DETERMINATION OF GLUCOSINOLATE IN INTACT SEEDS OF WINTER RAPE (B.NAPUS) BY NEAR INFRARED REFLECTANCE METHOD

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Modern plant breeding calls for fast methods for routine quantitative determination of various substances present in breeding material. One of such methods is NIR which allows for quick and complex determination of many substances. In breeding for improved rapeseed quality the NIR method can be used also for glucosinolates determination in whole intact seeds. Following the analysis, the undamaged seeds can be used to grow plants of next generations

Materials and methods

The NIR apparatus Infrapid 61 (Labor MIM,Hungary) renders possible measurements within the range of wavelength between 1300-2400 nm and facilitates calculations by different mathematical methods. To calibrate the apparatus a series of 50 rapeseed samples was employed. The samples represented various contents of four major glucosinolates (gluconapin, glucobrassicanapin ,progoitrin and napoleiferin) which ranged between 2-120 μmole/g fat free matter. The glucosinolate content was obtained by the GC and UV techniques (Byczyńska B. 1972 , Youngs Wetter 1967) .(Tab.1 item.2) Reflection spectra were taken off the samples and transferred to the computer memory. They were converted into second derivatives of optic density by the following formula:

$$D2OD\lambda=(2*\log r\lambda - \log r(\lambda-d\lambda) - \log r(\lambda+d\lambda))$$

$r\lambda$-reflection for wavelenght λ
$d\lambda$-wavelenght increment (16 nm)

As it is shown in Fig.2 the NIR spectra of whole intact seeds and of ground seeds seem to have been very much alike. On the basis of the latter observation, the whole intact seeds were used to take measurements of NIR. Screening for the most suitable wavelength was based on correlation analysis between the glucosinolate content and the second derivatives of optic density. The programme was developed to calculate correlation coefficients for all successive spectrum points. The curve of correlation coefficients for spectrum is given in Fig.3. The first wavelength charactereized by the highest correlation coefficient was selected on the basis of the above calculations. A regression equation was calculated for the selected wavelenght using the multiple regression coefficient programme (modified programme of Nowatech Katowice). The results are shown in Tab.1 item.3. The differential between the true and calculated data (dy1) were used for calculations of the correlation coefficient with a view to find the IInd wavelength. The above procedure was repeated to find further wavelengths (Tab.1 Item 4,6,8,10,12,14) Fig 4-8.

Results

Wavelengths were set up and coded in the Infrapid 61 apparatus. Standard samples were measured and regression coefficients calculated by internal programme. The coefficients introduced into the Infrapid 61 EPROM allows calculations of the total content of four main glucosinolates in rape seeds. The standard error of 6µmole/g f.f.m is permissible for samples with high glucosinolate level but is too big for good estimation of glucosinolate levels in low glucosinolate samples.Therefore the second calibration was made using the set of seed samples with glucosinolate content not exceeding 30 µmole /g fat free matter.A new regresion coefficients were calculated for the narrow range of glucoasinolate contents,which gives the estimation with standard error of only 4 µmole/g f.f.m.

Conclusions

The proposed method allows determination of glucosinolates in whole intact seeds. The precision of the method is satisfactory for selection .

Advantages

-fast and easy measurements
-not destructive for measured seeds
-no chemicals are needed
-not detrimental to the operator

