

Rapid Location of Ultimate Double Bonds in Triglycerides

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The present technique was worked out to simplify the location of ultimate double bonds in the main unsaturated fatty acids of seed oils. It is based on a rapid, isothermal gas-chromatographic determination of the main end carbon chains¹ split off as mono-carboxylic acids by permanganate oxidation of the oils in acetone solution during a few minutes at room temperature. The acids are liberated² by formic acid, and loss of volatile and water-soluble members is avoided by direct analysis of the crude oxidation supernatant. Major reaction products other than the mono-carboxylic acids are retained on the column. The solvent peak is small enough to allow detection of acetic acid. Nonanoic acid, which often is the longest mono-carboxylic acid formed by oxidative cleavage of seed oils, emerges in less than 10 min.

A polyester column incorporating phosphoric acid³ is used. It permits analysis not only of the mono-carboxylic acids, but also of the methyl esters from intact oils, both at the same temperature. Comparison of the two types of resulting gas chromatograms will usually allow easy correlation of their major peaks, and thus often of chain length and number of double bonds with end carbon chain. Relationships among minor peaks may be less clear, partly due to some over-oxidation producing lower homologues of the free acids.

The following general procedure, which may be scaled down if necessary, has been used to analyse widely different seed oils. Oxidations were carried out in small (5 ml) glass-stoppered test tubes by dissolving 10 mg of oil in 0.2 ml of acetone, adding 20 mg of finely powdered potassium permanganate and vibrating for 5 min. After addition of 20 μ l of formic acid (98–100% HCOOH) and vibrating again for 5 min, the supernatant appearing on standing or centrifugation was analysed by gas chromatography. Resulting chromatograms for some well-known seed oils

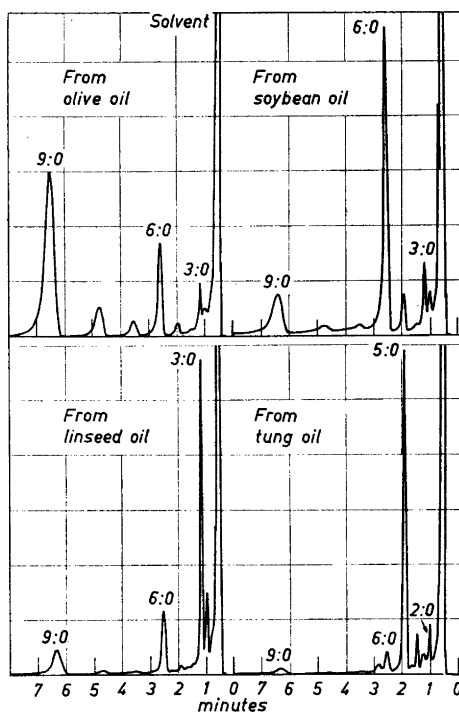


Fig. 1. Gas chromatograms showing mono-carboxylic acids formed by permanganate oxidation of seed oils in acetone during 5 min at room temperature. Column (old): 2 m \times 4 mm i.d. aluminium tube filled with a commercial⁴ packing of 10% (w/w) poly-adipate of diglycol (DEGA) and 2% phosphoric acid (85% H₃PO₄) on 60–80 mesh calcined and acid-washed diatomaceous earth (Gas-Chrom A). Temperature of column and flame ionization detector: ca. 200°C. On-column injections (ca. 1 μ l) eluted with argon. Inlet pressure (ca. 1.4 kg/cm²) adjusted to give a retention time of 1 min for acetic acid (2:0).

are reproduced in Fig. 1. They may be compared with Table 1 for results to be expected.

The mild oxidation procedure probably leaves double bonds intact, and might thus produce mono-carboxylic acids in amounts which are not strictly proportional to the number of the corresponding ultimate double bonds. But approximate data usually suffice for correlations to be made, and serious discrepancies between oxidation results and oil composition are not apparent. Minor peaks are sometimes stronger than expected from

Table 1. Seed oil composition.⁵

Analysed seed oils n_D^{25}	Main unsaturated acids (decreasing amounts)	End carbon chains
Olive oil 1.4676	18:1(9)	9
	18:2(9,12)	6
Soybean oil 1.4736	18:2(9,12)	6
	18:1(9)	9
	18:3(9,12,15)	3
Linseed oil 1.4799	18:3(9,12,15)	3
	18:2(9,12), 18:1(9)	6,9
Tung oil 1.5181	18:3(9,11,13)	5
	18:2(9,12), 18:1(9)	6,9

analysis of the unsaturated esters. In the case of acetic acid (2:0) this may partly be due to impure formic acid, as all samples so far examined have given a small peak at 2:0. More than trace amounts of acetic acid are apparently not formed by oxidation of acetone or by decarboxylation of malonic acid from skipped ⁶ systems. A propanoic acid (3:0) peak stronger than expected may indicate that the linolenate value is too low; substantial losses of polyunsaturated esters easily occur during gas chromatography.^{7,8} Over-oxidation should produce heptanoic acid (7:0) in amounts corresponding to about 10 % of 8:0. The present peaks are stronger, probably because the oils contain small amounts of 9-hexadecenoate and/or 11-octadecenoate.^{9,10} Some minor unidentified peaks are also seen. Carry-over^{11,12} of components from previous injections may occur, but can be recognized and minimized by blank injections prior to analysis or by repeated injections of the oxidation supernatant.

The present technique can also be used for the rapid location of ultimate double bonds in open-chain compounds other than triglycerides, and to locate ultimate skipped ⁶ systems. When applied to the free fatty acids from a seed oil hydrolysed during alkali isomerisation, a clean gas chromatogram of the mono-carboxylic acids formed by oxidation was obtained.

Comparison with the oxidation chromatogram of the original oil allowed immediate location of an ultimate skipped system, revealed by an increased amount of one acid and a corresponding decrease in the amount of its next lower homologue.

Rapid location of ultimate hydroxyl groups is also possible. When for example castor oil is oxidized, the main mono-carboxylic acid formed is heptanoic acid, a result of cleavage between the carbon atoms Nos. 11 and 12 in ricinoleic acid, 18:1(9,12-OH). Oxidation of compounds with ultimate triple bonds or oxo groups has not yet been tried.

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