

# Harmonized SOP for NIRS Acquisition on Fresh Intact and Mashed Cassava Roots using portable NIRS ASD

High-Throughput Phenotyping Protocols (HTPP), WP3

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<b>SOP: Protocol for NIRS Measurement on fresh intact and mashed cassava roots using portable ASD Near Infra-Red Reflectance Spectrophotometer.</b>		
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# ABSTRACT

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Cassava (*Manihot esculenta* Crantz) is a major root crop in the tropics and its starchy roots are significant sources of calories for more than 500 million people worldwide. Cassava breeders aim to produce many clones rapidly with desired agronomic traits. Near-infrared spectroscopy (NIRS) is a physical detection method that was developed in recent decades. This technique is based on the correlation between chemical properties and the absorption of light at specific wavelengths in the near-infrared region. A rapid and accurate method for determining dry matter (DM) content of cassava root will be valuable and help the breeders in selecting clones with good traits. The objectives of this SOP were to calibrate Near-infrared spectroscopy (NIRS) using a portable ASD spectrophotometer for analysis of fresh intact and mashed cassava roots, and to make a comparison between both methods so as to identify which gives the best prediction equation. Fresh intact and mashed cassava roots were analyzed for dry matter content. The near-infrared (400-2400 nm) spectra of the samples were measured. Calibration equation with cross validation were computed using partial least squares method. The quality of prediction was evaluated by root mean square (RMS) and  $r^2$  parameters between the predicted values from cross-validation. The dry matter content was predicted with 95% confidence ( $r^2 = 0.995$ ) for the mashed roots which gave mean RMS value of 19193 and 12135 for the two genotypes, and 77% ( $r^2 = 0.771$ ) for the intact roots which gave a mean RMS values of 47686 and 42255 respectively.

**Key Words:** cassava, NIRS, dry matter, mean, root mean square

## 1 SCOPE AND APPLICATION

This SOP discusses the method involved in NIRS analysis of fresh intact and mashed cassava root samples using a portable ASD NIRS device. The quantity (g) of samples used in this SOP are done to be representative of the whole lot of the samples to be analysed.

Fresh cassava root contains approximately 60 - 70% moisture, while the pulp accounts for about 10-15% of the root (Thongkratok *et al.*, 2010). Most processing procedure requires oven drying to reduce the moisture content and improve the shelf life of the intermediate or the final products. However, during quality traits phenotyping of cassava, the drying process before biochemical analysis tends to increase the overall time taken for laboratory analysis. Modern breeding programs require fast methods for analyzing samples. Hence, the feasibility of using intact fresh and mashed roots for NIRS analysis has been examined, and a sampling and sample preparation protocol developed.

## 2 DEFINITIONS

DM: Dry matter

G1: Genotype 1 (Game changer)

G2: Genotype 2 (Baba 70)

## 3 PRINCIPLE

Near infrared spectroscopy (NIRS) is one of the most important analytical techniques based on the vibrational properties of atoms in molecules (Stuart, BH. 2004). The Near infrared (NIR) spectroscopy is based on the absorption of electromagnetic (EM) radiation at wavelengths in the range 350 - 2,500 nm. The light interacts with the sample and the detector measures its transmittance and absorbance. Transmittance refers to the amount of light that passes completely through the sample and strikes the detector. Absorbance is a measurement of light that is absorbed by the sample. The absorptions measured by NIR spectroscopy correspond mostly to overtones and combinations of vibrational modes involving C–H, O–H, and N–H chemical bonds (Osborne *et al.*, 1993). Recording the electromagnetic radiation absorbed from those molecular bonds in the NIR wavelengths produces spectra, which are unique to a sample acting as a “fingerprint”. The collected spectrum includes data related to the chemical and physical properties of organic molecules in the sample and, therefore, important information on sample composition (dos Santos *et al.*, 2013).

## 4 APPARATUS

Portable vis/nirs device (qualityspec trek: s-10016) from ASD, PANalytical Products, Malvern Panalytical B.V, Lelyweg 1 (7602 EA), PO Box 13, Almelo 7600 AA, Netherlands



## 5 INSTRUMENTATION

### 5.1 Operating mode to Start instrument

- Turn on the instrument by pushing the start button at the right hand side of the instrument. (The power LED turns green to show the device is turning on).



Figure 1 ASD portable NIRS Device

- Place the white reference disk on the window of the portable ASD device and allow it run for about 2 – 3 minutes while the instrument does internal configuration and calibration.



Figure 2 Reference disk



Figure 3 Sampling cups with the white cover

- NIRS scan was conducted on intact and mashed cassava roots by placing the smooth surface (pulp part) of each section (proximal, central and the distal) and the filled sampling cups on the window of the portable ASD NIRS Instrument.
- After each scan, the window of the portable ASD device was cleaned with wet soft tissue.

### 5.2 Instrument Configuration

- Performance test was conducted for wavelength accuracy and noise level was conducted prior to spectra data collection. Before the performance test is conducted, the device operates a self-internal configuration and calibration. This takes about 2 – 3 minutes and is observed by the different start-up display on the screen of the device. The performance test is done by taking about two spectra of the white reference disk as a baseline for creating diffuse reflectance. It is used to check instrumental noise of the instrument (the ratio signal/noise).

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Stage 1: when the device was turned on, asking for reference disk



Stage 2: Internal instrument calibration after the reference disk is placed on the device.



Stage 3: Internal configuration of the instrument



Stage 4: Device ready for spectra capture after the start up processes

Figure 4 Different stages of the device start up



## 6 SAMPLE PREPARATION PROCEDURE

Mature, healthy and fresh roots of varying sizes; big, medium and small were selected for each genotype to obtain a representative of the field plots. The fresh roots were washed and dried with a paper towel to remove dirt. The intact roots were cut axially into proximal, middle and distal sections. The dimension of each section is subjective depending on the length and shape of the root. However, the stalk on the proximal end is cut off before dissecting into three parts. The surface of each section was cut smoothly with a stainless-steel knife, and then tagged with a labelled ribbon then placed in a clean whirl pack bag for analysis. NIRS scan using ASD Spectrometer was conducted by placing the smooth face (pulp part) of each section (proximal, middle and the distal) against the window of the portable ASD device. For the mashed cassava roots, 2 – 3 different quartz cups were filled with the mashed cassava samples (of about 8 – 10g). The two/three cups are chosen to serve as replicates for the samples during spectra collection. After filling the sampling cups, the already filled sampling cups are covered with the white cover (came alongside the portable NIRS device) and placed against the window of the portable NIRS device and scanned. Prior to scanning of the samples, spectra of the white reference disc is always taken. This helps check the accuracy of the spectrometer. (See fig 1 and 2 below).

### 6.1 Protocol of spectral measurement and sample codification

- The appropriate codes were used for the samples. The code used for each sample does not exceed 12 characters to prevent loss of sample codes when exporting spectra data. The codes used in this SOP are as follows:
  - G1BP1: Genotype 1, Big Proximal 1
  - G1BP2: Genotype 2 Big Proximal 2
  - G1BM1: Genotype 1 Big Middle 1
  - G1BM2: Genotype 1 Big Middle 2
  - G1BM3: Genotype 1 Big Middle 3
  - G1BM4: Genotype 1 Big Middle 4
  - G1BD1: Genotype 1 Big Distal 1
  - G1BD2: Genotype 1 Big Distal 2
 (Preceding genotypes continues.....G2, G3, G4 etc.).
- Duplicate spectra were collected for each of the mashed sample while twenty four (24) spectra data were collected on the intact roots,
- Spectra data collection takes about 5 seconds per scan

### 6.2 Procedures for spectra storage

- Spectra data were exported to the WinISI software stored in. nir format on the computer's hard drive. This was done by transforming the spectral data to log (1/R) using ViewSpec Pro software through the following steps:

