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Glyceride Composition of Processed Fats and Oils As Determined by Glass Capillary Gas Chromatography

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ABSTRACT

Refined and bleached fats and oils can be analyzed directly by high-temperature glass capillary column gas chromatography after derivatization of the fatty acids, mono- and diglycerides with BSTFA [(N,O)-bis(trimethyl silyl) trifluoroacetamide]. The intact glycerides are separated on the basis of volatility to provide characteristic carbon number profiles (CNP). Quantitative information on mono-, di- and triglycerides, as well as free fatty acids, is obtained from a single, rapid separation. Profile values are reported for 13 different processed fats and oils. The analysis performed in the split-injection mode maintains column integrity after numerous separations, while producing acceptable relative standard deviations.

INTRODUCTION

The gas chromatographic (GC) separation of triglycerides according to their total number of carbon atoms has been done for nearly 20 years (1-4). In this laboratory, a procedure has been established to extend the carbon number profile (CNP) analysis to mixtures of fatty acids, mono- and diglycerides after derivatization with BSTFA, in addition to the triglyceride profiling. This procedure has used short-packed columns with on-column injection, but recently has been replaced by glass capillary columns with split injection. Several workers have identified the advantages of glass capillary chromatography for this type of application (5-8).

We have reported previously the details on the analysis of soybean oil by capillary GC (8). The same procedure has been applied to the quantitative CNP analysis of 13 refined and bleached fats and oils, and the results are reported here.

EXPERIMENTAL

Equipment

A Hewlett-Packard Model 5880A (level 2) gas chromatograph, equipped with a flame ionization detector and capillary inlet system, was used for all separations. The chromatograph was interfaced to a Hewlett-Packard Model 3351B data system with 32K memory for data processing and report formating.

Reagents

BSTFA [(N,O)-bis(trimethyl silyl) trifluoroacetamide] (Pierce Chemical Company) was used as a derivatizing reagent for the free fatty acids, mono- and diglycerides to form the corresponding trimethyl silyl esters and ethers. Derivatization prevents both glyceride rearrangement side reactions and peak tailing. Reagent-grade methylene chloride (MC/B Manufacturing Chemists, Inc.) and all glyceride reference standards (Applied Science Division) were used as received.

Preparation of Glyceride Standard Mixtures

A primary mixture of glycerides used to determine response factors was prepared by dissolving known amounts of pure fatty acids, mono-, di- and triglycerides in 100 mL of methylene chloride according to Table I. The mixture is stable for several weeks under refrigeration. For samples containing components for which there were no corresponding reference standards, an average response factor obtained from neighboring reference peaks was assigned to the unknown. For example, the average response factor of the

TABLE I

Composition of Primary Glyceride Standard Mixture

Amount (mg)	Component	Notation
50	Palmitic acid	C16A
50	Stearic acid	C18A
50	Monopalmitin	C16M
50	Monostearin	C18M
50	Dipalmitin	C32D
50	Palmitostearin	C34D
50	Distearin	C36D
150	Tripalmitin	C48T
150	Dipalmitostearin	C50T
150	Distearopalmitin	C52T
150	Tristearin	C54T

triglyceride reference standards C48T to C54T was assigned to the C56T to C64T components in rapeseed oil samples. If these types of samples are to be analyzed on a more routine basis, the appropriate reference compounds should be obtained for accurate calibration.

A mixture of readily available glycerides and propylene glycol esters was prepared to serve as a secondary standard.

Chromatographic Conditions

All separations were performed using an 0.5 mm × 6 m glass CP-SIL5, WCOT capillary column (Chrompak). Chromatographic conditions used are given in Table II. An initial oven temperature hold time of 0.3 min was used to ensure complete separation of the methylene chloride solvent peak from palmitic acid. The hold time may vary slightly (± 0.1 min) from column to column, depending on small differences in column length and carrier gas-flow rate.

