Laboratory Standard Operating Procedure

Preparation of Cell Wall Material from Sweetpotato Roots

Biophysical Characterization of Quality Traits, WP2

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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.

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RTBfoods

WP2: Biophysical characterization of quality traits

SOP: Preparation of cell wall material from sweetpotato roots

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CONTENTS

Table of Contents

1 SCOPE AND APPLICATION

2 REFERENCES

Original Reference: Jardine, W.G., Doeswijk-Voragen, C.H.L., MacKinnon, I.M.R., van den Broek, L.A.M., Ha, M.A., Jarvis, M.C. and Voragen, A.G.J., 2002. Methods for the preparation of cell walls from potatoes. *Journal of the Science of Food and Agriculture*, *82*(8), pp.834-839.
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

3 DEFINITIONS

The cell walls of sweetpotatoes (SWPs) have an important role in the development of texture during cooking and influence the textural quality of every major processed sweetpotato product. They are also significant contributors to fiber intake in many diets.

4 PRINCIPLE

To study cell walls, one needs to isolate them. Preferably they should be isolated in pure form with the minimum of structural degradation. A de-starching step is necessary before beginning cell wall analysis.

5 REAGENTS

1. 50 mL conical tubes (Greiner Bio-One, skirted)
2. Ethanol (95 %)
3. Enzymatic starch degradation
    a. Amyloglucosidase (Sigma, catalogue number: 10102857001)
    b. α-Amylase (Sigma, catalogue number: 10102814001)
4. Ultrapure water (Milli-Q or equivalent)
5. Sodium acetate, anhydrous (Sigma, catalogue number: 32319-1KG-R)
6. Potassium Chloride (Sigma, catalogue number: P9541)
7. Calcium Chloride (Sigma, catalogue number: C8106)
8. Triton X-100 (Sigma, catalogue number: 9036-19-5
9. Lugol solution (Sigma, catalogue number: 32922-6X1L)

6 APPARATUS

1. Vortex mixer (Stuart scientific, UK)

2. Refrigerated centrifuge (capable of reaching a RCF of 5000 Xg)

3. Temperature Controlled Shaking Water bath

7 PROCEDURE

Details of the miniaturised cell wall method are:

The supplied milled freeze-dried SWP powders (2.5 g each) were weighed out then resuspended in 35 mL of ice-cold mixed cation buffer (MCB) consisting of 10 mM sodium acetate, 3 mM KCl, 2 mM $MgCl_2$, and 1 mM $CaCl_2$, pH 6.5 containing 0.5 % (v/v) Triton X-100 in a 50 mL tube. These were vortexed vigorously for 1 min then heated at 80 °C for 40 min with shaking (60 rpm) at 100 rpm to gelatinise starch.

The sample is then cooled to 40 °C then incubated at 40 °C for 3 hours with 100 units of amyloglucosidase from Rhizopus and 100 units of α-amylase from Bacillus to hydrolyse starch.

Then the samples were centrifuged (5,000 x g 10 min at 4 ^{0}C to separate the pelleted cell wall material (CWM) from the supernatants. The supernatants could then be precipitated at 5°C for 2 hr with 2 volumes of 95 % ethanol to obtain soluble polysaccharide samples. These may be recovered by centrifugation as above then freeze dried.

The CWM is routinely put through another two de-starching procedures until the material was free of residual starch as determined using the Lugol test.

The CWM material was washed with 70 % ethanol then resuspended in deionised water and freeze dried.

8 EXPRESSION OF RESULTS

Cell wall yields are expressed as % DM. The yields of CWM from SWP was routinely ~ 10% DW.

9 CRITICAL POINTS OR NOTE ON THE PROCEDURE

- The samples must contain a low residual starch (< 10% CWM).
- Three successive de-starching procedures usually is sufficient.
- The use of a high precision balance (0.01 mg) is imperative to ensure a good accuracy of measurement.

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