High-Resolution Gas-Chromatographic Determination of Diacylglycerols in Common Vegetable Oils

N. Frega*, F. Bocci and **G. Lercker**

Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Sezione di Industrie e Tecnologie Alimentari, Università di Firenze, 50144 Firenze, Italy

The gas-chromatographic determination of partial glycerides of sunflower (both high- and low-oleic acid varieties), peanut and extra virgin olive oil was studied with a polar capillary column characterized by its high thermal stability. This column allows for the determination of single diacylglycerols separated as a function of the position occupied by the individual fatty acids on the glycerin backbone as well as by the degree of unsaturation of the fatty acids.

KEY WORDS: Diacylglycerols, gas-chromatographic analysis, oil quality, vegetable oils.

The quantitative determination of partial glycerides (mono- and diacylglycerols) present in lipid materials in recent years has been the subject of numerous studies (1–11). This is explained in part by the established correlation between the content of mono- and diacylglycerols and the oxidative stability of the oils (12,13). Based on the content of diacylglycerols and their fatty acyl composition, it appears possible to develop analytical indices that will safeguard premium oils (*e.g.*, extra virgin olive oil) as well as indicate the state of a product's freshness (14–16,17). The present investigation was designed to further study the composition and structural characterization of diacylglycerols found in several common vegetable oils.

EXPERIMENTAL PROCEDURES

The oils analyzed were extra virgin olive oil (Carapelli, Firenze, Italy), sunflower oil (Sagra, Lucca, Italy) and peanut oil (Sagra, Lucca, Italy). Packaged products were obtained from retailers and wholesale suppliers and used as received.

Thin-layer chromatographic (TLC) separation of diacylglycerols from the oil were carried out on silica gel G plates (Stratocrom, Carlo Erba, Milano, Italy) with the eluent *n*-hexane/ethyl ether 60:40 (vol/vol). The plates were sprayed with a 0.2% alcohol solution of 2,7′-dichlorofluorescein (sodium salt) and detected under ultraviolet radiation at 254 nm. TLC bands, corresponding to 1,2- and 1,3-diacylglycerols ($R_f = 0.25$ and 0.18, respectively) (18,19) were scraped off separately and extracted with chloroform. The extracts were evaporated, the residue was treated with silylating reagent and the silyl esters were analyzed by high-resolution gas chromatography (HRGC).

A 50-mg oil sample, taken from a 1-g sample of oil, to which had been added 50 μL of a 10% solution of squalane ($C_{30}H_{62}$ internal standard) in benzene, was treated initially with several drops of diazomethane (CH_2N_2) in ethyl ether (20) to esterify free fatty acids to methyl esters, and then with trimethyl chlorosilane (TMCS) to convert alcohol groups to trimethylsilyl (TMS) derivatives as described by Sweeley *et al.* (21).

The composition of the diacylglycerols in the assayed oils was determined in a Mega 5160 (Carlo Erba, Rodano, Italy) gas chromatograph equipped with a fused silica capillary column (25 m × 0.32 mm i.d.), having a 50% phenyl-/50% methylpolysiloxane (TAP, Chrompack, Middleburg, The Netherlands) and 0.1 μm thickness of stationary phase, and interfaced to a Spectra Physics 4290 integrator (St. Albans, United Kingdom). GC operating conditions were: initial oven temperature 200°C, temperature programmed to 350°C at 3°C/min; 350°C final temperature for 20 min; injector and flame-ionization detector (FID) temperature 350°C; split-system sample introduction; 1:80 column/vent ratio; and He carrier gas at 0.8 mL/min. The composition of the diacylglycerols was also determined with the same instrument equipped with a fused silica capillary column (25 m × 0.32 mm i.d.) with SE 52 stationary phase (Mega, Milano, Italy). GC operating conditions were: initial oven temperature 180°C, temperature programmed to 350°C at 3°C/min; 350°C final temperature for 20 min; injector and detector (FID) temperature 350°C; split-system sample introduction; 1:80 column/vent ratio; and He carrier gas at 0.6 mL/min.

Peak identification was carried out by comparison of relative retention time with those of standard diacylglycerols (supplied by Sigma Chemical Co., St. Louis, MO, and Supelco, Inc., Bellefonte, PA) and from fatty acid analysis performed after transmethylation (22) of 1,2-diacylglycerol and 1,3-diacylglycerol bands collected by preparative TLC.

