NEW METHODS FOR THE ANALYSIS OF RAPESEED CONSTITUENTS

W. Thies

In table 1 the actual aims of the "quality rapeseed" breeding are summarized, which call for special analytical techniques.

oil	oil content > 45 %	erucic acid	(22:1)	< 1 %
		linoleic acid	(18:2)	> 40 %
		linolenic acid	(18:3)	< 3 %
meal		glucosinolates	(GSL)	< 8 µMol/g

Table 1: The present aims of "quality rapeseed" breeding

In the following a report is given on the methods we use in Goettingen for the different selections of desired genotypes.

Preselection of genotypes low in erucic acid and glucosinolate content

The technique of paper chromatography which has been used for several years in our laboratory (THIES, 1971) could furthermore be simplified. The homogenization and extraction of single seeds or cotyledons as well as the hydrolysis of the triglycerides are now accomplished in so called microliter trays made from polystyrene (Greiner und Söhne, D 744 Nürtingen) with 100 µl of a combined extraction and hydrolysis mixture (6 g KOH + 60 ml methanol + 30 ml water + 150 ml i-propanol), the following chromatography with the solvent acetone / water / acetic acid (70:30:1) and the formation of the copper soaps in 0.05 % copper acetate + 1.3 % sodium acetate. Though according to this procedure along with the fatty acids glycerol and KOH is transferred to the chromatography paper, the separations are not worse than the separations obtained according to our previously described sample preparations.

Preselection of genotypes low in linolenic acid and glucosinolate content

The photometric preselection of genotypes low in linolenic acid and glucosinolate content (RAKOW and THIES, 1972) has been replaced by a spot-test procedure according to the principle of an analysis street (THIES and NITSCH, 1974). A specially impregnated endless paperstrip is loaded in 2 cm distances with few microliters of an oil extract and is drawn after this through an UV-radiation field and an hot air reactor (fig. 1).

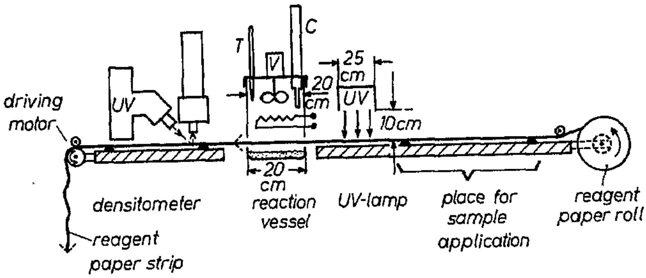


Figure 1: Schematic presentation of a paper spot test analysis street

The intensity of the resulting red-coloured spots is proportional to the content of linolenic acid. The latter therefore may be estimated by quantitative densitometry. From Fig. 2 one may recognize the good correlation between the 18:3-content estimated by gas chromatography (GLC) and the new spot test procedure (peak heights on the densitometer recorder). Figured is also the selection effectiveness if 5, 10, 20 or 30 % of the seeds are selected whose extracts have caused the weakest coloured spots (peak heights).

Gas chromatography (GC) of the fatty acids

With modern fractometers (e.g. Perkin-Elmer F 30/AS 41/PEP) GLC analysis is feasible in day and night operations. Instruments of this kind are capable of analysing hundreds of samples per day. In this case the sample preparation may easily become the limiting factor if simple extraction and derivatization procedures are not available. We homogenize single seeds or cotelydons in the above mentioned microtiter trays simply by stainless steel rods (fig. 3). To the grinded material we add 50 - 100 µl 0.5 n Na-methylate in methanol / i-octane (4:1) (extraction and transmethylation) and overlay this solution after 20 min with 50 µl of i-octane. After 20 minutes sufficient fatty acid methylesters have diffused in the upper layer and may be transferred to the aluminium capsules of the Perkin-Elmer autosampling system AS 41. For the separation we used packed 6 % BDS columns and H_2 as carrier gas. The oven temperature is so adjusted, that samples free from erucic acid are analysed within 3 min. In regular intervals the column quality is tested by determining the separation number. Under the mentioned separation conditions we normally find values lying between 1500 and 2000.

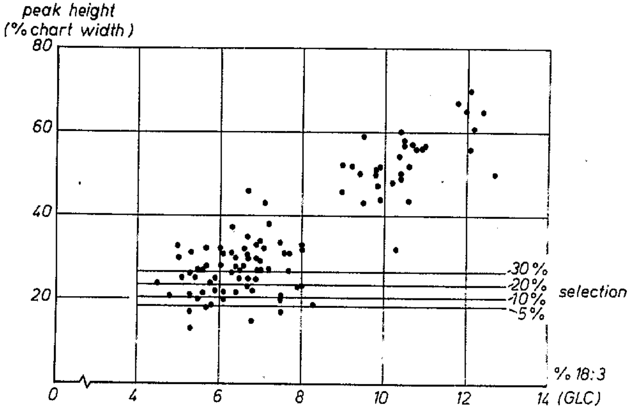


Figure 2: Preselection of rapeseeds with a low content of linolenic acid. Comparison of the results obtained by the new paper spot test analysis street (peak heights on the densitometer recorder) and by GLC

