Direct Gas Chromatographic Analysis of the Unsaponifiable Fraction of Different Oils with a Polar Capillary Column

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 analysis of the unsaponifiable fraction of several oil samples was performed by a new gas chromatographic technique with a polar column of high thermal stability. This column was adequate for fractionating sterols, methyl sterols and alcohols of the unsaponifiable matter, and allowed the detection of peaks usually unresolved with nonpolar columns.

KEY WORDS: Alcohols, gas chromatographic analysis, methyl sterols, oils, sterols, unsaponifiable fraction.

Studying the composition of minor components of the unsaponifiable fraction of oils is useful both to identify the sources of various fatty materials and to reveal adulteration of the most valuable ones. This is accomplished by fractionating the unsaponifiable matter in classes of compounds by thin-layer chromatography (TLC) and studying each class by gas chromatographic analysis. At present, the trend in lipid research is to improve the analytical procedures to reduce sample manipulation: the high-resolution power of analytical chromatographic techniques now supports this desire. Total lipid composition (1,2), triglyceride analysis (3–6), and a nearly fully-automated liquid chromatography-gas chromatography (LC-GC) method developed by Grob and collaborators (7) are some of the analytical methods recently modified to follow this trend.

This paper deals with the gas chromatographic study of the integral unsaponifiable fraction without the previous time-consuming TLC separation into classes of compounds. This procedure offers useful information about the mutual quantitative relationships between classes of minor components. Newly available polar stationary phases of high thermal stability are used that allow improved separation of triglycerides. The study of the whole unsaponifiable fraction was executed with nonpolar and polar columns.

EXPERIMENTAL PROCEDURES

The different lipid substrates analyzed were: extra virgin olive oil (Carapelli, Firenze, Italy), hazelnut oil (obtained from L.S. Conte, Ispettorato Prevenzione e Repressione delle Frodi Agroalimentari, Bologna, Italy), peanut oil (Sagra, Lucca, Italy), corn oil (Carapelli), soybean oil (Zucchi S.P.A., Cremona, Italy), and grapeseed oil (Carapelli). Packaged products from retailers and wholesale suppliers were used.

After the addition of 0.1% (w/w) of the internal standard, squalane [2,6,10,15,19,23-hexamethyltetracosane ($C_{30}H_{62}$); obtained from Merck, Darmstadt, Germany], total lipids were saponified according to the Norme Grassi e Derivati (NGD) C-12 method (8), and the unsaponifiable fraction was treated with diazomethane (CH_2N_2) (9) to transform acid groups to methyl esters. The trimethylsilyl (TMS) derivatives of alcohols, sterols, and methylsterols were obtained according to the method of Sweeley *et al.* (10). The fractionation of unsaponifiable matter was accomplished with silica-gel G TLC plates (Stratocrom, Carlo Erba, Rodano, Milano, Italy) and *n*-hexane/ethyl ether 60/40 (v/v) as the eluent. Plates were sprayed with 0.2% ethanolic solution of 2,7′-dichlorofluorescein (sodium salt). Bands were scraped off the plate and extracted twice with ethyl ether.

The composition of the whole unsaponifiable fraction was determined in a Carlo Erba HRGC Model 5160 Mega gas chromatograph (Carlo Erba). The instrument was equipped with a Mega 2 computing integrator and a fused silica capillary column (25 m × 0.25 mm i.d.) with a 50% phenyl-/50% methylpolysiloxane (TAP, Chrompack, Middleburg, The Netherlands) stationary phase. Carrier gas was He at 0.8 mL/min column flow rate and 1:80 split ratio, with an inlet pressure of 100 KPa. Injector and detector temperatures were 330°C; oven temperature was programmed from 200 to 300°C with a rate of 3°C/min. Furthermore, the gas chromatographic analysis of the different classes of compounds, fractionated by TLC, was performed under the same analytical conditions as previously described and 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). Carrier gas was He at 0.8 mL/min column flow rate and 1:80 split ratio, with an inlet pressure of 80 KPa. Injector and detector temperatures wre 320°C; oven temperature was programmed from 160 to 300°C with a rate of 5°C per min.