Procedure

Approximately 40 mg (2 drops) of sample of 1 mL of reference standard solution was added to a 1-dram (ca 3.7-mL) vial. Solid samples were melted gently on a steam bath and mixed well to ensure a representative sample. One mL of tricaprin solution (2 mg tricaprin/mL methylene chloride) was added to the vial and evaporated to dryness with a gentle stream of nitrogen gas. After evaporation was complete, 1 mL of BSTFA reagent was added to the sample to derivatize the free fatty acids, mono- and diglycerides. The vial was then sealed, shaken vigorously for a few seconds, and placed in a heating block at 100 C for 5 min. After derivatization, 1 μL was injected into the gas chromatograph for analysis. Results are reported on a percent-normalized basis using the previously determined reference standard response factors. Tricaprin was used as a retention time reference standard for peak identification by the HP-3351B data system and did not enter into the normalized calculations. Several representative chromatograms of typical processed oil samples are presented in Figure 1.

DISCUSSION

Column chromatography (CC), thin layer chromatography (TLC), periodic titrations, packed-column GC were used as independent techniques to establish the composition of the secondary standard mixture (8). This mixture was determined daily to test the accuracy and precision of the glass capillary GC analysis (Table III). The secondary standard was also analyzed at different concentrations to determine linearity of response. As an example, Table IV lists the results obtained for the 4 major triglycerides normally found in fats and oils. Excellent linearity was obtained for each of the triglycerides reported which ranged in concentrations between the glycerides from 0.5 to 27.8 mg/mL.

The results of the analyses of single lots of 13 refined and bleached fats and oils are reported in Table V. It should be noted that this technique is not applicable to coconut oil due to the presence of low-molecular-weight free fatty acids (which would elute under the solvent peak using the specified conditions) and severe overlapping of the di- and triglyceride regions. Also, the technique is not recommended for crude oils without some knowledge of the level and types of nonglyceride components present. The technique is, however, applicable to the analysis of the free fatty acids, mono- and diglycerides which presumably arise from partial hydrolysis in the processing of the oils.

The CNP technique is an excellent complement to the widely used GC-fatty acid composition (9) procedure for the identification of fats and oils. For example, the fatty acid composition cannot identify accurately a source oil after hydrogenation. However, hydrogenation will have no effect on the profile of triglycerides determined by this technique because separation is based on the total number

TABLE II

Chromatographic Conditions for Separation of Mixed Glycerides on 6-m CP-SIL5 Glass Capillary Column

Initial oven temperature	175 C
Initial hold time	0.3 min
Temperature program rate	25 C/min
Final oven temperature	350 C
Final hold time	5 min
Septum purge flow rate	1 mL/min
Inlet pressure	4 psi
Split vent flow rate	50 mL/min
Split ratio	75:1
Make-up gas flow rate	30 mL/min
FID hydrogen flow rate	30 mL/min
FID air flow rate	400 mL/min
Chart speed	1 cm/min

TABLE III

Statistical Analysis of Secondary Reference CNP Standard[a] on Glass Capillary Column

Component	Known (%)	Mean (%, n=7)	Std. dev.
C16A	0.2	0.2	0.0
C18A	0.5	0.5	0.0
PGMP	5.6	5.7	0.7
PGMS	5.6	7.3	0.6
C16M	7.3	7.3	0.8
C18M	9.5	9.6	1.1
C32D	3.9	4.0	0.2
C34D	9.0	9.1	0.4
C36D	7.6	7.5	0.4
C46T	0.1	0.1	0.0
C48T	1.1	1.1	0.1
C50T	4.9	4.9	0.2
C52T	14.9	14.8	0.7
C54T	26.9	26.7	1.4
C56T	1.3	1.1	0.1
C58T	0.3	0.2	0.1

[a]Known composition established by CC, TLC, periodic titrations, and packed-column GC.

TABLE IV

Analysis of Secondary Standard Triglycerides at Different Levels by Capillary GC

Triglyceride	Taken (mg)	Found (mg)
C50T	2.01	2.01
	2.54	2.53
	4.29	4.28
	5.07	5.06
C52T	6.0	6.1
	7.6	7.7
	12.8	12.9
	15.1	15.1
C54T	10.8	11.0
	13.6	13.8
	23.0	23.3
	27.2	27.4
C56T	0.53	0.51
	0.66	0.71
	1.12	1.18
	1.32	1.34

of carbon atoms and not on the degree of unsaturation of the fatty acid constituents. On the other hand, where the triglyceride profile is almost identical, as with sunflower and safflower oils, the fatty acid composition can distinguish easily the source oils by the linoleic acid content which is 66.0 and 77.0%, respectively.

