A Method To Determine the Monoglyceride Content in Fats and Oils

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ABSTRACT

A methodology was worked out to analyze monoglycerides present in fats and oils in low levels (<0.5%). The monoglycerides are enriched by acetonitrile extraction and further evaluated either by gas liquid chromatography or by a combination of thin layer chromatography-gas liquid chromatography. Results are presented showing the selectivity, yield, and reproducibility of the isolation step and the efficiency of the thin layer chromatography fractionation. Examples also are given demonstrating some possible applications of the proposed method.

INTRODUCTION

Monoglycerides exist in certain fat raw materials mainly as a result of enzymatic splitting of triglycerides. The monoglyceride levels are, however, rather low, less than 0.05% in most raw fats and up to at most 0.5% in fats like coconut oil, palm kernel oil, and palm oil.

Because of their emulsifying ability, the monoglycerides may influence rheological properties of emulsion products, such as margarine. Therefore, in special cases, it is desirable to know the effect of refining processes upon the monoglyceride level in processed fats.

Quantitative analysis of monoglycerides present in fat systems under the conditions given above is hardly ever described in the literature. An appropriate procedure involves isolation or enrichment of the monoglycerides connected with a subsequent evaluation method.

Isolation of monoglycerides has been performed by molecular distillation (1) and by various forms of selective adsorption (liquid-solid) and partition (liquid-liquid) chromatographic techniques (2-7). The methods referred to have, however, been applied to model mixtures of high monoglyceride content which give them a high degree of accuracy and reproducibility. Some of the cited techniques are also tedious and time-consuming.

Evaluation of total monoglyceride content has been carried out by periodic acid titration (α-monoglycerides only), gravimetrically (3,6), densitometrically (1), and spectrophotometrically as monoglyceride derivates (8,9). Monoglyceride components have been classified by gas liquid chromatography (GLC) (10,11) and by reversed phase chromatography (12).

The intention behind this article is to report a simple method to analyze monoglycerides of naturally existing concentrations in fats and oils.

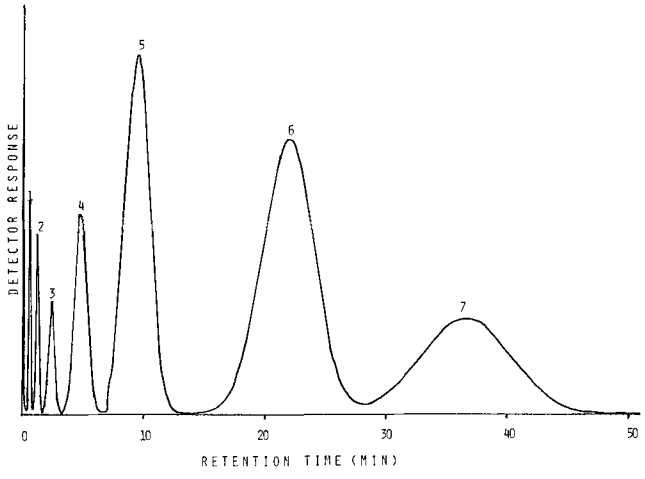

FIG. 1. The resolution of a calibration standard on the gas chromatographic column used. Column temperature: 175 C. For other details, see text. 1 = Monodecanoate, 2 = monolaurate, 3 = monomyristate, 4 = monopalmitate, 5 = monostearate, 6 = cholesterol, and 7 = monobehenate.

EXPERIMENTAL PROCEDURES

Chemicals and Reagents

Chemicals and reagents are: n-hexane, puriss; acetonitrile, for synthesis; hexamethyldisilazane (HMDS), for silylation, Analytical Standards AB, Gothenburg, Sweden; trimethylchlorosilane (TMCS), for silylation, Analytical Standards; pyridine, for silylation, Analytical Standards; cholesterol, for biochemistry, E. Merck, Darmstadt, Germany; and monoglycerides, purity > 99%, Nu Chek Prep, Elysian, Minn.

The silylation reagents and the pyridine were kept in an exsiccator over blue silica gel.

For silylation, a solution containing 3 ml TMCS and 6 ml HMDS diluted to 10 ml with pyridine was used. The solution is stable for weeks at room temperature.

Cholesterol was used as internal standard for GLC at a concentration of 50 mg/ml in pyridine. To calibrate the GLC system a standard mixture consisting of commercial monoglycerides and cholesterol was prepared in pyridine solution.

