OIL CROPS: BRASSICA SUBNETWORK

PROCEEDINGS OF THE THIRD WORKSHOP, QUALITY TRAINING, AND CHINESE PROJECT REPORTS, HELD IN SHANGHAI, PEOPLE'S REPUBLIC OF CHINA, 21–24 APRIL 1990

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Edited by
Abbas Omran
Technical Advisor, Oilcrops Network

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NEW METHODS OF MYROSINASE BIOREACTOR AND GLUCOSE SENSOR FOR RAPID AND ACCURATE ASSAY OF GLUCOSINOLATES IN RAPESEEDS

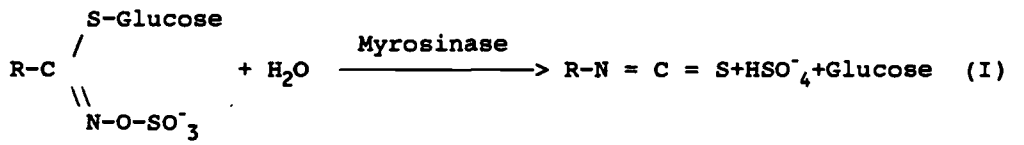
Zhong Yi Yuan, Xiao Jun Wang, Tian Min Zhu, Pei Ying Chen and Xin Song Ji*

Shanghai Institute of Biochem., Academia Sinica, and
***Shanghai Academy of Agricultural Sciences, Shanghai, China**

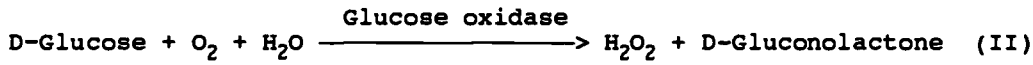
As the demand for protein is on the increase, the selection and development of rapeseed species with low glucosinolates is a challenge to the developing countries. A fast, simple, accurate, and precise method is essential for such a program.

Three years ago, we succeeded in constructing a glucose sensor, Fig. 1. An immobilized glucose oxidase membrane was attached on the top of Pt-Ag/AgCl electrode. The glucose sensor was also utilized for clinical diagnosis of blood sugar and monitoring of glucose change in the fermentation broth. The good linearity of glucose between 10^{-3} to 2×10^{-4} mol/L with r=0.999 and CV<3% made it possible to use 20 μl sample for each assay. Response time is 40 seconds. The glucose oxidase membrane possesses high operational stability that may be used for over 3000 assays, and also stable for storage at 4°C for 2.5 years or at 37°C for 4 months.

In the view of principle of enzyme catalysis, we believe that the combination of glucose sensor with myrosinase-hydrolysis is certainly an approach of promise for measurement of glucosinolate. This is illustrated as follows:



$$R\text{-}C(\text{S-Glucose})(\text{N-O-SO}^-_3) + H_2O \xrightarrow{\text{Myrosinase}} R\text{-}N=C=S + HSO^-_4 + \text{Glucose} \quad (I)$$



$$\text{D-Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose oxidase}} H_2O_2 + \text{D-Gluconolactone} \quad (II)$$

METHODOLOGY DEVELOPMENT

To determine the total glucosinolates in rapeseeds, we have been developing three types of combined techniques of bioreactor and biosensor:

1. Combination of free myrosinase and GOD electrode
   ↓
2. Combination of immobilized myrosinase and GOD electrode
   ↓
3. Co-immobilized myrosinase-glucose oxidase membrane/O_2 electrode

Fig. 2 indicates that pure sinigrin can be hydrolyzed by free myrosinase and the hydrolysates can be assayed on the glucose sensor to obtain a linear response between 1-5 μmoles/mL.

In the case of rapeseeds or de-fatted meal, glucosinolates were extracted from the crushed seeds or meal powder with boiling water, and then hydrolyzed by exogenous myrosinase for 5 minutes.