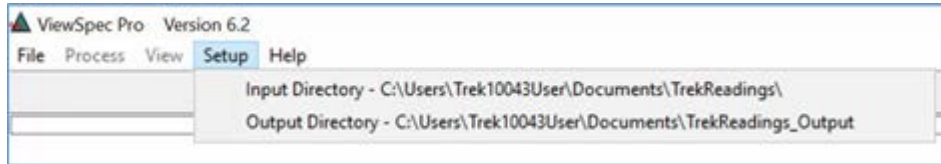
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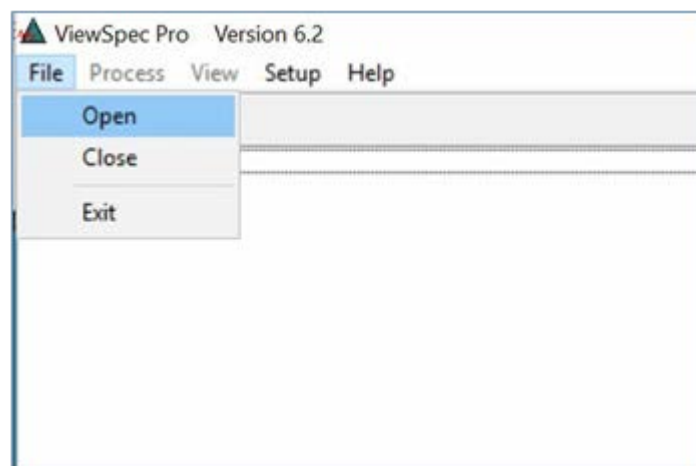
- Select the input directory for the spectra files in the ViewSpec pro (the input directory is the location where the spectra data files reside)



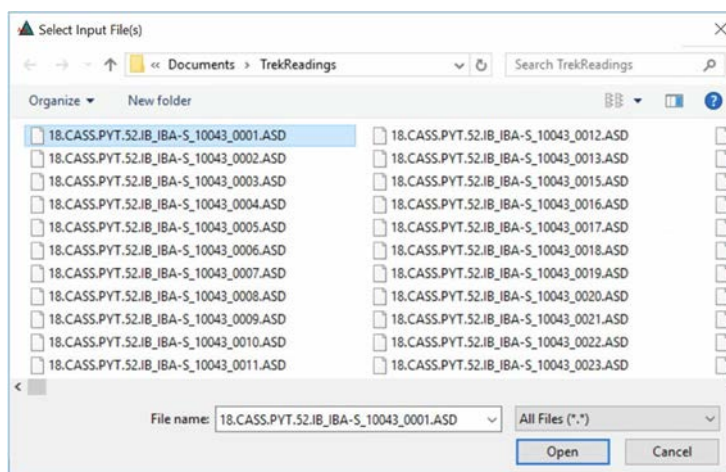
- Select the output directory for the spectra files (the input and output directories do not have to be the same directory). The image below describes the interface after the output directory has been chosen



- Select and open the 'file' icon on the main menu bar and then choose the files for post-processing;



- The following dialog box will be displayed, which will automatically taking you to the directory selected in the first step;



- Select the file(s) from the list available in the directory
- After selecting, then click 'open'

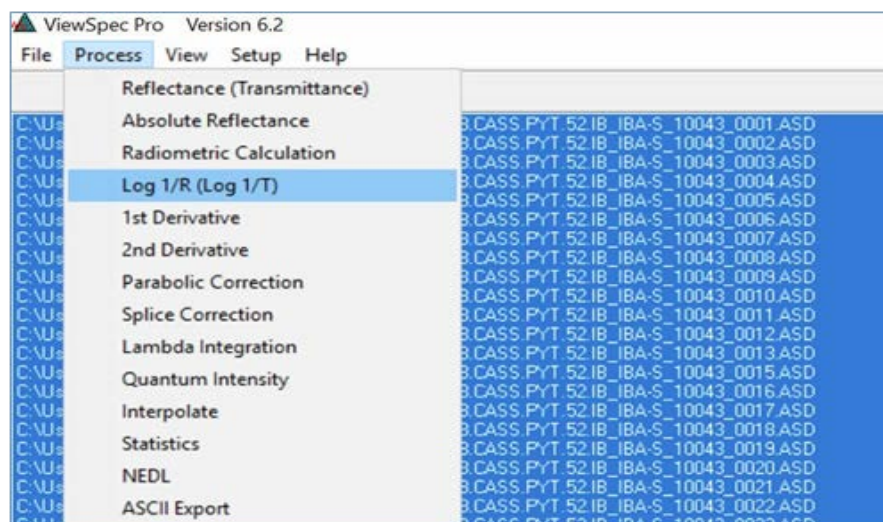
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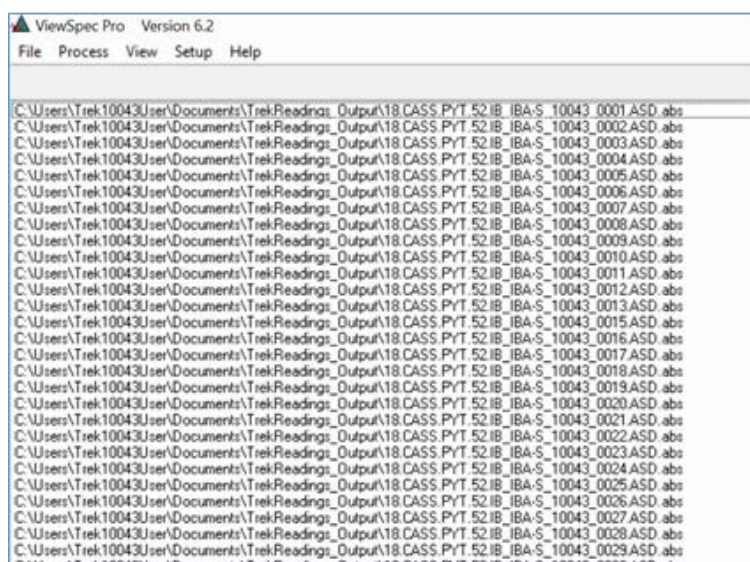
Release: 1

- Select all the files and from Process pull-down menu and then select log 1/R (1/T) option



log 1/R (1/T) option Converts reflectance or transmittance to absorbance;  
Absorbance =  $\log(1/\text{Transmittance})$ .

Now when this is done, all the .ASD files will be converted in to .abs files, as seen below:



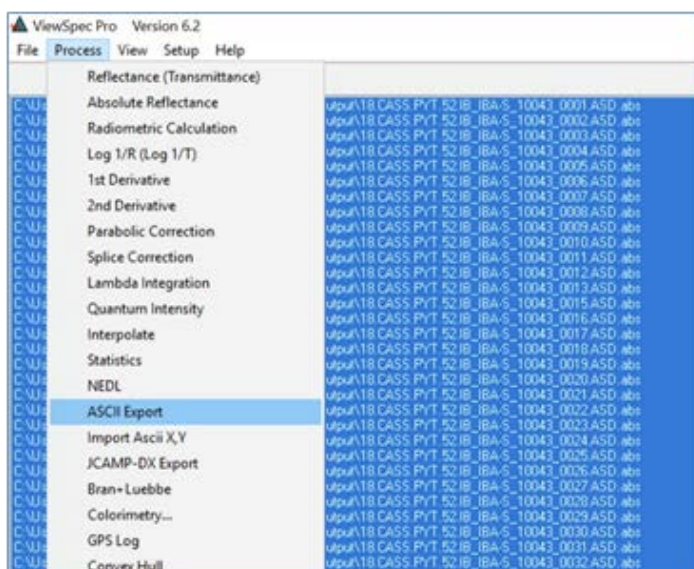
- In this step, Select all the .abs files and from 'Process' pull-down menu and choose ASCII Export option. This process will now convert data files into 'ASCII text files'.

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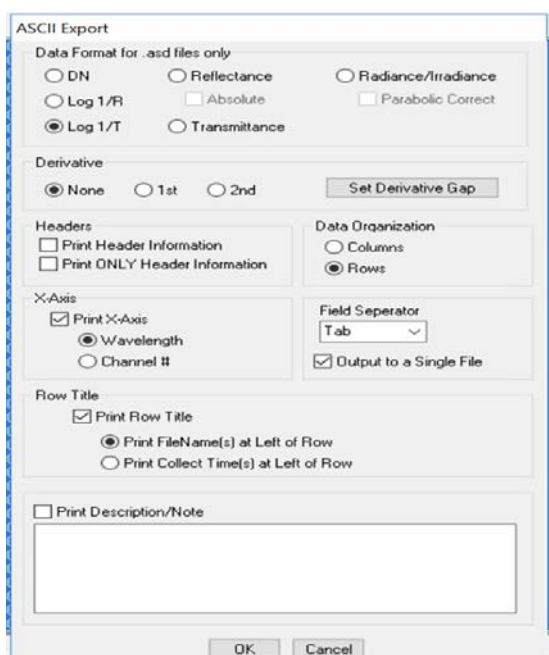
### SOP: Protocol for NIRS Measurement on fresh intact and mashed cassava roots using portable ASD Near Infra-Red Reflectance Spectrophotometer.