References

1.Byczyńska B. Biuletyn IHAR (5) 57-61 , 1972

2.Youngs C.G Vetter L.R. J.Am. Oil Chem. Soc. (44)
551-554 , 1967

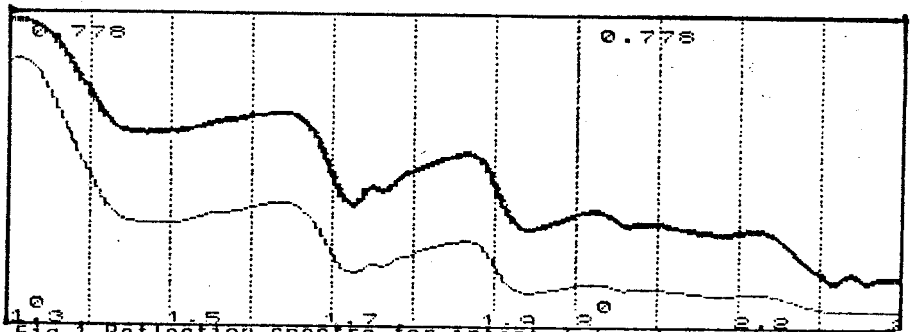

Fig.1 Reflection spectra for intact /—/ and /▬/ground seeds

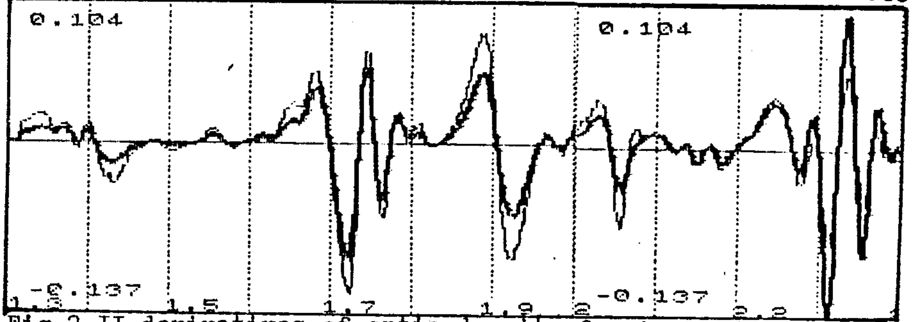

Fig.2 II derivatives of optic density for intact/—/ and ground/▬/ seeds /B.napus "Górczański"/

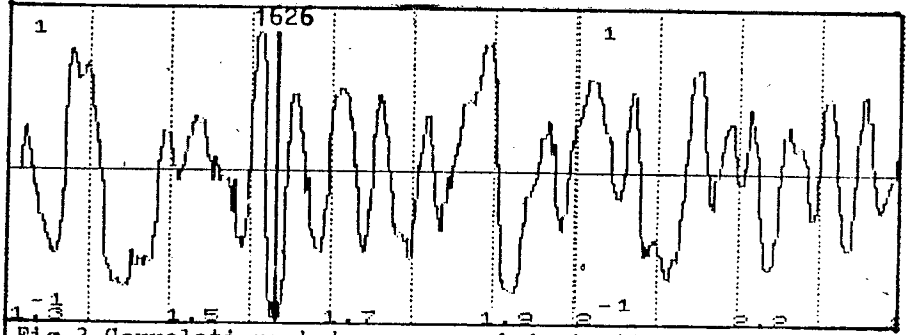

Fig.3 Correlations between second derivative spectrum/SDS/ and glucosinolate content /50 samples/

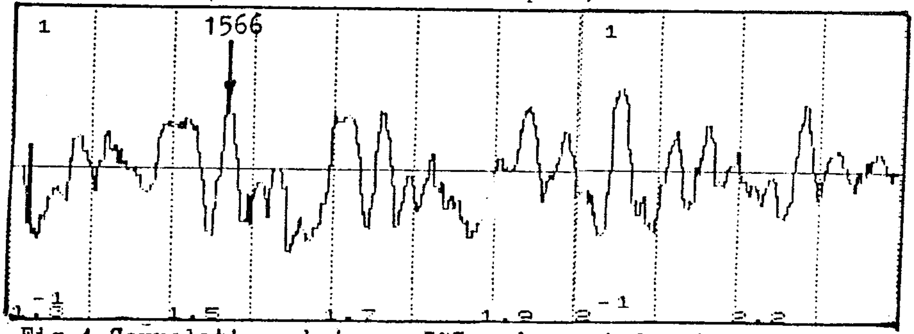

Fig.4 Correlations between SDS and remainder differences after regression with I wavelenght