For thin layer chromatography (TLC) analysis, the following were used: diethyl ether, puriss; petroleum ether (bp 40-60 C), puriss; DC-Alufolien Kieselgel, E. Merck, size 20 x 20 cm, layer thickness 0.25 mm; ethanol, 95%; acetone, puriss; potassiummetaperiodate, pro analysi; anis-

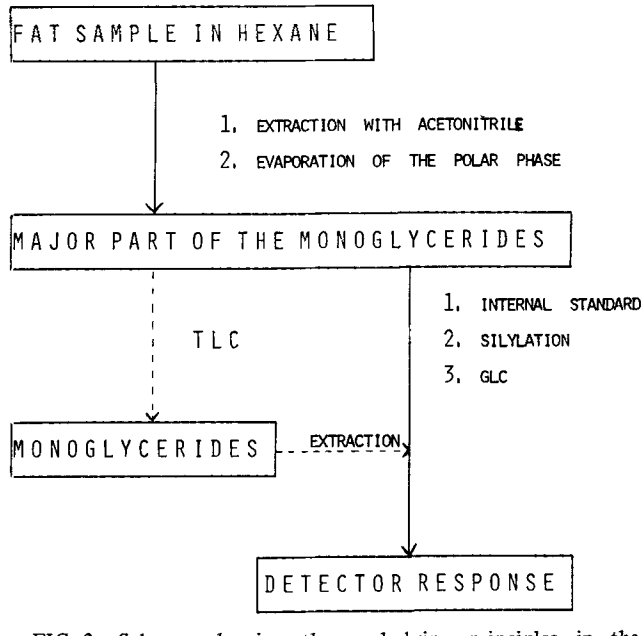

FIG. 2. Scheme showing the underlying principles in the described method. The dashed way is applied only on coconut oil and palm kernel oil.

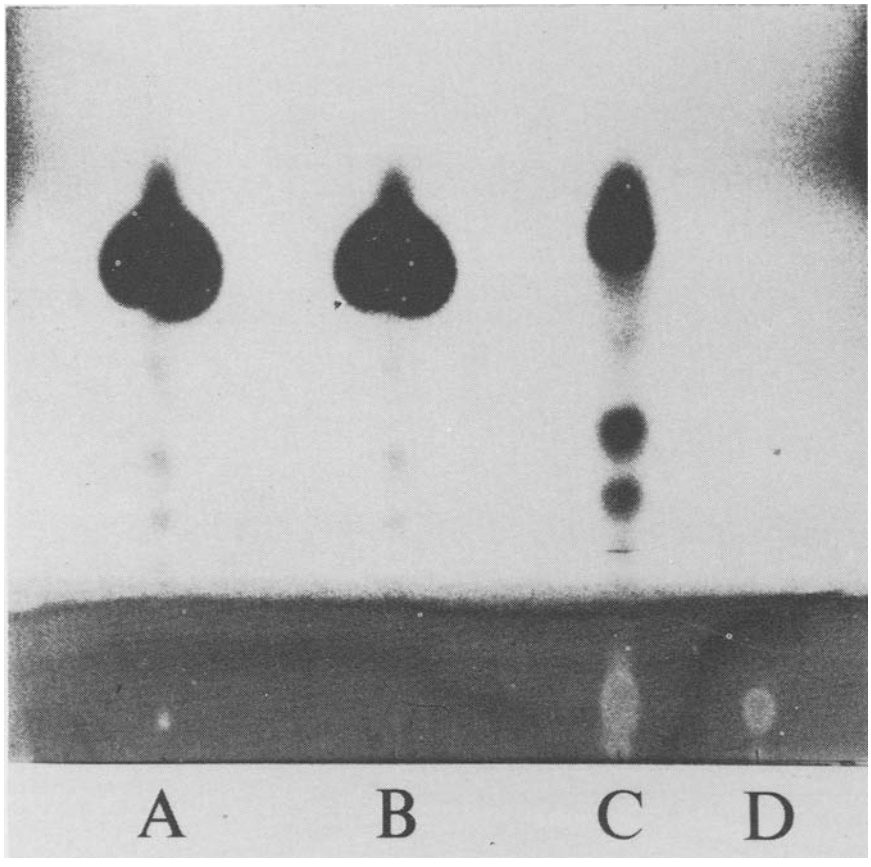

FIG. 3. Thin layer chromatography plate illustrating the effect of the extraction step. A = Hexane phase before extraction, B = hexane phase after extraction, C = acetonitrile phase after extraction, and D = monoglyceride standard. For details, see text.

idine, pro analysi; and 1 N hydrochloric acid.

