SOME REMARKS TO THE RAPID METHODS FOR AN ESTIMATION OF ERUCIC ACID AND GLUCOSINOLATES FOR WINTER RAPE SEEDS.

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The chemical laboratory of plant Breeding Station at Slapy u Tábora has to test all the winter rape breeding material for a content of both the erucic acid and the glucosinolates during a relatively short time from the rape harvest to the winter rape sowing.To fulfil this target, some rapid methods are used being adapted to workplace conditions.

A determination of the total constitution of fatty acids /according to ČSN 560 059/ is very complicated and tedious indeed,that is why it can be realised only for selected samples of picked generations.The tedious preparation of methyl esters for the gas chromatography measurements was adapted in the following way : The sample of 0,2 g or less of crushed or grinded stuff can be given to a test tube.Then 0.3 ml hexane p.a. will be added and the oil will be extracted for 1 hour in a closed tube at the room temperature.The sample must be shaked two times during this extraction.After 1 hour,2 ml 0.4 N solution of CH_3ONa will be added.Then test tubes must be closed again and they are to be stored in a refrigerator box at 5°C.

The proper estimation of an erucic acid content by the gas chromatography will be carried out next day.Methylesters of fatty acids obtained by using such a method can be used also for a determination of the fatty acids total constitution on the polar phase as well as for an estimation of the erucic acid only on the non-polar phase /by the rapid testing method/.

Analysis parameters for the rapid testing method are following ones :

-The gas chromatograph CHROM 5 /Laboratorní přístroje n.p. Praha,ČSSR/
-Chromatographic column-120 cm x 3 mm I.D.,glass
-Chromaton N AW-DMCS,granulation 0.160-0.200 mm + 3 % JXR
-Detector FID
-Gases - N_2 0.19 MPa
H_2 20 ml/min
air 500 ml/min
-Temperature of the injector 290°C
of the column 235°C
of the detector 250°C
-Microsyringe Hamilton 1 ul
-0.5 ul of a hexane phase
-Recorder TZ 4601,moving 3 cm/min.

The elution time of the erucic acid is about 3 minutes. After an elution of fatty acids methyl esters having 18 carbon atoms /C:18 acids/, it is necessary to increase the sensitivity of this apparatus hundred times.

Four main peaks will appear on the chromatogram. These peaks correspond to methylesters of fatty acids having 16, 18, 20 or 22 carbon atoms. The last mentioned zone can be divided into two parts - for the erucic acid and for the behenic one /see Fig. 3/. The following formula can be used for the erucic acid calculation, as far as this content does not exceed 10% /according to Mr. Svoboda, a representative of VÚTP Ústí nad Labem/:

$$\% \text{ KE} = \frac{A_{KE} \cdot 90}{A_{18} \cdot K}$$

where KE is the erucic acid denotation

A_{KE} ..... an area corresponding to the erucic acid

A_{18} ..... an area corresponding to C:18 fatty acids content

K ..... The factor of an apparatus sensitivity increasing

90 ..... an aproximate content of C:18 fatty acids /%/

The area of respective peaks can be calculated from their height and from the peak width in the height.

As all the present breeding materials have a low content of the erucic acid and the analyses are make like the control tests only, we do not use the mentioned formula at all.

The Table 1 shows the number of analyses and the content of erucic acid.

Table 1.

Year	Number of analyses	Percentage of samples containing erucic acid 1%	erucic acid over 1%
1984	2874	2.07	2.04
1985	3583	1.53	2.64
1986	3274	1.91	1.94

For a rapid estimation of the erucic acid content in an analysed sample, the content of the behenic acid is used because this value is almoust constant in all samples. The average content of the behenic acid calculated for the past year is then used as a base for all calculations in the present year. The average year values are shown in Table 2.

Table 2.

Year	Average percentage of behenic acid /%/
1984	0.42
1985	0.38
1986	0.34

If there is necessary to know the erucic acid content, the calculation can be carried out using the wave height for the erucic acid and for the behenic one, the erucic acid content being lower than 1%. Such a calculation is much more rapid than a calculation from the peak area of erucic acid and fatty acids having 18 carbon atoms. Usually, we do not calculate the erucic acid content but we do not use to recommend for the further selection such samples where the peak for erucic acid is the same or heigher than the peak for behenic acid.

Figure 2 shows an erucic acid content in 10 independently measured samples by using a calculation from the erucic acid peak height and C18 acids peak height and, on the other hand, from the erucic acid wave height and from the behenic acid wave height. Results of both the calculations have a very good consistency.

