Laboratory Standard Operating Procedure

Determination of Pectin Methylesterase (PME) Activity in Sweetpotato Roots

Dundee, UK, December 2020

Gordon McDOUGALL, The James Hutton Institute (JHI), Dundee, UK

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

To be cited as:

Gordon McDOUGALL, (2021) *Determination of Pectin Methylesterase (PME)Aactivity in Sweetpotato Roots*. Dundee, UK: RTBfoods Laboratory Standard Operating Procedure Report, 9 p.

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.

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: Determination of Pectin Methylesterase (PME) Activity in Sweetpotato Roots

Date: 18/12/2020	**Release: 1**

Written by: Gordon McDOUGALL

For information on this SOP please contact:

- Gordon McDOUGALL (Writer) gordon.mcdougall@hutton.ac.uk
- Christian MESTRES (WP2 Leader) Christian.mestres@cirad.fr

This document has been reviewed by:	
Agnès ROLLAND-SABATE (INRAE)	18/12/2020
Christian MESTRES (CIRAD)	21/12/2020
Final validation by:	
Christian MESTRES (CIRAD)	21/12/2020

CONTENTS

Table of Contents

ABSTRACT

Pectin methylesterase (PME) activity correlates well with texture in potatoes (Solanum tuberosum); higher PME activity is associated with increased firmness as the PME rapidly demethylates pectin in the cell walls and middle lamellae, allowing for hardening through cross linking with divalent cations. In order to understand cooked sweet potato root texture, an assay was developed for PME activity

PME removes methyl groups from pectin producing methanol and free acids. A simple and sensitive method for determination of methanol is important in the study of pectin methyl esterase (PME) in plant material. A determination of the amount of methanol produced by a particular plant tissue can be used to assess the level of PME activity or, indeed, determine the degree of methylation of purified pectins.

An approach to assay methanol is to oxidize the methanol to formaldehyde and then colorimetrically determine the formaldehyde. The oxidation is carried out by alcohol oxidase. This enzymatic method improves selectivity and also eliminates the use of hazardous chemicals. The formaldehyde produced is condensed with N-methylbenzothiazolinone-2-hydrazone (MBTH) under neutral conditions. When the medium is acidified and an oxidant (i.e., Fe3+) is added, this adduct then forms a blue formazan dye that can be determined colorimetrically.

Assess PME activity in roots of sweet potato genotypes known to have different textural properties.

Correlate PME activity against measurements of textural differences upon cooking to assess role of pectin modification in tissue softening.

Key Words (10 maximum): Pectin methylesterase, sweet potato, texture, genotypes, cooking properties

1 SCOPE AND APPLICATION

Pectin methylestyerase (PME) activity correlates well with texture in potatoes (*Solanum tuberosum*); higher PME activity is associated with increased firmness as the PME rapidly demethylates pectin in the cell walls and middle lamellae, allowing for hardening through cross linking with divalent cations. In order to understand cooked sweetpotato root texture an assay was developed for PME activity.

2 REFERENCES

Anthon, G.E. and Barrett, D.M., 2004. Comparison of three colorimetric reagents in the determination of methanol with alcohol oxidase. Application to the assay of pectin methylesterase. *Journal of agricultural and food chemistry*, *52*(12), pp.3749-3753.

3 PRINCIPLE

PME removes methyl groups from pectin producing methanol and free acids. A simple and sensitive method for determination of methanol is important in the study of pectin methylesterase (PME) in plant material. A determination of the amount of methanol produced by a particular plant tissue can be used as a way to assess the level of PME activity in that tissue. Quantitative determination of methanol can also be used to assay PME activity in vitro, or as a way to determine the degree of methylation of purified pectins.

An approach to assay methanol is to oxidize the methanol to formaldehyde and then colorimetrically determine the formaldehyde. The oxidation can be done enzymatically with alcohol oxidase (AO). The enzymatic procedure is preferable because it improves the selectivity of the procedure and because it eliminates the use of hazardous chemicals. The formaldehyde produced by the oxidation of methanol can be determined following condensation with *N*-methylbenzothiazolinone-2-hydrazone (MBTH) under neutral conditions. When the medium is acidified and an oxidant such as Fe^{3+} is added, this adduct then oxidatively couples with a second MBTH molecule to form a blue formazan dye that can be determined by measuring absorbance at 620 nm

4 REAGENTS & PREPARATION

4.1 Reagents

All commercially obtained chemical reagents, solvents and gases are listed in Table 1 and are used without further purification

