Gas Chromatography of Triacylglycerols in Palm Oil Fractions with Medium-Polarity Wide-Bore Columns

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Wide-bore (WBOT, 0.53 mm i.d.) capillary columns, operated with helium carrier gas, are a viable alternative to narrow-bore (0.25 mm i.d.) equivalents operated with hydrogen carrier gas in high-resolution gas chromatography (HRGC) of triacylglycerols (TAG). Resolution and quantitation proved to be similar for the two types of columns. WBOT HRGC with automatic cold-on-column injection has strong potential for precise real-time control of oils and fats processing and offers a reliable approach to automated HRGC of TAG. The technique has been used to characterize palm oil and palm oil fractions.

KEY WORDS: Automatic cold-on-column injection, capillary gas chromatography, triacylglycerol molecular species, wide-bore (WBOT) *vs.* narrow-bore (WCOT) columns.

High-resolution gas chromatography (HRGC) of triacylglycerols (TAG) on narrow-bore (WCOT) capillary columns coated with high-temperature-stable, medium-polarity phases has potentially important application to the characterization of oils and fats (1). It is possible to obtain rapid, detailed compositional information on molecular species of TAG, a capability with important possibilities for quality control (QC) and for determination of the composition of unknown fat blends (*e.g.*, detection/identification of cocoa butter equivalents in chocolate) (2). TAG are separated primarily according to carbon number (here defined as the number of carbon atoms in the combined acyl groups) but, more importantly, within each carbon number group there is a further separation based on polarity. Polarity and retention increase with the degree of unsaturation in the fatty acyl portion of the TAG (Ln >>> L >> O > S; linolenic, linoleic, oleic and stearic acids, respectively). Thus TAG of carbon number 54 (C54) are separated into SSS, SOS, SOO, OOO (etc.) types. This separation provides a powerful analytical tool, and gives information previously only available from combination techniques such as silver-phase high-performance liquid chromatography (HPLC)–carbon-number GC, from reversed-phase HPLC or from calculations based on overall and 2-position fatty acid composition (3–5). On-column injection, combined with dedicated capillary GC instrumentation, provides acceptable quantitation. For automated sample introduction into WCOT capillary columns, the outer diameter of the autosampler needle generally dictates that a 0.53 mm i.d. wide-bore (WBOT) inlet be coupled to the analytical column by means of a capillary connector. It has been our experience that at the high temperature required for HRGC of TAG (typically 360–365°C), both metal and glass press-fit column connectors prove to be unreliable, reducing the robustness of the technique and making it less suitable for use in a QC environment.

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Having successfully used 0.53 mm i.d. WBOT capillary columns for routine fatty acid methyl ester and carbon-number analyses with automatic on-column sample introduction, we were interested in the possibility of adopting a WBOT approach to HRGC of TAG. To this end, we have compared the performance of a 0.53-mm i.d. WBOT column (with helium as carrier) with that of a 0.25-mm i.d. WCOT column (with hydrogen as carrier). Both columns were 15 m long, coated with 0.1-μm films of methyl 65%-phenyl silicone (Quadrex Corp, New Haven, CT).

EXPERIMENTAL PROCEDURES

An HRGC 5300 MEGA series gas chromatograph (Carlo Erba, Milan, Italy) was used, fitted with a nonvaporizing, septumless, cold-on-column injector (after Grob), a flame-ionization detector, a secondary cooling control module OC516 and an automatic injector AS-550. Hydrogen carrier gas was used in conjunction with a Chrompack HSS4 hydrogen safety system (Chrompack b.v., Middleburg, The Netherlands). For this work, the cold-on-column injection system was operated in conjunction with a high oven temperature secondary cooling system (6), allowing sample introduction into the column oven zone at < 100°C while maintaining oven temperatures up to 350°C. Simply turning off the air curtain to this device allowed the injection zone to heat quickly to oven temperature (30 s typically). A significant reduction in analysis time was achieved by avoiding the need to ramp up from low initial oven temperatures. Samples were analyzed on 15-m, 0.1-μm film thickness, methyl 65%-phenyl silicone WCOT capillary columns of 0.53 mm and 0.25 mm i.d. (Quadrex Corp.). The detector temperature was set to 370°C. Helium carrier gas (20 kPa, 25 cm/s) was used with the 0.53 mm column, hydrogen carrier gas (65 kPa, 40 cm/s) with the 0.25 mm column.

