Journal of Chromatography, 100 (1974) 243–246

CHROM. 7760

Note

Detection of mustard seed, soya bean and safflower seed oils in groundnut oil by thin-layer chromatography

T. N. B. KAIMAL, V. V. S. MANI, K. T. ACHAYA* and G. LAKSHMINARAYANA
Regional Research Laboratory, Hyderabad (India)
(Received May 2nd, 1974)

Thin-layer chromatography (TLC) has become widely used for the detection of adulteration of oils and fats[1]. The presence of erucic acid in rape and mustard seed oils has been made use of to detect these oils in other oils[2–4]. Gas–liquid chromatography of the fatty acid methyl esters[5] and TLC of triglycerides[6] were used to detect soya bean oil in olive oil. Safflower seed oil is reported to contain an unidentified sterol or group of sterols[7] different from those present in groundnut oil. This difference is used to detect safflower seed oil in groundnut oil.

EXPERIMENTAL

Soya beans and mustard seeds were extracted with *n*-hexane. Other oils were reliable commercial samples. Mixtures of known composition were prepared for use in the study.

Purification

For the detection of soya bean oil in groundnut oil, a preliminary purification is necessary in order to remove autoxidation products, which if present might interfere in the detection of linolenate. This was accomplished by filtering without suction 200 ml of a solution of the oil (*ca.* 1.0 g) in a mixture of 5% of diethyl ether in light petroleum (b.p. 40–60°) through a bed of silica gel (chromatographic grade, 40 g) held in a sintered-glass funnel (diameter 6.5 cm). The first 100 ml of the filtrate were collected and used after removal of the solvent.

Preparation of methyl esters

Into a clean, dry test-tube fitted with a standard joint was added one drop of the oil followed by two drops of dry carbon tetrachloride or dry alcohol-free chloroform and 1.0 ml of 1% sodium methoxide solution (prepared by dissolving 0.5 g of sodium in 100 ml of anhydrous methanol). The tube was stoppered and heated on a hot water-bath for 3 min, taking care to release the pressure occasionally. Alternatively, the contents of the tube were shaken vigorously for 5 min, either by hand

* Present address: Protein Foods and Nutrition Development Association of India, Bombay, India.

or with a wrist-action shaker, until the turbidity had disappeared. The methanol solution was used directly for spotting. The methyl esters were also extracted in some instances with light petroleum (b.p. 40–60°) after dilution with water.

Isolation of unsaponifiable matter

Unsaponifiable matter was isolated by the official method Ca 6b-53 of the American Oil Chemists' Society.

Preparation of argentated silica gel C plates

Clean glass plates (20 × 10 cm) were coated with a slurry of silica gel C (silica gel containing 13% of calcium sulphate as binder: Acme Synthetic Chemicals, Bombay, India) in a 2.5% aqueous solution of silver nitrate (1:2, w/v) to a thickness of 300 μm. The coated plates were air-dried for 10 min, heated at 110° for 1 h and cooled in a desiccator.

Preparation of argentated silica gel C microscope slides

A slurry was prepared by shaking well a mixture of 30 g of silica gel C and a solution of 9.0 g of silver nitrate in 15 ml of acetonitrile and 60 ml of chloroform. Clean microscope slides were coated by dipping two of them together and withdrawing them immediately. After evaporation of the solvent, the slides were ready for use.

Detection of mustard seed oil

The methyl esters (*ca.* 100 μg) were spotted on argentated silica gel C plates and developed with light petroleum (b.p. 40–60°)–diethyl ether (94:6) to a distance of 12 cm in *ca.* 40 min. The developed plate was heated on a hot-plate at 220–240° for 20 min, when black spots appeared on a white background. Prolonged heating of the plate for more than 40 min or long exposure to light at room temperature tended to darken the plate.

Detection of soya bean oil

The methyl esters (*ca.* 100 μg) were spotted on silica gel C–silver nitrate plates and developed as above using light petroleum (b.p. 40–60°)–diethyl ether (140:10), and the spots were located as described above.

Detection of safflower seed oil

Unsaponifiable matter (*ca.* 100 μg) was spotted on silica gel C plates and developed with benzene–ethyl acetate (4:1), and the developed plates were sprayed with concentrated sulphuric acid–formaldehyde (4:1) and heated at 110° for 10 min.

RESULTS AND DISCUSSION

Fig. 1 shows the separation of methyl esters of mustard seed oil and groundnut oil and 5% of mustard seed oil in safflower seed, cottonseed and groundnut oils. A spot corresponding to erucate above that of oleate indicates the presence of mustard seed oil. This method can be used to detect mustard seed oil in any oil that does not contain erucic acid, which includes almost all common edible oils.

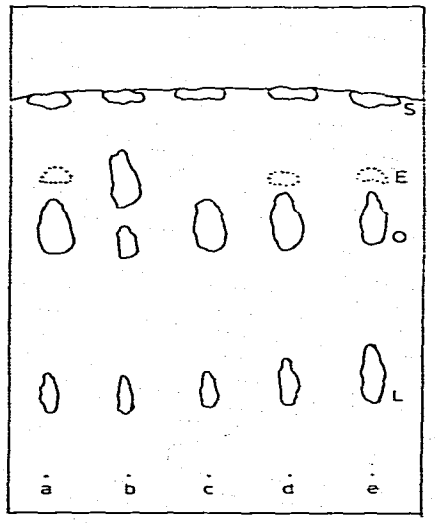

Fig. 1. Detection of adulterant mustard seed oil. Adsorbent: silica gel C–silver nitrate. Developer: light petroleum (b.p. 40–60°)–diethyl ether (94:6). The developed plate was heated at 220–240° for 20 min; no spray reagent was used. Methyl ester sample: (a) 5% of mustard seed oil in groundnut oil; (b) mustard seed oil; (c) groundnut oil; (d) 5% of mustard seed oil in cottonseed oil; (e) 5% of mustard seed oil in safflower seed oil. S, saturated; E, erucate; O, oleate and L, linoleate.