RESULTS AND DISCUSSION

Figures 1b and 1c show the high-resolution GC traces of 1,3- and 1,2-diacylglycerols, respectively, obtained from direct fractionation of sunflower oil (high oleic acid), while Figure 1a shows the GC trace of the mixture of the two diacylglycerol TLC bands. The HRGC separation achieved with the TAP stationary phase enabled the determination of individual diacylglycerols because they were separated as a function of both the location of individual fatty acids on the glycerine backbone and the extent of unsaturation of the fatty acid. The diacylglycerols with 34 and 36 carbon atoms are well separated by HRGC into distinct groups. Furthermore, the positional isomers of each group (1,2- and 1,3-diacylglycerols) with the same total carbon number are clearly separated (from each other) as a function of the degree of unsaturation. In contrast, the HRGC analysis with a nonpolar SE 52 stationary-phase column did not separate on the basis of the degree of unsaturation of diacylglycerols, as shown in Figure 2.

Figure 3 shows the gas-chromatographic separation, after CH_2N_2 and TMS treatments but without prior preparative TLC fractionation into classes of compounds, of extra virgin olive, sunflower (low oleic acid) and peanut oil diacylglycerols. The chromatograms also show aside from the diacylglycerols, free fatty acids, squalene, monoacylglycerols, tocopherols and free sterols. One may note the presence of several unknown peaks (labeled X). These

*To whom correspondence should be addressed.

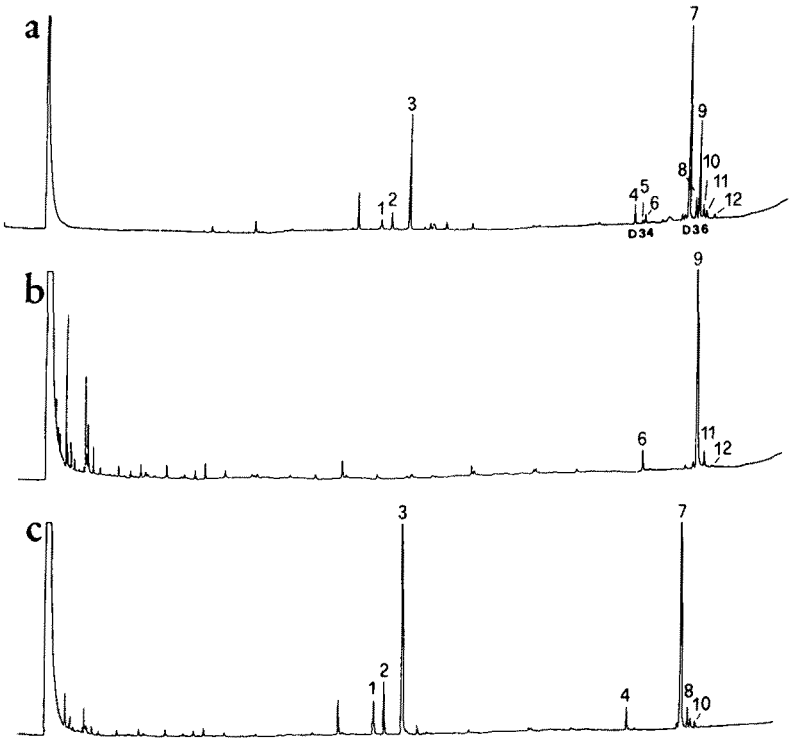

FIG. 1. Gas-chromatographic traces of sunflower oil (high oleic acid) diacylglycerols obtained with polar TAP column: (a) total diacylglycerols (mixture of b + c); (b) 1,3-diacylglycerols (from thin-layer chromatography (TLC) band with $R_f = 0.18$); (c) 1,2-diacylglycerols (from TLC band with $R_f = 0.25$). 1 = campesterol; 2 = stigmasterol; 3 = β-sitosterol; 4 = 1,2-PO; 5 = 1,2-PL; 6 = 1,3-PO; 7 = 1,2-OO; 8 = 1,2-OL; 9 = 1,3-OO; 10 = 1,2-LL; 11 = 1,3-OL; 12 = 1,3-LL. P = palmitic acid; O = oleic acid; L = linoleic acid. D34 = diacylglycerols with 34 carbon atoms; D36 = diacylglycerols with 36 carbon atoms; TAP, 50% phenyl-/50% methylpolysiloxane.