Solutions of oils and fats in isooctane (0.05–0.1% wt/vol) were introduced by autosampler onto the 0.53 mm column, or by hand onto the 0.25 mm i.d. column. Injection volumes were in the range of 0.1 to 0.2 μL. Solution strength was adjusted to give the best combination of resolution and sensitivity and was found to be sample-dependent. The secondary cooling was turned on for 2 min before injection and held on for a further 20 s into the run to allow solvent evaporation from the cooled inlet zone prior to chromatography. Temperature programming was used for all TAG separations. Samples containing TAG in the carbon number range 44–62 (*i.e.*, palm oil-based materials, Figs. 1 and 2) were analyzed with a temperature program of 345 to 365°C, ramped at 1°C/min. For this work, the WBOT capillary column was operated to reproduce as nearly as possible, within the same time-frame, the chromatography achieved with the WCOT column under optimum conditions.

For automated direct injection onto 0.25-mm i.d. capillary columns, a 0.53-mm i.d. inlet is coupled to the analytical column by means of a capillary connector. In high-temperature HRGC of TAG, repeated heating and

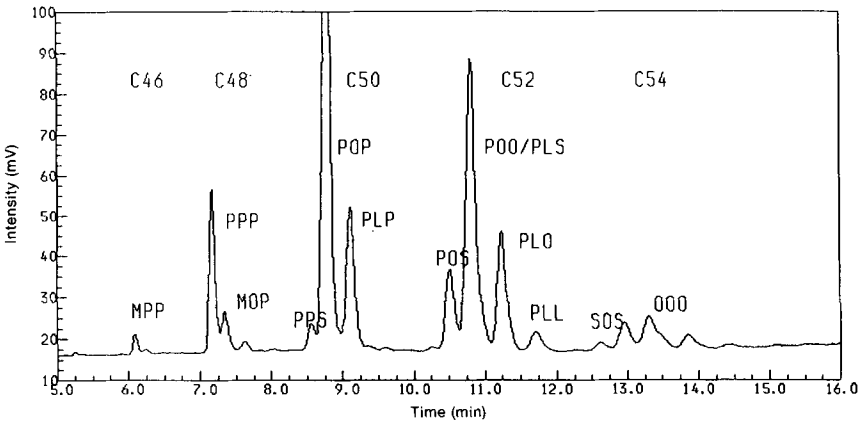

FIG. 1. Palm oil: 15 m × 0.53 mm i.d. methyl 65%-phenyl wide-bore capillary column; 345-365°C @ 1°C/min. Abbreviations: M, P, O, S, L = myristic, palmitic, oleic, stearic and linoleic acids, respectively.