Date: 08/12/2021

Release: 1



When ASCII Export is selected, the following Dialog Box will be displayed:



- Select log 1/T for Data Format for .asd files only
- Select None for Derivative
- Data organization should be Rows
- Under field separator check output to a single file option
- others will be left as default
- Click ok



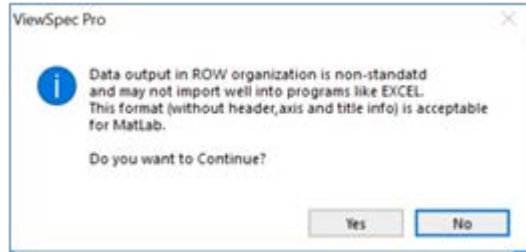
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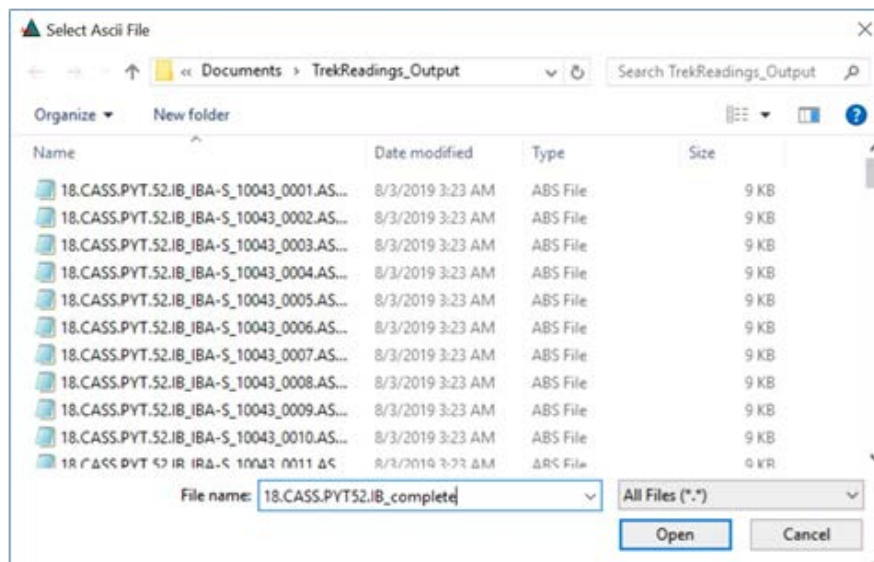
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When these are done, a warning message will appear; then click yes.



After this, provide a name for the single file and click ok. It saves the file in the selected location;



Next, open the saved file in excel (this is done by dragging the file to excel). It will include Sample number in the first column and spectra data for wavelength from 350 to 2500 in next columns as shown below. Each row corresponds to individual spectra selected:

A screenshot of an Excel spreadsheet titled "18.CASS.PYT52.IB\_complete - Excel". The first row (row 1) is labeled "Wavelength" and contains values from 350 to 2500 in increments of 5. The subsequent rows (rows 2 to 25) represent individual spectra, each starting with a sample ID (e.g., "18.CASS.PYT.52.IB\_IBA-S\_10043\_0001.AS...") followed by 36 columns of numerical data corresponding to the wavelengths in the first row.

Wavelength	350	351	352	353	354	355	356	357	358	359	360	361	362	363
18.CASS.PYT.52.IB_IBA-S_10043_0001.AS...	0.473897	0.473637	0.475764	0.477454	0.488081	0.483591	0.481890	0.481729	0.479401	0.478195	0.479474	0.480284	0.485731	0.4865
18.CASS.PYT.52.IB_IBA-S_10043_0002.AS...	0.440354	0.44898	0.449475	0.450413	0.450745	0.447253	0.449585	0.449678	0.45343	0.454859	0.452316	0.453576	0.456455	0.45319
18.CASS.PYT.52.IB_IBA-S_10043_0003.AS...	0.440921	0.439795	0.446546	0.44731	0.445418	0.444755	0.448564	0.451441	0.454721	0.457776	0.458006	0.455747	0.457353	0.45911
18.CASS.PYT.52.IB_IBA-S_10043_0004.AS...	0.468304	0.474378	0.473822	0.469345	0.473592	0.471093	0.47514	0.474288	0.478016	0.479787	0.480769	0.476962	0.482623	0.48168
18.CASS.PYT.52.IB_IBA-S_10043_0005.AS...	0.435895	0.44387	0.443916	0.442648	0.444341	0.44392	0.4486	0.443526	0.443992	0.448423	0.44953	0.449091	0.453613	0.45385
18.CASS.PYT.52.IB_IBA-S_10043_0006.AS...	0.446773	0.448348	0.446335	0.444568	0.446751	0.446811	0.453368	0.448017	0.447374	0.449382	0.44842	0.447636	0.451523	0.45075
18.CASS.PYT.52.IB_IBA-S_10043_0007.AS...	0.375903	0.379324	0.379747	0.378937	0.385124	0.383193	0.383218	0.381875	0.381117	0.384775	0.384548	0.38178	0.383906	0.38490
18.CASS.PYT.52.IB_IBA-S_10043_0008.AS...	0.375959	0.374781	0.374074	0.379793	0.381269	0.379183	0.38207	0.379098	0.381125	0.383953	0.382373	0.381571	0.381389	0.3834
18.CASS.PYT.52.IB_IBA-S_10043_0009.AS...	0.476467	0.475188	0.47226	0.475872	0.484723	0.481288	0.475749	0.475555	0.481355	0.482879	0.478111	0.478037	0.477927	0.47935
18.CASS.PYT.52.IB_IBA-S_10043_0010.AS...	0.411353	0.408505	0.407691	0.412107	0.415184	0.411861	0.414052	0.409411	0.412621	0.414749	0.410501	0.409464	0.412577	0.41348
18.CASS.PYT.52.IB_IBA-S_10043_0011.AS...	0.536893	0.538451	0.536916	0.537987	0.545364	0.541343	0.540626	0.541993	0.549269	0.552264	0.549929	0.543381	0.545858	0.54545
18.CASS.PYT.52.IB_IBA-S_10043_0012.AS...	0.527919	0.52419	0.522047	0.525865	0.534417	0.533024	0.526186	0.527151	0.530591	0.532956	0.53199	0.528337	0.532036	0.53305
18.CASS.PYT.52.IB_IBA-S_10043_0013.AS...	0.442193	0.446565	0.448452	0.449053	0.449416	0.447877	0.453794	0.452528	0.453082	0.457881	0.456541	0.452421	0.454899	0.4543
18.CASS.PYT.52.IB_IBA-S_10043_0014.AS...	0.432282	0.431232	0.434383	0.433308	0.43984	0.437946	0.439167	0.439007	0.437302	0.443184	0.440155	0.437278	0.437708	0.43860
18.CASS.PYT.52.IB_IBA-S_10043_0015.AS...	0.412438	0.416652	0.417934	0.417125	0.422469	0.419704	0.422231	0.421099	0.422712	0.427094	0.424009	0.422297	0.423661	0.42635
18.CASS.PYT.52.IB_IBA-S_10043_0016.AS...	0.415073	0.415328	0.418196	0.420969	0.425454	0.420239	0.42198	0.418037	0.422741	0.426693	0.424733	0.422641	0.424033	0.42395
18.CASS.PYT.52.IB_IBA-S_10043_0017.AS...	0.388745	0.385794	0.389143	0.391208	0.395397	0.394957	0.395724	0.392924	0.39265	0.396819	0.396547	0.395618	0.395973	0.39813
18.CASS.PYT.52.IB_IBA-S_10043_0018.AS...	0.420763	0.421338	0.41824	0.418023	0.42273	0.422494	0.418406	0.415496	0.422813	0.425358	0.421744	0.416406	0.419399	0.41874
18.CASS.PYT.52.IB_IBA-S_10043_0019.AS...	0.413511	0.412131	0.414156	0.414896	0.419281	0.4188	0.420604	0.415708	0.417718	0.421739	0.421614	0.419629	0.42255	0.42324
18.CASS.PYT.52.IB_IBA-S_10043_0020.AS...	0.370909	0.371958	0.370313	0.370755	0.375113	0.374692	0.378376	0.373579	0.374576	0.378252	0.379637	0.378732	0.379032	0.3816
18.CASS.PYT.52.IB_IBA-S_10043_0021.AS...	0.440332	0.43825	0.440847	0.439986	0.446906	0.4474	0.445989	0.444932	0.449965	0.451252	0.446971	0.443158	0.447491	0.44800
18.CASS.PYT.52.IB_IBA-S_10043_0022.AS...	0.422544	0.421681	0.423353	0.421297	0.426029	0.420489	0.423979	0.424461	0.426775	0.426139	0.424816	0.422943	0.421235	0.42612
18.CASS.PYT.52.IB_IBA-S_10043_0023.AS...	0.374177	0.371503	0.369651	0.371759	0.373328	0.368499	0.373903	0.376776	0.373993	0.376572	0.374058	0.373636	0.373849	0.37810
18.CASS.PYT.52.IB_IBA-S_10043_0024.AS...	0.275484	0.277592	0.276944	0.275598	0.279601	0.280152	0.282752	0.280621	0.277245	0.27997	0.282851	0.280608	0.282078	0.28369

