Laboratory Standard Operating Procedure

SOP for NIRS Measurement on Raw Fresh, Cooked and Freeze Dried Sweetpotato and Potato

High-Throughput Phenotyping Protocols (HTPP), WP3

Kampala, Uganda, 14/11/2022

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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: Near infrared spectroscopy (NIRS) acquisition on sweetpotato roots and potato tubers

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ABSTRACT

NIRS provides rapid, simple, non-invasive and non-destructive phenotyping for rapid selection of traits for breeding. Standardization of spectra collection is important for replicability and hence comparability of different experiments. This SOP highlights the basics in NIRS as well as spectra collection in sweetpotato roots and potato tubers. The aim is to describe the method for spectra collection in different sample types for easy replication of the activities.

Key Words: sweetpotato, potato, near infrared spectroscopy

1 SCOPE AND APPLICATION

This SOP describes the preparation of sweetpotato and potato root samples for NIR spectroscopy (NIRS) measurement. NIRS is widely used to follow the chemical, physical, technological, or physiological processes that affect the structure and composition of organic compounds found in many different organisms. NIRS provides rapid, simple, non-invasive and non-destructive phenotyping for rapid selection of traits for breeding. Electromagnetic radiation absorbed from functional groups C-H, N-H, S-H and O-H, which have specific absorbance patterns in the NIR region (800 -2500 nm) (Prenato et al; 2020, Nantongo et al; 2021) produce spectra which are unique to a sample acting as a “fingerprint”. The spectra can be subjected to qualitative and/or quantitative analysis in order to elucidate the chemical and physical properties of organic molecules in the sample. Over the past decades, the combined application of NIRS-based detection and chemometrics has been used to accurately measure plant primary and secondary organic compounds (Nantongo et. al 2021; Alamu et al 2020).

2 REFERENCES

Prananto, J. A., Minasny, B., & Weaver, T. (2020). Near infrared (NIR) spectroscopy as a rapid and cost-effective method for nutrient analysis of plant leaf tissues. Advances in Agronomy, 164, 1-49.

Nantongo et al (2021). Developing near infrared spectroscopy models for predicting chemistry and responses to stress in Pinus radiata (D. Don). Journal of Near Infrared Spectroscopy, 29, 245-256.

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3 INSTRUMENTATION

The FOSS XDS is used. This instrument uses near-infrared (NIR) spectral energy to illuminate the sample. By measuring the energy reflected off (or passing through) the sample, chemical information and composition may be determined. This information may be used for quantification of constituents, or for comparison to a library of known materials, providing identification and qualification of materials.

The XDS is turned on an hour before scanning is done. All the other configurations and tests are done as per the manufacturer’s manual such as powering the instrument, performance tests and procedure for scanning (e.g. closing the door of the sample chamber to exclude ambient light during sample scanning).

Figure 1: Preparation and scanning a raw-intact sweetpotato root using a FOSS XDS

4 PROCEDURE

Roots and tubers are collected from selected CIP and NaCCRI genetic trials. The roots or tubers from different plots are bulked but separated per genotype before transportation to the sample storage area. Then, health roots/tubers per genotype are randomly selected for NIRS scanning.

To scan the raw-intact roots/tubers, three health roots/tubers are selected. Each sample is washed thoroughly to remove debris before being taken to the NIRS lab. In the lab the root/tuber is again washed with distilled water, patted dry with a paper towel and peeled. The peeled sample is again washed with distilled water, dried with paper towel, cut cross-sectionally across the mid-section, and immediately scanned with the cut surface facing the lens (Figure 1) producing three spectra per genotype.

For the mashed sample (Figure 2), three roots/tubers cleaned as mentioned above are mashed together. Then, three sub samples are selected from the mix, put in cuvettes and scanned producing three spectra per genotype. The cuvettes are cleaned with ethanol between respective samples.

