GLUCOSINOLATE CONTENTS ANALYSIS IN POLISH BREEDING FORMS OF RAPE /BRASSICA NAPUS/

Piotr Kachlicki

Polish Academy of Sciences, Institute of Plant Genetics
Strzeszyńska 34, 60-479 Poznań, Poland

Introduction

The determination of glucosinolate contents and composition in rape seeds has become the major analytical task in this plant chemical studies since the erucic acid elimination by the breeding. Different analytical techniques for this purpose have been discussed recently /McGregor et al., 1983; Sørensen, 1985/. The progress made in this field gives us a range of methods differing in their precision, cost and instrumentation and an analyst must make a choice according to his needs. This report compares three of the routine methods used in this laboratory for the total glucosinolate determination. One of these methods - high performance liquid chromatography - allows the individual compounds quantitation as well.

Materials and Methods

The plant material for these experiments were 50 breeding forms of rape from the collection of Plant Breeding and Acclimatization Institute, Poznań.

Total glucosinolates were isolated and purified according to Brzeziński and Mendelewski /1984/ or Møller et al. /1985 a/ for determination with thymol or palladium chloride respectively. Quantitative analysis of individual compounds was carried out using high performance liquid chromatography according to Møller et al. /1985 b/. All the determinations of total glucosinolates were carried out in triplicate and with HPLC - in duplicate. The HPLC apparatus consisted of Octadecyl=Si 100 /Serva, FRG/

column and Liquochrom /Labor MIM, Hungary/ instrument with changeable wavelength UV monitor.

Results and Discussion

The results of the glucosinolate contents determination using Method A /based on the colorimetric measurement of the thymol - glucose reaction product/, Method B /Palladium - glucosinolate complex formation/ and HPLC of intact compounds are summarized in Table 1. As can be seen from these data, the results obtained using different methods are very close to each other. The respective correlation coefficients are:

- between methods A and B : 0.997
- between methods A and HPLC : 0.997
- between methods B and HPLC : 0.996

and the corresponding regression equations :

/1/ $Tgc_B = 1.026\ Tgc_A + 0.8$

/2/ $Tgc_{HPLC} = 1.046\ Tgc_A - 2.3$

/3/ $Tgc_{HPLC} = 1.015\ Tgc_B - 2.8$

where Tgc_A - Total glucosinolate contents measured with the method A

So high a correlation of both methods used for the total glucosinolate analysis indicates that they are equally acceptable in most applications. The results obtained using method B are generally slightly higher than these obtained with method A /Table 1, equation 1/. It seems to be likely that this difference comes from differences in the glucosinolate sample purification course in both methods. The critical step in this respect may be the lead acetate treatment of extracts used in method A while omitted in the procedure B. It is known that 4-hydroxy glucobrassicine is a very labile compound and may be hydrolised in the heavy ions presence. Such a phenomenon has been observed during HPLC analysis of purified rapeseed glucosinolates samples /data not shown/. The 4-hydroxy glucobrassicine share in the total glucosinolate amount decreased in these samples by about 20% upon the lead acetate treatment. In this light a minor modification in the

sample purification protocol of Brzeziński and Mendelewski /1984/ should be regarded.

The high performance liquid chromatographic analysis of rapeseed glucosinolates allows for quantitative determination of 10 compounds. In the described experiments some response factors were calculated using pure glucosinolates obtained from different plant materials in this laboratory. However, some of the compounds of interest /the indolyl glucosinolates and napoleiferin/ were not purified well enough to be chromatographic standards. In these cases the response factors of Møller et al. /1985 b/ have been adapted for the respective compounds contents calculations. The total glucosinolate contents from HPLC analysis was lower than this obtained using colorimetric methods, especially in the low glucosinolate rapeseeds /Table 1, equations 2 and 3/. On this basis it should be concluded that the literature response factors do not fit precisely to our HPLC system.

The glucosinolate contents in the same rapeseed samples were determined using the standard methods: gas - liquid chromatography of isothiocyanates plus spectrometric measurement of 5-vinyl-oxazolidine-2-thione /Michalski et al. 1987/. Those results obtained for double low rapes were sometimes much different from these presented here - in some cases their value equals to one fourth of our mean from the three methods. On the other hand, a good correlation of results was obtained in the high glucosinolate forms of rapeseed. Discussion of the identical problem has been presented at this Congress /Bjerg et al. 1987/.