TABLE 1. COMMERCIALLY OBTAINED CHEMICALS AND SOLVENTS

Liquid Nitrogen (supplier no. differs between countries)

100 mM Tris HCl PH 7.5 - Sigma – (CAS Number: 77-86-1)

Apple Pectin - Sigma 9000-69-5 (no CAS number)

Alcohol Oxidase solution from Pichia pastoris - Sigma A2404 (CAS Number 9073-63-6)

Sodium Sulphite - Sigma 239321 (CAS Number 7757-83-7)

Sodium Chloride - Sigma (CAS Number 7647-14-5)

N-methylbenzothiazolinone-2-hydrazone (MBTH) - Sigma 4338-98-1 (CAS Number 237402-29-8)

Sulfamic Acid - Sigma 5329-14-6 (CAS Number 5329-14-6)

Ferric Ammonium Sulphate - Sigma CAS Number 7783-83-7)

Sodium hydrogen phosphate (Sigma S3264; CAS number 7558-79-4) and sodium dihydrogen phosphate (Sigma S3139) (CAS Number 7558-80-7).

Polyvinylpyrrolidone; PVPP – Sigma PVP40 (MW 40000 kDa).

Methanol – HPLC grade

4.2 Solutions and reagents preparation

Sulfamic acid/ferric ammonium sulphate reagent was prepared by dissolving 0.5 g each of ferric ammonium sulfate and sulfamic acid in 100 mL of water.

5 APPARATUS

1. Eppendorf tubes (2 mL)
2. Mortar and pestle
3. Vortexer
4. End-over-end rotatory (blood) mixer
5. Centrifuge with cooling function
6. Spectrophotometer

6 PROCEDURE

The enzyme was extracted from frozen, ground sweet potato roots in five volumes sodium phosphate buffer (200 mM, pH7) containing 1 M NaCl, 10 mM sodium sulphite and 1% PVPP.

Sodium phosphate buffer (200 mM pH 7.0) was prepared from stock solutions of 1 M NaH_2PO_4 and 1 M Na_2HPO_4 as described in http://cshprotocols.cshlp.org/content/2006/1/pdb.rec8303.full.

This is normally done using 1.5 g for frozen ground material with 7.5 mL extraction buffer and vortex mixing. If freeze dried material is used, then we extract 150 mg pro-rata with 5 mL of extraction buffer with vortex mixing prior to extraction to assure wetting and complete extraction.

Extraction was carried out for 30 min with end-over-end mixing at 5 °C. Longer extraction times (up to 2 h) did not increase activity but reduced specific PME activity. After centrifugation (2500 g, 10 min, 5 °C), a 100 µL aliquot of the extract was added to 200 µL of 2 mg/mL apple pectin solution in ultra-pure water. 200 µL of 100 mM TrisHCl pH 7.5 containing 250 mM NaCl, 200 µL of 3 mg/mL MBTH, 2.5 units of alcohol oxidase and ultrapure water to make up the volume to 1 mL. The reaction was incubated for 30 min at room temperature (25 °C) and an aliquot (100 µL) removed and added to 100 µL of sulfamic acid/ ferric ammonium sulphate reagent and held for 30 min at room temperature. After the incubation, 800 µL ultrapure water was added. At the 1 h mark, another 100 µL aliquot of the reaction was removed and treated as above. The absorbance was read off at 620 nm. The enzyme activity was calculated based on a methanol standard curve.

6.1 Method of calculation and formulae

The absorbance value was used as the enzyme unit value.

This value is multiplied by 10 (conversion to mL) then 7.5 mL (total volume) then divided by 1.5 g to give units PME/g FW.

For freeze dried material, the calculation is absorbance unit X 10 X 5 for total volume then divided by 0.15 to give units PME/g DW. Values can be converted into µmoles of methanol using a suitable standard curve.

6.2 Repeatability

Repeatability was < 5 %.

7 Critical Points or Note on the Procedure

It is crucial that the roots are ground to a powder in liquid N_2 and kept cool throughout. Extraction must also be carried out at 5 °C to maintain PME activity.

8 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.

Institute: Cirad – UMR QualiSud

Address: C/O Cathy Méjean, TA-B95/15 - 73 rue Jean-François Breton - 34398 Montpellier Cedex 5 - France

Tel: +33 4 67 61 44 31

Email: rtbfoodspmu@cirad.fr

Website: https://rtbfoods.cirad.fr/