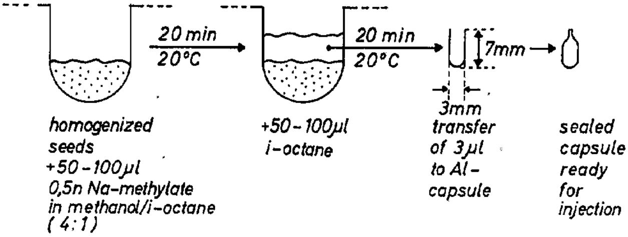


Figure 3: Schematic presentation of the sample preparation for GLC of fatty acids by the aid of microtiter trays and Al-capsules (Perkin-Elmer autosampling system AS 41)

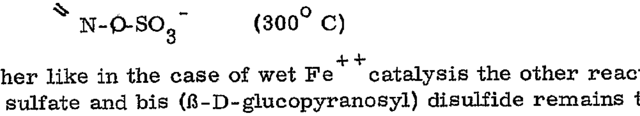
Gas chromatography of glucosinolates (GSL)

a) GLC as trimethylsilyl-(TMS)derivatives

According to UNDERHILL and KIRKLAND (1971) the intact GSLs may be analysed by GLC after transforming the GSL to their corresponding TMS derivatives. This procedure has three advantages against all other known methods: All GSLs are determined in one working operation and - as in the case of the fatty acids - automatic analyses are feasible. The use of this method for plant breeding purposes is however bound to different assumptions: Simplified sample preparations, lowest level of detector contamination (FID) by the used silylation reagent and good reproducibility of the measurements even after prolonged series of analyses. A simplified sample preparation is represented in fig. 4. For the homogenization and extraction of the GSLs only one vessel (5 ml-Potter-Elvehjem glass homogenizer)is used and the derivatization is performed in aluminium capsules of the Perkin-Elmer autosampling system AS 41 at 130° C within 20 min. UNDERHILL and KIRKLAND (1967) have used the silylation reagent hexamethyldisilazane (HMDS) that during serial analyses readily contaminates the detector. But HMDS may be replaced by N-methyl-N-trimethylsilyl-heptafluorbutyramide (MSHFBA) where by the danger of contamination is drastically reduced. A chromatogram obtained, using the described sample preparation, is shown in fig. 5. Because of the obviously relatively great instability of the TMS-GSLs instead of metal columns in every case glass columns have to be used. If the TMS-oligosaccharides (mainly raffinose and stachyose) accompanying the GSLs are not "baked out" after each analysis, the columns have a relative short lifetime and have to be refilled after ca. 200 analyses. Periodical controls of the column quality - e.g. by the aid of a test mixture with known GSL content - are recommended.
With the intent to overcome the above mentioned difficulties we looked for other analytical possibilities.

b) GLC as nitriles

According to YOUNGS and PERLIN (1967) GSLs soluted in water may be transformed to their corresponding nitriles by Fe^{++}catalysis. These nitriles like the TMS-GSLs may analysed by GLC. We have found that the nitril formation is also possible by dry heating (pyrolysis) of the GSLs:

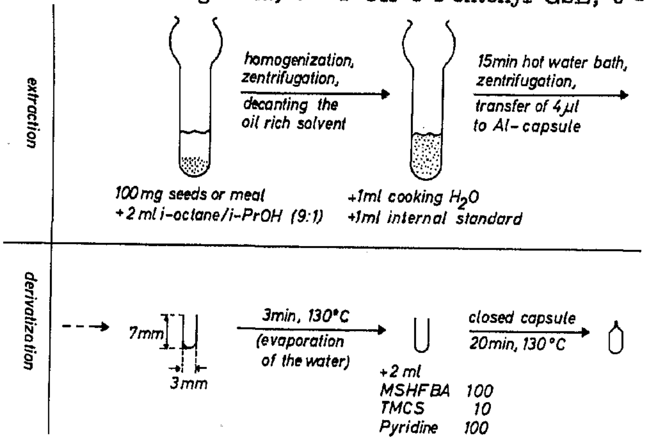


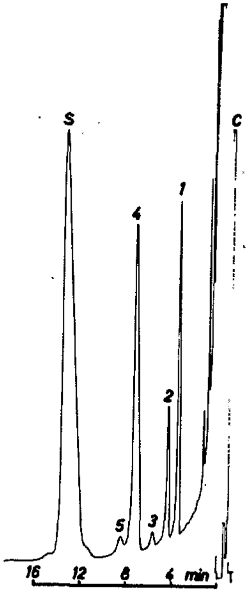
$$\mathrm{R{-}C}\begin{cases}\mathrm{S{-}Glu}\\ \mathrm{N{-}O{-}SO_3^-}\end{cases} \xrightarrow[(300^\circ\ \mathrm{C})]{\text{dry heat}} \mathrm{R{-}C{\equiv}N + X + Y}$$

