A METHOD FOR DETERMINATION OF GLUCOSINOLATES IN RAPESEED AS TMS-DERIVATIVES

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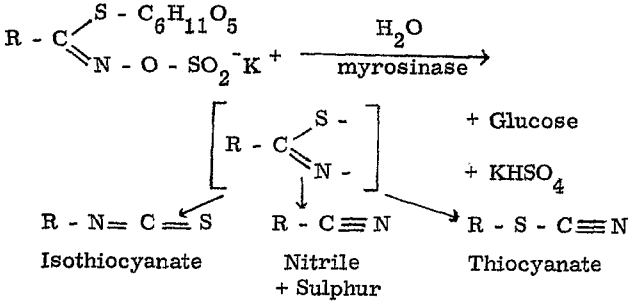
Thioglucosides, or as they nowadays should be called, glucosinolates, occur in all species of Cruciferae. The general formula for a glucosinolate is shown in fig. 1. It is the R-group that differentiates one glucosinolate from another.

About fifty different glucosinolates have been found in cruciferous plants.

Figure 1:



The glucosinolates are hydrolysed when wet, unheated plant material is crushed. The hydrolysis is catalyzed by enzymes, myrosinases, and the main split products can be isothiocyanates, nitriles and thiocyanates.

The most important glucosinolates in rapeseed, Brassica napus, and turnip rapeseed, Brassica campestris, are progoitrin, gluconapin, glucobrassicanapin and 2-hydroxy-4-pentenylglucosinolate (fig. 2).

Figure 2:

Trivial name	R-group	Systematic name
Gluconapin	CH_2=$CHCH_2CH_2$-	3-butenylglucosinolate
Glucobrassicanapin	CH_2=$CHCH_2CH_2CH_2$-	4-pentenylglucosinolate
Progoitrin	CH_2=$CHCHOHCH_2$-	2-hydroxy-3-butenyl-glucosinolate
	CH_2=$CHCH_2CHOHCH_2$	2-hydroxy-4-pentenyl-glucosinolate

It is difficult to determine the glucosinolates intact, because they are rather large molecules, with molecular weights of about 400, and as there are only minor differences between the glucosinolates there will be separation problems. Consequently, previous methods for the determination of glucosinolates in plant material have utilized split products formed during a controlled enzymatic hydrolysis.

The total glucosinolate content of a sample can be measured by determining how much glucose or sodium hydrogen sulphate is liberated during the hydrolysis.

For the determination of the amount of each glucosinolate in a sample, there are at least three methods, of which two measure the split products. For quantitative determination of the split products either gas-liquid-chromatography or UV-spectroscopy is used. The only method for glucosinolate analysis before hydrolysis is our so called TMS-(trimethylsilyl)-method. It is a further development of a method published by UNDERHILL and KIRKLAND in J. Chromatogr. 57 (1971). When we AB Karlshamns Oljefabriker and Alfa-Laval AB in cooperation started to develop a process for rapeseed protein concentrate we found it necessary to have a method more sensitive than previous methods for glucosinolate analysis. The TMS-method is thus specially designed for analysis of very low glucosinolate content in defatted meal of rapeseed and turnip rapeseed. A quantitative glucosinolate analysis of a defatted material with the TMS-method consists of the following steps (fig. 3).

Figure 3:

1) Milling
2) Leaching
3) Evaporating
4) Derivatising
5) Gas chromatographic determination
6) Calculation

The glucosinolates are leached from the plant material with ethanol:water 1:1 by volume at 110° C in a rotating test tube holder in a warming cupboard. In order to minimize the time of analysis we have optimized the leaching time. As you can see from Fig. 4, leaching for 40 minutes gives optimal response for progoitrin, gluconapin and glucobrassicanapin.

An aliquot of the liquid is then evaporated to dryness by means of a stream of nitrogen at 100° C. The residual moisture is removed by azeotropic evaporation with dichloromethane.

The trimethylsilyl derivatives of the glucosinolates are formed during reaction with a mixture of hexamethylenedisilazane:trimethylchlorosilane:pyridine in the proportions 2:1:10 by volume.

