Total Glucosinolate Content in Rapeseed Using Reflectance

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Abstract

A method has been developed for the determination of total glucosinolate content in rapeseed. Following selective hydrolysis of glucosinolates by the endogeneous myrosinase in rapeseed, the glucose produced is detected with commercially available glucose test strips. The intensity of the colour on the strips is measured using a portable reflectometer. This method is suitable for use by plant breeders and for the determination of glucosinolate content in commercial rapeseed loads.

Introduction

Several methods are available for the quantitative determination of glucosinolates in rapeseed. The High Pressure Liquid Chromatography (HPLC) method for separating desulfoglucosinolates can provide a measure of the content of individual glucosinolates including indole glucosinolates (1) . This method, however, is not the method of choice for most routine plant breeding purposes where a total glucosinolate figure is all that is required.

A number of methods are available for the estimation of total glucosinolate content in rapeseed for example, thymol (2), chloropalladate (3) and techniques based on glucose release (4). Some of the advantages and disadvantages of these methods are discussed at the end of this paper. The aim of this project was to develop a method for measuring total glucosinolate content which was rapid, simple, lacked any chromatographic steps and which did not require the use of expensive equipment.

Experimental Determination of Glucosinolate Content in Rapeseed.

1. Weigh 200 mg air-dried rapeseed into a 10 ml centrifuge tube.
2. Add 3.0 ml of 50 mM Glycine-NaOH buffer (pH 9.0) and homogenise thoroughly (15 seconds). Rinse the ultra turrax shaft with 2x1.0 ml aliquots of buffer solution dispensed through the top hole in the shaft.
3. Mix the tube gently and leave for 10 minutes.
4. Add 1.0 ml of chloroform, seal the tube and mix thoroughly by shaking.
5. Add 50ul 10% chlorohexidine diacetate in methanol and mix.
6. Add 1.0 ml of 100 mM Citric Acid/Sodium Citrate buffer pH 5.0

7. A scoop of activated charcoal (0.25g) is added and the tube is sealed and vortexed.

8. Centrifuge the sample at 4,000 g for 2 minutes. A clear colourless supernatant must be obtained.

9. Dip a Clinistix strip into the supernatant (4 seconds). Shake off excess liquid. Leave for 2 minutes for colour to develop.

10. Calibrate the meter setting the high end at 90 using a strip dipped in 1.0mM glucose and the low end at 0 using water.

11. Determine the reflectance of the sample and read the corresponding glucose concentration from the standard curve. Convert to umole glucosinolate /g seed by multiplying by the factor 30 (i.e. Glucose mM x 30 = Glucosinolate umole /g seed.) The appropriate correction for the moisture content of the seed sample can be applied.

Standard Curve Construction.

Preparation of curve A (fig. 1) involves aliquoting 27, 45, 90, 135 and 180 ul of the 5.56 mM standard glucose into separate vials containing 166 ul of 0.1 M Citric Acid / Sodium Citrate buffer pH 5.0. The total volume in each is adjusted to 1.0 ml giving 0.10, 0.25, 0.50, 0.75, and 1.0 mM glucose.

Preparation of curve B (fig. 1) is as follows:

1. Weigh 200 mg rapeseed into a 10 ml centrifuge tube.

2. Immerse in a boiling water bath and add 3.0 ml of boiling water, leave for at least 10 minutes.

3. Homogenise in an ultra turrax for 15 seconds. Wash shaft with 2 x1.0 ml aliquots of water.

4. The extract is then treated according to steps 4-8 of sample preparation.

5. Prepare standards using 5.56 mM glucose as described previously and adjust total volume to 1.0 ml using the extract.

INSTRUCTIONS FOR GLUCOSINOLATE ANALYSIS.

A. CALIBRATION

Two solutions will be required.
For Zero adjustment : Take a six times dilution of 0.1 M Citric Acid / Sodium Citrate buffer pH 5.0.
For Gain adjustment : Prepare a 1.0 mM glucose solution by taking 180 ul of the 5.56 mM glucose into a vial and add 166 ul of 0.1 M Citric Acid/ Sodium Citrate buffer pH5.0 and adjust the final volume to 1.0 ml.

Meter Adjustment.

1. Turn on with switch at back of the meter. Press RUN.

2. Insert a test strip which has been moistened with buffer. Close the cover and press READ. Adjust the ZERO control (Right knob) while repeatedly pressing the READ, until a value of 0 mM is displayed.

3. Take a fresh test strip and place a drop of 1.0 mM standard glucose wait 5seconds and shake off the excess moisture. Wait 2 minutes. Insert into the meter as before. Press READ. Adjust the GAIN control (left Knob) while repeatedly pressing READ until a value of 1.0 mM is displayed.

4. Repeat Steps 2 to 3.

5. The meter is now calibrated for glucosinolate determinations.