Comparative results for a mixed glyceride standard obtained by packed and capillary column techniques (previously reported in ref. 8) show excellent agreement, thus indicating the absence of any significant discrimination during split injection.

These results also showed improved precision with the capillary procedure, but further improvement can be made by injecting the sample directly onto the capillary column as demonstrated by Grob (5). It should be realized that on-column capillary systems are not widely available and require special instrumental modification (7).

In addition to improvements in accuracy and precision, the capillary approach inherently exhibits many other advantages over packed-column technology. Reproducibility of retention times is improved significantly (10) and is not affected severely by the concentration or size of the peaks, allowing more consistent component identifications by the data system. Furthermore, the long column lifetime and the ability to perform the separation rapidly provides a greater number of analyses per column.

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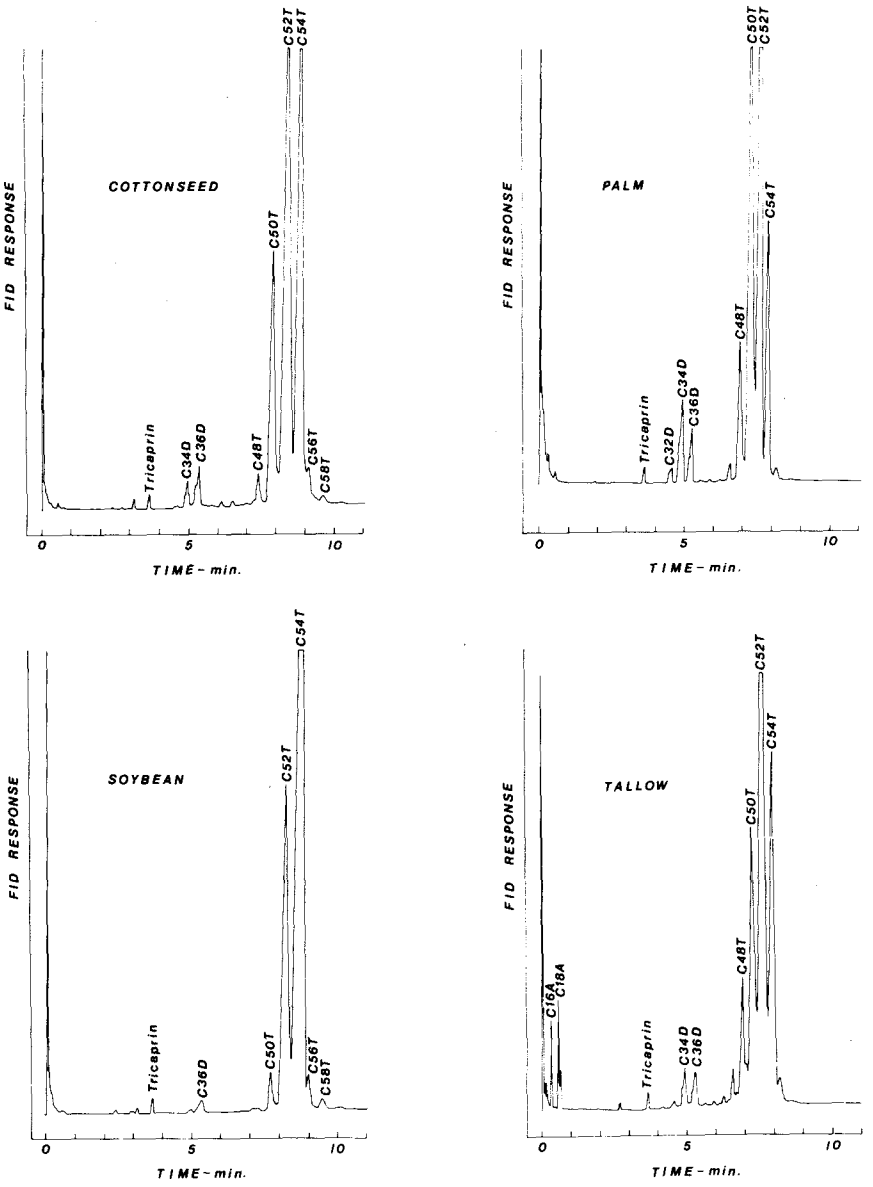

