THE EFFECT OF METHANOL-AMMONIA-WATER TREATMENT ON THE DEGRADATION OF GLUCOSINOLATES IN CANOLA AND AS ISOLATED COMPOUNDS

F. Shahidi, Department of Biochemistry, Memorial University of Newfoundland, St. John's, Newfoundland, A1B 3X9, Canada, and J.E. Gabon and L.J. Rubin, Department of Chemical Engineering, University of Toronto, Toronto, Ontario M5S 1A4, Canada

INTRODUCTION:

Canadian rapeseed varieties low in glucosinolates (less than 3 mg/g) and erucic acid (less than 2%) content called CANOLA, have presented a more attractive oilseed processing than previously grown rape cultivars. However, these levels of glucosinolates are still too high to allow their incorporation into food products. Methods have been utilized to inactivate the enzyme, myrosinase, which is primarily responsible for glucosinolate hydrolysis (Maheshwari et al., 1981), thus leaving intact glucosinolates in the proteinaceous meal after oil extraction. However, these glucosinolates are still capable of inducing undesirable effects by forming toxic aglucones in the gastro-intestinal tract (Fenwick et al., 1983). Recently, a novel process for their removal from canola (Rubin et al., 1984) and from Midas rapeseed and mustard seed (Naczk et al., 1986) was developed. The crushed seeds were treated with a two-phase extraction solution. The first phase was ammonia in methanol containing 5% water, and hexane which extracted the oil was the second phase. An improved meal with a light colour and bland taste was obtained, and its glucosinolate content was below the detection limit of Wetter and Youngs' method (Wetter and Youngs, 1976). However, the fate of extracted glucosinolates in this process was not known.

In this investigation, the effect of $CH_3OH/NH_3/H_2O$ treatment on the concentration of different glucosinolates in canola is reported. The nature of major breakdown products of glucosinolates, either in canola or in pure form, is also reported.

MATERIAL AND METHODS:

Canola seed (Altex variety) was obtained from POS Pilot Plant Corporation in Saskatoon, Saskatchewan. Allyl glucosinolate was obtained from Aldrich Chemical Company and benzyl glucosinolate was acquired through the Canola Council of Canada, Winnipeg, Manitoba. Gluconapin and progoitrin were kindly supplied by Dr. G. R. Fenwick, AFRC Food Research Institute, Norwich, U. K. Glucoiberin was generously provided by Dr. H. Sørensen, Chemistry Department, Royal Veterinary and Agricultural University, Copenhagen, Denmark.

The derivatizing reagents, N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA) and trimethylchlorosilane (TMCS), and pyridine (silylation grade) were obtained from Pierce, Rockford, Illinois. Other chemicals were purchased from Sigma Chemical Company.

Canola meal, hexane extracted or $CH_3OH/NH_3/H_2O$ treated, was prepared as described elsewhere (Rubin et al., 1984). The glucosinolate

content of meal or solutions of pure glucosinolates was determined by gas chromatography using the method of the Canadian Grain Commission, as developed by Heaney and Fenwick (1980) and modified by Daun and McGregor (1981), with slight modifications.

TMS derivatives of desulphoglucosinolates were separated on a fused silica SPE-20 column (30m x 0.25mm ID) using a Hewlett-Packard gas chromatograph model 5890A equipped with flame ionization detector and a 3392A Hewlett-Packard area integrator. Helium was used as the carrier gas. The separation of underivatized desulphoglucosinolates was achieved on a Hewlett-Packard ODS- Hypersil (C18) reversed-phase column (5 um packing; 200mm x 4.6mm ID) using a HP 1090A HPLC coupled to a diode array detector at a wavelength setting of 227.5nm (Minchinton et al., 1982). A two-component solvent system consisting of water and acetonitrile was employed. The identity of desulphoglucosinolates was confirmed by electron impact (EI) and ammonia chemical ionization (CI) mass spectra of their TMS derivatives.

Glucone degradation products were identified as their TMS derivatives. Volatile degradation products were extracted into CH_2Cl_2 and were analyzed by gas chromatography at a split ratio of 30, using helium as the carrier gas. Both non-polar (SPB-20) and polar (Supelcowax 10) GC columns were used (30m x 0.25mm ID).

The thiocyanate ion content was determined using the procedure of Johnstone and Jones (1966) as modified by McGregor (1978).

RESULTS AND DISCUSSION:

The content of individual glucosinolates in the meals prepared from hexane-extracted and $CH_3OH/NH_3/H_2O$ treated canola (Altex variety) was determined by both GC and HPLC. Results in Table 1 show a marked reduction in the amount of glucosinolates in the treated meals. From the HPLC analysis, a better quantitation of indole glucosinolates was possible and the total glucosinolate content determined by this method was greater than those determined by GC.

The breakdown products of glucosinolates from Altex meal are given in Table 2. Results show that the glucosinolates were mainly extracted, in the intact form, into the polar phase which also included most of the degradation products. Some breakdown products were also found in the oil and in the proteinaceous meal. No oxazolidinethrione was detected in the meal, in the extracted oil, or in the gums. The equivalent sulphur content of the oil from breakdown products was less than 1 ppm.

Pure glucosinolates - allyl, 3-butenyl, 2-hydroxy-3-butenyl, 3-methylsulphinylpropyl, and benzyl - which were treated with $CH_3OH/NH_3/H_2O$ in model experiments showed a maximum of 13.4% decomposition (Table 3) and the major aglucone breakdown products were their corresponding nitriles. Thioglucose and its dimer were the major sugars resulting from the decomposition of glucosinolates.