Literature:

Bjerg B., L. M. Larsen, H. Sørensen, 1987. Reliability of Analytical Methods for Quantitative Determination of Individual Glucosinolates and Total Glucosinolate Content in Double Low Oilseed Rape. Proceedings of 7th International Rapeseed Congress, Poznań.

Brzeziński W., P. Mendelewski, 1984. Determination of Total Glucosinolate Content in Rapeseed Meal with Thymol Reagent.

Z. Pflanzenzuchtg. 93: 177 - 183

McGregor D.I., W.J. Mullin, G.R. Fenwick 1983. Analytical Methodology for Determining Glucosinolate Composition and Content. J. Ass. Offic. Analyt. Chem. 66: 825 - 849.

Michalski K., J. Krzymański, B. Byczyńska, 1987. Determination of Glucosinolate in Intact Seeds of Winter Rape /B. napus/ by Near Infrared Reflectance Method. Proceedings of 7th International Rapeseed Congress, Poznań

Møller P., A. Pløger, H. Sørensen 1987a. Quantitative Analysis of Total Glucosinolate Content in Concentrated Extracts from Double Low Rapeseed by the Pd - Glucosinolate Complex Method. In: Advances in the Production and Utilization of Cruciferous Crops /Ed. H. Sørensen/ Martinus Nijhoff/Dr. W. Junk Publ., Dodrecht/Boston/Lancaster. pp. 97 - 110

Møller P., O. Olsen, A. Pløger, K.W. Rasmussen, H. Sørensen 1985b. Quantitative Analysis of Individual Glucosinolates in Double Low Oilseed Rape by HPLC of Intact Glucosinolates ibid. pp. 111 - 126.

Sørensen H. 1985. Limitations and Possibilities of Different Methods Suitable to Quantitative Analysis of Glucosinolates Occuring in Double Low Rapeseed and Products Thereof. ibid. pp. 73 - 84.

Table 1.
Total glucosinolate contents in 50 samples of rape determined using different methods[§]

Sample No	Method A	Method B	HPLC total	Sample No	Method A	Method B	HPLC total
1	12.0	13.1	11.6	26	26.6	27.8	22.2
2	19.2	19.9	16.8	27	45.8	48.1	42.4
3	22.0	20.6	20.6	28	106.2	114.1	118.6
4	26.0	23.4	27.1	29	108.4	117.6	105.8
5	11.8	14.2	15.1	30	105.0	114.1	109.6
6	13.8	15.2	14.1	31	125.6	121.6	131.2
7	27.5	25.8	25.5	32	123.2	131.5	127.2
8	14.8	14.4	14.1	33	102.7	111.2	104.7
9	23.2	25.1	21.1	34	155.2	148.1	161.2
10	42.9	46.7	40.4	35	143.1	150.4	152.1
11	19.6	18.1	15.0	36	17.3	19.2	14.8
12	33.7	31.8	27.8	37	142.0	138.1	141.2
13	28.2	32.6	27.8	38	140.0	141.2	146.2
14	22.5	25.0	26.2	39	130.6	137.1	135.7
15	11.0	11.7	11.2	40	118.1	125.7	121.4
16	16.4	18.0	13.2	41	131.4	126.4	128.6
17	14.0	14.4	9.7	42	94.7	112.2	97.3
18	12.3	13.9	10.3	43	114.6	124.1	127.2
19	12.5	12.1	12.1	44	121.8	130.0	127.3
20.	13.5	15.1	12.4	45	134.0	141.1	138.9
21.	15.3	14.1	13.1	46	132.7	133.1	137.2
22	22.7	25.9	25.0	47	28.1	30.0	26.0
23	31.0	37.7	27.7	48	19.1	18.5	16.3
24	19.6	18.4	16.5	49	16.8	16.5	14.9
25.	23.6	24.1	26.5	50	128.1	130.0	117.3

[§]All the glucosinolate contents are expressed in micromoles per gram of dry, defatted meal.