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Note

Rapid method for the determination of fatty acid profiles from fats and oils using trimethylsulphonium hydroxide for transesterification

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To evaluate their fatty acid compositions, glyceride fats and oils are generally converted into fatty acid methyl esters by base catalyzed transesterification using sodium methoxide^{1,2} or by saponification followed by acid catalyzed esterification³⁻⁵. Quaternary ammonium hydroxides such as trimethyl(*m*-trifluorotolyl)ammonium hydroxide (TMTFTA^H)⁶ or tetramethylammonium hydroxide (TMAH)⁷ have recently been used as transesterification catalysts for triglycerides. In addition to the triglycerides, free fatty acids which may be present in the sample are converted into their quaternary ammonium salts, which are then alkylated pyrolytically to the corresponding methylesters.

Trimethylsulphonium hydroxide (TMSH) has been shown to be an even more powerful pyrolytic alkylation reagent⁸. In this paper some results of the transesterification with TMSH are reported and the advantages of this method are discussed.

MATERIALS AND METHODS

Chromatographic equipment and conditions

A Model L 402 gas chromatograph (Siemens, Karlsruhe, G.F.R.) equipped with a flame ionization detector and 50 m × 0.32 mm quartz capillary column coated with OV-351 (Orion, Finland) was used for the analytical work. The injection port temperature was 250°C, the detector temperature 300°C. Hydrogen with a linear flow-rate of 40 cm/sec was the carrier gas and the splitting ratio was 1:50. The fatty acid profiles of almond oil were evaluated isothermally at 220°C, with manual (needle) injection of 2- μ l volumes. Fatty acid compositions of other fats and oils (sesame oil, olive oil, butter) were measured using an automatic injection system (Autosampler, AS 180, Siemens) equipped with a pneumatically driven, flash heated piston having an annular groove of 2 μ l: injection port temperature, 210°C; piston heating time, 0.5 sec to 250°C. The temperature program for the chromatography of fatty acids of sesame and olive oil was 2 min at 140°C and then at 5°C/min to 230°C; that for butter was 2 min at 100°C then at 5°C/min to 230°C.

Reagents

A 0.2 mol/l solution of TMSH in methanol was prepared by reaction of trimethylsulphonium iodide (Ventron, G.F.R.) with silver oxide (E. Merck, Darm-

stadt, G.F.R.) at room temperature. The reaction was allowed to proceed until no iodide could be detected in the reaction solution. Sodium methoxide in methanol (0.5 mol/l) and TMTFTAHA (0.2 mol/l in methanol, Meth-Prep II) were both obtained from Applied Science Labs. (State College, PA, U.S.A.).

Transesterification procedures

Sodium methoxide. Sodium methoxide solution (1 ml) and 1 ml toluene were added to 10 μ l (or 10 mg) of fat or fatty oil and heated to 75°C for 20 min. After cooling, 1.5 ml toluene and 1.5 ml water were added and the mixture was shaken vigorously. After phase separation the water was removed with a pipette and another 1.5 ml water were added and the mixture was shaken again. After phase separation an aliquot of the toluene phase was used for gas chromatography.

TMTFTAHA. Ten microlitres (10 mg) of fat or fatty oil were dissolved in 0.5 ml toluene, 250 μ l Meth-Prep II solution were added and after 30 min the solution was ready for fatty acid analysis.

TMSH. The same procedure as for TMTFTAHA was applied, using TMSH instead of Meth-Prep II. It is possible to avoid solvents like toluene by adding the methanol solution of TMSH directly to the fat or oil. The reaction is complete upon dissolution of the fat.

TABLE I

FATTY ACID COMPOSITIONS OF THREE OILS OBTAINED BY TRANSESTERIFICATION WITH TMSH, SODIUM METHOXIDE HYDROLYSIS-METHYLATION AND TRANSESTERIFICATION WITH TMTFTAHA.

The normalized peak areas and S.D. values (in parentheses) are given. For reaction conditions see Materials and methods.

	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	n
<i>Almond oil</i>									
NaOCH ₃	7.55 (0.43)	0.49 (0.06)	1.45 (0.20)	64.35 (1.09)	25.91 (0.88)	0.24 (0.07)			11
TMTFTAHA	8.66 (0.54)	0.63 (0.06)	1.37 (0.22)	62.84 (1.48)	25.96 (1.13)	0.28 (0.02)			12
TMSH	8.34 (0.97)	0.68 (0.20)	1.42 (0.17)	64.52 (2.03)	24.88 (1.31)	0.27 (0.03)			12
<i>Sesame oil</i>									
NaOCH ₃	10.72 (0.11)	4.70 (0.05)	35.94 (0.28)	47.91 (0.14)	0.29 (0.01)	0.37 (0.02)	0.05 (0.03)		5
TMTFTAHA	13.59 (0.54)	4.76 (0.21)	35.08 (0.13)	45.49 (0.71)	0.31 (0.02)	0.38 (0.02)	0.38 (0.02)		7
TMSH	12.94 (0.27)	4.67 (0.06)	35.30 (0.17)	46.19 (0.15)	0.34 (0.02)	0.45 (0.04)	0.11 (0.03)		7
<i>Olive oil</i>									
TMTFTAHA	16.15 (0.25)	0.87 (0.05)	2.80 (0.06)	68.89 (0.29)	9.88 (0.08)	0.62 (0.02)	0.39 (0.04)	0.60 (0.04)	10
TMSH	15.95 (0.33)	0.81 (0.06)	2.90 (0.07)	69.02 (0.33)	9.86 (0.06)	0.71 (0.04)	0.42 (0.04)	0.31 (0.01)	12

