

GLUCOSINOLATE MEASUREMENT IN RAPESEED USING REFLECTANCE.  
THE TRUBLUGLU METER

R.J.W. Truscott (1), J. Tholen (1), G. Buzza (2), D.I. McGregor (3)

- (1) Department of Chemistry, University of Wollongong  
Wollongong, N.S.W. 2500, AUSTRALIA
- (2) Pacific Seeds Ltd., Toowoomba, Qld., 4359, AUSTRALIA
- (3) Agriculture Canada Research Station, 107 Science Place,  
Saskatoon, Saskatchewan, CANADA S7N 0X2

INTRODUCTION

A simple and inexpensive method has been developed to estimate the total glucosinolate (GSL) content in rapeseed. The assay is based on the selective hydrolysis of GSLs by endogenous myrosinase at pH 9 followed by measurement of the released glucose using Clinistix strips and a newly developed portable reflectance instrument, the TRUBLUGLU meter (Fig. 1). The meter, which is calibrated prior to use with a standard glucose solution, can display a digital readout in units of either mM glucose or  $\mu\text{mol}$  GSL/g seed.

METHOD

Stepwise determination of glucosinolate content is performed as follows:

Weigh 200 mg air-dried rapeseed into a 10 mL centrifuge tube.

Add 3.0 mL of 50 mM glycine-NaOH buffer (pH 9.0).

Homogenize thoroughly (15 seconds) with a high speed homogenizer (i.e. Ultra-turrax or Polytron homogenizer).

Rinse the shaft 2 times with 1.0 mL glycine-NaOH buffer.

Incubate at room temperature for 10 minutes.

Add 1.0 mL chloroform and mix.

Add 50  $\mu\text{L}$  10% chlorohexidine diacetate and mix.

Add 1.0 mL 100 mM citrate buffer (pH 5.0).

Add one scop (ca 250 mg) activated charcoal.

Mix and centrifuge ( $>1000 \times g$  for 2 minutes).

Immerse Clinistix strip in clear supernatant.

Shake of excess liquid after 5 seconds.

Let stand for 2 minutes.

Insert strip into TRUBLUGLU meter and press "READ" button.

The TRUBLUGLU meter is calibrated before use, using a Clinistix strip dipped in water to set the low end at 0.00 mM (ZERO KNOB) and a Clinistix strip dipped in 1.00 mM glucose to set the high end at 1.00 mM (SPAN KNOB). After calibration the 'UNIT DISPLAY' button is pressed to alter the digital display from mM glucose to  $\mu\text{mol}$  glucosinolate/g seed. In this mode, allowance is made for the free glucose content in seed by incorporation of an offset in the standard curve.

A key step is the myrosinase-catalysed hydrolysis of seed glucosinolates at pH 9. At this pH, other seed glycosidase enzymes are inactive.

### RESULTS

Clinistix strips are marketed by Armes Division, Miles Laboratories Inc. for the visual estimation of glucose in urine. We have found that they can also be used to provide an accurate quantitative measure of glucose concentration when used together with the TRUBLUGLU meter (Fig. 2).

Despite the lack of a chromatographic step, comparison of the TRUBLUGLU meter results with those obtained by GC, HPLC and thymol methods show a high degree of agreement, using both Canadian and Australian rapeseed cultivars (Table 1).

Some features of the method:

- 1). Expensive reagents and chromatographic media are not required and the method requires little laboratory expertise.
- 2). Clinistix strips costs approx. 4 cents (U.S.) each, are readily available and can be stored in an unopened container for approx. 2 years.
- 3). The meter is portable and can be powered by batteries or from an outlet using either a 220/240 V or 110/120 V transformer.
- 4). The TRUBLUGLU meter has considerable potential for use at seed receival depots as well as plant breeding stations, especially those which do not have ready access to laboratory facilities where more time-consuming and expensive methods of analysis can be performed.
- 5.) The TRUBLUGLU meter can be purchased for approximately U.S. \$800. Enquiries should be directed to Dr. R.J.W. Truscott or Dr. D.I. McGregor.

### REFERENCES

DAUN, J.K. and MCGREGOR, D.I. 1989. Glucosinolates analysis of rapeseed (canola). Method of the Canadian Grain Commission Grain Research Laboratories. Dec. 15, 1981, revised Sept. 30, 1983, Sept. 1, 1989.

MCGREGOR, D.I. 1985. Determination of glucosinolates in Brassica seeds. Eucarpia Cruciferae Newsletter No. 10, pp. 132-136.

McGREGOR, D.I. and DOWNEY, R.K. 1986. Determination of total glucosinolate content in seed meal of rapeseed using thymol. Proc. of the Third Oil Crops Network held in Addiss Ababa, Ethiopia. Oct. 6-10. pp. 242-250.

