Journal of Chromatography, 115 (1975) 652–654

CHROM. 8676

Note

Screening for fatty acids in oilseed breeding programmes using lipase and paper chromatography

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(Received August 5th, 1975)

The paper chromatographic method of Thies[1] has been widely used for early generation screening of rapeseed crosses for the absence of erucic acid. This method requires overnight saponification with methanolic alkali (performed in a very small glass vial) followed by acid extraction into hexane before spotting a 5-μl aliquot onto a paraffin-pretreated paper. Development in 95% acetic acid is followed by detection with copper acetate and dithiooxamide. Despite accurate measurement of reagents and sample aliquot for chromatography, the method remains qualitative.

The major disadvantage of the method is in the saponification step. If, as is usual, some hundreds of single seed analyses are being performed each day, the contribution of washing up to the work load is disproportionately large and it was found in this laboratory that contamination and sample confusion could also be problems.

This paper describes a method by which the hydrolysis of the expressed oil is carried out on the chromatographic paper by a lipase enzyme preparation. This innovation has eliminated the major part of the manipulative work associated with analysis.

Rice bran lipase was selected because of its ready availability in this area. The preparation used is similar to the "crude enzyme solution" of Funatsu *et al.*[2]. In the technique described, lipases from most sources could be used. Oat lipase, prepared as described, was also found to be as effective, and would probably be more easily obtained in rapeseed-growing areas. It is important, however, to use a true lipase. Some commercial preparations examined, whose activity was quoted in triacetin or tributyrin units, did not cause hydrolysis of rape oil.

EXPERIMENTAL AND RESULTS

Rice bran lipase was prepared from fresh twice-hexane-extracted rice bran by extraction with four parts 10^{-2} *M* calcium acetate (adjusted to pH 6.7 with NaOH)

for 30 min. After initial extraction the preparation was filtered through cheese cloth, centrifuged and the supernatant taken to 60% saturation with ammonium sulphate. The precipitate was collected by re-centrifuging and dissolved in about one quarter the original volume of 10^{-2} *M* calcium acetate pH 6–7, dialysed at 4° against the same solution for 48 h and then re-centrifuged: the resulting supernatant was used as the lipase preparation. Prepared in this fashion and refrigerated, the lipase enzyme has remained active for well over seven months. Rice bran lipase is known to be a calcium ion dependent enzyme[3,4]. It has a pH optimum of 7.5–8.0 and an optimum reaction temperature of about 37° (ref. 4). The enzyme has been shown to be relatively specific for hydrolysing fatty acid ester bonds at the 1,3 position. This is not a disadvantage as rape oil binds virtually all the erucic acid at the 1,3 position[5]. 1,3-Specific lipases have often been used in studies of positional fatty acid content of triglycerides.

The following chromatographic conditions gave consistent identification of

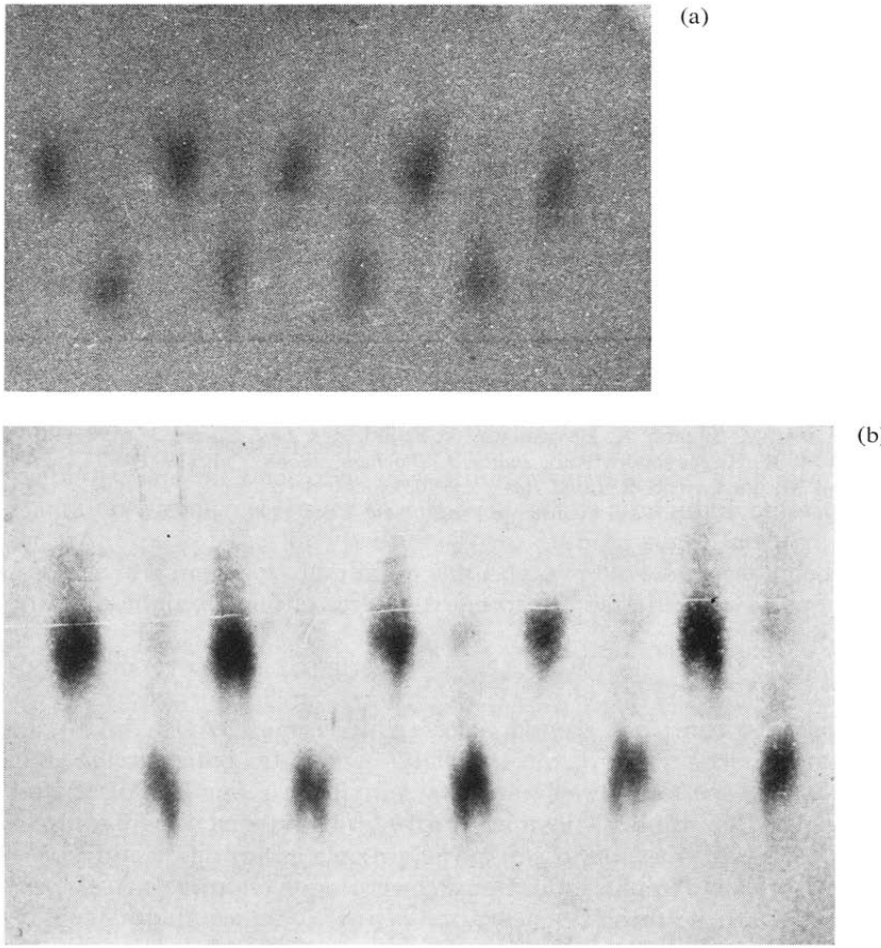

Fig. 1. Paper chromatogram of five seeds of var. Tower (zero erucic) interspersed with five seeds of var. Target (high erucic). (a) Analysis by the lipase method: one half of each seed was used. (b) Analysis by the method of Thies[1]; the other halves of the seeds were used.

oil samples containing erucic acid. The method of Thies[1] was modified by using a paraffin impregnation of 7.5% instead of 15%. This gave a 20-cm front in 2 h using Whatman No. 4 paper compared with the 7-cm front obtained using the original conditions.

The seed cotyledon was smeared onto the paper at the origin, and two drops of lipase preparation (approximately 0.02 ml) added from a dispensing syringe. The papers were placed in a tank of 100% relative humidity in an incubator at 37° overnight. During this period enzymic hydrolysis of the oil occurred. Development with 95% acetic acid and detection using copper acetate and dithiooxamide was carried out the next day according to Thies[1]. Washing was made more complete after copper treatment by hanging the papers vertically in a 22.5-l container and running water in the bottom and out the top for 40 min.

Figs. 1a and b show photographs of papers immediately on removal from the dithiooxamide. Fig. 1a is the chromatogram of half seeds of var. Tower and var. Target by the lipase method and Fig. 1b that of the other half of the same seeds by the method of Thies[1].

The method is in routine use in the Wagga Wagga A.R.I. laboratories for erucic acid screening of breeder's crossbreds of both *B. napus* and *B. campestris*. It has also been used for examination of other *Brassica* spp. such as *B. tournefortii* and *B. oleracea*.

1,3-Specific lipases have often been used in studies of the positional fatty acid content of triglycerides[6]. In other oil seeds use of the method of Thies[1] and the present lipase method on the same oil sample should easily differentiate substitutions on the 1, 3 and 2 positions.

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