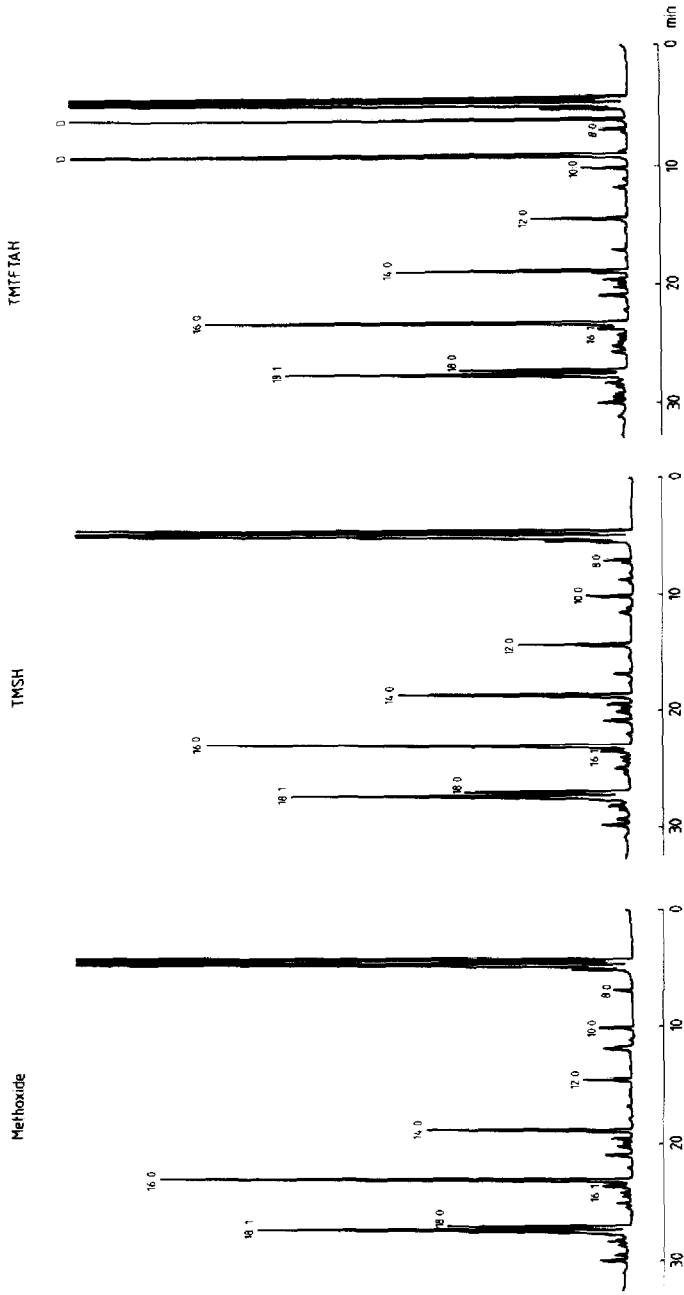


Fig. 1. Gas chromatographic profiles of fatty acids from butter obtained by different transesterification procedures. For conditions see Materials and methods.

RESULTS AND DISCUSSION

The method generally used to obtain fatty acid methylesters from triglycerides, *i.e.*, alkali-catalyzed methanolysis followed by acid-catalyzed esterification, has several disadvantages. It is time-consuming, involving many steps such as backwashing, drying, evaporating and transferring reagents and reaction products⁹. One-step transesterification procedures were therefore developed using sodium methoxide^{1,2} or quaternary ammonium hydroxides such as TMTFAH⁶ or TMAH⁷. An additional extraction step is needed when sodium methoxide is used.

Table I presents a comparison between transesterification with sodium methoxide and TMTFAH and TMSH for almond, sesame and olive oils. The results of the TMTFAH and the TMSH procedures are identical within the limits of error. Slightly different results are obtained with sodium methoxide because it does not convert fatty acids initially present into the corresponding methyl esters². In other words, the TMTFAH and the TMSH procedures yield the total fatty acid composition of the oils, whereas with sodium methoxide only the fatty acids of the triglycerides are considered. The greater standard deviations for almond oil compared to sesame and olive oils are due to the use of manual injection. TMAH transesterification was not included in this comparison as this method needs a neutralization step if the total fatty acid content is desired⁷.

Transesterification with TMSH and TMTFAH seems to give equivalent results for fats and oils bearing only long chain fatty acids. The fatty acid profiles of butter (Fig. 1) show the limitations of the TMTFAH procedure. Degradation products formed in the pyrolytic methylation with TMTFAH, such as *m*-trifluorotolyl dimethylamine, show long retention times and may disturb the chromatographic separation of fatty acids of medium chain lengths (see D peaks, Fig. 1). The by-product of the pyrolytic methylation of TMSH is dimethyl sulphide, b.p. 37°C. It is eluted with the solvent peak and does not disturb the chromatographic separation of fatty acids.

The advantages of the TMTFAH method have already been mentioned⁶: room temperature transesterification; no extraction or additional treatments and low injection port temperature. These are also true of TMSH transesterification. Thus, because TMTFAH is very expensive (\$500 per 100 ml)⁷ and its by-products cause interference, TMSH seems to be the reagent of choice for the evaluation of fatty acid profiles using a one-step transesterification procedure.

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