Laboratory Standard Operating Procedure

SOP for Analysis of Monosaccharide Composition of Sweetpotato Cell Wall Material/Polysaccharides after Acid Hydrolysis by High Performance Anion Exchange Chromatography (HPAEC)

Biophysical Characterization of Quality Traits, WP2

Dundee, UK, October 2021

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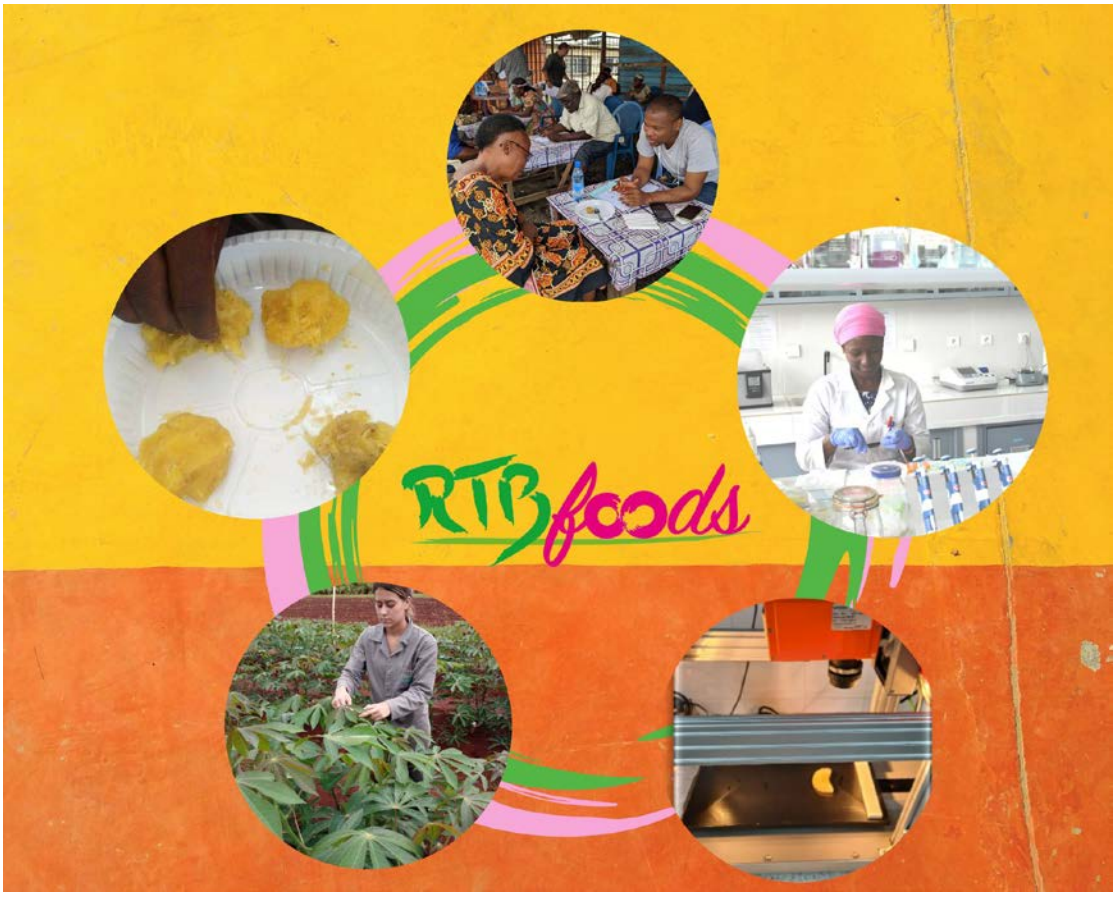

This report has been written in the framework of RTBfoods project.

To be cited as:

Gordon McDOUGALL, Agnès ROLLAND-SABATE, Christian MESTRES (2023). *SOP for Analysis of Monosaccharide Composition of Sweetpotato Cell Wall Material/Polysaccharides after Acid Hydrolysis by High Performance Anion Exchange Chromatography (HPAEC). Biophysical Characterization of Quality Traits, WP2.* Dundee, United-Kingdom: RTBfoods Laboratory Standard Operating Procedure, 9 p https://doi.org/10.18167/agritrop/00704

Ethics: 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.

Acknowledgments: This work was supported by the RTBfoods project https://rtbfoods.cirad.fr, through a grant OPP1178942: Breeding RTB products for end user preferences (RTBfoods), to the French Agricultural Research Centre for International Development (CIRAD), Montpellier, France, by the Bill & Melinda Gates Foundation (BMGF).