We have studied the temperature and time dependence of the derivatisation reaction. Fig. 5 shows that silylation for 60 minutes at 100° C and higher temperature gives constant response for the glucosinolates sinigrin, progoitrin and gluconapin and fig. 6 shows the time dependence of

Figure 4: Relative response versus leaching time
Leaching temperature 110° C
Internal standard Sinigrin

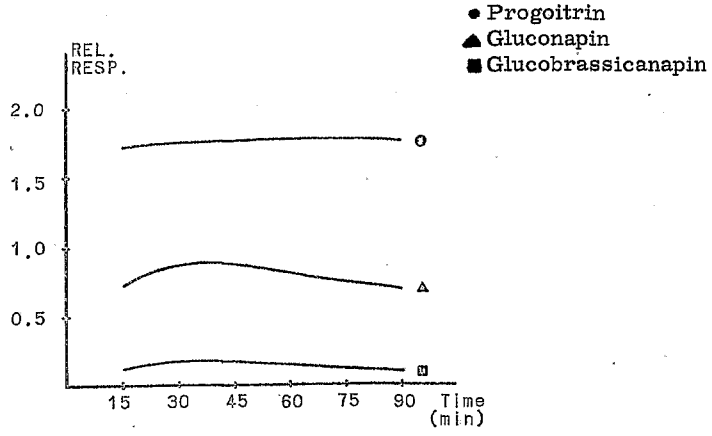


Figure 5: Relative response versus derivatisation temperature
Derivatisation time 60 min
Internal standard Tricosane

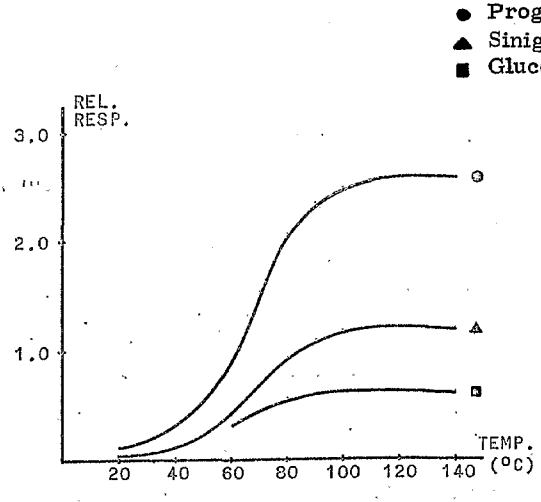
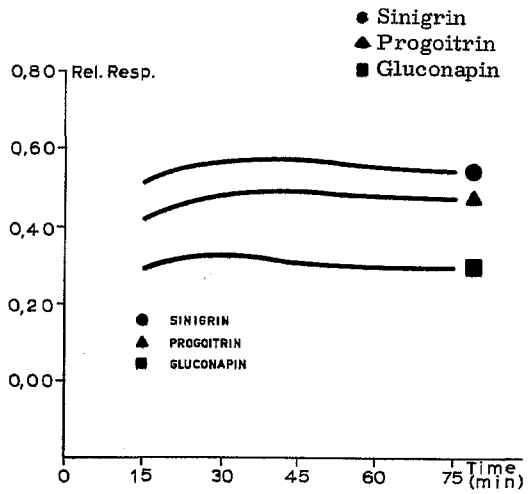


Figure 6: Relative response versus derivatisation time
Derivatisation temperature 110° C
Internal standard Tricosane



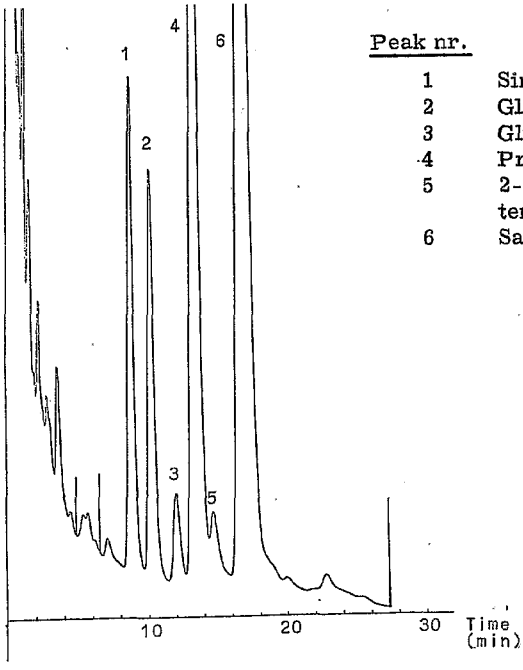
the reaction at 110° C. As you can see there is maximal response after 40 minutes of reaction. The silylation reaction is carried out in the same test tube-holder and warming cupboard as the leaching.