Now Winisi software accepts inputs in the following format;

- No heading information – the first row of the file has to be deleted,

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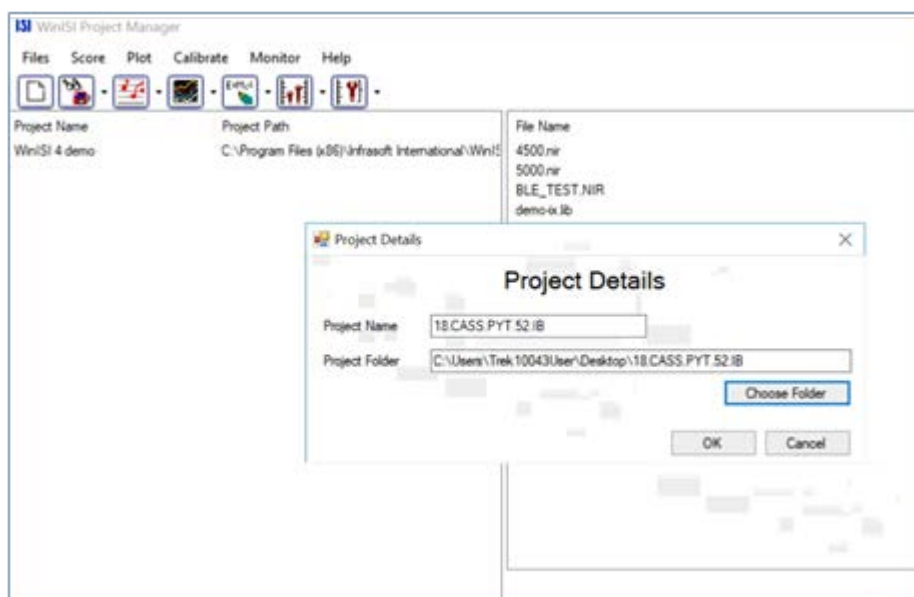
Release: 1

- Sample number is a 12-character alpha/numeric identifier - add a new column with 12-character Sample number and delete the original Sample numbers. (note : It is recommended to save the original Sample number and changed Sample number in a separate file for future reference).

After editing the sample codes, you save the file as .txt

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
1	Exp1_0001	0.473897	0.473637	0.475764	0.477454	0.488061	0.483591	0.481896	0.481729	0.479401	0.478195	0.479474	0.480284	0.485731	0.486559	0.480896	0.478144	0.480311
2	Exp1_0002	0.449354	0.448898	0.449475	0.450413	0.450745	0.447253	0.449585	0.449678	0.453443	0.454859	0.452316	0.453576	0.456455	0.453196	0.449737	0.452182	0.456692
3	Exp1_0003	0.440921	0.439795	0.446546	0.44731	0.445418	0.444755	0.448564	0.451441	0.454721	0.457776	0.458006	0.455747	0.457353	0.459117	0.460074	0.461097	0.463411
4	Exp1_0004	0.468104	0.474378	0.473822	0.469345	0.473592	0.471093	0.47514	0.474288	0.479016	0.479787	0.480769	0.476962	0.482521	0.481688	0.480107	0.479734	0.482821
5	Exp1_0005	0.455895	0.442387	0.443916	0.442648	0.444341	0.44392	0.4486	0.443526	0.443992	0.448423	0.44953	0.449091	0.453613	0.453858	0.453766	0.45277	0.453752
6	Exp1_0006	0.446773	0.448348	0.446335	0.444568	0.446751	0.446811	0.453368	0.448017	0.447374	0.449382	0.44842	0.447636	0.455523	0.450757	0.450487	0.446694	0.449899
7	Exp1_0007	0.375903	0.379324	0.379747	0.378937	0.385124	0.383193	0.383218	0.381875	0.381117	0.384775	0.384548	0.38178	0.383906	0.384907	0.384874	0.384371	0.385332
8	Exp1_0008	0.375959	0.374781	0.374074	0.379793	0.381269	0.379183	0.38207	0.379098	0.381125	0.383953	0.382373	0.381571	0.381389	0.38348	0.382608	0.381868	0.38324
9	Exp1_0009	0.476467	0.475188	0.47226	0.475872	0.484723	0.481288	0.475749	0.475555	0.481355	0.482879	0.478111	0.478037	0.477927	0.479354	0.477801	0.477133	0.479252
10	Exp1_0010	0.411353	0.408505	0.407691	0.412107	0.415184	0.411861	0.414052	0.409411	0.412621	0.414749	0.410501	0.409464	0.412577	0.413481	0.411634	0.411209	0.410822
11	Exp1_0011	0.536893	0.538451	0.536916	0.537987	0.545384	0.541343	0.540626	0.541993	0.549269	0.552264	0.549929	0.543381	0.545858	0.545453	0.545766	0.545165	0.545122
12	Exp1_0012	0.527919	0.524619	0.522047	0.525865	0.534417	0.533024	0.526186	0.527151	0.530991	0.532956	0.53199	0.528337	0.532036	0.533056	0.532796	0.52985	0.5288
13	Exp1_0013	0.442193	0.446565	0.444652	0.449053	0.449416	0.447877	0.453794	0.452528	0.453082	0.457981	0.456541	0.453421	0.454899	0.454434	0.455681	0.454195	0.455794
14	Exp1_0015	0.432282	0.431232	0.434383	0.433308	0.430964	0.437546	0.439167	0.435007	0.43702	0.443184	0.440155	0.437278	0.437708	0.438602	0.439428	0.440871	0.438325
15	Exp1_0016	0.412438	0.416652	0.417934	0.417125	0.422469	0.419704	0.422231	0.421099	0.422712	0.427094	0.424009	0.422297	0.423661	0.426355	0.427025	0.423832	0.426943
16	Exp1_0017	0.415073	0.415328	0.418196	0.420969	0.425454	0.420239	0.42198	0.418037	0.422741	0.424693	0.424733	0.422641	0.424033	0.423955	0.424622	0.424725	0.425766
17	Exp1_0018	0.388745	0.385794	0.389143	0.391208	0.395397	0.394957	0.395724	0.392924	0.39265	0.396819	0.396547	0.395618	0.395973	0.398133	0.398423	0.396939	0.397202
18	Exp1_0019	0.420763	0.421338	0.41824	0.418023	0.42273	0.422404	0.418406	0.415496	0.422813	0.425358	0.421744	0.416406	0.419399	0.418745	0.420094	0.417404	0.416784
19	Exp1_0020	0.413511	0.412131	0.414156	0.414896	0.419281	0.4188	0.420604	0.415708	0.417718	0.421739	0.421614	0.419629	0.422255	0.423245	0.422191	0.421194	0.420324
20	Exp1_0021	0.370909	0.371958	0.370313	0.370755	0.375113	0.374692	0.378376	0.373759	0.374576	0.378252	0.379637	0.378732	0.379032	0.381608	0.381851	0.380734	0.381676
21	Exp1_0022	0.440132	0.438205	0.440647	0.439986	0.446906	0.4474	0.445989	0.444932	0.449965	0.451252	0.446971	0.443158	0.447491	0.448664	0.444373	0.441376	0.442438
22	Exp1_0023	0.422544	0.421681	0.423353	0.42197	0.426029	0.420489	0.423979	0.424461	0.426775	0.426139	0.424816	0.422943	0.421235	0.426126	0.424845	0.422841	0.426834
23	Exp1_0024	0.374177	0.371503	0.369651	0.371759	0.373328	0.368499	0.373903	0.376776	0.373993	0.376572	0.374058	0.373636	0.378349	0.378106	0.377441	0.37532	0.376814
24	Exp1_0025	0.275484	0.277592	0.276944	0.275598	0.279601	0.280152	0.282752	0.280621	0.277245	0.27997	0.282851	0.280608	0.282078	0.283691	0.281981	0.279491	0.280915
25	Exp1_0026	0.264791	0.264545	0.266144	0.266728	0.268216	0.268804	0.269113	0.268907	0.268184	0.271097	0.272582	0.270733	0.270577	0.271104	0.269624	0.270163	0.271975

Then go to winisi, Open the Winisi software, create a new project.



