GAS CHROMATOGRAPHIC DETERMINATION OF INDOLE GLUCOSINOLATES - A REEXAMINATION

B.A. Slominski and L.D. Campbell

Introduction

In recent years, gas-liquid chromatography (GLC) has been used extensively to analyze for glucosinolates as hydrolysis products following treatment with myrosinase enzyme or as trimethylsilylated (TMS) desulpho-derivatives. This methodology has not been considered totally satisfactory for the determination of the indole glucosinolates. Poor recovery of indoles is indicated from the fact that the detector response factor used for the major indole, 4-hydroxyglucobrassicin is two-fold that calculated from the TMS-glucosinolate carbon number. The objective of the current study was to investigate the factors contributing to the underestimate of indole glucosinolates by GLC.

Experimental Methods

Samples of commercial canola (low glucosinolate rapeseed) meal were obtained from crushing plants and canola seed samples were provided by B.R. Stefansson of the Department of Plant Science, University of Manitoba, Winnipeg. Individual glucosinolates were determined according to the method of Thies as modified by Heaney and Fenwick (1980, 1982) and by Daun and McGregor (1983). For extraction of glucosinolates and inactivation of myrosinase enzyme, 100 mg commercial canola meal or defatted canola seed was weighed into a test tube which was then placed in a boiling water bath for 10 minutes. Subsequent to the addition of 1 ml of hot (95°C) water, the contents were mixed well and heat-treated for an additional 3 minutes. This standard heat-treatment/extraction procedure was used for all glucosinolate analyses, except for those experiments in which heat-treatment parameters were varied.

Following heat-treatment, the sample was cooled immediately and 1 ml of internal standard solution (1 μmol ml^{-1} allyl or benzyl glucosinolate) was added. Benzyl glucosinolate was used as the internal standard for analysis of all commercial canola meal. Prior to centrifugation (2000g for 10 min) 125 μl of a 1:1 (v/v) mixture of 0.5 M barium acetate and 0.5 M lead acetate were added. The resulting supernatant (0.5 ml) was applied to a DEAE Sephadex A-25 column (pyridine acetate form, 8 mm x 5 mm i.d.) which was then washed with 1 ml of 0.02 M pyridine acetate. A purified sulphatase solution (50 μl) was added to the column and the contents allowed to stand overnight at room temperature. The desulphoglucosinolates were then eluted with water (4 x 0.5 ml) into a 2 ml sealed-cap vial. The standard procedure involved the use of 2 ml of water for the elution step

except for experiments in which the elution procedure was varied.

Following drying under a stream of nitrogen at 60°C, 100 µl of pyridine, 50 µl MSTFA and 10µl TMCS were added to each vial which was then capped, mixed and heated at 120°C for 20 minutes. The derivatized desulphoglucosinolates were separated using a Varian Vista 6000 gas chromatograph equipped with a flame ionization detector and a Vista 402 computer. A glass column (1.2 m x 2 mm i.d.) packed with 2% OV-7 on Chromosorb W,AW-DMCS (100-120 mesh) was used with helium gas at a flow rate of 40 ml min^{-1}. The oven temperature was kept at 200°C for 4 min, then increased at 5°C min^{-1} to 275°C. Injection port and detector temperatures were 280°C and 300°C, respectively. Relative response factors (RRF) were calculated from the ratios of the internal standard TMS carbon number and the respective glucosinolate TMS carbon numbers with the exception of the RRF for 4-hydroxyglucobrassicin which was determined experimentally.

A study was conducted to determine the extent of decomposition of glucosinolates as a consequence of the heat-treatment used to inactivate myrosinase enzyme in the glucosinolate-analysis procedure. The influences of dry and wet heat-treatments were tested independently. To test the effect of dry heat-treatment, test tubes containing a 100 mg sample of commercial canola meal were placed in a boiling water bath for varying time periods (0, 2.5, 5, 10 and 15 minutes). One ml of hot water (95°C) was then added and the samples were maintained in the water bath for a further 5-minute time-period. The influence of wet heat-treatment was determined by subjecting samples (100 mg commercial canola meal) to a constant dry heat-treatment (5 minutes in a boiling water bath). One ml of hot water (95°C) was then added to each sample and the heat-treatment was continued for varying time periods (0, 2.5, 5, 10 and 15 minutes),. Glucosinolate analysis using benzyl glucosinolate as the internal standard was conducted on all samples as described above.