Peak identification was carried out by comparison of relative retention time with those reported in the literature (11–13) and with retention times of standards (supplied by Sigma Chemical Co., St. Louis, MO, and Supelco Inc., Bellefonte, PA).

Quantitative analyses were performed with squalane as the internal standard and adopting the corrected area normalization method.

All analytical determinations were carried out in duplicate and all data are expressed as mean values.

RESULTS AND DISCUSSION

It is widely recognized that the different quantitative relationships between the components of both saponifiable and unsaponifiable fractions enable the analyst to ascertain the origin of lipids (14–17).

Figure 1 shows high-resolution gas chromatographic (HRGC) traces of the unsaponifiable fraction of some common seed oils. In this figure, comparison between olive (A) and hazelnut (B) oils, for example, underlines which analytical differences could be used to determine the presence of hazelnut oil illegally mixed with olive oil.

The utilization of a polar column in the analysis of the total unsaponifiable fraction led to the identification of a higher number of constituents than would have been

*To whom correspondence should be addressed.

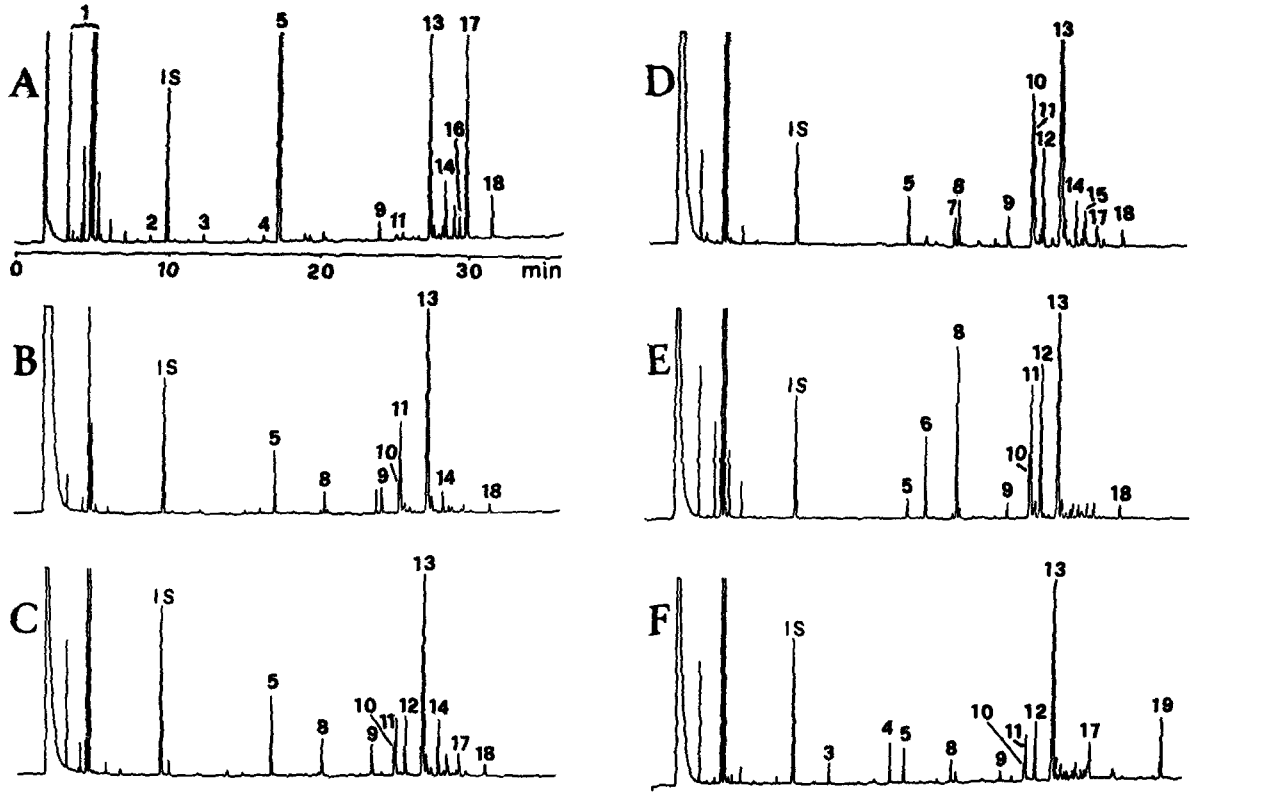

FIG. 1. Gas chromatographic traces of the unsaponifiable fraction of common oils. A = olive oil; B = hazelnut oil; C = peanut oil; D = corn oil; E = soybean oil; F = grapeseed oil. 1 = methyl esters; IS = internal standard (squalane); 2 = docosanol; 3 = tetracosanol; 4 = hexacosanol; 5 = squalene; 6, 7, 8 = unknown; 9 = *α-tocopherol*; 10 = unknown; 11 = campesterol; 12 = stigmasterol; 13 = β-sitosterol; 14 = Δ^5-avenasterol; 15 = cycloartenol; 16 = unknown; 17 = 24-methylenecycloartanol; 18 = citrostadienol; 19 = oleanolic acid.