In order to check the results obtained by the mentioned rapid testing method, 10 parallel determinations were carried out using each of these methods, i.e. for the determination according to ČSN, for the determination on a polar phase /Chromaton N-AW-DMCS/15% Ethylene glycol adipate/ and for the determination on a non-polar phase /Chromaton N-AW-DMCS/ 3% JXR Silicone/ by using the rapid methode respectively. The results of all three methods are listed in Figure 1. The relative standard deviations /i.e. coefficients of variance/ for particular methods are shown in Table 3.

Table 3.

Method	s_r /%/
ČSN	5.15
polar phase	7.57
non-polar phase /rapid method/	10.64

Described results show that the modified method for the methylesters preparation and a rapid evaluation of results for the erucic acid content are in a good agreement with the results obtained according to ČSN. This rapid method enables to test 100 samples in one working shift of one operator. In such a way, two operators can analyse all the breeding material for the content of erucic acid during the time from the harvest to the sowing.

Also the rapid testing of the breeding material for the total glucosinolates amount can be carried out by so called Glucose-specific test by using the diagnostic strips Glukophan.Some elected samples can be analysed according to the standard ISO/DP 5504.

We have been interested in an experimental error occuring by using the mentioned Glucose-specific test.A random set of 321 samples was tested by using this Glucose-specific test and the same samples were simultaneously analysed according to the ISO standard.The results obtained by both the methods were then compared.

The diagnostic strip Glukophan /production by Lachema n.p.Brno/ will be adapted by cutting the indication field for reducing compounds /near to the indication field for glucose/.Five seeds of one sample will be tested,each of them independently.A seed will be crushed on a clear work table,then 3 drops of distilled water will be added and a hydrolysis will take place during 10 minutes.After this time,the adapted /by cutting/ diagnostic strip will be inserted and after an application of 30 seconds the colour intensity at the respective field of a diagnostic strip will be compared with the standard colour scale and the glucose content will be evaluated in such a way.The intensity of colour can be estimated by a point-scale 0, 1, 2, 3, 4 according to the producer's instruction.Such a scale was more enlarget by us by using the point 0-1.Such samples only were recommended to further selection which had the points 0 or 0-1,and not more than 1 seed can be estimated with the point 1.An example of such a testing of the breeding material is given in Table 4.

Table 4.

Sample	Glucose-specific test /points/	ISO standard /umol g^{-1} of the deffatted meal solid content/
1	0, 0, 0, 0, 0	8.0
2	0, 0, 0, 0, 0-1	15.2
3	0, 0, 0, 0-1, 0-1	22.2
4	0, 0, 0-1, 0-1, 0-1	24.9
5	0, 0, 0, 0-1, 1	30.2
6	0, 0-1, 0-1, 0-1, 1	35.9
7	0, 0, 0, 1, 2	47.6
8	0, 0, 2, 2, 3	86.6
9	2, 2, 2, 2, 2	116.0
10	2, 2, 3, 3, 4	140.2

Samles being tested for a glucosinolates content were simultaneously tested according to the standard ISO/DP 5504. According to the above mentioned criterions for the estimation of breeding materials by using the diagnostic strips Glukophan,the results of both the methods have been found concident for the limit 20 umol g^{-1} of the defatted meal solid content in 87.9 % of events,for the limit 25 umol g^{-1} in 91.0 % events and for 40 umol g^{-1} for 93.2 % events.It was found 0.31 % of distant results only.

In the case of selecting samples having been estimated for all 5 seeds by the point 1 or less, the reliability of choice will increase to 96.3 % for the limit 40 umol g^{-1} of the defatted meal solid content. This mentioned mode of choice cannot be used for the GSL values 25 and 20 umol g^{-1} respectively, as for the defatted meal solid content of a sample.

The Glucose specific test is used as a screening method and its advantage is in its simplicity and expeditivity. One working woman can test 90 samples, i.e. 450 seeds, during 8 hours of a working shift. Nevertheless, There is necessary to take into account errors in 6.8 % events and that is why all the selected and sowed material will be then /in winter months/ analysed according to the standard ISO/DP 5504.

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FIGURE 1.

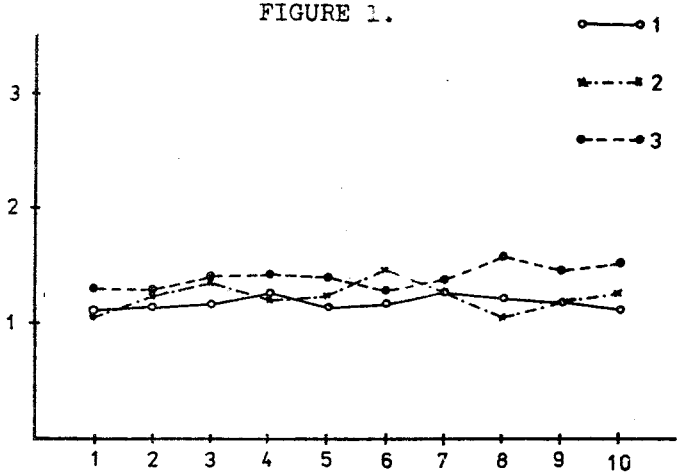

Fig.1 shows a content of erucic acid for 10 parallel samples:
1.Measured according to ČSN standard
2.The rapid testing method
3.Total constitution of fatty acids

FIGURE 2.