A subsequent study was conducted to determine the volume of water required to accomplish complete elution in the glucosinolate-analysis procedure of all glucosinolates from the DEAE-Sephadex A-25 column. Samples of commercial canola meal were analyzed for glucosinolates according to the procedure described above except that the volume of water used to elute the desulphoglucosinolates from the Sephadex column was varied. The volumes of water studied included; 0.5, 1, 1.0, 1.5, 2, 2.5 and 3 ml.

In a final study the glucosinalbin and indole glucosinolate contents of 11 samples of commercial canola meal and 7 samples of defatted canola seed were determined by two methods of analysis. Results according to GLC methodology as described above were compared with those estimated from thiocyanate ion (SCN) release following treatment with myrosinase enzyme (Heaney and Fenwick 1982).

Results and Discussion

Analysis of gas chromatograms of pre-trimethylsilylated desulphoglucosinolates from commercial canola meal and defatted canola

seed indicated that in addition to the common glucosinolates known to be present in rapeseed, the glucosinolates, glucosinalbin and sinigrin, were shown to be present in samples of canola meal. The data for canola seed and to a lesser extent that for canola meal indicated the presence of relatively high contents of indole glucosinolates.

The influence of heat-treatment on the glucosinolate content of commercial canola meal is shown in Figures 1 and 2. Dry heat-treatment for varied time-periods followed by 5 minutes of wet heat-treatment resulted in loss of indole glucosinolates but did not affect glucosinalbin or any of the aliphatic glucosinolates (Figure 1). The percent loss of indole glucosinolates was constant over all time-periods and averaged approximately 15%. Constant dry-heat treatment (5 minutes) followed by varying time-periods of wet heat-treatment resulted in substantial loss of indole glucosinolates that varied with time, but had little or no effect on aliphatic glucosinolates or glucosinalbin (Figure 2). The effect of wet heat-treatment on indole glucosinolates increased from no effect at the 0 time-period to a loss of about 30% at 15 minutes of heat-treatment. Wet heat-treatment influenced the level of free SCN in the samples and the response was dependent on time-period. The SCN content varied from a low of 0.04 $\mu mol\ g^{-1}$ at the 0 time-period to 0.9 $\mu mol\ g^{-1}$ at 15 minutes of heat-treatment.

The volume of water used to elute the desulphoglucosinolates from the DEAE-Sephadex column had a marked influence on the amount of glucosinolates eluted. Recovery of glucosinolates was dependent on the volume of water used and approached 100% only when large volumes (i.e. > 1.5 to 2 ml) were used (Figure 3). The effects were most pronounced for indole glucosinolates and differed depending on the internal standard used.

A number of samples of commercial canola meal and samples of defatted canola seed representing several varieties (cultivars) were analyzed for SCN-releasing glucosinolates by two methods. The data presented in Table 1 indicate that values obtained using GLC methodology compared well with those estimated from the determinations of SCN released following the treatment of samples with mynosinase enzyme. Glucosinalbin was shown to be present in commercial meal at a variable concentration but was not detected in seed samples.

Conclusions

The results indicate that all glucosinolates, including the indole glucosinolates, were well separated with the chromatographic conditions used in the GLC method. The heat treatment required for the inactivation of myrosinase in the extraction step of the analysis procedure was shown to affect the analysis of glucosinolates-particularly that of indole glucosinolates. In addition, the elution of glucosinolates from a DEAE sephadex column was shown to cause variability in analytical results. This effect was dependent upon the volume of water used as well as upon the internal standard used and was most pronounced for indole glucosinolates. With modifications,

the GLC methodology was shown to be adequate as a routine procedure for the analysis of glucosinolates in canola meal and seed.