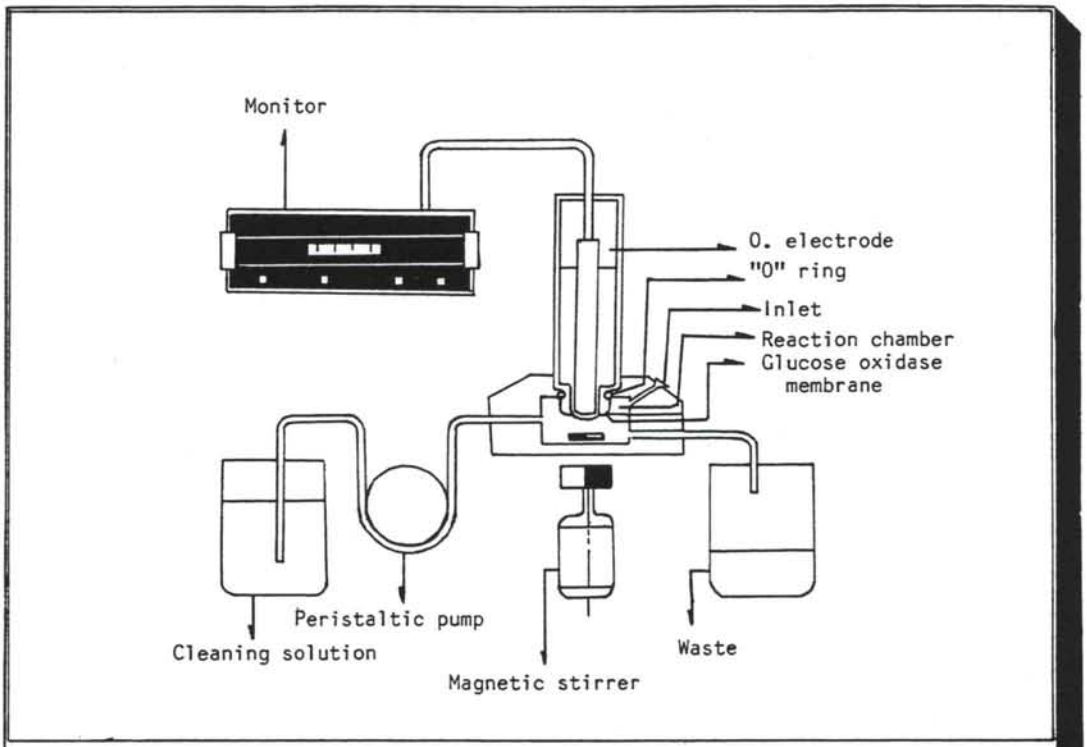


Fig. 1. Schematic diagram of the glucose sensor.

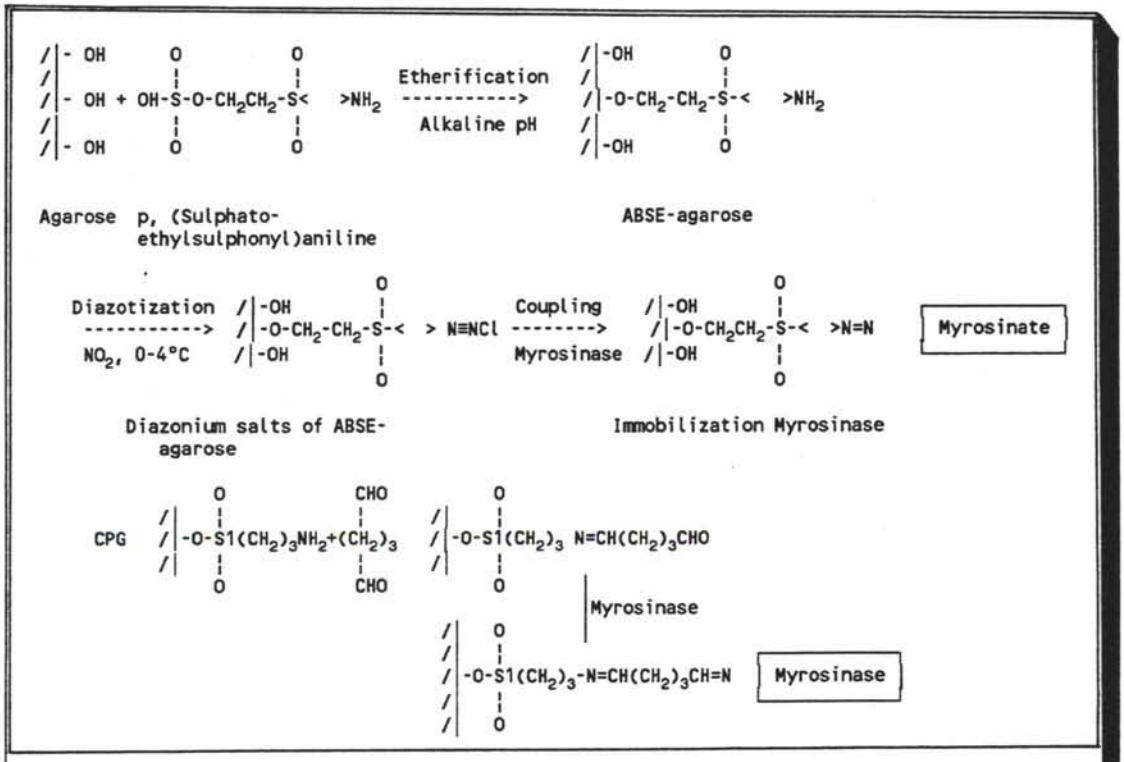


Fig. 2. Immobilization of Myrosinase

Finally, withdrew aliquots of hydrolysate and injected into glucose sensor. The response is obtained in one minute. As shown in Fig. 3, glucosinolate contents between 3 to 130 μmoles/g were linearly related to the response data. The precision and accuracy of the procedure are good as data listed in Tables 1 and 2.