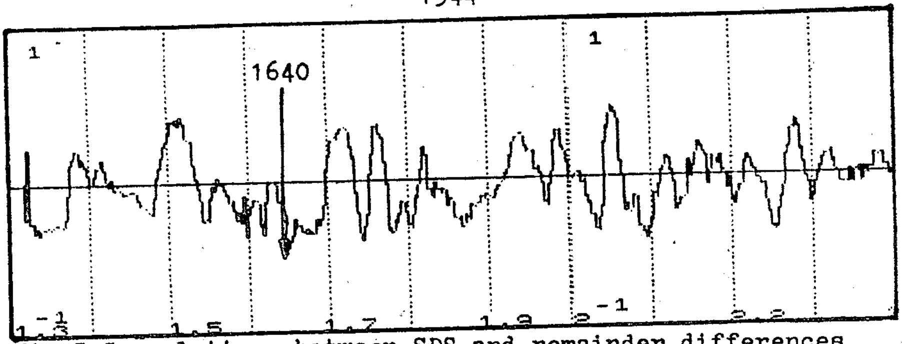

Fig.5 Correlations between SDS and remainder differences after regression with I and II wavelenghtes

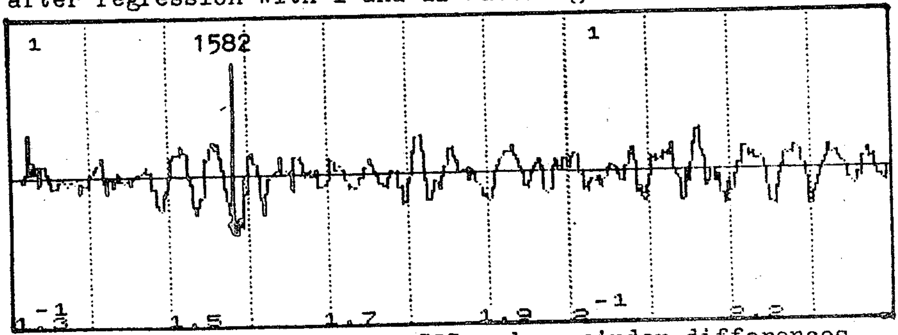

Fig.6 Correlations between SDS and remainder differences after regression with I,II and IIIwavelenghtes

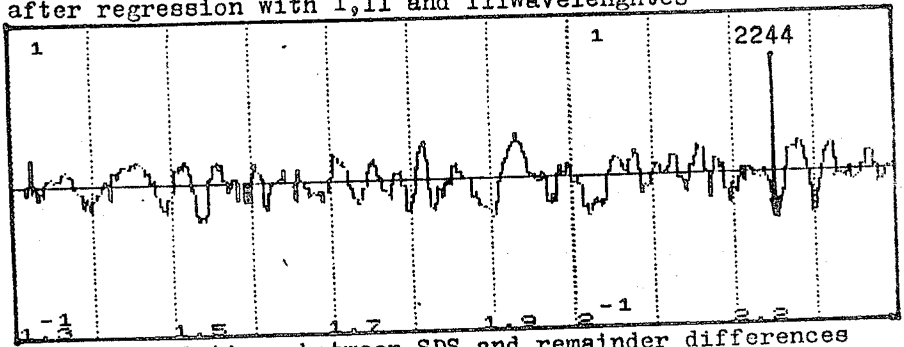

Fig.7 Correlations between SDS and remainder differences after regression with I,II,III,IV wavelenghtes

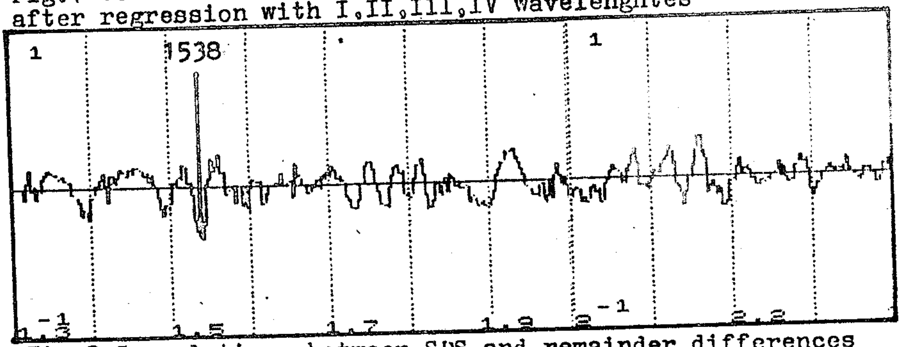

Fig.8 Correlations between SDS and remainder differences after regression with I,II,III,IV,V wavelenghtes

