Laboratory Standard Operating Procedure

MIRS Measurement on Cassava Cell Walls and Flours

High-Throughput Phenotyping Protocols (HTPP), WP3

Montpellier, France, November 2020

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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: MIRS Measurement on Cassava Cell Walls and Flours

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ABSTRACT

The purpose of this SOP is to use MIRS to predict the cooking behaviour of cassava from different varieties by using spectra of corresponding cassava cell walls or flour. For this purpose, eighty-nine samples of cell walls from 37 different varieties (2 or 3 roots per genotype) provided by CIAT (Colombia) were analysed by using Thermo Scientific™ Nicolet™ iS50R Research FTIR Spectrometers in the 4000-400 cm-1 region.

For the repeatability test, 8 spectra of the same flour sample were acquired in order to determine the optimum number of MIRS measurements (replicates) required to be representative of the sample. The RMS obtained for 5 combinations of two replicates (2-3, 1-2, 3-5, 3-7 and 6-8) are 457, 1066, 710, 1352 and 614 µabs respectively. The RMS values between 2 replicates, randomly selected, were lower than the RMS mean for all the replicates. These results indicate that two replicates of the sample result to a spectral dispersion (variability) similar to the one obtained with 8 repetitions.

Key words: MIRS, Cassava cell walls, Cooking behaviour.

1 SCOPE AND APPLICATION

One of the fingerprinting techniques widely used to characterize food ingredients and detect possible adulterants is Fourier transform infrared spectroscopy (FT-IR) in the middle range (400–4000 cm−1, FTMIR). FT-MIR monitors the fundamental vibrational and rotational stretching modes of molecular bonds, which produce a chemical profile of the sample and provide a greater amount of chemical information than FT-NIR (Lohumi, Lee, Lee,& Cho, 2015). Thus, FT-MIR has been used to classify cereal flours, such as wheat, oats and buckwheat (Cocchi et al., 2004), to discriminate different types of wheat flours from rye and triticale flours (Sujka,Koczoń, Heglińska, Reder, & Ciemniewska-Żytkiewicz, 2017) or to determine components of quinoa flour (González-Muñoz et al., 2016). Trans-fatty acids in cereal products were determined by (Kim, Himmelsbach, et al, 2007). Suchowilska, Kandler, Wiwart, and Krska (2012) could differentiate between species of wheat by applying Principle Components Analysis to MIR spectra. Tamaki and Mazza (2011) quantified different types of polysaccharides, ash, and extractives in straw, albeit with mixed results.

The purpose of this SOP is to use MIRS to predict the cooking behaviour of cassava from different varieties by using MIRS spectra of corresponding cassava cell walls or flour. This SOP describes the MIR measurement on Cassava cell wall and flour and how can we discriminate between good and bad cooking time by using MIR spectra data. In this protocol, cell walls analysis are taken as an example.

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Rapid Determination of Carbohydrates, Ash, and Extractives Contents of Straw Using Attenuated Total Reflectance Fourier Transform Mid-Infrared Spectroscopy | Journal of Agricultural and Food Chemistry https://pubs.acs.org/doi/10.1021/jf200078h (accessed Oct 21, 2020).

3 PRINCIPLE

Mid-infrared spectroscopy measures the absorption of radiation in the frequency range from 4000 to 400 cm^{-1}. The absorption involves transitions between vibrational energy states and rotational substates of the molecule. A selection rule applies to these transitions, and absorption of infrared light can occur only if the vibration causes a change in the dipole moment of molecules. It is possible to assign absorptions to specific functional groups, making IR spectroscopy very useful in structural elucidation. Because the intensity of the absorption is proportional to the concentration of the absorbing species, quantitative analysis is also possible (Kačuráková and Wilson, 2001).

MIRS analysis involves some steps. First, the spectrum of the air is collected as a reference spectrum (background) and then MIR spectra are collected and corrected with reference spectrum. The pre-treatment and multivariate analysis can be performed by using any chemometrics software.

4 PROCEDURE

4.1 MIRS measurements

Eighty-nine samples of cell walls from 37 different varieties (2 or 3 roots per genotype) were provided by CIAT (Colombia) in September 2019. The protocols of preparation of flours and cell walls are detailed in the WP2 SOPs “WP2_SOP _flours starch_finalyzed” and “WP2_SOP_CellWalls_tt” respectively.

Spectra are collected using Thermo Scientific™ Nicolet™ iS50R Research FTIR Spectrometers in the 4000-400 cm-1 region and resolution of 0.09 cm-1 (fig.1). The acquisition system is controlled by Thermo Scientific™ OMNIC™ Series Software. This software provide not only the acquisition and saving of spectra but also to set the parameters of acquisition such as spectral range, number of scans, offset, gain, integration time, resolution, spectral path.

