Laboratory Standard Operating Procedure



Fourier Transform Infra-Red Spectroscopy Analysis of Cell Walls from Sweetpotato Roots

Biophysical Characterization of Quality Traits, WP2

Dundee, UK, October 2022

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<u>Ethics</u>: The activities, which led to the production of this manual, 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



WP2: Biophysical characterization of quality traits

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1 SCOPE AND APPLICATION

Fourier Transform infra-Red (FTiR) spectroscopy is a powerful and rapid technique which can be applied for analyzing cell wall components and putative cross-links, which is able to non-destructively recognize polymers and functional groups and provide abundant information about their *in muro* organization. FTiR spectroscopy has been reported to be a useful tool for non-destructive monitoring of cell wall changes occurring as a result of various factors, such as growth and developmental processes, mutations or biotic and abiotic stresses. The absorbances across the mid-IR spectral range can be compared to those from standard reference compounds and related to changes in (say) polysaccharide composition. Of particular value in this project, is the use of FTiR spectroscopy to investigate differences in the degree of esterification of pectin, a major cell wall component by examining the absorbance at specific wavelengths known to be related to pectin methylation (Ross et al., 2011).

2 REFERENCES

Ross H.A. et al. 2011. Potato tuber pectin structure is influenced by pectin methyl esterase activity and impacts on cooked potato texture, Journal of Experimental Botany, Volume 62(1) pp 371–381, https://doi.org/10.1093/jxb/erq280

3 DEFINITIONS

Infrared absorption spectroscopy is a rapid, economical, and non-destructive physical method, universally applicable to the characterisation of inorganic and organic compounds, including biological materials; Many sample types can all be analysed by a judicious choice of sampling technique and access to a comprehensive library of reference spectra.

4 **PRINCIPLE**

A source of infrared radiation is directed into a RockSolidTM interferometer where the beam strikes a beamsplitter. About half of the light is reflected and is directed on to a fixed mirror. The remainder is transmitted through the beamsplitter and is directed on to a moving mirror. When the beams recombine, constructive or destructive interference occurs depending on the position of the moving mirror relative to the fixed mirror. The modulated beam is then reflected from the mirrors to the sample, where selective absorption takes place. From the sample, the beam travels to the detector which translates the beam into an electrical signal. The cosine waves produced by the source and modulated by the interferometer appear to the detector as an interferogram, a signature of intensity versus mirror position. The signal from the detector is converted into an infrared spectrum, using a dedicated computer, through a mathematical formula, called Fourier Transform. Absorption of infrared radiation by different functional groups at characteristic frequencies, represents a fingerprint of the unknown compound. Comparison of the spectrum with reference spectra allows classification





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of the compound type to be achieved. REFS: BRUKER manual & Kemp, W (1975) Organic Spectroscopy. 3rd edition; MacMillaN Press, Edinburgh).

5 REAGENTS

- 1. Analar grade ethanol for swabbing and cleaning the DATR sample holder between samples.
- 2. Suitable standard pectins with different degrees of methylation can be used for comparison.

6 **APPARATUS**

Bruker Vertex 70 Fourier Transform Infrared Spectrometer has the option of a potassium bromide beamsplitter which allows a scanning wavenumber range of 7500-370 cm⁻¹ (Mid IR) or a Silicon beamsplitter which allows a scanning wavenumber range of 680-30 cm⁻¹ (Far IR). The spectrometer has a standard resolution of 4 cm⁻¹, with a deutero-triglycine sulphate (DTGS) detector. The spectrometer is purged constantly with dry air (Dew point – 70°C) to reduce water vapour and carbon dioxide levels, which are strong absorbers of infrared radiation. This also protects the KBr beamsplitter which is moisture sensitive.



Vortex 70 FT-IR spectrometer at the Hutton





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7 **P**ROCEDURE

The diamond attenuated total reflectance (DATR) sampling accessory is used for rapid universal sample handling of solids. A polystyrene standard sample forms part of the internal validation unit in the instrument and is used, in conjunction with the spectrum of water vapour, to provide a measure of wavelength accuracy. The photometric precision of the instrument is tested by comparing the spectrum of a glass filter (also part of the internal validation unit) to reference spectra. The signal should also be checked and recorded every day to ensure that it passes QC tests.

When recording DATR spectra, using the DATR/KRS-5 experimental file, then scan the background spectrum by using the Background Single Channel command in the Basic tab.

Place the freeze dried sample (milled to pass 0.5 mm sieve) on the DATR window, and screw down the magnetic compression tip on the universal press until it has clicked into place and the solid sample is in good contact with the window.

The sample can then scanned to produce a spectrum. Routinely, 100 scans are collected per sample and triplicate samples are scanned. The internal data collection software will reject scans if the quality does not meet pre-set criteria. The spectrum generated are automatically saved under the recorded filename in the relevant folder. When suitable spectra have been obtained, that sample can be removed and the DATR is cleaned with a tissue and ethanol if required for the next sample.

8 EXPRESSION OF RESULTS

Interpretation of the infrared spectra is achieved by a combination of assignment of peaks from first principles, using correlation tables, and often most importantly, comparison with spectral libraries of known purified components. Some standard samples (e.g., pectins with different published degrees of methylation can be re-run within the sample set to ensure that the spectra are completely comparable. A range of in-house spectral libraries, together with several commercial libraries of infrared spectra are also available. However, care must be taken to ensure that spectral libraries are used appropriately, especially where spectra are of complex mixtures or the standards were poorly defined in their purity and structures. The raw data are saved as spectral files in appropriate directories stored in the FT-IR computer's hard drive and backed up to drives in JHI secure cloud systems. Examination and comparison of spectra can be achieved using Omnic software and spectra can be coped into Microsoft software formats such as Word or Powerpoint. Peak intensities at specific cm⁻¹ values can be obtained using the Omnic software and used to cross compare with spectra from standard components of defined composition. Further statistical tests can be carried out on the raw spectra.







FT-IR signals associated with methylation status in sweetpotato tuber cell walls



Ratio of signals at 1730 and 1625 cm⁻¹ and 1415 and 1235 cm⁻¹ have been associated with the degree of pectin methylation in potato cell walls and textural properties of the tubers (Ross et al., 2011). These can be used to discern the relative level of pectin methylation.

9 CRITICAL POINTS OR NOTE ON THE PROCEDURE

The samples **must be** freeze-dried and kept dry as residual moisture will produce overlapping spectra and greatly reduce resolution. If required, samples should re-dried by freeze drying.

10 TEST REPORT

The test report shall indicate the method used and the results obtained. In addition, it shall mention all operating conditions not specified in the international procedure, or regarded as optional, as well as any circumstances that may have influenced the results.

The test report shall include all details necessary for the complete identification for the sample.







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