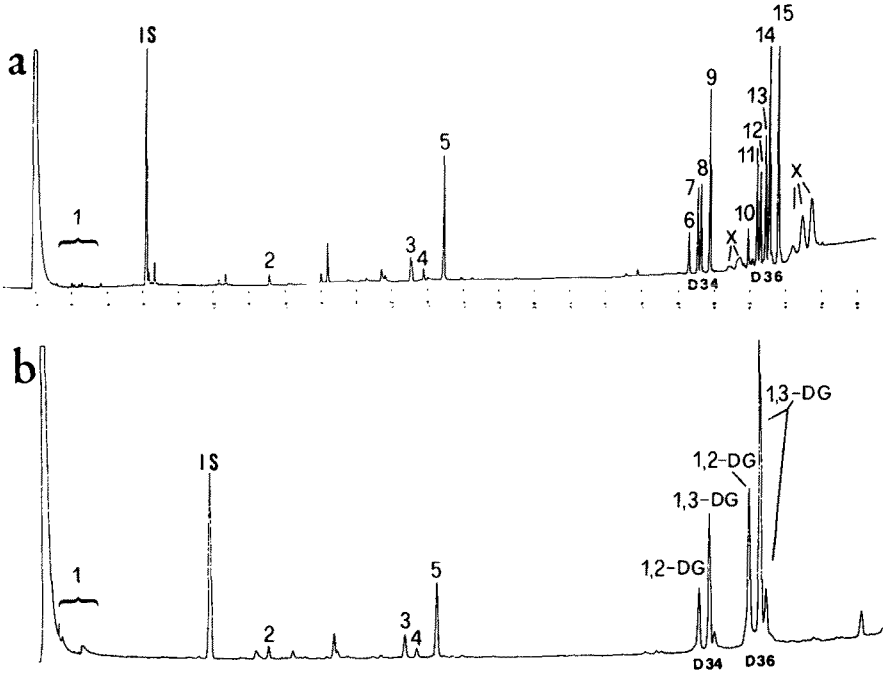

FIG. 2. Gas-chromatographic traces of sunflower oil (low oleic acid) diacylglycerols obtained with polar column, TAP (a) and a nonpolar column SE 52 (b). 1 = free fatty acids; IS = internal standard (squalane); 2 = squalene; 3 = campesterol; 4 = stigmasterol; 5 = β-sitosterol; 6 = 1,2-PO; 7 = 1,2-PL; 8 = 1,3-PO; 9 = 1,3-PL; 10 = 1,2-OO; 11 = 1,2-OL; 12 = 1,3-OO; 13 = 1,2-LL; 14 = 1,3-OL; 15 = 1,3-LL; X = unknown; DG = diacylglycerols. TAP, 50% phenyl-/50% methylpolysiloxane.

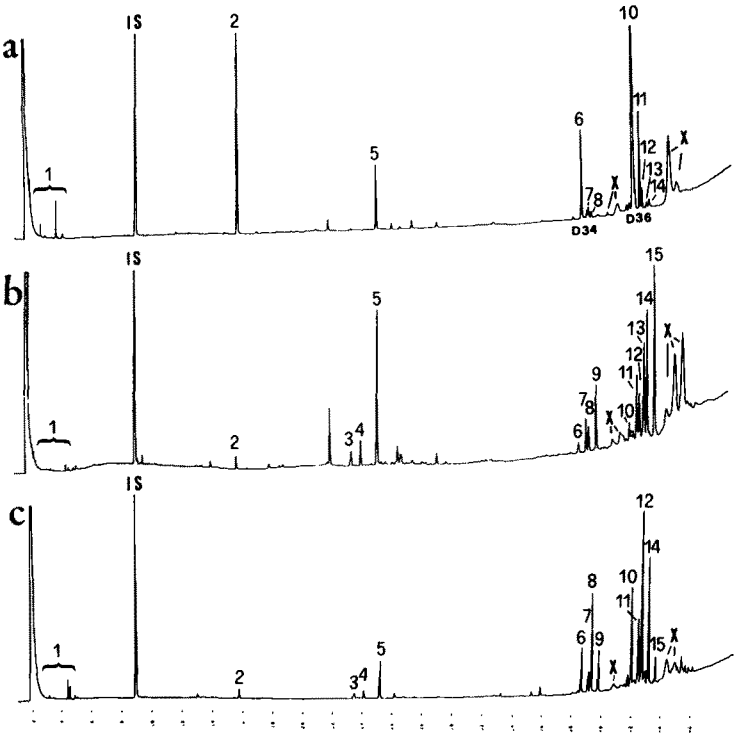

FIG. 3. Gas-chromatographic traces of olive oil (a), sunflower oil (low oleic acid) (b), peanut oil (c) diacylglycerols. See Figure 2 caption for identification of peak numbers.

substances, which have not been reported heretofore, were found in all assayed samples; their identification is now in progress.

Diacylglycerols are commonly present to the extent of 1–3% in oils (6,8,9,18), and are found as 1,2- and 1,3-isomers (3,15–17,23). The presence of 1,2-diacylglycerols is known to be due to the incomplete biosynthesis of triglycerides, whereas the 1,3-diacylglycerols are derived mainly from triglyceride hydrolysis and, as such, increase with increasing free fatty acid (FFA) content in the oil (16–18).

The qualitative and quantitative determination of diacylglycerols is thus a viable method, in conjunction with FFA content, for establishing the original (*i.e.*, before refining) quality of crude oils. Whereas free fatty acids and most of the monoacylglycerols are eliminated during various processing steps, the diacylglycerols are only partially removed and, hence, constitute an important index for not only the original quality of the oil but also for the resulting refined oil.

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