Microreactor for methanolysis of triglycerides before gas–liquid chromatography

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SUMMARY A rapid, accurate microtechnique has been developed for gas chromatographic determination of the fatty acid composition of small (2–3 μl) samples of vegetable oils. This microtechnique combines transesterification and sample injection into a single operation. The fatty acid compositions of soybean, linseed, and safflower oils thus determined are compared with those obtained by the usual two-step procedure.

KEY WORDS microtechnique · gas–liquid chromatography · fatty acid composition · vegetable oils

Procedures currently used to determine the fatty acid composition of triglycerides are actually preparative methods modified to make them more amenable to analytical operations. Consequently, these procedures generally involve multiple-handling techniques and consume relatively large quantities of sample and time.

The microreactor apparatus (MRA) recently described for locating double bonds by ozonization–pyrolysis (1) has now been adapted for use with a simple transesterification–injection procedure for microscale analyses of triglyceride oils by GLC. This improvement facilitates operations, saves time, and permits the use of microsamples.

Apparatus. The MRA is made by modifying an ordinary soldering gun (Fig. 1). A copper-encased, $^1/_8$ inch o.d., stainless steel loop is fitted between the poles of the gun to form the connection customarily made by a replaceable tip. A 20-gauge hypodermic needle is attached to one end of the steel loop and a Swagelok tee to the other. The side-arm of the tee is fitted with a gas connection and the remaining opening is closed with a silicone rubber septum.

The chromatograph was a temperature-programmed Aerograph, Wilkins Model 350-B, equipped with a standard filament cell. Dual analytical columns were of coiled $^1/_4$ inch o.d. aluminum tubing, 30 inches long, containing 30% diethylene glycol succinate polyester on 40–60 mesh Chromosorb W.

Quantitative results with the Applied Science counterpart of National Heart Institute Fatty Acid Standard "D" agreed with the stated composition data with a rela-

Abbreviations: GLC, gas–liquid chromatography; MRA, microreactor apparatus.

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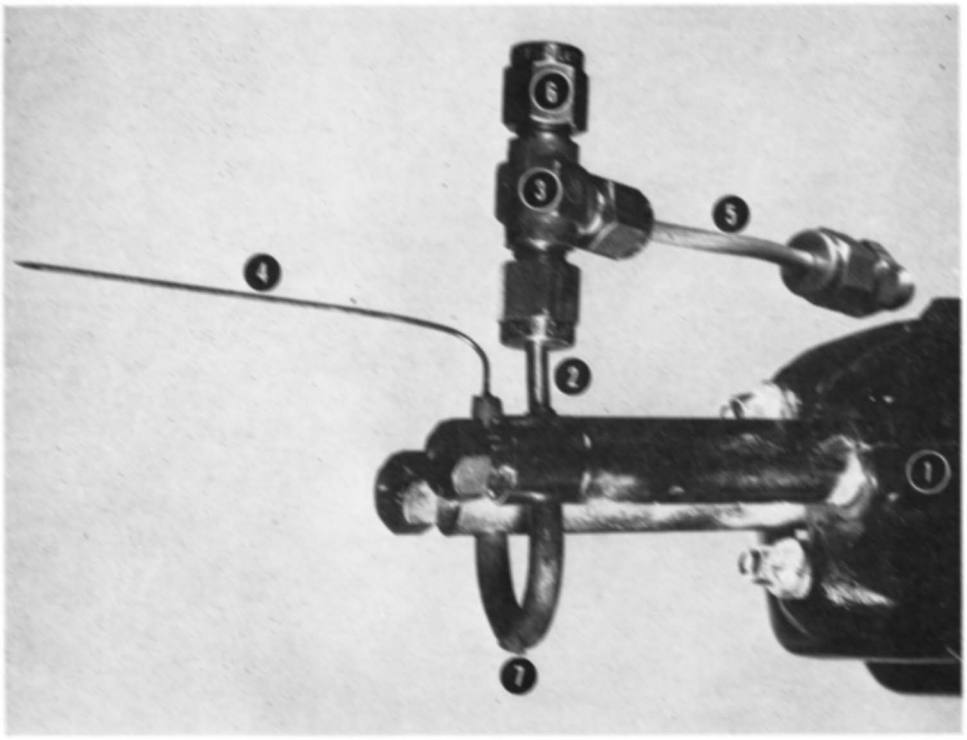

FIG. 1. Soldering gun modified to form a microreactor apparatus. *1*, Soldering gun; *2*, copper-encased, 1/8 inch O.D. stainless steel loop; *3*, Swagelok tee; *4*, needle, 20 gauge; *5*, gas connection; *6*, fitting for silicone rubber septum; and *7*, position of thermocouple attachment.