Select 'Convert' from Files pull-down menu

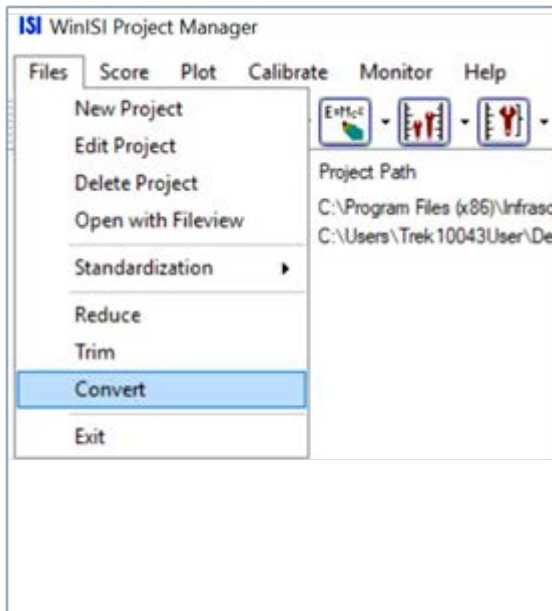


## RTBfoods-WP3

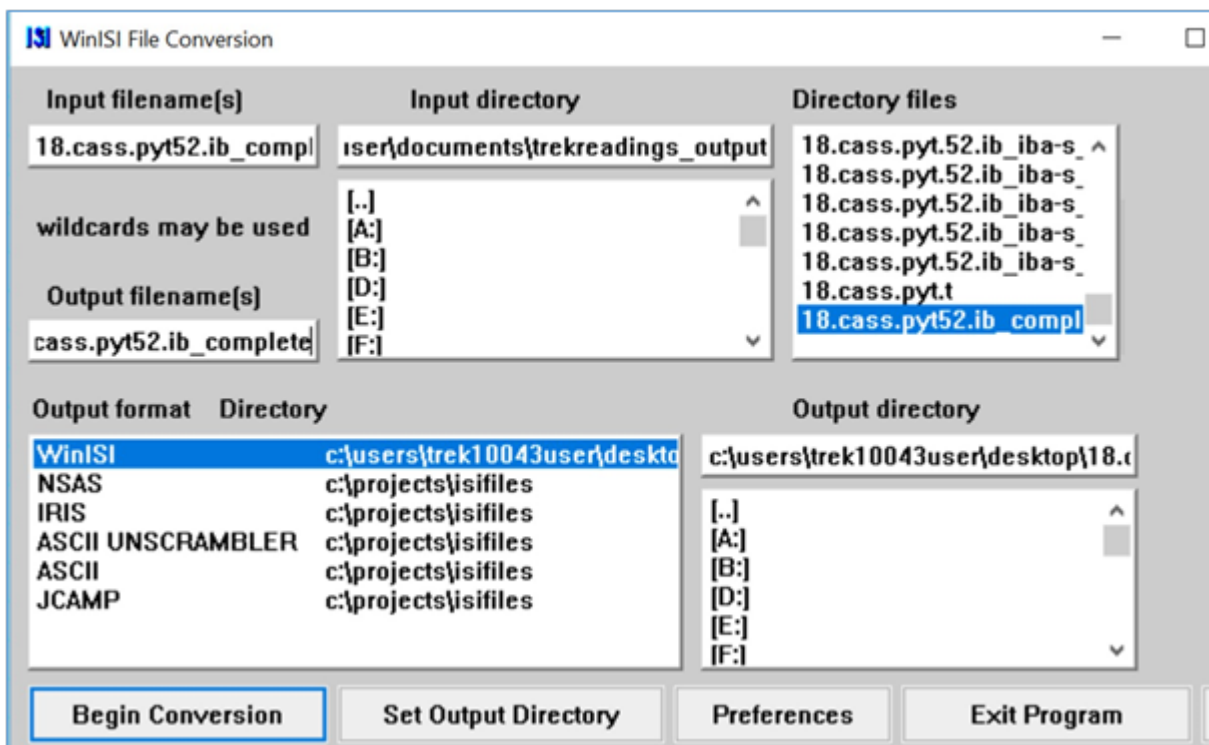
### SOP: Protocol for NIRS Measurement on fresh intact and mashed cassava roots using portable ASD Near Infra-Red Reflectance Spectrophotometer.

Date: 08/12/2021

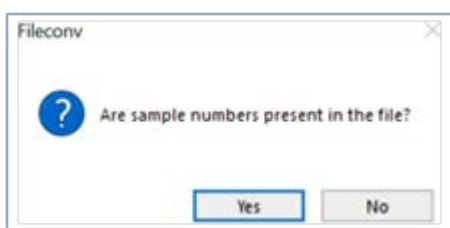
Release: 1



Select the input file (.txt) and give output file name and directory to save



A prompt to check if the sample number is present in the file will pop up, select 'Yes'



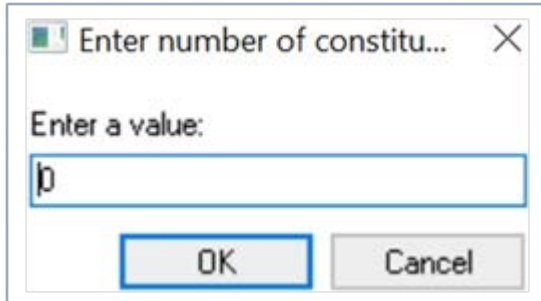
### RTBfoods-WP3

#### SOP: Protocol for NIRS Measurement on fresh intact and mashed cassava roots using portable ASD Near Infra-Red Reflectance Spectrophotometer.

Date: 08/12/2021

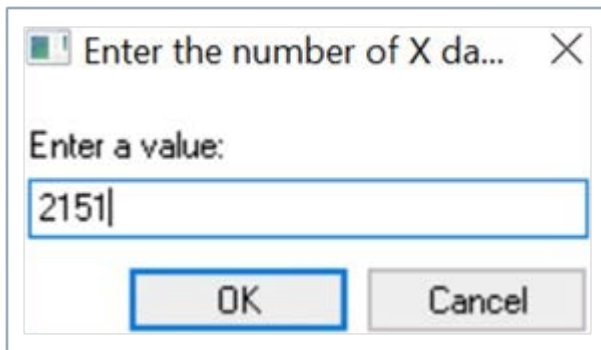
Release: 1

Enter number of Constituents – 0



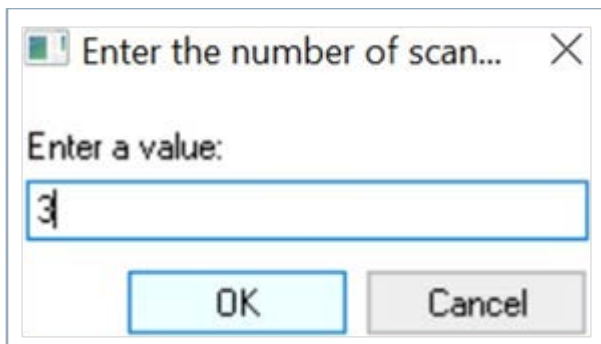
A screenshot of a Windows-style dialog box. The title bar reads "Enter number of constitu...". Inside, there is a label "Enter a value:" followed by a text input field containing the number "0". At the bottom, there are two buttons: "OK" and "Cancel".