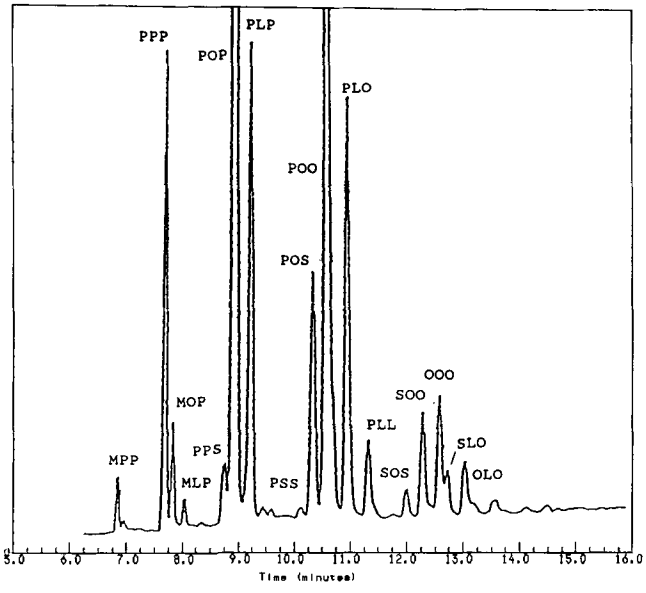

FIG. 2. Palm oil: 15 m × 0.25 mm i.d. methyl 65%-phenyl wide-bore capillary column; 350-360°C @ 1°C/min. Abbreviations: M, P, O, L, S = myristic, palmitic, oleic, linoleic and stearic acids, respectively.

cooling cycles subjected this connection to considerable stress. As a consequence, the joint became impossible to make leak-tight and in many cases failed after a relatively short time. In a QC environment, these problems preclude such an approach.

RESULTS AND DISCUSSION

Visual comparison of chromatographic resolution between the 0.53-mm i.d. and 0.25-mm i.d. capillary columns can be made from Figures 1 and 2 (palm oil). The separation achieved on the WBOT column is comparable to that on the WCOT column. However, the WBOT column does not resolve the C54 TAG pairs SOO/SLS and OOO/SLO to the degree obtainable with the WCOT column. The methyl 65%-phenyl phase essentially gives a separation based on the total number of double bonds present in the TAG but does not fully differentiate between OO and L fatty acyls (*i.e.*, two double bonds in each case). Our experience confirms that the extent of resolution achieved for these TAG pairs is dependent upon the relative amounts present in the sample.

Reproducibility data obtained for palm oil on the 0.53-mm i.d. capillary column is given in Table 1. Peak identifications have been applied logically and follow Sandra's scheme (1). Table 2 compares quantitation for a palm oil chromatographed on both WCOT and WBOT formats. The data show consistent quantitation between the two columns.

TABLE 1

Reproducibility Data for Palm Oil[a]

Carbon number	TAG	Mean	SD	SD (%)
46	MPP/MOM	1.0	0.08	8.0
48	PPP	7.0	0.06	0.9
	MOP	2.0	0.04	2.0
	MLP	0.5	0.04	8.0
50	PPS	1.5	0.04	2.7
	POP	31.8	0.18	0.6
	PLP	9.2	—[b]	—
52	PSS	0.3	0.05	16.7
	POS	5.6	0.05	0.9
	POO/PLS	22.5	0.11	0.5
	PLO	8.2	0.13	1.6
	PLL	1.6	0.11	6.9
54	SSS	—[c]	—	—
	SOS	0.6	0.04	6.7
	SOO/SLS	2.4	—[b]	—
	OOO	3.9	0.04	1.0
	SLO	1.3	0.05	3.9
	OLO	0.3	0.04	13.3

[a]Data are given in area%, n = 5; 15 m × 0.53 mm i.d. methyl 65%-phenyl wide-bore capillary column. Abbreviations: M, P, S, O and L are acyl groups derived from myristic, palmitic, stearic, oleic, linoleic acids, respectively. TAG, triacylglycerols. SD, standard deviation.
[b]Replicates are the same to first decimal place.
[c]Not detected.

TABLE 2

Comparative Data (area%) for a Palm Oil with WCOT and WBOT Methyl 65%-Phenyl Capillary Columns[a]

TAG	0.53-mm i.d.	0.25-mm i.d.
MPP	0.7	0.7
MOM	0.3	0.3
PPP	6.8	6.6
MOP	1.9	1.8
PPS	1.4	1.4
POP	30.7	29.6
PLP	8.8	9.8
PSS	0.3	0.3
POS	5.7	5.8
POO + PLS	22.5	22.8
PLO	8.6	9.2
PLL	1.8	2.0
SOS	0.9	0.8
SOO + SLS	2.8	2.8
OOO + SLO	4.5	4.4
OLO	1.8	1.9
OLL	0.5	0.5

[a]Abbreviations: WCOT, narrow-bore capillary columns; WBOT, wide-bore capillary columns. For other abbreviations, see Table 1.