Acknowledgements

Partial funding provided by the Natural Sciences and Engineering Research Council of Canada and by the Canola Utilization Assistance Program of the Canola Council of Canada is gratefully acknowledged.

References

Heaney, R.K.and G.R. Fenwick, 1980. The quantitative analysis of indole glucosinolates by gas chromatography - The importance of the derivatization conditions. Journal of the Science of Food and Agriculture 31, 593-599.

Heaney, R.K. and G.R. Fenwick, 1982. The analysis of glucosinolates in Brassica species using gas chromatography. Direct determination of the thiocyanate ion precursors, glucobrassicin and neoglucobrassicin. Journal of the Science of Food and Agriculture 33, 68-70.

Daun, J.K. and D.I. McGregor, 1983. Glucosinolate analysis of rapeseed (canola). Agriculture Canada, Winnipeg, pp.1-32.

Table 1. The contents in commercial canola meal and defatted canola seed of SCN-releasing glucosinolates[1] determined by two different methods (μmole g^{-1})

	Gas chromatography method				
Sample	Gluco-sinalbin	Gluco-brassicin	OH-Gluco-brassicin	Total	Thiocyanate ion method
Meal(11)[2]	4.88	0.44	2.55	7.87	7.41
Seed(7)	n.d.[3]	0.42	7.56	7.98	8.90

[1]SCN-releasing glucosinolates = glucosinalbin, glucobrassicin and 4-hydroxyglucobrassicin.

[2]Number of samples analyzed.

[3]n.d. = not detected.

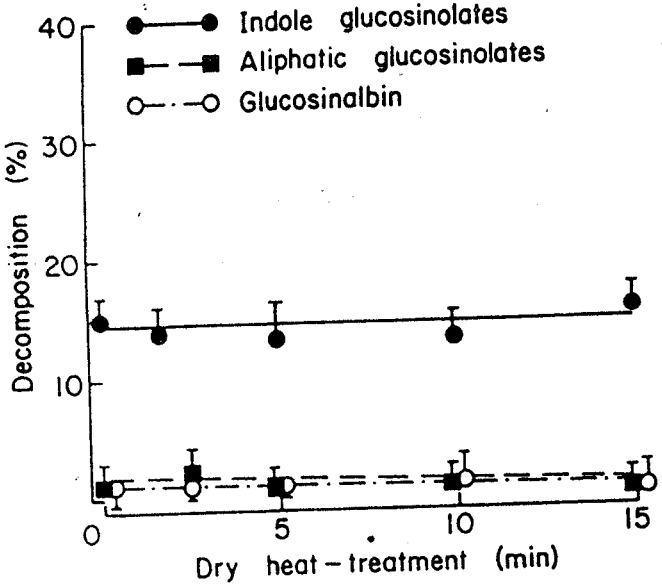

Figure 1. Effect of varying times of dry heat-treatment (95°C) and a constant amount of wet heat-treatment (95°C for 5 min) on the contents of glucosinolates in a sample of commercial canola meal.