Table 1. Analytical recovery rate of sinigrin in measurement of glucosinolates using the enzyme-enzyme electrode procedure.

Concntration of gluco-sinolate in rape-seeds (μmoles)	Added amount of Sinigrin (μmoles)	Detected amount of total gluco-sinolates (μmoles)	Recovery rate of Sinigrin (%)
	16.0	38.5	103.1
22.0	74.5	92.5	94.6
("86.66")	131.5	153.0	99.6
	16.0	109.5	96.9
94.0	74.5	176.0	110.1
("909.2")	131.5	236.5	108.4

Table 2. Precision of measurement of glucosinolates in rapeseeds using the combined procedure of enzyme and glucose sensor.

Sample	Times of measurement	Mean content of gluco-sinolates (μmol/g)	SD (%)	CV (%)
"24144-3"	10	7.5	0.27	3.6
"902-2"	9	105.7	1.51	1.4

Immobilization of enzymes is a well known technique to stabilized enzyme. We separated and purified myrosinase from seeds of white mustard (*Sinapis alba*) by ethanol frac-tionation, ion exchange chromatography and affinity chromatography on Con A-Agarose. Based on the developed methods and supports of enzyme-immobilization in our laboratory, the high activity of myrosinase was covalently bound on ABSE-Agarose (CL) and porous glass beads. This is illustrated as follows:

In the second procedure of determination of glucosinolates, the extract mentioned above was added in a bioreactor (a column containing 1 g immobilized myrosinase) and shaked for 5 minutes. An aliquot of effluent was injected into the glucose sensor. The relation between sinigrin degradated by immobilized enzyme and response on glucose sensor is shown in Fig. 4. Immobilized myrosinase could be repeatedly used for 500 times of hydrolysis. In the case of determination of total glucosinolates in rapeseed, a satisfactory correlation between immobilized myrosinase-glucose sensor method and the routine TMS-gas chromatography was obtained Fig. 5. We are pleased to know that this procedure was confirmed at Jiang Su Academy of Agriculture sciences.

In comparison with the conventional methods of glucosinolate assay, these combined procedures of bioreactor and biosensor have many advantages, such as accuracy, precision, simplicity, time saving, and cost saving, Table 3. Encouraged by those results, we are now turning our attention to prepare a Co-immobilized bi-enzyme system on the same membrane. In the preliminary experiments, we found that only extraction of glucosinolates is needed before assay on glucose sensor.

Table 3. A comparison of different methods for glucosinolates detection.

Method	Time
Enzyme-Enzyme electrode	15 min.
Gravimetric	6 hr.
Thiourea-UV	6 hr.
Palladium Chloride	3 hr.
GOD Kit	3 hr.
GOD paper	20 min.
TMS-GC	36 min.

ACKNOWLEDGEMENT

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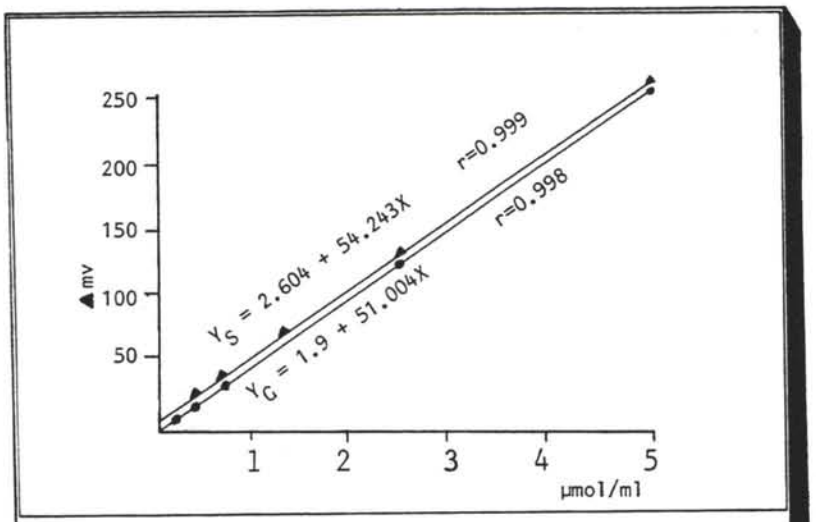

Fig. 3 Response-concentration relationship of glucose and sinigrin on GLUCOSE SENSOR. A sample containing various amounts of glucose or myrosinase-hydrolyzed sinigrin was injected into the GLUCOSE SENSOR.