Table 1

T test		t(1)=31.023		t(1)=38.26 t(2)=5.102		t(1)=44.634 t(2)=4.799 t(3)=4.357		t(1)=44.123 t(2)=3.961 t(3)=5.559 t(4)=3.761		t(1)=45.955 t(2)=3.942 t(3)=5.187 t(4)=4.18 t(5)=2.392		t(1)=47.693 t(2)=3.092 t(3)=5.506 t(4)=4.903 t(5)=3.214 t(6)=2.768	
correlation coefficient		0.975		0.984		0.989		0.991		0.992		0.993	
standard deviation		10.6		8.7		7.4		6.5		6.2		5.8	
determination coefficient		0.951		0.968		0.978		0.983		0.985		0.987	
Sample	GC/UV deter. content	content after regression for wave 1626	dy1 (3)-(2)	content after regression for wave 1626 1566	dy2 (5)-(2)	content after regression for wave 1626 1566 1640	dy3 (7)-(2)	content after regression for wave 1626 1566 1640 1582	dy4 (9)-(2)	content after regression for wave 1626 1566 1640 1582 2244	dy5 (11)-(2)	content after regression for wave 1626 1566 1640 1582 2244 1538	dy6 (13)-(2)
1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	5.2	4.5	-1.2	-3.9	-8.6	9.1	4.3	6.5	1.8	2.4	-3.2	10.7	5.9
2	10.7	11.2	0.9	15.4	5.1	21.6	11.4	17.0	7.7	20.7	10.5	12.7	2.4
3	15.3	-15.2	-29.0	9.7	-5.0	8.0	-6.7	10.5	-5.2	10.3	-5.4	12.9	-2.8
4	9.0	-1.4	-10.0	3.7	-6.7	2.7	-7.7	8.9	-1.5	3.9	-6.6	4.2	-6.2
5	6.8	14.4	8.1	14.4	8.1	20.6	14.3	18.2	11.9	11.8	5.5	8.7	2.4
6	7.6	-16.8	-23.9	-13.0	-21.2	-7.3	-14.4	-1.2	-8.4	2.7	-5.3	5.2	-2.8
7	15.8	-3.4	-18.7	4.2	-11.0	8.6	-7.6	8.2	-7.0	9.7	-6.5	10.9	-5.3
8	6.9	3.1	-4.2	-2.4	-8.8	4.7	-2.6	3.1	-4.2	4.8	-2.5	4.2	-3.1
9	13.4	25.7	12.7	25.2	12.2	19.0	6.0	21.6	8.7	19.9	6.0	18.0	6.1
10	41.1	51.4	10.8	43.7	2.0	46.5	5.9	44.1	3.5	45.8	5.2	47.0	7.3
11	12.7	7.0	-5.2	17.3	4.0	13.0	1.8	19.5	7.2	17.4	5.1	18.2	5.0
12	28.3	34.2	6.4	23.8	-4.9	21.4	-7.3	25.7	-2.0	24.9	-3.8	23.8	-4.9
13	24.8	35.0	11.6	32.7	8.4	26.0	2.6	22.6	-2.7	20.3	-4.9	18.0	-6.2
14	18.1	38.8	21.1	33.3	15.6	24.8	7.2	28.8	11.1	30.2	12.5	30.6	12.9
15	7.7	13.6	6.4	11.2	3.9	12.4	5.1	12.1	4.8	12.3	4.0	10.2	2.9
16	9.1	14.3	5.7	10.2	1.5	6.9	-2.7	8.9	-0.6	7.4	-2.2	5.2	-4.4
17	6.0	11.2	4.6	8.8	2.2	10.9	4.3	9.2	2.7	9.4	2.8	9.5	2.9
18	5.3	2.6	-3.2	-2.1	-6.0	-3.2	-7.0	-2.0	-7.9	-0.8	-5.7	-1.8	-6.7
19	3.0	6.4	2.9	4.4	0.9	7.5	3.0	7.9	4.3	9.6	6.1	9.8	6.3