Characterization of palm oil fractions. The C48 TAG content of palm-based materials (usually monitored by carbon-number GC) is often used as a measure of trisaturated TAG (*e.g.*, PPP); HRGC of TAGs shows that the C48 band contains MOP and MLP types in addition to PPP. To use the total C48 level as a representation of the trisaturated TAG content is therefore inaccurate, especially because the level of PPP within the C48 TAG group varies with the type of fraction (Table 3).

Table 4 gives more comprehensive data for a range of palm fractions and indicates the detailed compositional information available from HRGC of TAG. It is important to be aware that the range of TAG within each carbon-number group and the compositional differences between stearine and oleine fractions are much more complex than suggested by regular carbon-number data.

In any palm oil-based fraction, HRGC (and HPLC) techniques show that most carbon-number bands contain both stearine and oleine TAG. Thus, in palm fractions, an inaccuracy is introduced by using data for C48 as a measure of trisaturated TAG, or C50 as a measure of POP in midfractions, or C52 as a measure of POO in oleines.

TABLE 3

C48 Contents of Palm Fractions—Carbon-Number Data *vs.* High-Resolution TAG Data[a]

Palm fraction	Total percentage C48 carbon number	TAG composition		
		PPP	MOP	MLP
Palm oil	9.6	7.1	2.0	0.5
Mid-fraction	5.1	3.0	2.0	0.1
Wet-fractionated oleine	3.3	0.6	2.0	0.7
Dry-fractionated oleine	2.9	0.5	1.9	0.5
Dry-fractionated stearine	24.0	22.0	1.6	0.3

[a]See Table 1 for abbreviations.

TABLE 4

TAG Composition for a Range of Palm Oil-Derived Materials as Determined on a Methyl 65%-Phenyl WBOT (area %)[a]

Carbon number	TAG	Oil	Mid-fraction	Wet oleine	Dry oleine	Dry stearine
46	MPP/MOM	1.0	0.8	0.4	0.3	1.9
48	PPP	7.0	3.0	0.6	0.5	22.1
	MOP	2.0	2.0	2.0	1.9	1.7
	MLP	0.5	0.1	0.7	0.5	0.3
50	PPS	1.5	3.8	n.d.	n.d.	n.d.
	POP	31.8	63.2	16.9	31.2	30.0
	PLP	9.2	6.2	11.2	10.1	7.1
52	PSS	0.3	n.d.	n.d.	n.d.	0.4
	POS	5.6	13.0	2.1	5.9	4.4
	POO/PLS	22.5	5.0	38.0	27.6	16.3
	PLO	8.2	0.8	12.7	9.8	5.5
	PLL	1.6	n.d.	2.2	2.0	1.0
54	SOS	0.6	3.3	0.2	0.6	0.4
	SOO/SLS	2.4	0.9	3.6	2.8	1.5
	OOO/SLO	3.9	0.5	6.7	4.8	2.6
	OLO	1.3	n.d.	2.0	1.6	0.8
	OLL	0.3	n.d.	0.4	0.3	n.d.
56	AOS	n.d.	0.2	0.3	n.d.	n.d.

[a]Abbreviations: See Table 1 for abbreviations; n.d. = not detected. A, acyl group derived from arachidic acid.

