Technical

On the Composition of Iranian Olive Oil

V. PAGANUZZI, Laboratorio Chimico Dogane ed Imposte Indirette, via Rubattino 6, 16126 Genoa, Italy, and **E. LEONI**, Istituto Tecnico Industriale per Chimici A. Gastaldi, via Milano, 16126 Genoa, Italy

ABSTRACT

Two samples of virgin olive oil and one sample of hexane-extracted husk oil coming from Iran were examined. The analyses included physical and chemical characteristics, the composition of total fatty acids and fatty acids at the glyceride 2-position by gas liquid chromatography (GLC) of methyl esters, the triglycerides composition calculation according to Vander Wal theory, the separation of the alcoholic fractions (sterols, 4-methylsterols, triterpene alcohols, triterpene dialcohols and aliphatic alcohols) of the unsaponifiable matter by thin layer chromatography (TLC), the quantitation and the composition of these fractions by GLC of TMS derivatives. The results were in line with data from literature for olive oils of different origin, with the exception of: a high content of unsaponifiable matter (1.75 and 1.95% for virgin oils, 5.33% for husk oil); a high amount of sterols for husk oil (562 mg/100 g oil); a low content of SE 30 apparent β-sitosterol for husk oil (91.1%); a low amount of triterpene dialcohols (1 mg/100 g oil) and triterpene alcohols (78 and 91 mg/100 g oil) for virgin oils; a content of cycloartenol (60.2-66.9%) higher than the 24-methylenecycloartanol one (22.8-26.6%; a content of C_{24} linear saturated alcohol (33.9-38.0%) slightly higher than the C_{26} alcohol one (29.3-32.8%).

INTRODUCTION

Approximately 95% of the world olive oil production is concentrated in the Mediterranean basin, and for this reason most of the analytical data in the literature concerns olive oil originating from this area, while little or no information is available on oil produced in other regions.

One of the minor producing countries outside the Mediterranean basin is Iran. The number of olive trees in this country is estimated to be 675,000 (1), which represents only 0.14% of world production (2). This figure is expected to increase because of efforts to increase the cultivation of olive trees in Iran.

We have not been able to find analytical data in the literature on characteristics and composition of the Iranian olive oil. Having obtained samples of this oil, we have therefore carried out an extensive characterization, taking into account, besides the traditional tests and indexes, those analytical determinations which have become routine in the last ten years (fatty acids at the glyceride 2-position and sterols) and other unsaponifiable fractions only rarely reported in the literature (4-methylsterols, triterpene alcohols, triterpene dialcohols, and aliphatic alcohols).

EXPERIMENTAL PROCEDURES

Materials

Three samples of Iranian olive oil, from the 1977-78 crop, have been examined: a commercial virgin olive oil, product of Sefid-roud oil mill, Rudbar; a virgin oil obtained by laboratory-pressing Iranian olives; an oil from the hexane extraction of the husk, residue of the laboratory-pressed Iranian olives.

TABLE I

Physical and Chemical Characteristics of Iranian Olive Oils and Composition of Their Unsaponifiable Matter

Oil characteristics	1 Commercial virgin oil	2 Laboratory virgin oil	3 Laboratory extracted husk oil
Refractive index at 25 C	1.4676	1.4671	1.4674
Acidity (oleic, %)	1.96	5.99	10.78
Saponification value (mg KOH/g oil)	193.5	193.5	191.8
Iodine value, Hanus (g I_2/100 g oil)	77.0	79.8	79.1
Bellier index (C)	14.5	14.5	23.5
Bellier – Carocci Buzi test	neg.	neg.	pos.
Spectrophotometric values:			
K_{232}	2.663	1.538	2.826
K_{268}	0.357	0.203	0.810
K_{270}	0.360	0.200	0.810
ΔK (Italian method)	0.010	0.001	0.023
ΔK (E.E.C. method)	0.016	0.003	0.021
Triglycerides (%)	71.1	64.6	55.8
Unsaponifiable (%)	1.75	1.98	5.33
Sterols (mg/100 g oil)	111	104	562
Triterpene dialcohols (mg/100 g oil)	1	1	29
Triterpene alcohols (mg/100 g oil)	78	91	329
4-methylsterols (mg/100 g oil)	20	18	70
Linear satd. alcohols (mg/100 g oil)	38	16	593
Phytol (mg/100 g oil)	12	6	18

Physical and Chemical Characteristics

Unless otherwise stated, physical and chemical characteristics were determined according to Italian standard methods (3). Spectrophotometric values were measured according both to Italian official methods (3) and to E.E.C. Regulation No. 1058/77 (4). Triglyceride percentages were obtained by a method described elsewhere (5).

Lipid Composition

The fatty acids' composition has been determined by gas chromatography (GLC) of fatty acids methyl esters for total oil, triglycerides, and glycerol 2-position.

For total oil and triglycerides, methyl esters were prepared by boiling fatty matter with 1% H_2SO_4 (W/V) in methanol (5). Triglycerides were obtained as described previously (5). 2-Monoglycerides and related methyl esters were prepared by Italian official methods (3) on triglycerides obtained as mentioned above.

GLC analysis of methyl esters was carried out on a Carlo Erba Model GD-AI apparatus with flame ionization detector. The column was a 2 m x 2 mm stainless steel tube packed with 60-80 mesh acid-washed Chromosorb W, coated with 20% Carlo Erba diethylene glycol succinate LAC 728. The temperatures were 197 C for the column and 300 C for the injector and the detector. The flow rate of nitrogen carrier gas was 15 ml/min.