In Fig. 2, the separation of the methyl esters of groundnut oil and soya bean oil and groundnut oil containing 10% of soya bean oil is shown. The spot corresponding to methyl linolenate indicates adulteration of the groundnut oil. The lowest limit of detection is 5% for adulterant soyabean oil of Indian origin which contains *ca.* 4.0% of linolenic acid[8] and 2% for samples of American origin containing about

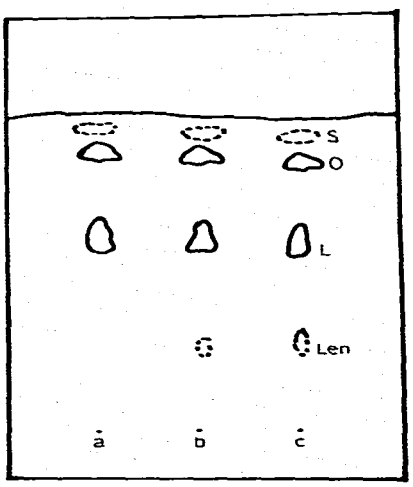

Fig. 2. Detection of soya bean oil in groundnut oil. Adsorbent: silica gel C–silver nitrate. Developer: light petroleum (b.p. 40–60°)–diethyl ether (140:10). Location of spots as in Fig. 1. Methyl ester sample: (a) groundnut oil; (b) 5% of soya bean oil in groundnut oil; (c) soya bean oil. S, saturated; O, oleate; L, linoleate and Len, linolenate.

8.0% of linolenic acid[8]. Linseed oil contains over 50% of linolenic acid[9] and, if present as an adulterant, it cannot be differentiated from soya bean oil. In fact, even 1.0% or less of adulterant linseed oil can be detected. The test can also be carried out on microscope slides coated with silica gel C–silver nitrate using light petroleum (b.p. 40–60°)–diethyl ether (60:40) as the solvent. This method can be used to detect soya bean oil or linseed oil in any oil that does not contain linolenic acid, such as groundnut, safflower seed, sunflower or sesame oil.

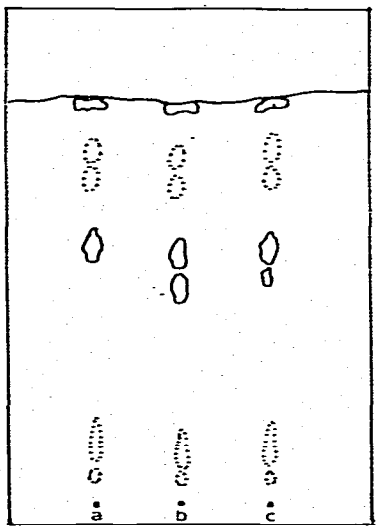

Fig. 3. Detection of safflower seed oil in groundnut oil. Adsorbent: silica gel C. Developer: benzene–ethyl acetate (4:1). Spray reagent: sulphuric acid–formaldehyde (4:1). Unsaponifiable matter sample: (a) groundnut oil; (b) safflower seed oil; (c) 5% of safflower seed oil in groundnut oil.

The separation of the constituents of the unsaponifiable fraction of groundnut oil and safflower seed oil and groundnut oil containing 5% of safflower seed oil is shown in Fig. 3. An extra spot caused by the unidentified sterol which occurs in safflower seed oil[7] indicates the presence of the latter in groundnut oil.

These potentially useful methods are very simple, and it is hoped that their scope and limitations in practice will be determined by analytical and trade laboratories.

REFERENCES

1 V. V. S. Mani and G. Lakshminarayana, *Chromatogr. Rev.*, 10 (1968) 159.
2 P. S. Nataraja Sarma and V. V. Nithyanandan, *Curr. Sci. (India)*, 35 (1966) 43.
3 G. Gorbach and G. L. Weber, *Fette, Seifen, Anstrichm.*, 65 (1963) 989.
4 G. Lakshminarayana and T. N. B. Kaimal, *J. Proc. Oil Technol. Ass. (India)*, 21, Part II (1966) 77.
5 M. Vitagliano, A. M. Leone and A. Vodret, *Olearia*, 14 (1960) 177; *C.A.*, 55 (1961) 8896e.
6 J. Sliwiok, *Mikrochim. Acta*, (1965) 294; *C.A.*, 62 (1965) 14939e.
7 J. Eisner and D. Firestone, *J. Ass. Offic. Agric. Chem.*, 46 (1963) 542.
8 G. K. Viswanadha Rao, M. R. Subbaram and K. T. Achaya, *Indian J. Technol.*, 9 (1971) 265.
9 T. P. Hilditch and P. N. Williams, *The Chemical Constitution of Natural Fats*, Chapman & Hall, London, 1964, p. 210.