found with a nonpolar column. Figure 2 compares gas chromatograms of the unsaponifiable fraction of corn oil obtained with a polar (TAP, Chrompack) column and with a nonpolar one (SE 52). In the gas chromatogram obtained with the polar column, campesterol (peak 11) is accompanied by another unknown sterol (peak 10). These two compounds appear as a single peak in the analysis performed with a nonpolar column. These two components are present in all oil samples examined, but at a different concentration ratio. The characterization of these unknown constituents is in progress. Furthermore, we can observe a specific component (Fig. 1A, peak 16), which is present only in olive oil.

Table 1 shows the composition (expressed as mg/100 g of oil) of the main constituents of the unsaponifiable fraction for the six samples examined. Squalene is present in substantial amounts in olive oil, accompanied by significant quantities of 24-methylenecycloartanol and β-sitosterol. Soybean oil shows small quantities of squalene, with consistent amounts of campesterol, stigmasterol, and β-sitosterol.

In order to evaluate the repeatability of the determination of each compound, gas chromatographic analysis of the unsaponifiable fraction of extra virgin olive oil was replicated 24 times. Table 2 shows that the repeatability is good.

Figures 3 and 4 report gas chromatograms of the unsaponifiable fraction of olive oil obtained with TAP and SE 52 columns, respectively. Under each gas chromatogram the related gas chromatograms of sterols, alcohols, and

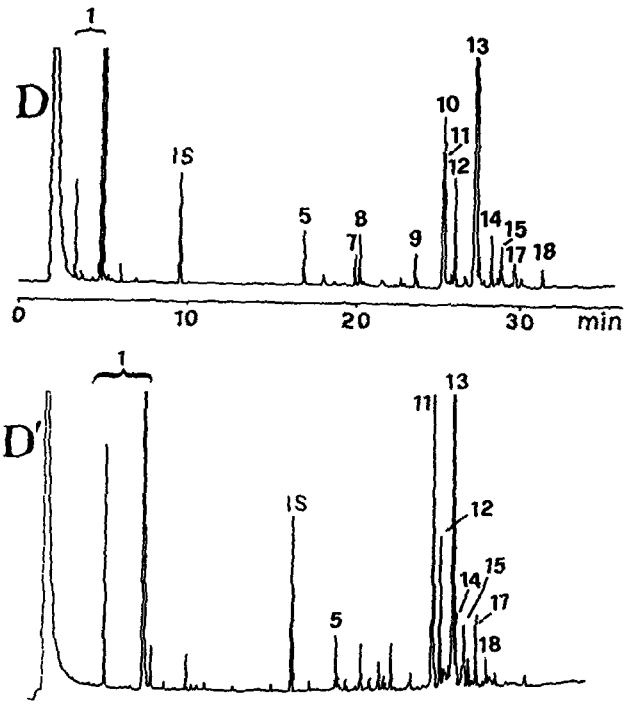

FIG. 2. Gas chromatographic traces of the unsaponifiable fraction of corn oil with a polar column, TAP (D), and with a nonpolar column, SE 52 (D'). See Figure 1 caption for identification of peak numbers.

