IITA Transforming African Agriculture CGIAR	**Crop:** Cassava **Function:** High throughput phenotyping using NIRS	**SOP #**	IITA-CS-SOP09
		Revision #	IITA-CS-SOP09-01
		Implementation Date	5/6/2024
Page #	1 of 10	**Last Reviewed/Update Date**	5/6/2024
SOP Owner	Mbanjo-Edwige Gaby-Nkouaya	**Approval Date**	5/28/2024

Standard Operating Procedure for High throughput Phenotyping using NIRS

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1. Introduction

The near-infrared spectroscopy is a non-destructive and rapid method for predicting the qualitative and quantitative properties of samples. The NIRS spectral regions, which are located between the visible and infrared ranges (800-2500 nm; 12500-4000 cm-1) contain a wealth of useful information. The development and optimization of NIRS

prediction models could enable reliable, high-throughput, and low-cost analyses of high-value traits. Hundreds of samples could be quickly screened, and multiple parameters could be evaluated in a single test.

2. Purpose

This standard operating procedure (SOP) provides clear guidance on the steps involve in capturing NIRS data using the portable ASD QualitySpec® Trek (QST) (350–2500 nm), the pocket-sized SCiO™ molecular sensor (SCiO) (740–1070 nm), and the benchtop FOSS XDS Rapid Content™ Analyzer (BT) (400–2490 nm) spectrometers.

3. Scope

The document covers the process of spectra data acquisition in different cassava products, including processed cassava roots (gari and flour).

4. Definition of terms

QST Trek – ASD QualitySpec® Trek Portable Spectrometer

5. Roles and Responsibilities

The personnel responsible for implementing this SOPs are listed below:

Breeder: Selects the trials to be harvested and scanned and analyzes spectra data

Data Manager: Curates and uploads scan data to Cassavabase

Supervisor: Oversees spectra data collection procedure

Technicians: Prepare sample and collects spectra data using different devices

Field workers: Involve in different activities from harvesting to sample preparation for spectra data collection.

6. Procedure/Protocols

6.1 Sample preparation for NIRS scan

6.1.1 Preparation of fresh cassava roots

- Harvest the storage roots of the selected plots from selected trials and keep in a properly labelled sample bag.
- The bags are transported to the laboratory.
- Select 6 healthy roots of varying sizes (small, medium, and large to ensure root plot representativeness). Selected roots should be free of defects (decomposed, diseased, or bruised)
- Wash and peel the selected roots and cut off the roots' proximal and distal ends.
- Shred each selected root's top, middle, and bottom parts with a (3 mm) hand grater.

6.1.2 Preparation of gari samples

- Collect already processed gari samples (refer to standard operating procedure for postharvest evaluation) from selected trials.
- Pulverize gari samples to a fine and uniform particle (< 0.1 microns) using an electronic milling machine (Fig 2).
- Pack and package milled sample for each plot into a well-labelled whirl pak sample bag for easy identification (Fig 3).
- Homogenized milled samples are then filled into samples cups (Quartz glass)

6.1.3 Preparation of cassava flour

- Collect already processed flour samples from selected trials.
- Pulverize flour samples to a fine and uniform particle (< 0.1 microns) using an electronic milling machine (Fig 2)

Figure 2. Labconco laboratory milling machine (to grind samples to fine particle size)

- Pack and package milled sample for each plot into a well-labelled whirl pak sample bag for easy identification. (Fig 3)

Figure 3: Labeled samples in Whirl pak

- Homogenized milled samples are then filled into samples cups (Quartz glass)

6.2 Instruments setup

6.2.1 SCIO setup

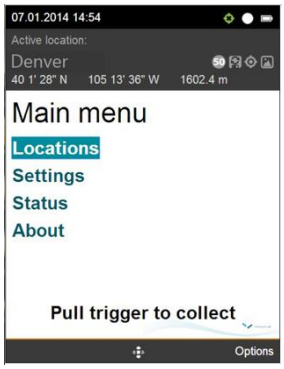
- Install the SCiO online application on a smartphone. This is necessary for gathering and storing data.
- Synce the SCiO sensor with the mobile device to allow communication between the two devices and data and information to flow from the sensor to the cloud through the installed application.
- Calibrate the SCiO sensor before sample capture as needed using a built-in reference standard in the SCiO case.
- Connect the SCiO optical shade to SCiO molecular sensor.

6.2.2 QST setup

1. Make sure the white reference disk is clean before placing it on the instrument
2. Start Trek.
   - Make sure the instrument window is clean.
   - Open the disk cover and make sure the disk is clean.
   - Place the white side over the window.
   - Press the power button.
   - Watch the startup process on the screen.
   - The power LED turns green. The startup process is displayed on the screen. The startup process takes about two minutes to complete.
   - When you see the Main menu, remove the spectralon white reference disk from the window and store in a safe place
   - Set the correct location to active
   - The instrument is now ready to collect data

White reference disk

Placing the white Reference disk on the instrument



Main menu displayed on the instrument

6.2.3 Instrument preparation for Benchtop (FOSS XDS, Rapid Content Analyzer)

- 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.

6.2.3.1. Instrument configuration

- The performance test is conducted for wavelength and noise level accuracy before spectra data collections following instrument manual.

6.3 Spectra acquisition

6.3.1 Spectra acquisition using QST

- Place each quartz glass against the instrument window and hold both the sample and instrument steady
- Pull the instrument trigger once and release. Keep the sample pressed against the window and hold both the sample and instrument steady until the instrument chime again. Each quartz glass containing sample would be scanned twice.
- Each quartz glass should be washed and wiped dry before use for another sample.

6.3.2 Spectra acquisition using SCIO

- Place the thoroughly mixed shredded cassava roots were in quartz cell glasses.
- Connect SCiO optical shade to SCiO molecular sensor
- Place the device on top of the cell quartz with the optical head facing down for spectra capture.
- Scan each cell quartz 3 times in different positions by rotating the quartz cell glass and average the results for chemometric analyses.
- Each accession is measured in three technical replicates (i.e. three independent samples).
- Send the reflectance spectra directly to the SCiO cloud database via Bluetooth.
- The spectra can be downloaded as comma-separated value files from the SCiO cloud database for further analyses.

6.3.3 Spectra acquisition using Benchtop

- Transfer the thoroughly mixed shredded cassava roots into the moving coarse cup of the NIRS machine for spectra data collection.
- Close the chamber and collect triplicate spectra data for each sample. Spectra data collection takes 60 secs per scan.
- After each scan, clean the sample compartment with wet soft tissue.

7. References

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