FIG. 1. Example chromatograms obtained using a 0.5 mm × 5 m, WCOT CP^{tm} Sil 5 glass capillary column.

TABLE V

Glyceride Composition by Capillary Gas Chromatography (% w/w)

	Soybean oil	Cottonseed oil	Palm oil	Corn oil	Sunflower oil	Safflower oil	Peanut oil	Sesame oil	Olive oil	Rapeseed oil	Cocoa butter	Tallow	Lard
Fatty acid	–	–	0.1	–	0.1	–	–	0.1	0.1	0.1	0.6	2.3	0.2
Monoglyceride	–	–	–	–	–	–	–	–	0.2	0.1	0.2	–	–
Diglyceride	1.0	3.1	5.8	2.8	2.0	2.1	2.2	2.6	5.5	0.8	2.2	3.8	1.3
Triglyceride	97.9	95.0	93.1	95.8	95.6	96.0	93.3	95.0	93.3	96.8	96.0	89.6	97.9
Other	1.1	1.9	1.0	1.4	2.3	1.9	4.5	2.3	0.9	2.2	0.9	4.3	0.6
Fatty acid													
C16A	–	–	–	–	–	–	–	–	–	–	0.2	1.0	0.1
C18A	–	–	0.1	–	0.1	–	–	0.1	0.1	0.1	0.4	1.3	0.1
Monoglyceride													
C16M	–	–	–	–	–	–	–	–	–	0.1	–	–	–
C18M	–	–	–	–	–	–	–	–	0.2	–	0.1	–	–
C20M	–	–	–	–	–	–	–	–	–	–	–	–	–
C22M	–	–	–	–	–	–	–	–	–	–	–	–	–
Diglyceride													
C30D	–	–	–	–	–	–	–	–	–	–	–	–	–
C32D	–	0.1	0.6	–	–	–	–	–	0.1	–	0.1	0.3	0.1
C34D	0.1	1.0	3.0	0.7	0.2	0.3	0.5	0.5	1.4	0.1	0.8	1.6	0.6
C36D	0.9	2.0	2.2	2.1	1.8	1.8	1.7	2.1	4.0	0.7	1.3	1.9	0.6
C38D	–	–	–	–	–	–	–	–	–	–	–	–	–
C40D	–	–	–	–	–	–	–	–	–	–	–	–	–
Triglyceride													
C46T	0.2	0.1	0.6	0.2	–	0.2	0.1	0.5	0.1	0.7	–	1.1	0.4
C48T	0.1	1.7	6.6	1.2	0.6	0.3	0.1	0.1	0.3	0.9	0.3	5.2	1.9
C50T	2.2	14.4	35.0	3.0	2.0	1.6	3.0	2.7	6.2	1.5	16.9	16.4	13.4
C52T	24.2	39.7	37.6	25.2	16.9	16.8	22.2	21.3	33.2	4.5	43.2	43.4	59.1
C54T	68.2	35.7	12.3	63.4	72.0	73.9	52.4	66.6	51.1	11.8	32.7	21.3	20.7
C56T	2.2	2.8	1.0	2.5	2.6	2.5	9.3	3.2	2.1	13.3	2.3	2.2	2.4
C58T	0.8	0.6	0.1	0.3	1.5	0.7	6.2	0.6	0.3	17.4	0.6	–	–
C60T	–	–	–	–	–	–	–	–	–	19.0	–	–	–
C62T	–	–	–	–	–	–	–	–	–	26.4	–	–	–
C64T	–	–	–	–	–	–	–	–	–	1.3	–	–	–

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