RTBfoods *WP2: Biophysical Characterization of Quality Traits*	

SOP: Analysis of monosaccharide composition of sweet potato cell wall material/ polysaccharides after acid hydrolysis by high performance anion exchange chromatography (HPAEC)

Date: 27/09/2022	**Release: 1**

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ABSTRACT

Protocol describing how to perform the analysis of monosaccharide composition of cell walls from sweetpotato roots using the high-performance anion exchange chromatography (HPAEC) method. It has been developed at the James Hutton Institute laboratory in the framework of the RTBfoods project with the objective to understand biochemical drivers of the most important quality traits of boiled sweetpotato roots

Key Words: Monosaccharide, Sweetpotato, Cell wall material, Polysaccharides, High Performance Anion Exchange Chromatography, HPAEC

1 SCOPE AND APPLICATION

This method is for the analysis of the monosaccharide composition of cell walls from sweet potato roots, or derived polysaccharide samples.

2 DEFINITIONS

The monosaccharide composition of the cell walls of sweet potatoes (or their derived polysaccharides) can be informative about the types and amounts of polysaccharides present. This information can be correlated back to different textural properties noted in different genotypes or varieties.

3 PRINCIPLE

Cell wall material (CWM) and polysaccharide solids are hydrolyzed by heating in 2 M trifluoracetic acid at 120 0C for 1 h. Liquid chromatography-based methods can be used to detect both neutral and acidic sugars in hydrolyzed cell wall samples. High-pressure, anion-exchange chromatography (HPAEC) coupled with electrochemical detection (ECD) allows for direct analysis of monosaccharides and oligosaccharides without derivatization or labeling. It uses high pH (pH 12–13) to partially deprotonate the sugar hydroxyl groups, yielding sugar anions that can be separated on anion-exchange columns designed to function at high pH (Dionex Ltd).

Reference for acid hydrolysis: Fry, S.C. (1988) The Growing Plant Cell Wall: Chemical and Metabolic Analysis; Wiley, London: p120

Author Reference: 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

4 REAGENTS

1. 2 mL screw-cap hydrolysis tubes; (Sarstedt, catalogue number: 72.694.006))
2. Eppendorf centrifuge tubes (2 mL)
3. 1000 µL autosampler vials (Sigma, catalogue number: 27422)
4. Autosampler vial lids (Sigma, catalogue number: 24757)
5. Fine metal spatula
6. CarboPac PA20 column (3 x 150 mm, Thermo Fisher Scientific, catalogue number: 060142)
7. CarboPac PA20 guard column (3 x 30 mm, Thermo Fisher Scientific, catalogue number: 060144)
8. Monosaccharide analysis standards:
    a. L-fucose (Sigma, catalogue number: F2252-5G)
    b. D-glucose (Sigma, catalogue number: G7528-1KG)
    c. D-galactose (Sigma, catalogue number: 48260)
    d. D-xylose (Sigma, catalogue number: W360600-SAMPLE)
    e. D-mannose (Sigma, catalogue number: 63579)
    f. L-arabinose (Roth, catalogue number: 5118.2)
    g. L-rhamnose (Sigma, catalogue number: W373011-SAMPLE-K)

h. D-galacturonic acid monohydrate (Sigma, catalogue number: 48280)
i. D-glucuronic acid (Sigma, catalogue number: G5269-10G)

9. Sodium hydroxide, 50% solution in water (Sigma, catalogue number: 415413)
10. Sodium acetate, anhydrous (Sigma, catalogue number: 32319-1KG-R)
11. Ultrapure water (Milli-Q or equivalent)

5 APPARATUS

1. Microcentrifuge (Eppendorf, model: 5424 R)
2. Microbalance (Mettler Toledo MX5)
3. Micro-centrifuge tube shaker (Eppendorf ThermoMixer F1.5)
4. Speed-vacuum centrifuge (Eppendorf Concentrator Plus)
5. Heating block (Stuart, model: SBH130D)
6. Autosampler (Dionex, model: AS-1)
7. Dionex ICS-5000 (Dionex, model: DC-5)
    a. ED Electrochemical Detector (without cell, product number: 072042)
    b. ED Cell (no reference or working electrode, product number: 072044)
    c. Gold (Au) on Polytetrafluororethylene (PTFE) Disposable Electrode (product number: 066480)

All product numbers are valid as date of document but subject to change by manufacturer/ supplier.