REFERENCES:

Daun, J. K. and McGregor, D. I. 1981. Glucosinolate analysis of rapeseed (canola). Method of Canadian Grain Commission, Grain Research Laboratory, Canadian Grain Commission, Winnipeg.

Fenwick, G. R., Heaney, R. K. and Mullin, W. J. 1983. Glucosinolates and their breakdown products in foods and food plants. CRC Critical Reviews in Food Science and Nutrition 18:123.

Heaney, R. K. and Fenwick, G. R. 1980. The analysis of glucosinolates in Brassica species using gas chromatography. Direct determination of the thiocyanate ion precursors, glucobrassicin and neoglucobrassicin. Journal of Science of Food and Agriculture 31:593.

Johnstone, T. D. and Jones, D. I. H. 1966. Variation in the thiocyanate content of Kale varieties. Journal of Science of Food and Agriculture 17:70.

Maheshwari, P. N., Stanley, D. W. and Gray, J. I. 1981. Detoxification of rapeseed proteins. Journal of Food Protection 44:459.

McGregor, D. I. 1978. Thiocyanate ion, a hydrolysis product of glucosinolates from rape and mustard seed. Canadian Journal of Plant Science 58:795.

Minchinton, I., Sang, J., Burke, D. and Truscott, R. J. W. 1982. Separation of desulphoglucosinolates by reversed-phase high-performance liquid chromatography. Journal of Chromatography 247:141.

Naczk, M., Shahidi, F., Diosady, L. L. and Rubin, L. F. 1986. Removal of glucosinolates from Midas rapeseed and mustard seed by methanol-ammonia. Canadian Institute of Food Science and Technology Journal 19:75.

Rubin, L. J., Diosady, L. K. and Philips, C. R. 1984. Extraction of oil bearing seeds. U. S. Palent 4 460 504.

Wetter, L. R. and Youngs, C. G. 1976. A thiourea-UV assay for total glucosinolate content in rapeseed meals. Journal of the American Oil Chemists' Society 53:162.

Table 1. Glucosinolate content in Altex meals before and after $CH_3OH/NH_3/H_2O$ Treatment as determined by GC and HPLC. [a]

Glucosinolate	-R	GC		HPLC	
		Hexane-extracted	Treated	Hexane-extracted	Treated
Sinigrin	Allyl	0.41±0.03	0.07±0.00	0.51±0.07	0.23±0.05
Gluconapin	3-Butenyl	2.43±0.11	0.20±0.02	2.41±0.16	0.22±0.04
Glucobrassicanapin	4-Pentenyl	0.23±0.22	0.02±0.00	0.25±0.05	0
Progoitrin	2-OH-3-Butenyl	4.96±0.22	0.52±0.06	4.61±0.41	0.62±0.11
Gluconapoleiferin	2-OH-4-Pentenyl	0.33±0.03	0.03±0.00	0.40±0.04	0.20±0.04
Glucoerucin	4-CH_3S-Butyl	0.13±0.01	0.03±0.00	0.10±0.00	0
Gluconasurtiin	Phenethyl	0.27±0.02	0.06±0.01	0.39±0.06	0
Sinalbin	p-OH-Benzyl	0.10±0.01	0	-	-
Glucobrassicin	3-Indolylmethyl	0.31±0.02	0.03±0.00	3.15±0.22	0.52±0.08
4-OH-Glucobrassicin	4-OH-3-Indolylmethyl	2.40±0.23	0.05±0.00	6.43±0.51	0.91±0.11
TOTAL		11.57±0.70	1.01±0.09	18.25±1.51	2.73±0.43

[a] Values are expressed in umol/g of defatted, moisture-free meal ± standard deviation for duplicate injections of three extracted samples. Solvent-to-seed ratio of 6.7.

Table 2. Glucosinolates and Their Decomposition Products in $CH_3OH/NH_3/H_2O$ Treatment of Altex Seed.[a]

Compound	Meal	Oil	Gums
glucosinolates	12.54	0.0	273.22
desulphoglucosinolates	0.21	0.0	5.33
glucose	0.22	0.0	2.81
thioglucose	0.49	0.0	3.65
thioglucose dimer	1.63	0.0	28.19
nitriles [b]	0.88	0.73	12.56
oxazolidinethione	0.00	0.0	4.03
isothiocyanates	0.11	0.16	2.31
furfuryl alcohol	0.05	0.0	0.56
thiocyanate ion	0.0	0.0	4.10

[a] The original hexane-extracted meal (28.24g seed weight) contained a total of 324.19 umoles of glucosinolates.

[b] Including hydroxy-and epithionitriles.

Table 3. The Degree of Decomposition of Glucosinolates Treated with $MeOH/NH_3/H_2O$ after 24 hours. [a]

Glucosinolate	-R	Decomposition %	Major Product [b]
Sinigrin	Allyl	13.4	3-Butenylnitrile
Gluconapin	3-Butenyl	10.2	4-Pentylnitrile
Progoitrin	2-OH-3-Butenyl	3.8	1-CN-2-OH-3-butene
Glucoiberin	3-CH_3-S-propyl	4.8	4CH_3S-Butylnitrile
Glucotropaeolin	Benzyl	9.60	Phenethylnitrile

a GC results.

b The yield of major aglucone products was >90%. The major glucone products were thioglucose and its dimer.