OLSSON, K., THEANDER, O. and AMAN, P. 1976. Determination of total glucosinolate content of rapeseed and turnip rapeseed meals by gas liquid chromatography. Swedish J. Agric. Res. 6: 225-229.

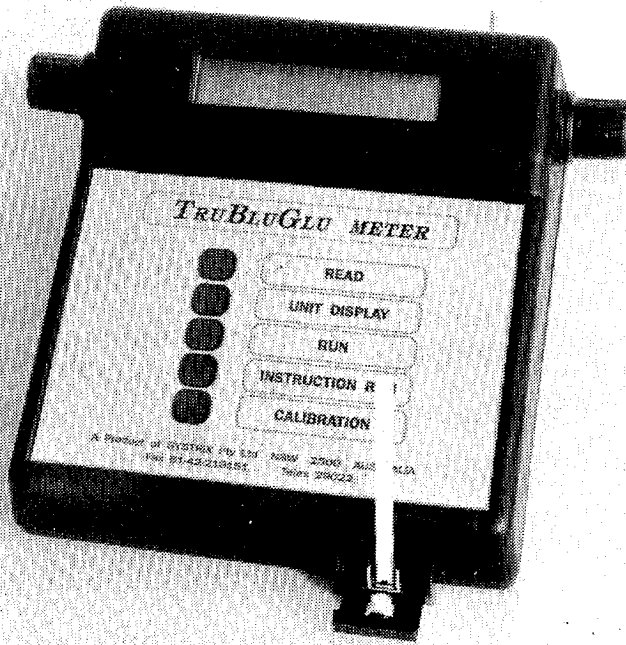


Fig. 1. TRUBLUGLU meter.

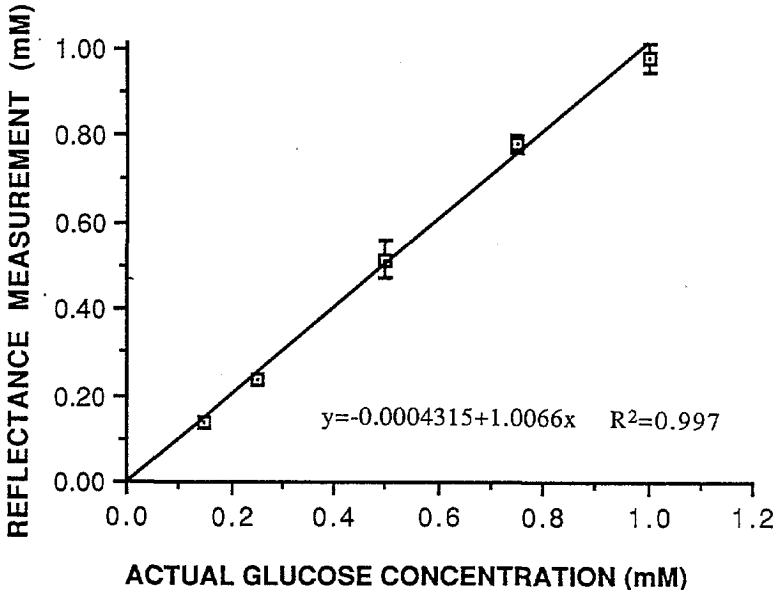


Fig. 2. Standard curve for quantitation of glucose using the TRUBLUGLU meter. Means and standard deviations are shown for 10 separate determinations at each glucose concentration.

Table 1. Relative accuracy and precision of the TRUBLUGLU meter compared with four other methods for determining the total glucosinolate content of rapeseed.

Sample	TRUBLUGLU Meter	THYMOL <sup>(1)</sup>	TMS GLUCOSE <sup>(2)</sup>	CGC GLC-TMS <sup>(3)</sup>	HPLC <sup>(4)</sup>
BC86-18	9.2	4.2	5.5	4.7	5.1
Tobin (S)	17.4	14.7	15.8	14.1	14.0
Westar	33.9	25.2	28.1	28.2	24.2
Tobin(Cert)	26.5	27.6	27.5	25.3	23.1
BL802	47.2	51.2	51.5	47.9	42.4
S.E. (5)	0.65	0.58	0.40	0.41	0.73
Overall mean	26.8	24.5	25.7	24.0	21.8

(1) McGregor and Downey 1986.

(2) Olsson et al. 1980.

(3) Daun et al. 1989.

(4) McGregor 1985.

(5) Pooled standard error of the means for  
3 determinations.

All values expressed in  $\mu\text{mol/g}$  oil-free meal.