# Improvement in Hydrolytic Procedure for GLC Determination of Non-Indolyl Glucosinolates in Rapeseed as Isothiocyanates

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**Some methods determine the non-indolyl glucosinolates content in rapeseed as their hydrolysates: the isothiocyanates and the oxazolidinethiones. These methods in their present form underestimate the amount of the glucosinolates content. In this investigation, a modified method was developed to give a better quantitative estimate, indicating a glucosinolate level five times that obtained by a typical existing method.**

Isothiocyanates (ITC) and oxazolidinethiones (OZT) result from the hydrolysis of non-indolyl glucosinolates (will be referred to as glucosinolates) of rapeseed by the action of thioglucoside glucohydrolase E.C.3.2.3.1 (myrosinase). Some methods that formerly were widely used determine the glucosinolate content of rapeseed as these two hydrolysates. In such methods the seed is subjected to thermal treatment prior to hydrolysis, to inhibit the formation of the nitriles (1,2) which are a competing hydrolysis product. However, this treatment deactivates the endogenous myrosinase; thus, to catalyze the hydrolysis, an exogenous myrosinase is used instead. This approach often underestimates the amount of glucosinolates present in rapeseed (3,4).

The authors felt that possible causes for this underestimation could be that since rapeseed glucosinolates are specific to their endogenous myrosinase (5), the use of an exogenous enzyme might result in an incomplete hydrolysis of the glucosinolates (6,7), or that adding the hydrolyzing solution before the extracting non-polar solvent might allow part of the formed ITC to react with the amino and sulfydryl groups of the seed protein to form substituted thioureas and dithiocarbamates (8,9).

## EXPERIMENTAL METHODS

Because changes in hydrolysis conditions have the same effect on the formation of ITC and OZT (1,2), for simplicity, the experimentation was concentrated on ITC only. The Youngs and Wetter method (10) for the determination of the glucosinolates was used for comparison. In this method the defatted dehulled Tower seed (*B. Napus*) was heated at 100 C for 16 hr. One g (all weights are dry basis) of the heated seed was placed in a 20-ml culture tube. Five ml of the hydrolyzing solution containing an exogenous myrosinase (from *Sinapis alba*) at a concentration of 6 mg/ml were then added, followed by 2 ml of methylene chloride containing 40 mg/l n-butyl isothiocyanate as an internal standard. The contents of the tube which were at pH 6-7 were mixed, with the aid of a 6 mm glass bead, in a rotary shaker revolving at 30 rpm in a 23 C water bath. After 2 hr mixing, the emulsion was broken by centrifuging at about 450 × g. One μl of the methylene chloride layer was then injected into a gas chromatograph fitted with stainless steel column (180 cm long and 4.5 mm inside diameter), packed with 20% FFAP on a 60/80 mesh Anakrom ABS. Helium was the carrier gas at a flow rate of 32 ml/min. Temperatures of the injection port, oven and FID were 250, 180 and 300 C, respectively. The formed ITC were identified by gas chromatography/mass spectrometry (GC/MS). The amounts of the identified ITC in mg/g were calculated according to Youngs and Wetter (10) and then converted to μmol/g. The amount of the released cyano-epithiobutane (threo and erythreo) and 1-cyano-2-hydroxy-3-butene (will be referred to as nitriles) were determined by the method of Daxenbichler et al. (11).

In the modified method under investigation, the defatted dehulled seed was not heated. To investigate the effectiveness of increasing the water-to-dehulled defatted seed (W/S) ratio in inhibiting the formation of the nitriles during the hydrolysis by the endogenous enzyme (12), ratios of 5:1, 10:1 and 150:1 ml/g were used. For the first two ratios 1.0 g seed was weighed in each culture tube, while for the last ratio only 0.1 g was used. In reverse order to that of the Youngs and Wetter method, the 2 ml methylene chloride containing the standard was added to the seed before the water. Another determination was carried out at a W/S ratio of 150:1 where the order of addition was the same as in the Youngs and Wetter method. The contents of all tubes were processed as described above for this method.

## RESULTS AND DISCUSSION

The formed ITC were identified to be mainly 3-butenyl isothiocyanate (BIT) and 4-pentenyl isothiocyanate (PIT). Also found were traces of 2-phenylethyl-. 4-methylthiobutyl- and 5-methylthiopentyl-isothiocyanates. Results of the determinations are presented in Table 1. From this table, it can be seen that at the same W/S ratio of 5:1, the modified method having a TITC (BIT + PIT) of 3.58 μmol/g is better than the Youngs and Wetter method, which gave 2.70 μmol/g. Increasing the W/S ratio to 10:1 further improved the determination to 4.38 μmol/g with the formation of only 0.3 μmol/g of nitriles as compared to 1.0 μmol/g for the W/S of 5:1 of the modified procedure. The ratio 150:1 gave the best result of 13.30 μmol/g TITC. The study was not extended beyond the ratio of 150:1 because at that ratio no nitriles were formed. The results indicate that the change in the order of addition and/or the use of the endogenous myrosinase at high W/S ratio improved the glucosinolate determination procedure. By comparing the result of 13.30 μmol/g obtained at the W/S ratio of 150:1 with that of 10.40 μmol/g obtained at the same ratio but with the order of addition as in the Youngs and Wetter method, it is concluded that both hydrolysis conditions contributed to the improvement.

This investigation has the following shortcomings which could be a subject of further study: It was carried out only on Tower rapeseed and at least the high- and

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**TABLE 1**

**Determination of ITC**

| | W/S, ml/g | BIT, μmol/g | PIT, μmol/g | TITC[a], μmol/g | S.D., μmol/g | Nitriles, μmol/g |
|---|---|---|---|---|---|---|
| As in Youngs and Wetter | 5:1 | 2.70 | 0 | 2.70 | 0.38 (3) | N.D. |
| Modified Method[b] | 5:1 | 3.50 | 0.08 | 3.58 | 0.08 (3) | 1.0 |
| Modified Method | 10:1 | 4.30 | 0.08 | 4.38 | 0.005 (5) | 0.3 |
| Modified Method | 150:1 | 12.40 | 0.90 | 13.30 | 0.60 (3) | 0.0 |
| Order of addition as in Youngs and Wetter | 150:1 | 10.40 | 0 | 10.40 | 0.11 (3) | 0.0 |

[a]TITC = BIT + PIT.

[b]Methylene chloride is added to defatted dehulled seed followed by water.

low-glucosinolate varieties of *B. napus* and *B. campestris* species should have been tested. Results obtained should have been compared with those of currently used methods which were developed to avoid giving low results, such as the trimethylsilylated desulfoglucosinolates method (3,4). The usefulness of the high W/S ratio in the analysis of commercial meals should have been studied (this would require an exogenous myrosinase).

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