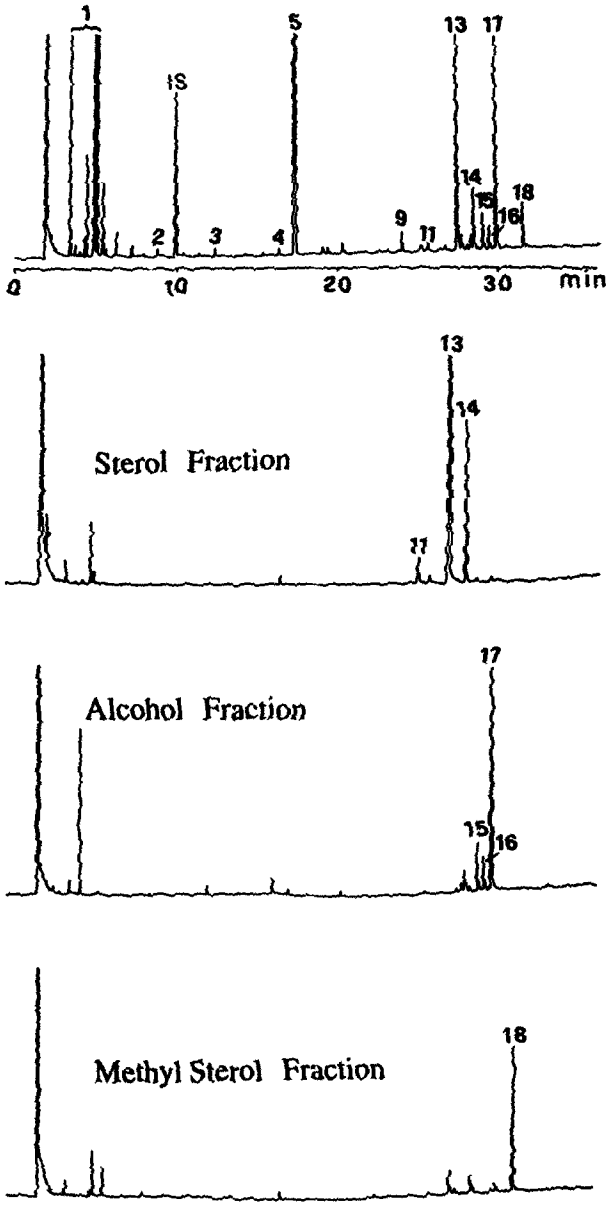

FIG. 3. *Gas chromatographic traces of the unsaponifiable fraction of olive oil (top) and sterol, alcohol, and methyl sterol fractions. Polar column TAP. See Figure 1 caption for identification of peak numbers.*