Enter the number of X data (wave length) : 2151 (as it is from 350 to 2500)



A screenshot of a Windows-style dialog box. The title bar reads "Enter the number of X da...". Inside, there is a label "Enter a value:" followed by a text input field containing the number "2151". At the bottom, there are two buttons: "OK" and "Cancel".

Enter the number of scanings : 3



A screenshot of a Windows-style dialog box. The title bar reads "Enter the number of scan...". Inside, there is a label "Enter a value:" followed by a text input field containing the number "3". At the bottom, there are two buttons: "OK" and "Cancel".

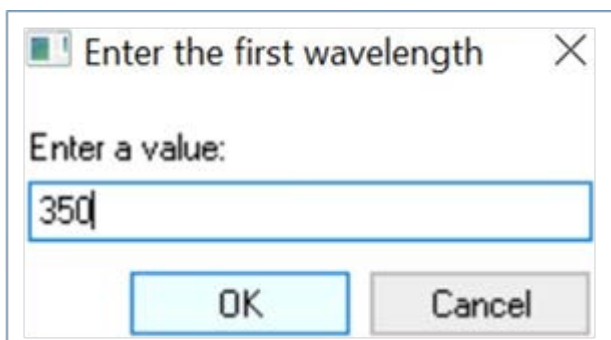
Enter the first wavelength : 350

### RTBfoods-WP3

**SOP: Protocol for NIRS Measurement on fresh intact and mashed cassava roots using portable ASD Near Infra-Red Reflectance Spectrophotometer.**

**Date: 08/12/2021**

**Release: 1**

A screenshot of a software dialog box titled "Enter the first wavelength". It has a close button (X) in the top right corner. Below the title bar, it says "Enter a value:". There is a text input field containing the number "350". At the bottom, there are two buttons: "OK" and "Cancel".

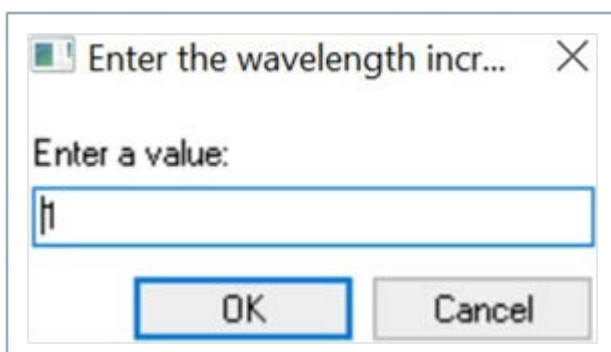
Enter the first wavelength

Enter a value:

350

OK Cancel

Enter the wavelength increment : 1

A screenshot of a software dialog box titled "Enter the wavelength incr...". It has a close button (X) in the top right corner. Below the title bar, it says "Enter a value:". There is a text input field containing the number "1". At the bottom, there are two buttons: "OK" and "Cancel".

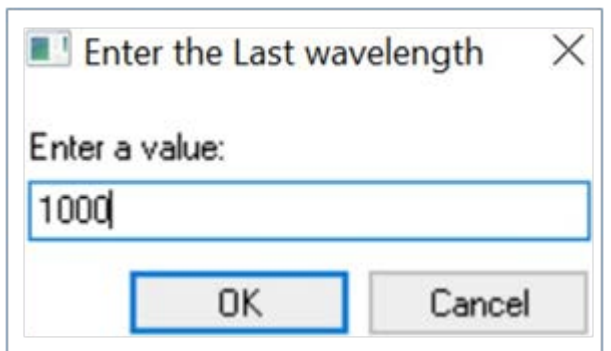
Enter the wavelength incr...

Enter a value:

1

OK Cancel

Enter the last wavelength : 1000

A screenshot of a software dialog box titled "Enter the Last wavelength". It has a close button (X) in the top right corner. Below the title bar, it says "Enter a value:". There is a text input field containing the number "1000". At the bottom, there are two buttons: "OK" and "Cancel".

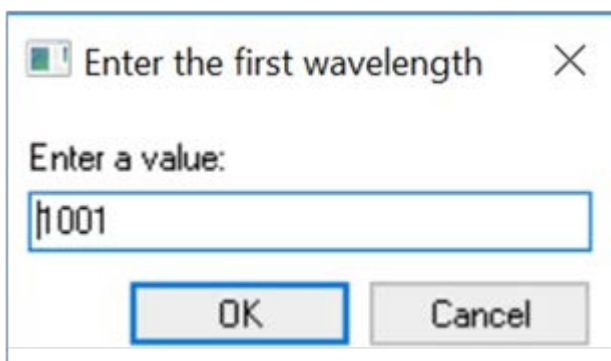
Enter the Last wavelength

Enter a value:

1000

OK Cancel

Enter the first wavelength : 1001

A screenshot of a software dialog box titled "Enter the first wavelength". It has a close button (X) in the top right corner. Below the title bar, it says "Enter a value:". There is a text input field containing the number "1001". At the bottom, there are two buttons: "OK" and "Cancel".

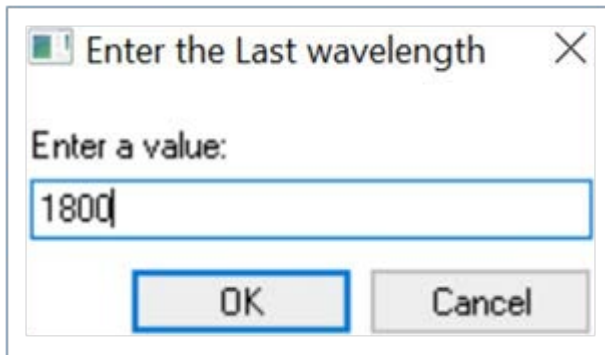
Enter the first wavelength

Enter a value:

1001

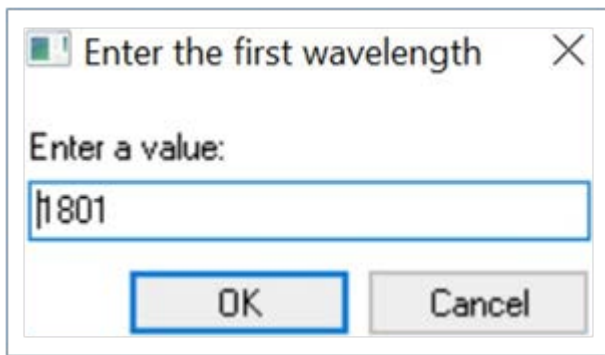
OK Cancel

Enter the last wavelength : 1800



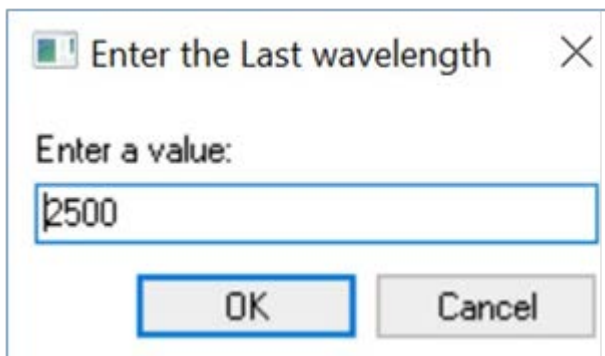
A screenshot of a Windows-style dialog box titled "Enter the Last wavelength". It contains a text input field with the value "1800" and two buttons at the bottom: "OK" and "Cancel".

Enter the first wavelength : 1801



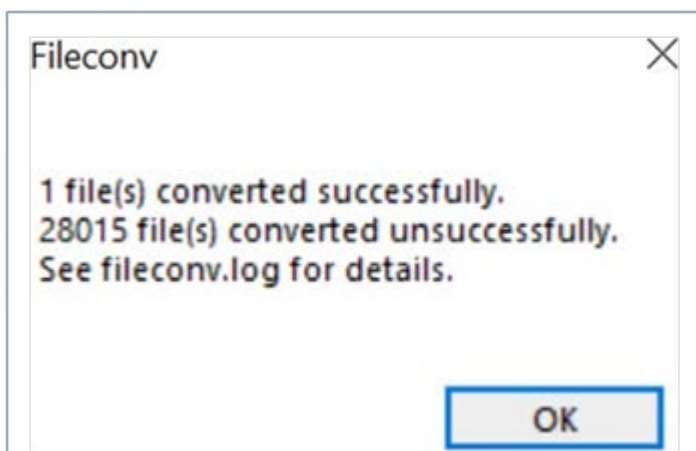
A screenshot of a Windows-style dialog box titled "Enter the first wavelength". It contains a text input field with the value "1801" and two buttons at the bottom: "OK" and "Cancel".