Apparatus

The following apparatus were used: separating funnel, 100 ml; round bottom flask, 100 ml; equipment for GLC; and equipment for TLC.

In this work, a Varian Aerograph 2 100 gas chromatograph equipped with a flame ionizer detector was used. The gas chromatograms were registered by a Servoriter II recorder.

The GLC work was carried out on a 1 ft x 1/4 in. stainless steel column filled with Chromosorb W (AW-DMCS), 80-100 mesh, coated with 3% OV-1.

The column temperature was varied between 175 and 200 C isothermally, the injector temperature was 325 C, and the detector temperature was 300 C.

Nitrogen was used as carrier gas at a flow rate of 75 ml/min. The resolution of a standard mixture on this column is demonstrated in Figure 1.

METHODS

The monoglycerides were enriched from the fat system and analyzed according to the principles in Figure 2.

The fat sample (10.0 g) was dissolved in 30 ml hexane and transferred to a 100 ml separation funnel. The hexane solution was extracted with 3 x 15 ml hexane-saturated acetonitrile, whereupon the polar phase was evaporated in a 100 ml round bottom flask in vacuum at 80-90 C.

The residue was, depending upon the type of fat investigated, treated in one of the following two ways.

All Fats and Oils Except Coconut Oil and Palm Kernel Oil

To the isolated residue was added 0.50 ml cholesterol standard and 0.5 ml silylation standard. The silylation was completed by warming the round bottom flask in a burner for ca. 1 min. The pyridine solution was then ready for GLC analysis under the given conditions. After evaluation of the recorder response, the detected monoglycerides were quantitated according to the following formula: If the concentration of a monoglyceride i in the fat sample is C_i mg/g and f_i is a response factor, and A_i is the area of the analyzed monoglyceride peak, and A_{chol} is the area of the cholesterol peak in the sample solution, then:

$$C_i = \frac{f_i \cdot A_i \cdot 25}{A_{chol} \cdot 10}$$

TABLE I

Results Obtained in Extractions Experiments with 0.10% Each of Monolaurate, Monomyristate, Monopalmitate, and Monostearate Dissolved in Soya Oil[a]

Monoglyceride	Extraction yield (%)[b] X	S_X
Monolaurate	94	5.9
Monomyristate	91	4.7
Monopalmitate	86	3.4
Monostearate	82	6.6

[a]The results are based upon five experiments.
[b]X = mean value and S_X = standard deviation.

TABLE II

Examples Showing the Monoglyceride Increase in Some Interesterification Experiments

Experiment scale	Amount of catalyst used (% Na-ethylate)	Monoglyceride level increase (%)
Factory	0.4	0.5
Pilot plant	0.2	0.1
Laboratory	0.3	0.2

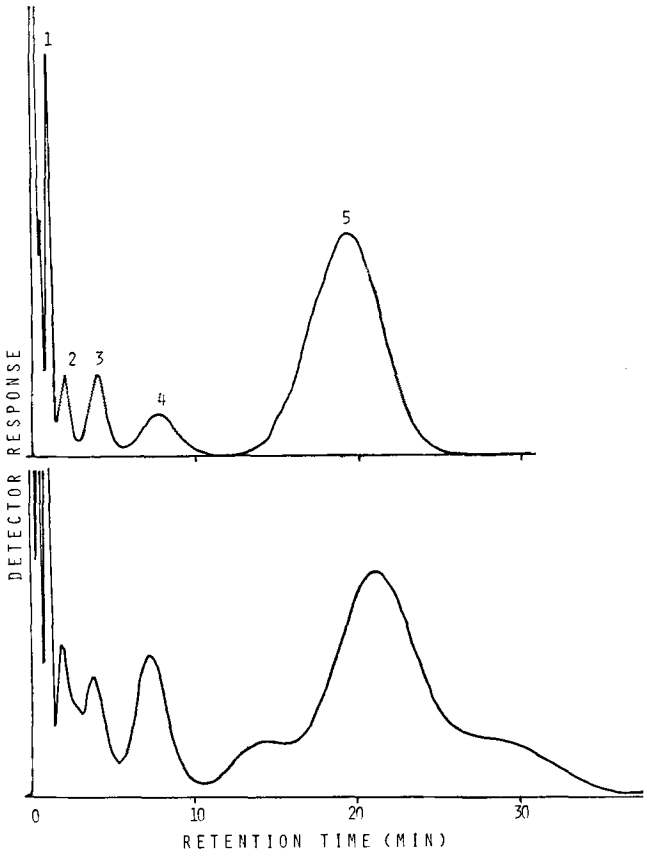

FIG. 4. Gas chromatograms demonstrating the effect of the thin layer chromatography prefractionating technique. The extracted fat sample, interesterified coconut oil, and hardened palm oil (45/55) were analyzed partly by thin layer chromatography-gas liquid chromatography (upper chromatogram) and partly by gas liquid chromatography alone (lower chromatogram). For a closer description, see text. 1 = Monolaurate, 2 = monomyristate, 3 = monopalmitate, 4 = monostearate, and 5 = cholesterol.