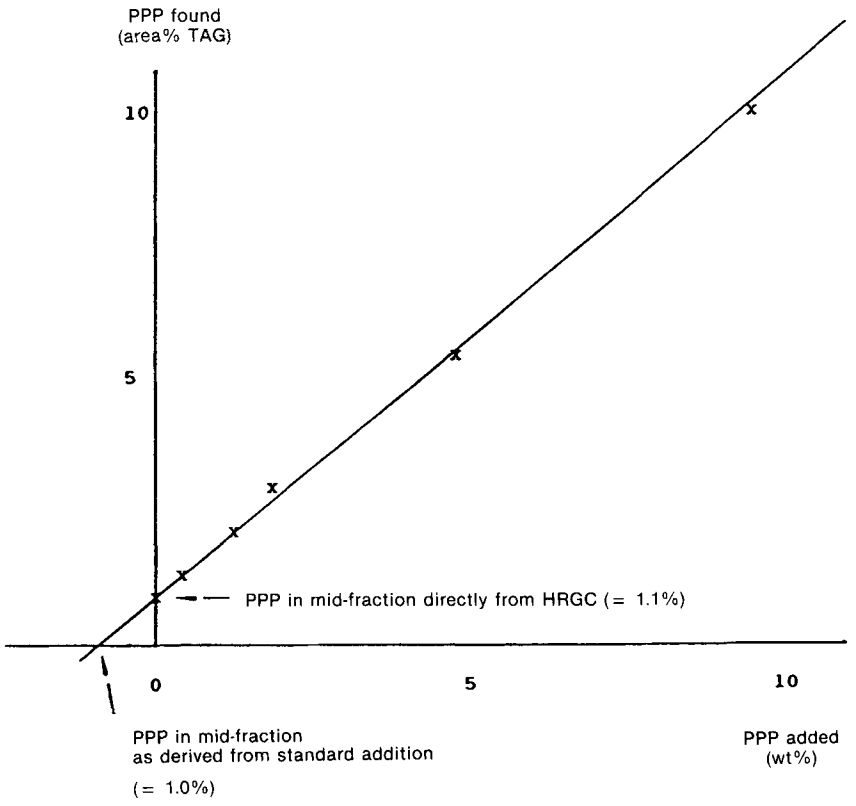

FIG. 3. Linearity of response and quantitative accuracy for PPP (15 m × 0.53 mm i.d. methyl 65%-phenyl wide-bore capillary column. Abbreviations: P = palmitic acid; TAG, triacylglycerols; HRGC, high-resolution gas chromatography.

To investigate whether HRGC on WBOT capillary columns could accurately determine the level of PPP in palm fractions, a mid-fraction (containing known additions of PPP) was analyzed to determine the linearity of response up to the 10% level. Figure 3 shows the data graphically, and the line obtained indicates good linearity of response. The y intercept (PPP level by HRGC = 1.1%) and the x intercept (PPP by standard addition = 1.0%) are in close agreement and indicate reasonably precise absolute quantitation of PPP in the original fraction. Each of the points on the graph is a mean of three replicates. The scatter on the triplicate determination was small and within the scale of the plotted symbol.

In many applications, WBOT HRGC of TAG is a viable alternative to WCOT HRGC and to silver-phase HPLC (the latter, however, has the advantage of positional isomer resolution—not yet available with HRGC). WBOT HRGC separates TAG on the basis of a combination of carbon number and the number of double bonds. As an automated technique, it is more robust than WCOT HRGC, allowing reliable injection and making it a good choice for use in process and QC environments. Helium (nonflammable) can be used as carrier gas, and resolution is not grossly inferior to that obtainable from WCOT separations. Quantitation is the same within experimental error. The technique offers a better way of characterizing palm oil fractions, because it differentiates stearine and oleine TAG, and thus provides information not directly available from routine carbon-number data.

The merits and demerits of WBOT columns have been subject for debate over a number of years (7-10). Our findings suggest that for TAG, 0.53-mm i.d. columns (phase ratio 1325, operated with helium carrier) can approach the performance of 0.25-mm columns (phase ratio 625, operated with hydrogen carrier gas). On safety grounds alone, nonflammable helium is a good compromise for the QC laboratory. WBOT capillary columns allow direct automated injection without the need for retention gaps. Faster, more reliable column installation procedures allow more robust injection techniques to be employed (almost *de rigeur* within a QC laboratory).

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