Wether like in the case of wet Fe^{++} catalysis the other reaction products are sulfate and bis (ß-D-glucopyranosyl) disulfide remains to be proved. If one makes use of the above mentioned automatic sampling device AS 41 the derivatization may take place during the sampling ("injection") moment. For this purpose the injection block has to be heated at a temperature of 300° C. The sample preparation is restricted to the evaporation of

Figure 4: Homogenization, extraction and TMS-derivatization of glucosinolates for GLC analysis by the aid of the capsule injection technique

Figure 5: A chromatogram obtained after GLC of seed extracts treated according to fig. 4 (6 ft. glass column, 1,2 % SE 52/ Chrom. W-AW-DMCS, oven: 190° C, injection: 250° C, carrier gas: H_2, 50 ml/min)
Abbreviations: C = capsule identification marks,
1 = Sinigrin, 2 = Gluconapin, 3 = Glucobrassicanapin,
4 = Progoitrin, 5 = 2-OH-4-Pentenyl-GSL, 6 = Saccharose





the solvent (water) from the still unsealed aluminium capsules in an oven (3 min, 130° C). After the "injection" of the sealed capsules the separation of the nitriles (formed during this moment) is best performed with glass columns filled with 10 % UCON LB 1715 on Chromosorb W-AW-DMCS or Chromosorb T (Teflon) at 130° C or with a temperature program (110° C to 210° C, 39° C/min). For the detection of the separated nitriles a N-specific detector is advantageous. From fig. 6 one may recognize the good reproducibility of the nitril formation under the described conditions.

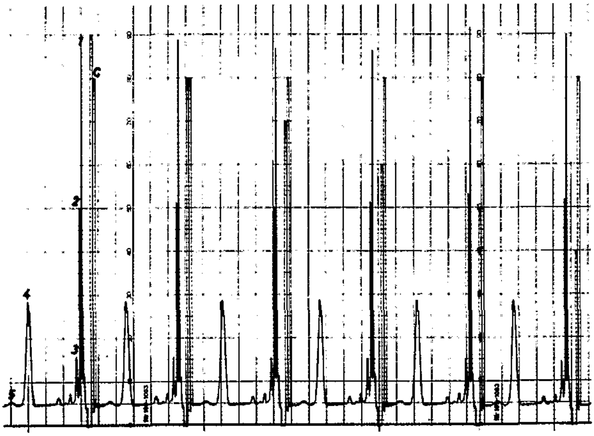


Figure 6: Repeated GLC of 4 µl water extract each from defatted rapeseed meal. The GSLs are chromatographed as their corresponding nitriles after dry heating in the moment of capsule "injection" using the Perkin-Elmer autosampling system AS 41 (6 ft. glass column, 10 % UCON LB 1715/Chrom., oven: 130°, injection: 300° C, carrier gas: H_2, 50 ml/min). Abbreviations: see fig. 5

Fig. 7 shows the result of GLC separations of extracts from rapeseed genotypes with a low (Lesira) and a high (Erglu) GSL content. For the quantitative evaluation sinigrin may be used as internal standard. Because pure GSL compounds for comparison purpose are not yet at our disposal, this method is still in the developing stage. But we hope that this procedure can one day replace the GLC of TMS-derivatives.

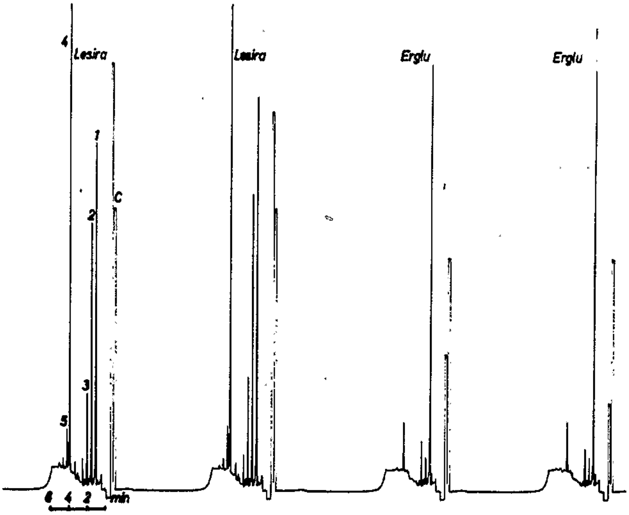


Figure 7: GIC of GSLs as their nitriles derived from defatted rapeseeds with a low (var. Erglu) and a high (var. Lesira) GSL content. Conditions as given in fig. 6. Exception: Temperature program 110° C to 210° C, 39° C/min. Abbreviations: see fig. 5

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