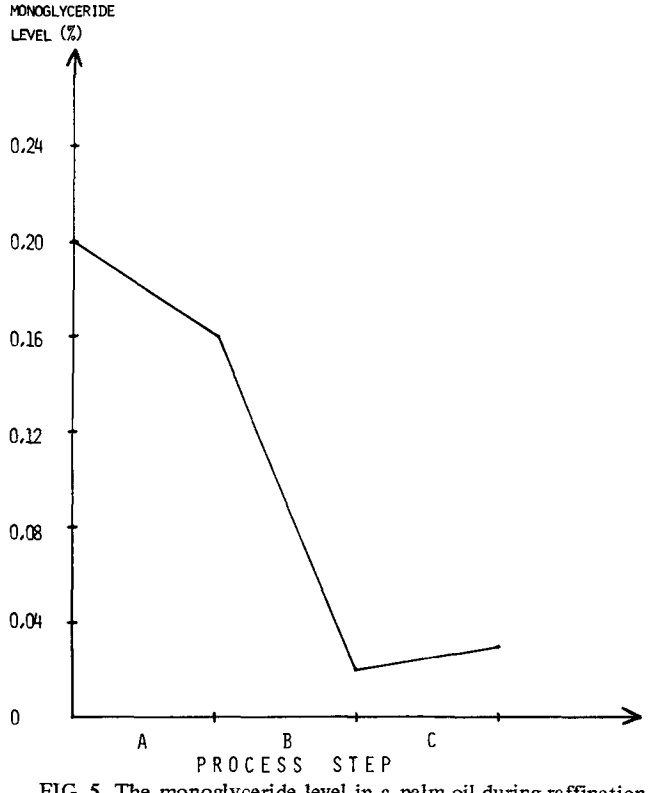

FIG. 5. The monoglyceride level in a palm oil during raffination according to the Alfa-Laval process. A = Neutralizaton, B = bleaching, and C = deodorization.

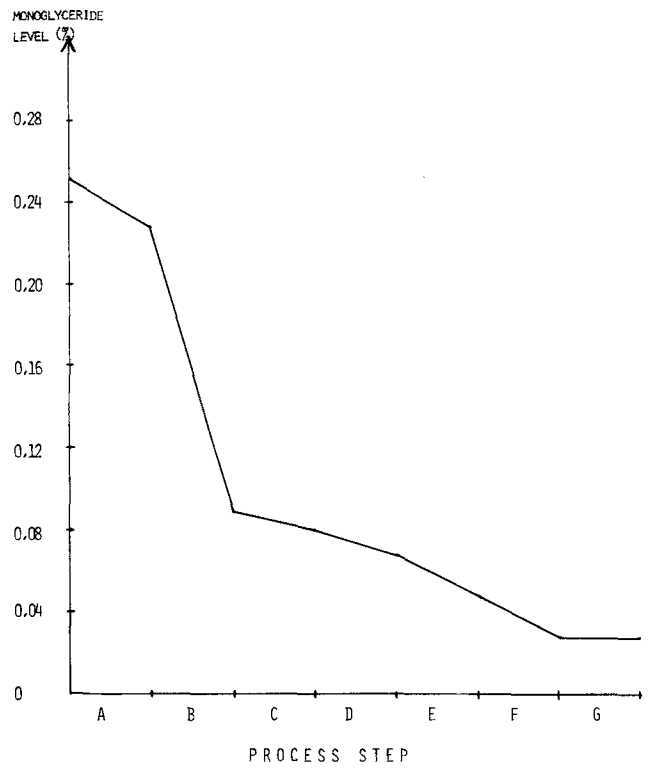

FIG. 6. The monoglyceride level in a palm oil during raffination according to the Zenith process. A,D = phosphoric acid treatment, B,E = neutralization, C,F = bleaching, and G = deodorization.

f_i is derived from the following expression:

$$f_i = \frac{A'_{chol}}{A'_i}$$

where A'_{chol} and A'_i are the relative peak areas (on the same wt basis) of cholesterol and the i monoglyceride from an appropriate calibration standard.