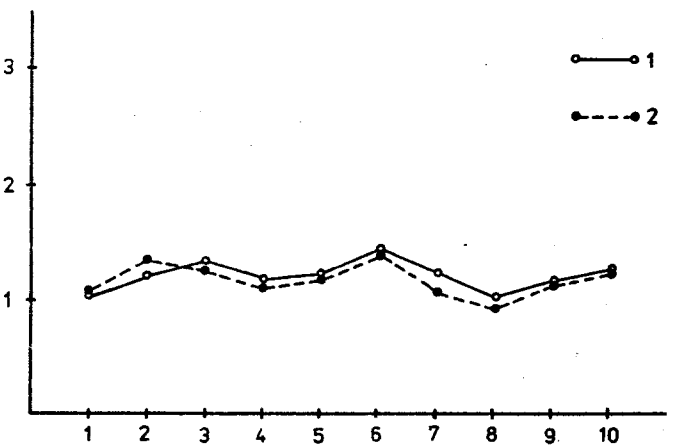

Fig.2 shows a calculation of an erucic acid content:
1.From peak areas for erucic acid and C18 acids
2.From wave heights for erucic acid and behenic acid

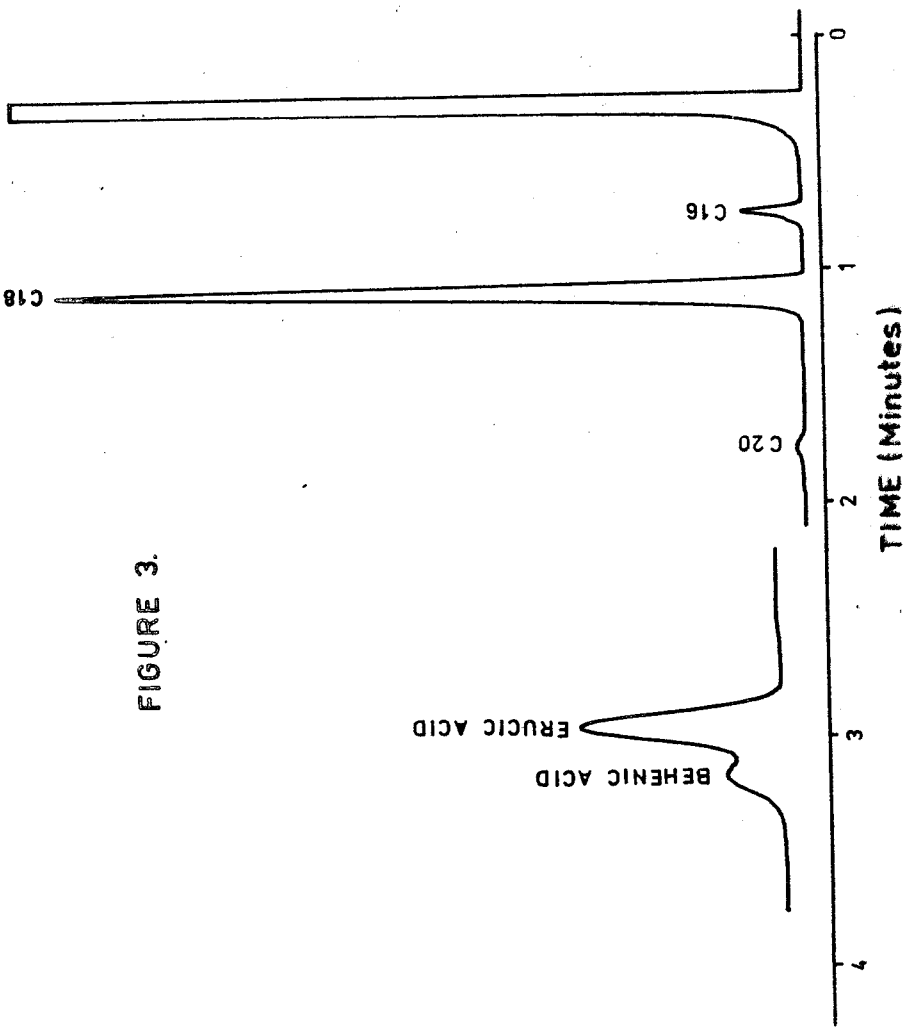

FIGURE 3.