Figure 1 : Mid infrared spectrometer system

Background spectrum of the air is collected before spectra acquisition. This spectra is used as reference for all measured spectra. Therefore, by using a small spatula, milled cell walls samples (~0.1 g) are placed directly on the surface of the diamond ATR crystal by pressing the powder sample onto the crystal using a pressure clamp and spectrum was collected (fig.2). Three Spectra were collected per sample.

Figure 2 : MIRS measurement of cassava cell walls

4.2 Repeatability

Eight spectra of the same flour sample were acquired in order to determine the optimum number of MIRS measurements (replicates) required to be representative of the sample. Then, the calculation of the root mean square error (RMS) is performed for the spectral range: 1400-800 cm-1 (fig.3). The individuals RMS are first calculated for the 8 measurements and then all the RMS are calculated for 5 combinations of 2 measurements randomly selected. The objective is to verify if the RMS value of any random of two samples put together remains of the same order than the RMS observed for 8 replicates.

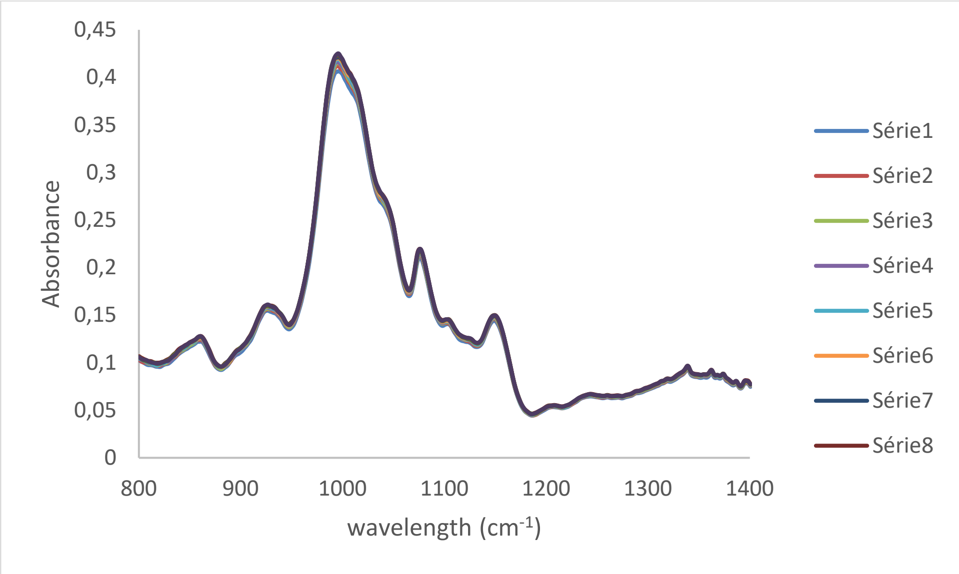


Figure 3: eight spectra measured on the same sample of cassava flour

The average RMS value obtained for the 8 replicates is 1607 uabs, ranged from 277 to 2493 µabs with a standard deviation of 1101 µabs. All of these values are within the confidence interval of 1607 ± 3SD.

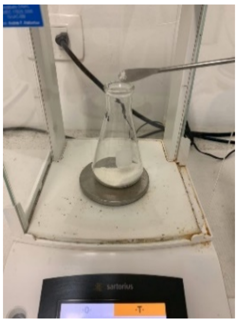
The RMS obtained for 5 combinations of two replicates (2-3, 1-2, 3-5, 3-7 and 6-8) are 457, 1066, 710, 1352 and 614 µabs respectively. The RMS values between 2 replicates, randomly selected, were lower than the RMS mean for all the replicates. These results indicate that two replicates of the sample result to a spectral dispersion (variability) similar to the one obtained with 8 repetitions.

5 Critical points or Note on the procedure

- To avoid the moisture absorption, store flours or cell walls samples in a dry place and closed plastic vials.
- To avoid the light diffusion on the spectra, place the sample on all the surface of the diamond ATR crystal.
- Clean the crystal and pressure clamp by using ethanol and paper towel after each measurement.

6 APPENDICES

6.1 Appendix 1: Procedure of extraction of cell walls from cassava flour


Weight the cassava flour


Add 60 mL water


Homogenize


Add alpha-amylase


Add 10 mL Milli-Q water


Clean Turrax's dispersion rod


Incubate 70℃, 17 hrs


Adjust pH


Add amyloglucosidase

Pour sample

Centrifuge

Iodine test

Take out supernatant

Incubate 60℃, 3 hrs

Add 10 mL of Ethanol

Vortex

Centrifuge

Take out supernatant

REPEAT PREVIOUS 4 STEPS WITH EACH ETHANOL SOLUTION

Add liquid nitrogen

Freeze dry

Grind

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