Enter the last wavelength : 2500



A screenshot of a Windows-style dialog box titled "Enter the Last wavelength". It contains a text input field with the value "2500" and two buttons at the bottom: "OK" and "Cancel".

Then finally, a File converted prompt displays to show the file has been converted successfully



A screenshot of a Windows-style dialog box titled "Fileconv". It contains the following text: "1 file(s) converted successfully.", "28015 file(s) converted unsuccessfully.", and "See fileconv.log for details." There is an "OK" button at the bottom right.

### RTBfoods-WP3

## SOP: Protocol for NIRS Measurement on fresh intact and mashed cassava roots using portable ASD Near Infra-Red Reflectance Spectrophotometer.

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→ Stage 1: Randomization of fresh healthy roots for selection



→ Stage 2: Selection of Big, Medium, and Small root per genotype



→ Stage 3: Cut roots into proximal (top), central and distal (bottom)



→ Stage 4: Smoothen the surface with knife



→ Stage 5: Label with ribbon and ready for scanning



→ Stage 6: Scanning with the portable ASD device

Figure 5 Work flow of intact cassava roots measurements using portable ASD device

### RTBfoods-WP3

## SOP: Protocol for NIRS Measurement on fresh intact and mashed cassava roots using portable ASD Near Infra-Red Reflectance Spectrophotometer.

Date: 08/12/2021

Release: 1



→ Stage 1: Randomization of fresh healthy roots for selection



→ Stage 2: Selection of Big, Medium, and Small root per genotype



→ Stage 3: Stainless steel knife for peeling the roots



→ Stage 4: Peeled roots ready for blending



→ Stage 5: Blending of Cassava roots



→ Stage 5: Blended fresh Cassava roots





Stage 6: Collecting spectra on blended sample with portable ASD NIRS

Figure 6 Work flow of mashed cassava roots measurements using portable ASD device

## 7 LIMITS FOR SPECTRA REPEATABILITY

### 7.1 Method of calculation and formulae

The repeatability test must be carried out by taking at least ten readings on the intact/ mashed roots (the measurements must be taking from the same part and point). And, the average of 10 spectra are obtained and compared between them. The mean ( $\bar{x}$ ) and standard deviation ( $s$ ) of the absorbance for the average spectra were calculated for each wavelength, and the root mean square error (RMS) was calculated using the equation below:

$$RMS(i) = \sqrt{\frac{\sum_j^p (X_{ij} - \bar{X}_j)^2}{p}}$$

Where:

$\bar{X}_j$  average of absorbance of wavelength  $j$

$p$  number of wavelengths ( $j$  variate from 1 to  $p$ ).

$X_{ij}$  is an absorbance value of spectra  $i$  for wavelength  $j$

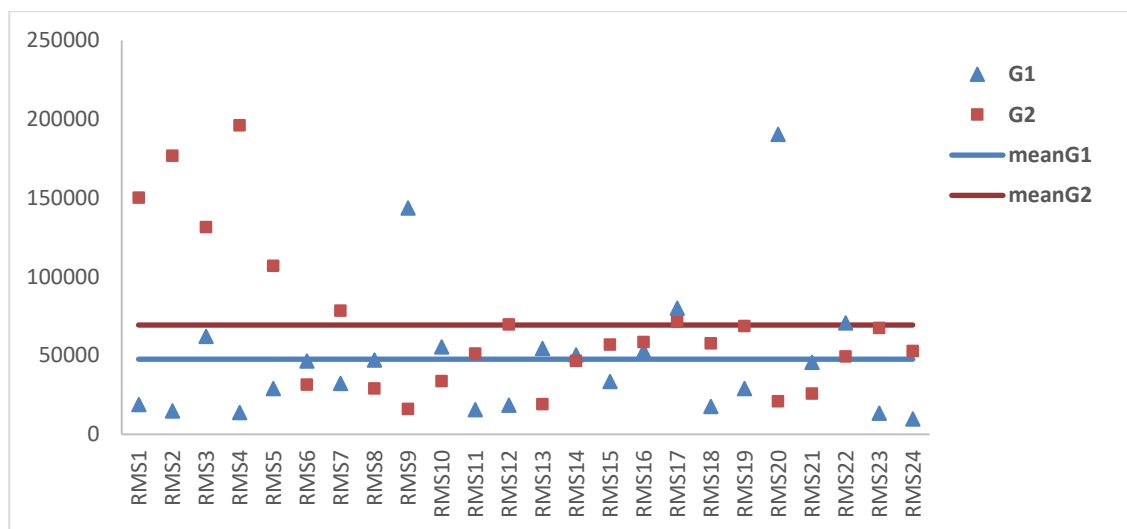


Figure 7 Scatter plot for the RMS values of the different portions of the intact roots

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SOP: Protocol for NIRS Measurement on fresh intact and mashed cassava roots using portable ASD Near Infra-Red Reflectance Spectrophotometer.	
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The graph (Fig.7) presented above shows the root mean square (RMS) values of the different portions of the intact roots. From the graph, the result shows that the total mean and standard deviation for genotype 1 (G1) gave 47686 and 42255 respectively while the total mean and standard deviation for genotype 2 (G2) gave 69378 and 49059. The overall mean values for the two genotypes analysed gave 58532 while the overall standard deviation gave 45657.

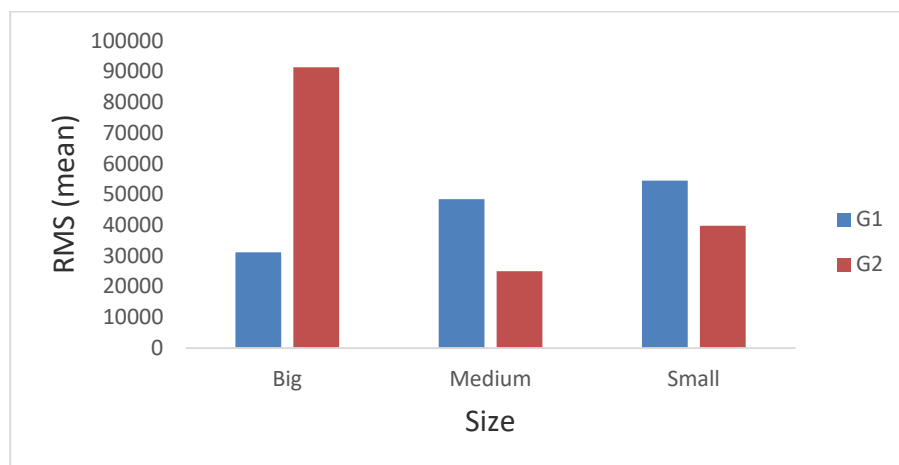


Figure 8 Histogram showing the RMS of the different sizes of the intact roots

The graph (Fig.8) presented above shows the histogram presentation of the root mean square values (RMS) for the different sizes of the intact roots. From the graph, the result shows that the total mean for the big, medium and small sizes of genotype 1 (G1) are 3114, 48495 and 54463 respectively. For genotype 2 (G2), the total mean for the big, medium and small sizes are 91333, 25023 and 39806 respectively.

