

CHROM. 7475

## Note

### Thin-layer chromatographic examination of various seed oils

A. BEVENUE\*

*Laboratorio di Chromatografia del C.N.R., Via Romagnosi 18/A, 00196 Rome (Italy)*

(Received April 8th, 1974)

Stahl<sup>1</sup> outlined a relatively simple thin-layer chromatographic procedure for the characteristic identification of fatty oils. In the present work, it is believed that the procedure has been simplified by incorporating certain modifications. Also, the usefulness of the technique has been extended into investigations on linseed oil *per se* and materials containing linseed oil.

### EXPERIMENTAL

Thin-layer chromatography (TLC) was carried out with pre-coated plastic sheets (20 × 20 cm) coated with a 0.1-mm layer of microcrystalline cellulose (Polygram Cel 400 UV<sub>254</sub>; Macherey-Nagel & Co., Düren, G.F.R.). Prior to use, the sheets were coated with liquid paraffin by ascending chromatography for a distance of 15 cm using a solution of 5% liquid paraffin in light petroleum (b.p. 40–70°). The air-dried sheets were then pre-washed with glacial acetic acid by ascending chromatography. The sheets were air-dried thoroughly prior to the application of the samples to be examined. One microlitre samples of the oils (2–10 µg) were applied on a line 2 cm from the edge of the TLC sheet and the sheets were developed in glacial acetic acid to a distance of about 10–11 cm. The development time was 2–3 h at room temperature (20–25°). The sheets were removed from the developing tank, air-dried, and then transferred into a closed chamber containing iodine vapour. Immediately after the removal of the sheets from the iodine tank, after a period of contact of about 5 min, the sheets were sprayed with a 4% aqueous solution of soluble starch.

Plastic sheets pre-coated with a 0.1-mm layer of cellulose (Polygram Cel 300) and prepared for use as described above were also used in similar experiments. However, they were less efficient in the separation of the various components of the oil samples and less sensitive to detection by the iodine–starch reaction.

The following additional experiments were included in this study: (1) putty samples, exposed for at least 4 years to the local atmosphere in Rome were obtained from an exterior window sash; (2) linseed oil films of about 2 mm thickness were exposed to the atmosphere in the laboratory at room temperature (20–25°) for a period of 54 days; (3) a compositional mixture was prepared so as to simulate

\* On leave from the Department of Agricultural Biochemistry, University of Hawaii, Honolulu, Hawaii, U.S.A.

an artist's painting. A layer of a mixture of pigment, basic lead carbonate and linseed oil was superimposed upon a layer of a mixture of plaster of Paris and glue. The film was then exposed to ultraviolet light for a period of 144 h.

Samples of the putty and the paint mixture were pulverized by means of a glass mortar and pestle. The pulverized samples and samples of the exposed linseed oil were then leached with carbon tetrachloride at room temperature for 24 h and the extracts were examined by TLC as described above.

## RESULTS AND DISCUSSION

A typical example of the results obtained with some oils is shown in Fig. 1. Repeated experiments indicated that the procedure was remarkably reproducible and the degree of sensitivity, or limit of detection, was about  $2\mu\text{g}$  for each oil, and even lower for the individual triglyceride components observed.

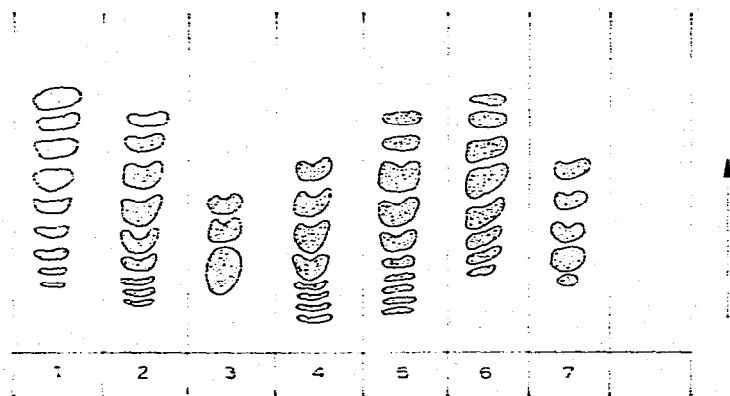


Fig. 1. TLC of various oils: (1) linseed, uncooked; (2) "mixed seeds"; (3) olive, freshly pressed; (4) groundnut; (5) poppy seed; (6) walnut; (7) oleomargarine. Amount of each oil used,  $5\mu\text{g}$ .

Drying oils, such as linseed oil, after a lengthy time of exposure to air and light, undergo chemical changes involving polymerization and some breakdown to a variety of small molecules<sup>2</sup>. This compositional change is illustrated markedly in Fig. 2. The spots (triglycerides) will decrease in intensity and additional spots will be observed near the origin and near the solvent front of the chromatogram. With increased time or accelerated exposure to ultraviolet radiation, the original spots will disappear completely with the formation of a new substance(s), which will appear in the area of the solvent front and the origin of the chromatogram.

The modification to the Stahl procedure included the elimination of the preparation and heat-treatment of the TLC plates prior to use. The use of commercially available plastic TLC sheets minimized the difficulty of preparing plates of uniform composition and constant performance characteristics. Some increase in the limit of detection was also observed. Washing the sheets with glacial acetic acid prior to their use eliminated the problem of iodine-sensitive contaminants at the solvent front and thereby eliminated the problems of interpreting any spots observed in the area of the solvent front.

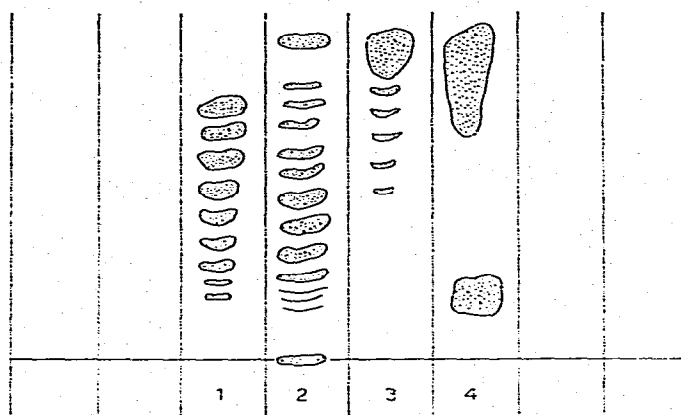


Fig. 2. TLC of "aged" linseed oil: (1) fresh, uncooked; (2) 2-mm oil film exposed to air and light at 20–25° for 54 days; (3) sample of putty exposed to the atmosphere for at least 4 years, obtained from exterior window sash; (4) simulated artist's painting composed of plaster of Paris, glue, pigment, basic lead carbonate and linseed oil; irradiated for 144 h under ultraviolet light (250–280 lux).

In addition to Stahl's original purpose to apply this procedure to the evaluation of "natural drugs and their mixtures and preparations," the technique is also useful for the preliminary screening of oils or materials that contain oils (such as works of art) for the purpose of identifying the oil and/or to determine the relative age of the oil or oil-bearing material.

#### ACKNOWLEDGEMENT

I am indebted to Prof. Paolo Mora, Istituto Centrale del Restauro, Rome, for the preparation of the artist's paint mixture.

#### REFERENCES

- 1 E. Stahl (Editor), *Drug Analysis by Chromatography and Microscopy*, Ann Arbor Sci. Publ., Ann Arbor, Mich., 1973, Ch. 7, p. 85.
- 2 N. Stolow, *Application of Science in Examination of Works of Art*, Boston Museum of Fine Arts, Boston, Mass., 1967, p. 172.