometer used was a FOSS 2500. The recommended operating conditions for spectra acquisition are as follows:

- Wavelength range: 400 to 2498 nm.
- Acquisition step: 2nm
- Number of scans per capsule: 32
- Store data as rlog (1/R), with R the reflectance at each wavelength.

Figure 2: Sample reading by NIRS

7 CRITICAL POINTS OR NOTE ON THE PROCEDURE

- After the NIRS capsules are filled with mashed sample, the NIRS spectra acquisition must be done quickly (within 10 minutes) to limit chemical or biochemical changes due to the rasping.

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