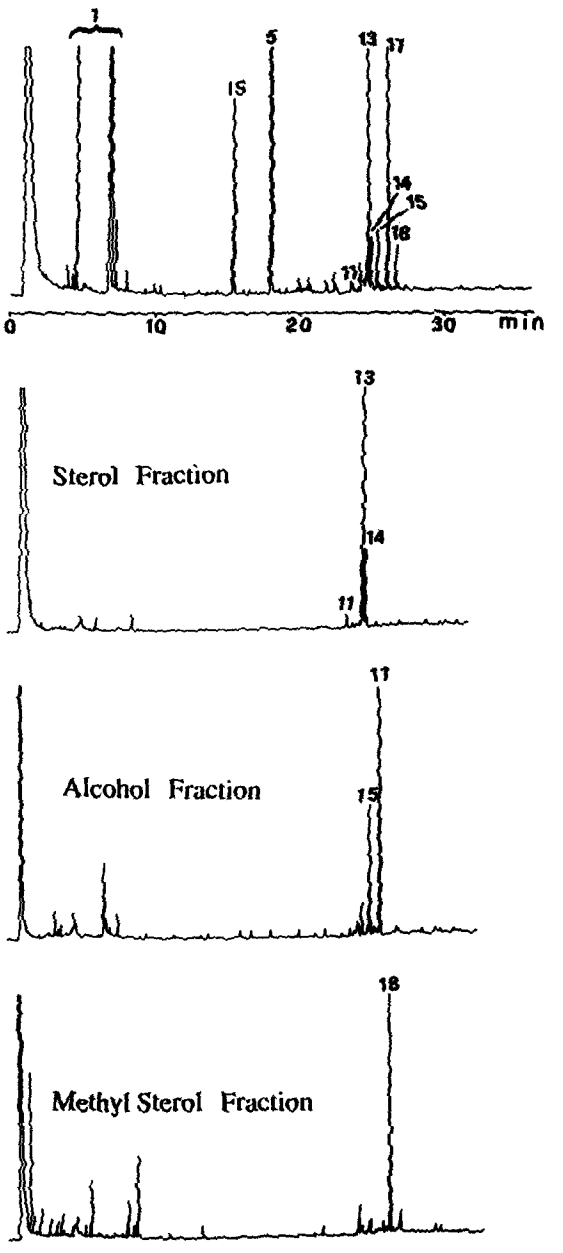

FIG. 4. *Gas chromatographic traces of the unsaponifiable fraction of olive oil (top) and sterol, alcohol, and methyl sterol fractions. Nonpolar column SE 52. See Figure 1 caption for identification of peak numbers.*

TABLE 1

Composition of the Unsaponifiable Fraction for the Different Lipidic Substrates Analyzed (mg/100 g)[a]

	Peak #																	
Oil	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Corn oil				30.6		21.7	30.8	23.5	136.7	72.7	71.2	722.3	34.5	36.2		13.9	14.1	
Peanut oil				27.4			13.9	12.2	12.7	22.2	23.3	155.4	23.4	12.7		10.8	5.6	
Hazelnut oil				27.9			10.3	11.9	12.7	47.2	4.5	222.4	10.5	4.5		4.6	10.8	
Grapeseed oil	4.0	7.6	15.5	14.1		9.2	4.7	4.7	6.9	20.8	27.7	215.0	5.4	11.8	5.4	6.9	8.8	30.8
Soybean oil				9.9	40.3		87.1	7.9	37.2	75.7	90.7	229.8	8.1	7.3	8.1	10.3	9.5	
Olive oil	1.7	2.8	2.5	564				8.4		3.5		124.6	28.8	18.3	10.2	105.6	19.5	

[a]*See Figure 1 caption for identification of peak numbers.*

TABLE 2

Repeatability of the Gas Chromatographic Determination of Unsaponifiable Fraction Components[a] of Extra Virgin Olive Oil[b]

Peak	2	3	4	5	9	11	13	14	15	16	17	18
Mean	0.2	0.3	0.3	66.9	1.1	0.3	12.6	2.8	1.8	1.0	10.6	1.9
SD[c]	0.02	0.02	0.02	2.13	0.33	0.03	0.81	0.21	0.12	0.07	0.73	0.16
CV[d]	9.5	7.4	6.9	3.2	31.5	7.8	6.4	7.3	6.5	7.3	6.8	8.6

[a]Values deduced from HRGC areas (%).

[b]On 24 samples. See Figure 1 caption for identification of peak numbers.

[c]Standard deviation.

[d]Coefficient of variation (%).

methyl sterols previously fractionated by TLC are reported. The separation between β-sitosterol (peak 13) and Δ^5-avenasterol (peak 14) can be obtained with the polar column (Fig. 3) but not with the SE 52 column (Fig. 4).

The fraction of triterpenic (peaks 15, 16, 17) alcohols shows a good separation between cycloartenol (peak 15) and 24-methylenecycloartanol (peak 17). In all cases, the improved separation between constituents of the unsaponifiable fraction with definition retention time (RT) values up to the RT of β-sitosterol is particularly significant. This resolution permits the detection of an unidentified peak (peak 16) present in the olive oil and unresolved by nonpolar columns. This identification will be the object of further studies.

Polar columns of high thermal stability have proved more efficient in this sort of analysis. Furthermore, polar columns improve the separation of components of single classes of compounds (*i.e.,* sterols, methyl sterols, alcohols). A capillary TAP column allows the detection of new components that are not detected with nonpolar gas chromatographic columns (SE 52, SE 54).

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