6 PROCEDURE

Samples (3 X 10 mg ; exact weight to 0.1 mg noted) are placed in labelled hydrolysis vials and 1 mL of 2 M TFA added, then vortexed well. The vials are transferred to suitable heating block pre-heated to 120 °C then incubated for 1 hour. The samples are then allowed to cool to room temperature then are centrifuged (5000 g, 10 min, 5 °C). The supernatants are then decanted into 2 mL Eppendorf tubes. The samples are then dried to completely remove the TFA solution using a Speed-Vac concentrator. The dried samples are stable for >6 months at -20 °C. When required, the dried samples are resuspended in 1 mL Ultra-Pure Water and are routinely diluted to 1 in 20 for analysis of monosaccharide content.

Monosaccharide analysis of hydrolysates was determined by HPAEC on a Dionex ICS3000 system with a Dionex AS autosampler fitted with a 25 □L loop. The standard monsaccharides fucose, rhamnose, arabinose, galactose, glucose, xylose and mannose, and the uronic acids galacturonic acid and glucuronic acid were separated on 3 X 150 mm PA-20 column with a 3 X 30 mm guard column. Eluents were ultrapure water (18.2 M Ohm) (eluent A), 200 mM NaOH (eluent B) and 1 M sodium acetate (eluent C). Flow rate was 0.4 mL/min and the following gradient was applied: 0 min, 97.5% A, 2.5% B; 25 min, 97.5% A, 2.5% B; 26 min, 72.5% A, 2.5% B, 25% C; 30 min, 72.5% A, 2.5% B, 25% C; 35 min, 47.5% A, 2.5% B, 50% C; 36 min, 2.5% B, 97.5% C; 37 min, 2.5% B, 97.5% C; 38 min, 97.5% A, 2.5% B; 40 min, 97.5% A, 2.5% B; 41 min, 100% B; 45 min, 100% B; 46 min, 97.5% A, 2.5% B; 55 min 97.5% A, 2.5% B. Arabinose and rhamnose are often not completely separated using this gradient but if crucial, they can be analysed on 4 X 250 mm PA-100 column with a 4·X 50 mm guard column eluted isocratically at 1 mL/min with 200 mM NaOH for 15 min. Detection is achieved using a standard quad carbohydrate waveform using a disposable gold electrode. Sugars and uronic acids were identified by co-elution with authentic standards and quantified from standard curves constructed using dilutions between 2 and 25 □g/mL. min. Dionex software can be programmed to automatically assign peaks and calculate peak areas.

7 EXPRESSION OF RESULTS

The response factors (peak area/mg) for each monosaccharide are calculated from their standard curves and the amount of each sugar in the hydrolysates quantified. Each sample hydrolysate is run in triplicate from the separate hydrolysates. Quantities of monosaccharide are then calculated taking into account the dilution factor applied to ensure that the samples fitted into the central area of the standard curves.

For example, the recovery of cell wall material from a sweetpotato root sample may be ~5 % of the total dry weight, and recoveries can differ between varieties and between raw and cooked regimes. Therefore, the recovery must be worked out empirically for each sample.

Next, the efficiency of hydrolysis of specific polysaccharide components by the TFA method is different. Certain components of pectic polysaccharides such as the neutral sugars, rhamnose, galactose and arabinose are hydrolyzed readily and recovered at high % (80 – 95 %). However, the recovery of galacturonic acid is lower because the TFA is not as effective in hydrolyzing the inter chain bonds and the released galacturonic acid itself is less stable to the acid conditions. For these reasons, *the monosaccharide composition of cell walls is only ever qualitative* and is only comparable within a set of similar samples (e.g., sweetpotato roots).

Taking these provisos on board, the amount of monosaccharide in any sample is calculated by comparison to standard curves using the response factors. The amount of monosaccharide in the triplicate sample hydrolysates is averaged then multiplied by the dilution factor (often X 20 or 25) used to get the sample within the standard curve range. The standard error for the hydrolysis can also be calculated. Then, the total amount of each monosaccharide can be expressed as ug per mg of dried cell wall material. This can also be expressed as ug or mg per g of original dried sweetpotato tuber using the empirical yield of cell wall material per g of tuber.

8 CRITICAL POINTS OR NOTE ON THE PROCEDURE

The acid hydrolysis must be carried out under the exact conditions described here in order to avoid incomplete hydrolysis which would lead to an underestimation of galacturonic acid and neutral sugar contents. Care must be taken that the samples are diluted to the correct extent so that the peak areas fit in the middle part of the standard curve for each standard sugar. This may mean quantifying the same sample at two different dilutions to ensure that the most abundant and least abundant monosaccharides are also in scale.

The response of the detector will vary between different runs but if the response noted for the standards drops by more than 20 % then maintenance/ replacement of the gold electrode is required.

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