Table 1 - cont'd

1	2	3	4	5	6	7	8	9	10	11	12	13	14
20	7.6	6.0	-1.1	3.4	-4.7	0.9	-7.2	-2.6	-9.7	-0.6	-7.7	-2.8	-9.0
21	5.3	15.7	10.9	9.4	4.5	7.3	2.5	7.2	2.4	7.6	2.8	6.8	1.9
22	22.2	36.7	14.9	21.0	-0.7	17.6	-5.1	18.1	-4.6	18.8	-3.8	20.6	-1.0
23	31.7	45.6	14.0	41.1	9.8	36.6	5.3	30.5	-1.6	31.1	-0.0	32.5	1.3
24	26.1	30.7	5.1	34.8	9.2	29.0	4.4	25.7	-0.8	26.8	1.2	26.7	1.1
25	24.8	26.8	2.5	35.6	11.3	29.9	5.6	24.8	0.5	27.2	2.9	27.6	3.3
26	28.3	31.0	4.2	35.8	7.0	30.4	2.6	25.1	-3.6	24.1	-4.6	24.8	-3.9
27	49.0	51.9	2.3	53.6	3.0	48.0	-1.5	49.9	-0.5	48.5	-1.0	47.7	-2.8
28	102.6	98.9	-4.1	106.0	4.9	109.9	7.8	107.6	5.5	108.1	5.0	108.2	6.1
29	112.6	105.3	-7.8	102.9	-10.1	102.5	-10.5	105.2	-7.8	105.6	-7.4	107.3	-5.7
30	93.0	105.4	11.9	100.5	6.0	100.9	7.4	105.5	11.9	104.9	11.4	103.3	9.7
31	111.1	108.3	-3.2	110.6	-0.9	111.0	1.4	107.2	-4.3	109.2	-2.3	109.0	-1.5
32	109.0	115.6	6.1	110.8	1.3	107.5	-2.9	109.3	-1.1	107.0	-2.4	106.4	-3.0
33	107.4	103.3	-4.6	100.8	-7.1	99.3	-8.5	100.6	-7.2	100.7	-7.2	101.9	-5.0
34	122.7	122.2	-0.9	132.3	10.1	130.3	8.1	124.8	2.5	123.6	1.4	124.9	2.7
35	125.7	125.2	-0.9	126.9	1.7	129.0	4.8	132.3	7.1	132.8	7.6	132.6	7.4
36	6.7	2.9	-4.3	0.9	-6.2	4.0	-2.2	5.3	-1.8	5.7	-1.5	6.5	-0.6
37	114.5	102.9	-11.0	110.8	-4.2	109.1	-5.8	111.4	-3.6	110.4	-4.5	110.3	-4.6
38	123.4	112.8	-10.0	119.4	-4.4	119.3	-4.5	124.1	1.2	123.4	0.5	120.6	-3.2
39	115.0	94.2	-22.3	102.5	-13.0	102.9	-13.6	105.9	-10.6	108.2	-8.2	111.2	-5.2
40	112.6	93.3	-19.7	87.0	-24.0	88.4	-24.6	88.8	-24.2	91.3	-21.7	94.7	-18.3
41	111.2	115.6	4.9	114.3	3.5	118.0	8.3	114.8	4.1	110.1	-1.5	108.6	-2.0
42	83.4	103.9	20.0	102.4	19.4	95.5	12.6	90.0	8.1	89.7	6.8	92.3	9.4
43	107.1	104.5	-3.1	103.0	-3.6	104.7	-2.8	110.5	3.9	113.6	6.9	115.1	8.4
44	114.1	116.0	3.4	112.5	-1.0	111.5	-2.0	111.9	-2.6	112.0	-1.5	113.2	-1.3
45	125.9	125.0	0.6	125.7	-0.6	128.4	2.0	123.3	-2.0	122.4	-3.9	122.2	-4.1
46	122.7	113.1	-9.0	112.5	-10.6	123.1	0.9	126.3	4.1	125.4	3.2	122.6	-0.5
47	14.7	24.2	9.0	24.1	9.9	14.8	0.5	18.0	4.8	17.0	3.8	17.7	3.4
48	5.5	12.0	7.0	13.8	8.8	14.0	9.0	11.2	6.1	13.0	8.9	12.4	7.4
49	6.9	7.2	0.8	4.9	-2.4	6.7	-0.6	7.2	0.8	5.7	-1.7	5.6	-1.7
50	103.4	95.9	-7.0	96.8	-6.0	102.3	-1.5	104.9	1.0	107.3	4.3	104.7	1.8