TABLE 1 COMPARISON OF MACRO- AND MICROTRANSESTERIFICATION OF FOUR VEGETABLE OILS

Methyl Ester	RSBO*		SBO		LSO		SFO	
	Macro	Micro	Macro	Micro	Macro	Micro	Macro	Micro
	weight % of methyl esters							
Palmitate	13.1	13.0	12.5	12.6	6.6	5.7	6.7	7.5
Stearate	2.7	2.7	3.1	2.4	2.7	2.4	1.6	1.7
Oleate	23.8	23.2	20.5	19.7	19.0	19.6	11.4	12.0
Linoleate	54.7	55.8	58.5	60.3	15.5	15.8	80.3	78.8
Linolenate	5.6	5.3	5.4	5.0	56.3	56.5	—	—

* RSBO, reconstituted soybean; SBO, soybean; LSO, linseed; SFO, safflower oils.

tive error less than 9% for major components and less than 8% for minor components.

Procedure. The MRA is connected, as previously reported in detail (1), to a separate helium cylinder from which both the flow and pressure of the gas are regulated to match closely those of the helium carrier gas of the gas–liquid chromatograph. The helium flow is temporarily discontinued when the MRA is completely filled with inert gas.

A 3 μl sample of 2.7 M sodium methoxide in methanol is drawn into a 10 μl Hamilton microsyringe followed by a small air space and a 2 μl sample of the oil to be analyzed.

TABLE 2 COMPOSITION OF RECONSTITUTED SOYBEAN OIL MIXED WITH INTERNAL STANDARDS

	With Laurate			With Margarate			With Both		
		Experimental			Experimental			Experimental	
	Theory	Macro	Micro	Theory	Macro	Micro	Theory	Macro	Micro
	weight % of methyl esters								
Laurate	17.8	16.8	21.7	—	—	—	11.8	11.1	12.7
Margarate	—	—	—	15.1	14.5	15.3	9.7	9.3	8.6
Palmitate	10.8	10.9	9.3	11.2	11.2	10.4	10.4	10.4	10.1
Stearate	2.3	2.3	2.3	2.4	2.3	2.0	2.2	2.2	2.0
Oleate	20.1	19.8	21.5	20.7	20.4	23.4	19.2	18.9	19.2
Linoleate	44.1	45.6	41.4	45.6	46.9	45.2	42.1	43.6	39.8
Linolenate	4.9	4.7	3.8	5.0	4.8	3.7	4.6	4.5	4.6

This sample is immediately injected, without mixing, through the septum into the reaction tube of the MRA. Activating the switch on the soldering gun raises the temperature of the reaction tube, as measured by a thermocouple, to 50°C, where it is maintained for 20–30 sec. (The temperature may be continuously controlled by the use of a variable transformer connected to the MRA; the MRA need not be switched on and off to keep the temperature constant.) The helium is again allowed to flow through the MRA and promotes evaporation of excess methanol. A 4 μl sample of formic acid is immediately injected and the temperature of the reaction tube raised to 100°C. When moistened indicator paper shows that no acid remains in the gas flowing from the needle, the needle is inserted through the inlet port septum of the gas chromatograph and the sample is vaporized onto the analytical column. Holding down the trigger of the soldering gun raises the temperature of the loop to 250°C. If flow through the MRA closely matches that through the chromatograph, sample injection is accomplished without appreciably affecting the balance of the thermal conductivity cell: the shapes of the peaks are identical with those obtained by standard methods of injection.

For cleaning, the MRA is disconnected from the helium source and connected to a vacuum line. Water, methanol, and acetone are successively drawn through it. The cooled MRA is then ready to be reconnected to the helium source for introduction of the next sample.

Results and Discussion. Initial investigations were conducted on a soybean oil reconstituted from its distilled methyl esters to give a pure, completely randomized, triglyceride of known composition. The use of this triglyceride simplified calculations and provided an additional measure of quantification.

Samples of the oil were treated by both the usual macrotransesterification procedure and by the MRA procedure. The results for each procedure and those originally calculated for the reconstituted oil were in quantitative agreement (Table 1). Macro- and microprocedures gave identical results also for soybean oil, linseed oil, and safflower oil (Table 1).

From the original composition of the soybean oil, an average molecular weight was computed. This value formed the basis on which to calculate weight percentages of three samples, each containing one or two additional chromatographically pure methyl esters as internal standards. Methyl laurate was added to one sample, methyl margarate to a second, and both esters were used in the final sample. Results of analyses by macro- and micromethods are shown together with the calculated values in Table 2. Agreement of the values for triglyceride-derived esters with those for standard esters indicates that the recovery and extent of reaction are comparable for both procedures.

The MRA technique proved as effective for analytical transesterification as the macrotransesterification procedure. In addition, the MRA eliminated processing samples in excess of the amounts necessary for GLC analysis, reduced total time involved in sample analysis, and prevented potential losses associated with multiple-handling techniques.

Reconstituted soybean oil was prepared and supplied by R. L. Hoffmann; W. F. Kwolek assisted in statistical evaluations.

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Reference

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