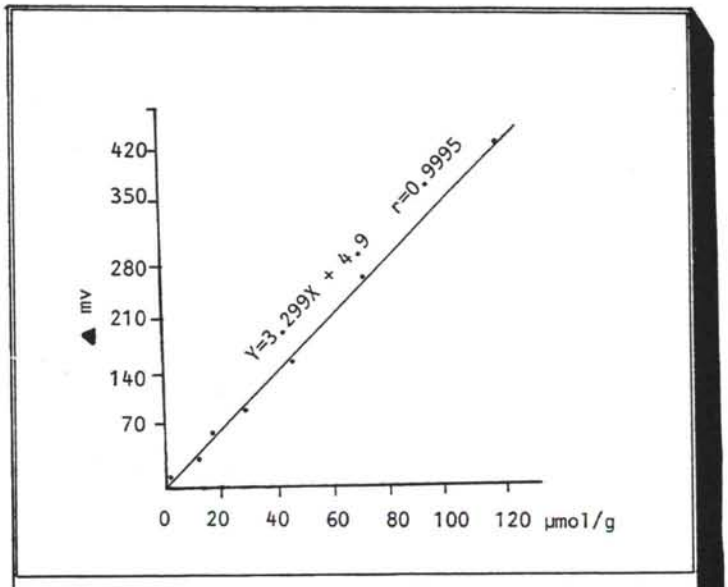

Fig. 4 Glucosinolate content in myrosinase hydrolysate and response on glucose sensor.

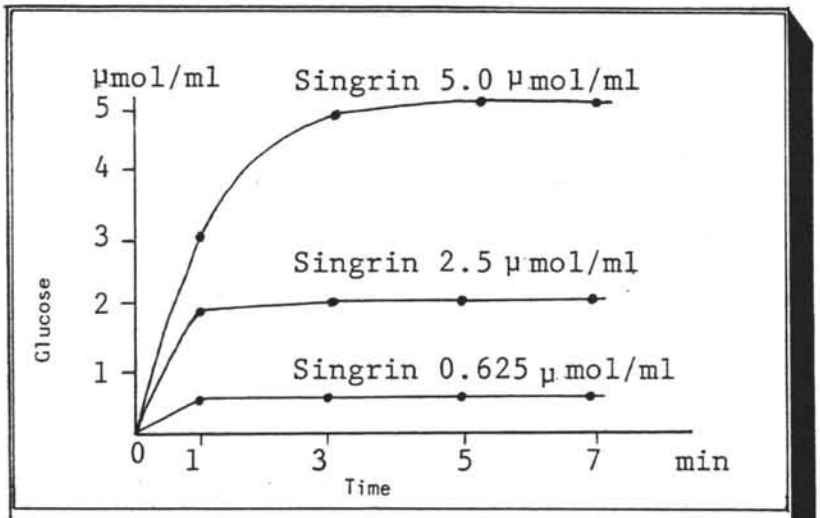

Fig. 5 . Relationship between Hydrolysis of Sinigrin by Immobilized Myrosinase & Release of Glucose.

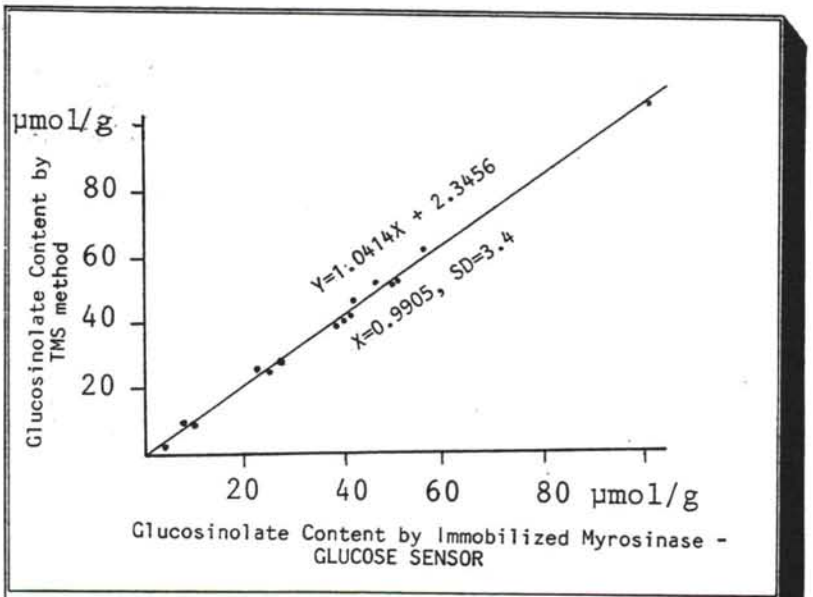

Fig. 6 . Correlation between Immobilized Myrosinase-Glucose Sensor Method and A Routine TMS Method.

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