The compositions of the standards used regarding the monoglyceride (-s) - cholesterol concentration ratio (-s) were chosen to imitate ca. the conditions in the sample solutions. This approach assures an accurate value of f_i.

Coconut Oil and Palm Kernel Oil

In this case, the residue from the acetonitrile phase was treated as described below.

The residue was dissolved in 2.0 ml acetonitrile. 500 µliter of this solution was spotted in a streak (4-5 cm) on activated silica gel (coated on Al-foil). As a reference 10 µliter of the remaining solution also was spotted on the starting line. After development of the chromatogram with diethylether-petroleum ether (3:2), as mobile phase, the monoglyceride zone was detected by spraying the reference with 0.1% water solution of KIO_4 and a solution consisting of 2.8 g anisidine dissolved in 80 ml 96% ethanol, 70 ml deionized water, 30 ml acetone, and 1.5 ml 1 N HCl.

By this treatment, the monoglycerides were detected as a white zone against a deep red background. The corresponding sample zone was cut out with a pair of scissors, chopped into pieces, and put into a test tube. After addition of 1.5 ml pyridine, 0.50 ml cholesterol standard, and 1 ml silylation reagent to the test tube, the solution was boiled for 1 min. The pyridine solution then was ready for GLC analysis as described above.

RESULTS AND DISCUSSION

The described method was tested concerning the selectivity, yield, and reproducibility of the extraction step and

concerning the yield of the TLC step. The silylation reaction is known to be quantitative.

Selectivity of the Extraction Step

In these experiments, a refined and deodorized soya oil, containing 0.10% each of monolaurate, monomyristate, and monopalmitate was used. The extracted monoglycerides were quantitated by GLC, as described above. The compositions of the extract and the hexane phase before and after extraction are illustrated by the TLC plate shown in Figure 3. Two spray reagents were used here, one to detect lipids in general and one for α-monoglycerides. In this case, the concentration ratio between the monoglycerides and the triglycerides was ca. 1:300 in the hexane layer before extraction and 1:2.5 in the extract, i.e. the monoglycerides were, by the extraction, enriched ca. 120 times relative to the triglycerides.

As can be seen from the TLC plate, other substances than monoglycerides are extracted into the acetonitrile phase. With a few exceptions, these substances do not influence the analyses of the monoglycerides.

Extraction Yield and Reproducibility

These tests were carried out on refined and deodorized soya oil containing 0.10% each of monolaurate, monomyristate, monopalmitate, and monostearate. Extraction yields with deviations are expressed in Table I.

The yields obtained are high but decrease with increasing chain length which also is to be expected. The deviations are fairly small. Regarding the nonquantitative silylation of the monoglycerides, GLC results found ought to be corrected according to Table I.

Yield of the TLC Procedure

This step was introduced to separate the monoglycerides from short chained diglycerides in fats, such as coconut oil and palm kernel oil, because of their interference in the GLC analysis.

After separation on the TLC plate, the monoglycerides are desorbed and silylated in one step by the referred treatment. The yield of this treatment was tested with acetonitrile extracts from fat samples containing 0.3% standard monoglyceride mixture. The monoglyceride content was evaluated by GLC analysis on one-half of the extract and compared with the result obtained by the TLC-GLC procedure on the other half. The overall yields in these experiments varied within 85-95%. The effect of the TLC step is reflected in Figure 4. The upper gas chromatogram shows the monoglyceride pattern after TLC separation, and the actual extract composition is shown in the lower gas chromatogram.

The interacting substances are short chained diglycerides from the coconut part of the interesterified fat.

APPLICATIONS

The main applications of the proposed method are probably in the area of fat refining processes, of which some examples are given.

The monoglyceride level in a palm oil was followed during refining and deodorizing according to two different process techniques. Figures 5 and 6 illustrate the result of Alfa-Laval and Zenith raffination, respectively. Evidently, in the Alfa-Laval process, the bleaching has the greatest influence upon the monoglyceride level in palm oil. It is reduced by ca. 70% in this step. In the Zenith raffination, however, the monoglyceride level in palm oil is most efficiently reduced by neutralization. Ca. two thirds of the original amount is lost in this process.

Table II demonstrates the effect of interesterification on the increase of the monoglyceride level in some fats. As should be expected, the monoglyceride concentration in the fats after processing are related closely to the amount of catalyst used.

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