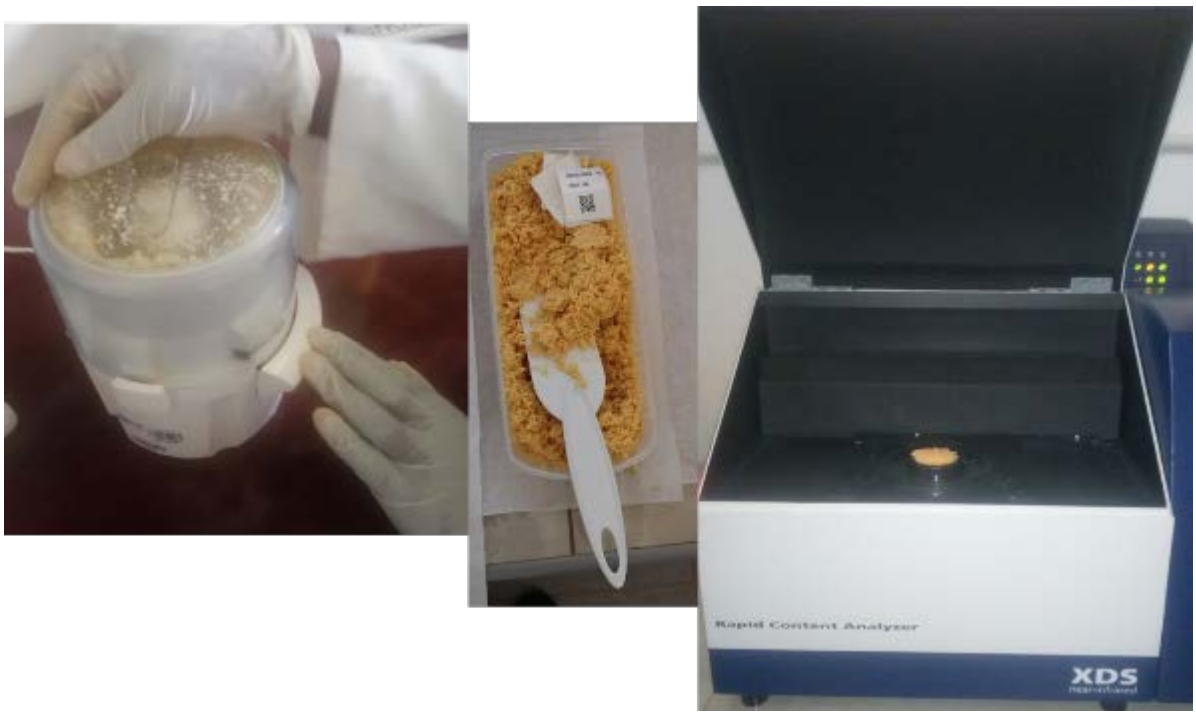

Figure 2: Mashing process and scanning a raw-mashed sweetpotato root in a cuvette using a FOSS XDS

For cooked roots, the remaining half of the raw-intact root/tuber is steamed with distilled water until ready. A folk is used to test whether all the roots/tubers are well cooked (Figure 3). The cooked sample is left to cool before mashing. Each cooked sample is mashed separately, then scanned in a cuvette to produce three spectra per genotype.

Figure 3: Mashing of cooked samples

After scanning the samples above, they are prepared for freeze drying, where, the intact root/tuber is sliced into smaller pieces. The pieces or the mashed samples are weighed out into 50gm subsamples, packed in a polyethene bag and taken to a -20oc freezer in preparation for freeze-drying. However, the potato samples are first snap frozen in liquid nitrogen to reduce oxidation before being taken to the freezer. Freeze-drying is done for 72 hrs. The freeze-dried samples are then reweighed and milled into a fine powder (Figure 4) before scanning in a cuvette, producing three spectra per genotype.

Figure 4: Freeze-dried and milled samples of sweetpotato root ready for NIRS scanning using a FOSS XDS

The spectra are collected with the NIRS XDS (FOSS North America) using ISIscan software (Infrasoft International, State College, PA, USA) at wavelength range 400–2500 nm at 2 nm intervals (Figure 2). They are labelled according to the genotype and root number (e.g., Naspot8_R1, is the first root of Naspot8 scanned). The genotype number corresponds to the one in the field trial. To avoid errors in entering the number, the sample number is scanned by a barcode scanner and directly recorded along the spectrum being collected. The spectra are stored on the working computer, with folders separated per year, season and genetic trial. The spectra are stored as log (1/R), with R the reflectance at each wavelength. Most downstream analyses are don in R.