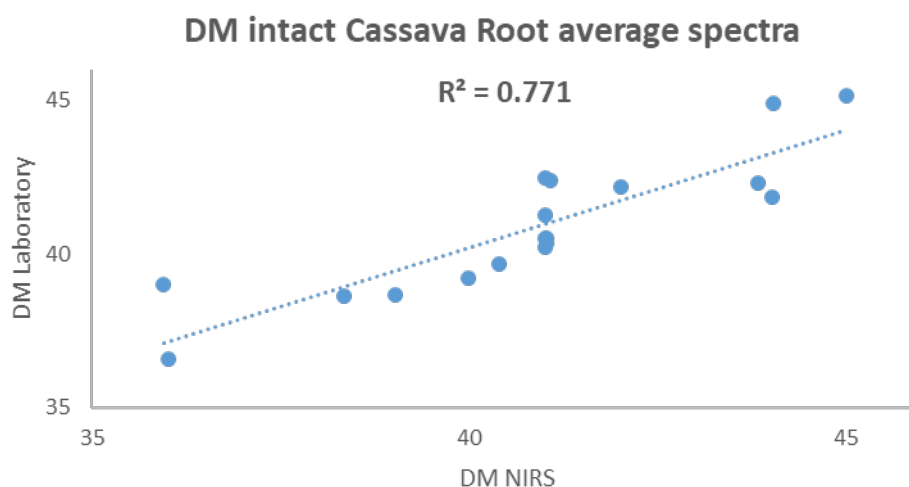


Figure 9 Scatter plot predicted on DM by NIRS versus reference on DM for intact roots

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The scatter plot (fig.9) above shows the prediction of DM by NIRS versus the reference on the DM. The intact cassava root gave a good prediction ( $R^2$ ) values of 0.771. This indicates the level of accuracy of using the intact root method for trait prediction on cassava.

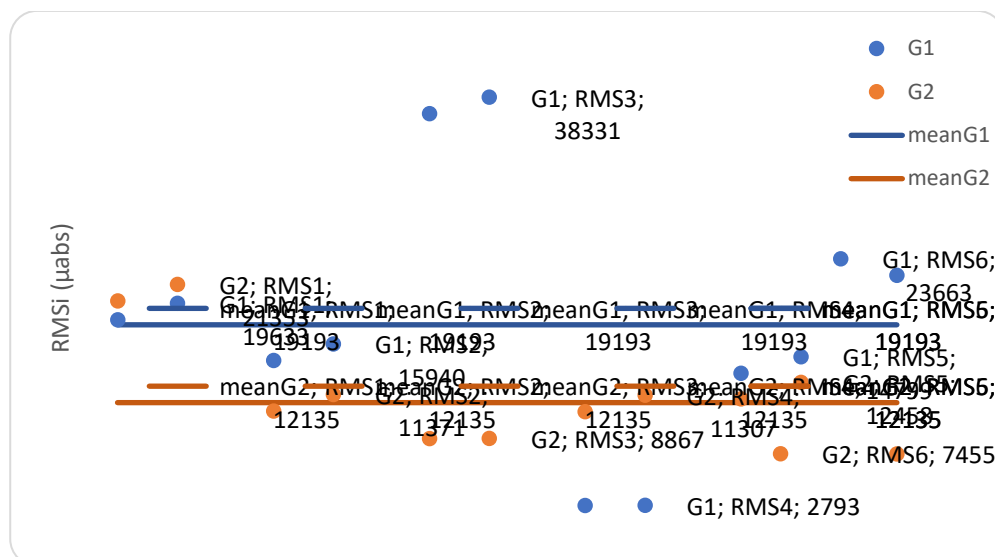


Figure 10 Line graph for the RMS of the mashed cassava genotypes

The graph (Fig.10) above shows the line graph presentation of the root mean square values (RMS) for the different sizes of the mashed roots. From the graph, the result shows that the mean for the big, medium and small sizes of genotype 1 (G1) range from 15940 to 19633, 2793 to 38331 and 14799 to 23663 respectively. For genotype 2 (G2), the mean for the big, medium and small sizes ranges from 11371 to 21353, 8867 to 11307 and 7455 to 12458 respectively.

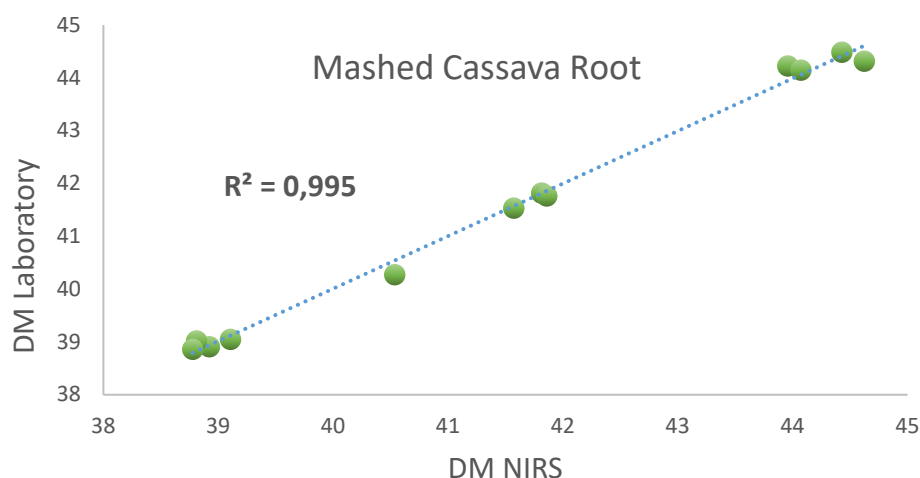


Figure 11 Scatter plot predicted on DM by NIRS versus reference on DM

The scatter plot (fig.12) above shows the prediction of DM by NIRS versus the reference on the DM. The mashed cassava root gave a high prediction ( $R^2$ ) values of 0.995. This indicates the level of accuracy of using the mashing method for trait prediction on cassava.

## 8 RESULTS AND DISCUSSION

From the results, it could be seen that the RMS values of the twenty-four spectra representing the different portions of the intact root, from 400 nm to 2400 nm acquired on Genotype 1 (G1) of the intact fresh roots ranged from 9681 to 190249  $\mu$ abs with a mean value of 47686  $\mu$ abs, and 15915 to 195978  $\mu$ abs with a mean value of 42255  $\mu$ abs acquired on Genotype 2 (G2) for the different portions of the intact fresh cassava. However, the RMS values ranged between 2793 and 38331  $\mu$ abs with a mean value of 19193  $\mu$ abs for the Genotype 1 (G1); and between 7455 to 21353 with a mean value of 12135  $\mu$ abs for Genotype 2 for the six spectra representing the different sizes of the cassava roots from 400nm to 2400nm collected on the blended fresh cassava roots. According to these two measurements, it could be seen that the RMS values for the mashed cassava roots were much lower than that for the intact roots. In addition, the  $R^2$  value of the DM for NIRS versus reference of the mashed root genotypes (0.995) was seen to be higher than that of the intact root genotypes (0.771). Although the intact root gave a good  $R^2$  value, the mashed cassava roots SOP could be used for more accurate prediction of traits on the crop.

## 9 CRITICAL POINTS OR NOTE ON THE PROCEDURE

1. Ensure that good and healthy fresh cassava roots are selected for the analysis.
2. The roots sub-sampled should be the right representative of the lot to be evaluated.
3. It should be made sure that sharp knives are used to cut the cassava roots to get good surfaces for good spectra.
4. The surface of the roots to be scanned must be smooth to avoid light scattering
5. There must be precautions to ensure quantitative transfer of the content after blending into the NIRS sample cup
6. Ensure that the surfaces of the roots to be scanned are well placed against the window of the NIRS device (for both the mobile handheld and stationary NIRS devices) so as to get good spectra.
7. The window of the NIRS device must be wiped with mild solvent intermittently as the spectra are being collected.

## 10 REFERENCES

ASD. ViewSpec Pro TM User Manual. ASD Doc 600555 Rev A. 2008; Available: <http://support.asdi.com/Document/Viewer.aspx?id=31>

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Fox GP, O'Donnell NH, Stewart PN, Gleadow RM. Estimating hydrogen cyanide in forage sorghum (*Sorghum bicolor*) by near-infrared spectroscopy. J Agric Food Chem. 2012; 60: 6183–6187. <https://doi.org/10.1021/jf205030b> PMID: 22594883

<b>RTBfoods-WP3</b> <b>SOP: Protocol for NIRS Measurement on fresh intact and mashed cassava roots using portable ASD Near Infra-Red Reflectance Spectrophotometer.</b>	
<b>Date: 08/12/2021</b>	<b>Release: 1</b>

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Stuart BH. Infrared Spectroscopy: Fundamentals and Applications [Internet]. Methods. 2004. <https://doi.org/10.1002/0470011149>

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## 11 REVISION RECORD

Date	Responsible person	Description of change
19/01/2022	Karima Meghar	Reviewing



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