Studies on pure gluconapin progoitrin and sinigrin have shown that the degree of derivatisation after 40 min at 110° C varies between 95 to 98 %.

When silylation is completed the sample is ready for quantitative determination of each glucosinolate by gas-liquid chromatography. We separate the glucosinolates gluconapin, glucobrassicanapin, progoitrin and 2-hydroxy-4-pentenyl glucosinolate on a glas column, 2 m long with 2 mm inside diameter, packed with about 1.5 % OV 7 on Anakrom ABS 70/80 mesh. The carrier gas is helium at a flow rate of 25 ml/min. We use temperature programming with 2°/min between 170 and 200° C in order to avoid peak broadening of the most nonvolatile components. The chromatogram shown in fig. 7 is typical for rapeseed, and as you can see all glucosinolates are well separated except progoitrin and 2-hydroxy-4-pentenyl-glucosinolate, between which there is some overlapping. For the quantitative determination of the glucosinolates in rapeseed and turnip rape, sinigrin is used as an internal standard and is added to the sample before the leaching.

It is possible to determine as low as 0,03 mg of each glucosinolate per gram of sample or 0.001 mg of each split product per gram of sample with a relative standard deviation of about 15 %, and it is possible to de-

Figure 7: Chromatogram of silylated glucosinolates extracted from rapeseed. Column: Glas 2 m long i.d. 2 mm. Packed with 1,5 % OV 7 on Anakrom ABS 70/80 mesh
Carrier gas: He
Column temperature: Programming between 170 and 200° C with 2°/min
Injector temperature: 210° C
Detector temperature: 230° C
Paper speed: 5 mm/min



Peak nr.	Compound
1	Sinigrin
2	Gluconapin
3	Glucobrassicanapin
4	Progoitrin
5	2-hydroxy-4-pentenyl-glucosinolate
6	Saccharose

tect about 0.005 mg/g, but then the quantitative determination is uncertain.

The accuracy of the TMS-method has been verified by recovery studies. We added meal with known glucosinolate content to a sample with very low glucosinolate content. The sample was then analysed by the TMS-method, and as you can see from Table 1 the recovery is 100 %.

We have analyzed aliquots of the same sample with the TMS-method, with the method of YOUNGS and WETTER and with the method of APPELQVIST and JOSEFSSON, and as you can see from table 2, the results differ quite a lot, especially for the oxazolidinethiones.

Table 1: Recovery studies after addition of meal with "known" glucosinolate content

Glucosinolate	Added (mg)	Recovered (mg)	Yield (%)
Gluconapin	0.49	0.51	104
	0.48	0.49	100
Glucobrassicanapin	0.12	0.12	100
	0.11	0.11	100
Progoitrin	1.95	2.03	104
	1.90	1.82	95

Table 2: Result of analysis of rapeseed meal by the methods of Youngs and Wetter, the method of Appelqvist and Josefsson and the TMS-method. The results are given in mg of split products per gram of sample

Split product	Youngs and Wetter	Appelqvist and Josefsson	TMS-method
Butenylisothiocyanate	1.89		2.13
	2.45	3.03	2.64
Pentenylisothiocyanate	0.56		0.51
Vinyloxazolidinthione			8.90
	11.2	9.22	9.45
Allyloxazolidinthione			0.55

The relative standard deviation for butenyl- and pentenylisothiocyanate is about 3.5 % for both the method of Youngs and Wetter and the TMS-method but for the oxazolidinthions is the relative standard deviation about 2 % for the TMS-method and 6 % for the method of Youngs and Wetter.

We have used this method for routine analysis of glucosinolates in rapeseed and rapeseed protein concentrate from the Karlshamns-Alfa-laval process for more than one year, and it works very well.