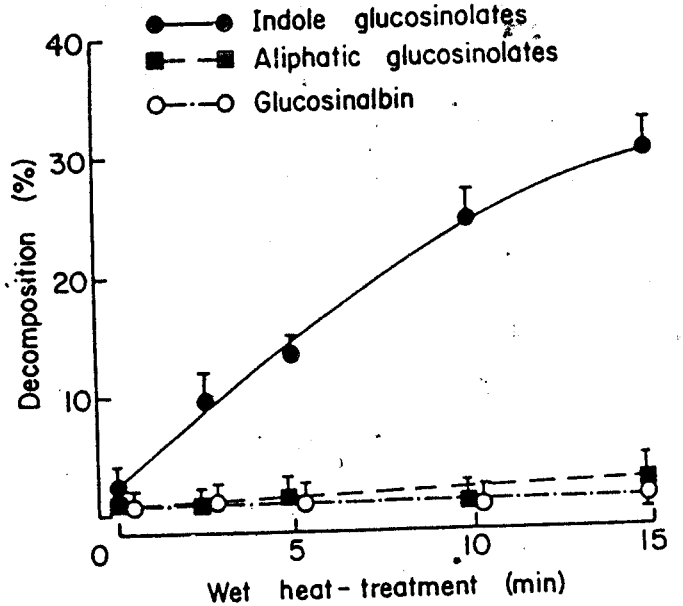

Figure 2. Effect of varying times of wet-treatment (95°C) and a constant amount of dry heat-treatment (95°C for 5 min) on the contents of glucosinolates in a sample of commercial canola meal.

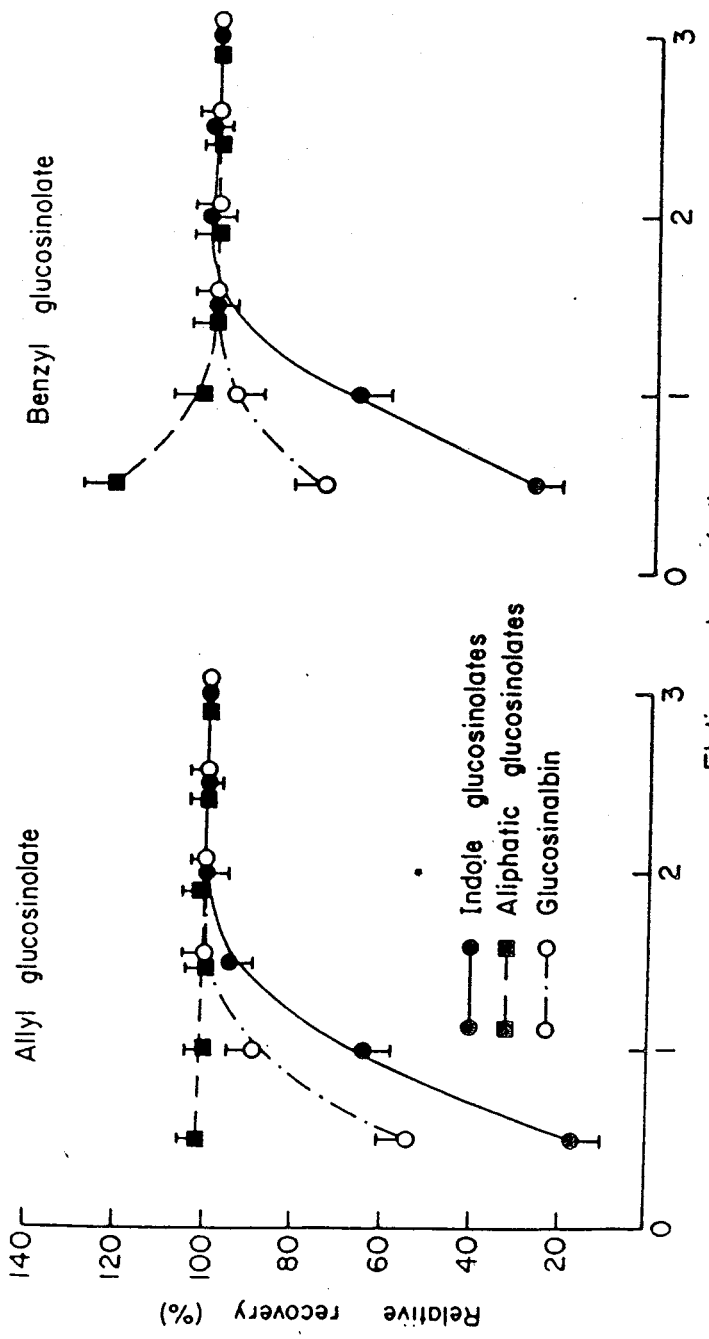

Figure 3. Effect of different volumes of water on the relative recovery of glucosinolates from the DEAE-Sephadex column using allyl and benzyl glucosinolate as internal standards.