5 LIMITS FOR SPECTRA REPEATABILITY

These spectra are compared with others from the same samples to identify consistence of the spectra. To check repeatability, a sample was scanned 10 times, and the spectra plus associated statistics compared. For the genotype U0188-14, for example, we noted consistence in all the statistical parameters among the spectra (Figure 2; Table 1). Significance of the differences among means of the wavelengths is checked in XLSTAT

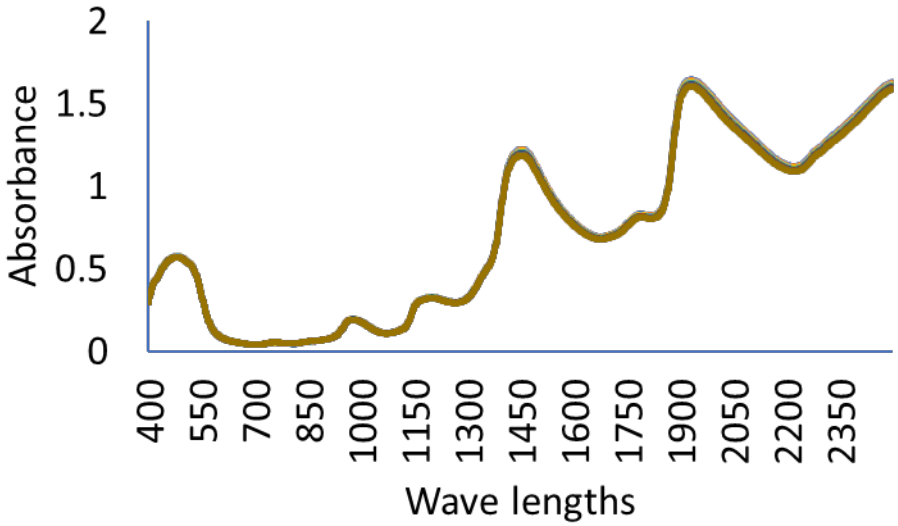

Figure 5: Ten sweetpotato spectra collected from the same sample to check repeatability

Table 1: Statistics associated with 10 sweetpotato spectra collected from the same sample

Statistic	U0188-14_R	U0188-14_R	U0188-14_R	U0188-14_R	U0188-14_R	U0188-14_R	U0188-14_R	U0188-14_R	U0188-14_R	U0188-14_R
Nbr. of observations	1050	1050	1050	1050	1050	1050	1050	1050	1050	1050
Minimum	0.039	0.039	0.040	0.040	0.040	0.041	0.041	0.041	0.041	0.041
Maximum	1.646	1.641	1.636	1.632	1.627	1.622	1.618	1.613	1.609	1.604
1st Quartile	0.182	0.181	0.181	0.181	0.180	0.180	0.180	0.180	0.180	0.179
Median	0.712	0.710	0.708	0.706	0.704	0.702	0.700	0.698	0.696	0.695
3rd Quartile	1.207	1.203	1.199	1.195	1.191	1.187	1.183	1.178	1.174	1.170
Mean	0.724	0.722	0.720	0.717	0.715	0.713	0.711	0.709	0.707	0.705
Variance (n-1)	0.281	0.278	0.276	0.274	0.272	0.270	0.268	0.266	0.264	0.262
Standard deviation (n-1)	0.530	0.528	0.526	0.524	0.522	0.520	0.518	0.516	0.514	0.512

6 CRITICAL POINTS OR NOTE ON THE PROCEDURE

- The raw samples should be scanned in the shortest time possible after preparation to reduce oxidation and water loss.
- The samples should be well parted dry to avoid excess water on the samples.
- The mashed samples should be as uniform as possible.
- Samples should be taken for cold storage for other subsequent analyses as soon as spectra collection is completed.
- The samples should be stored in the freezer after milling until scanning.

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