For each fatty acid the proportion (i.e., the percentage of a given fatty acid which is located in glycerol 2-position) was calculated according to Mattson and Volpenhein (6):

$$\text{proportion} = \frac{\text{\% fatty acid in 2-position}}{\text{\% fatty acid in triglycerides x 3}} \times 100$$

Finally, the triglycerides composition was determined following the 1,3 random, 2 random distribution proposed by Vander Wal (7).

Unsaponifiable

The unsaponifiable matter was prepared according to the Italian official method (3) and submitted to two different TLC procedures using 20 x 20 cm plates precoated with a 0.25 mm layer of Merck Silica Gel 60 and activated 2 hr. at 110 C. About 10 mg of the unsaponifiable matter were applied uniformly along a line 1.5 cm from one edge of the plate. The plates were developed as follows:

I. Development with benzene/acetone (95:5 v/v), followed by spraying with 1% 2′,7′-dichlorofluorescein solution in ethanol. Two zones were cut off:
   a. zone including band with R_f 0.21 (sterols), alone for OV 17 GLC and together with band with R_f 0.13 (triterpene dialcohols) for SE 30 GLC;
   b. zone including bands with R_f 0.29 (triterpene alcohols) and R_f 0.25 (aliphatic alcohols). As pointed out by Karleskind (8) and Wolff (9), these two fractions are not easily separable. The zone was used only for GLC determination of aliphatic alcohols.

II. Double development with hexane/ethyl acetate (85:15 v/v), followed by spraying with the above mentioned solution of 2′-7′-dichlorofluorescein, according to the method suggested by Sawicki and Mordret (10). Two bands were cut off:
   a. band with R_f 0.35 (triterpene alcohols);
   b. band with R_f 0.30 (4-methylsterols).

   Each band was extracted with chloroform and, after evaporation of the solvent, each residue submitted separately to a new, identical double development, in order to obtain purified fractions.

The four fractions obtained from the cromatoplates, through extraction of the bands with chloroform and

TABLE II

Fatty Acids Composition (%) and Proportion of Iranian Olive Oil[a]

Fatty acids	1 Commercial virgin oil				2 Laboratory virgin oil				3 Laboratory-extracted husk oil			
	Total oil	Triglycerides	Glycerol 2-position	Proportion	Total oil	Triglycerides	Glycerol 2-position	Proportion	Total oil	Triglycerides	Glycerol 2-position	Proportion
$C_{16:0}$	10.7	10.0	1.0	3	10.5	10.2	1.2	4	11.3	10.4	1.0	3
$C_{16:1}$	0.8	0.8	0.5	21	0.8	0.8	0.5	21	0.8	0.8	0.4	17
$C_{17:0}$	tr.	tr.	--	0	tr.	tr.	--	0	tr.	0.1	--	0
$C_{17:1}$	tr.	tr.	tr.	(33)	tr.	tr.	tr.	(33)	tr.	tr.	tr.	(33)
$C_{18:0}$	2.8	2.6	0.3	4	3.1	2.8	0.2	2	2.9	2.9	0.2	2
$C_{18:1}$	76.7	78.2	86.3	37	76.1	77.1	85.5	37	73.9	75.0	83.9	37
$C_{18:2}$	8.3	7.9	11.3	48	8.5	8.4	11.7	46	9.9	9.6	13.1	45
$C_{18:3}$	0.4	0.2	0.6	100	0.6	0.4	0.9	75	0.6	0.6	1.4	78
$C_{20:0}$	0.2	0.2	--	0	0.2	0.2	--	0	0.4	0.4	--	0
$C_{20:1}$	0.1	0.1	tr.	(17)	0.2	0.1	tr.	(17)	0.2	0.2	tr.	(9)

[a]Between parentheses: approximate values obtained assuming tr. = 0.05%.

TABLE III

Composition (%) of Sterol Fractions (OV 17) and Relative Content (% referred to the Sum Sterols + Triterpene Dialcohols) of Triterpene Dialcohol Fractions (SE 30) of Iranian Olive Oils

Compounds	1 Commercial virgin oil	2 Laboratory virgin oil	3 Laboratory-extracted husk oil
Sterols			
Cholesterol	0.9	0.7	1.2
Brassicasterol	---	tr.	0.1
Campesterol	3.2	3.1	3.6
Stigmasterol	1.3	2.1	3.4
β-sitosterol	83.2	88.8	88.9
Δ_5-avenasterol	10.1	4.4	2.2
Δ_7-stigmastenol	0.7	0.5	0.2
Δ_7-avenasterol	0.6	0.4	0.4
Triterpene dialcohols			
Erythrodiol	0.8	0.7	5.0
Uvaol	tr.	tr.	tr.

evaporation of the solvent, were trimethylsilylated according to Italian official methods (3), and the TMS derivatives analyzed by GLC using a Carlo Erba Model GI gas chromatograph with flame ionization detector and direct-in-column injection system. The column was a 3 m x 4 mm glass tube packed with 80-100 mesh Gas Chrom Q coated with 1.5% OV 17. The analysis of the fraction sterols-triterpene dialcohols was performed also with a different stationary phase, SE 30 1%, to avoid the partial overlap of Δ_7 avenasterol and erythrodiol peaks which occurs working with OV 17. Operating conditions for all the fractions but the aliphatic alcohols one were: column 242 C, injector and detector 300 C, nitrogen carrier gas 50 ml/min, β-sitosterol retention time about 45 min. Aliphatic alcohol GLC was carried out in two ways: isothermal conditions, column temperature 200 C; programmed temperature, for the short chain alcohols ($<C_{22}$) detection and the phytol content evaluation: initial temperature 150 C, final temperature 220 C, rate 0.667 C/min. Peak areas were calculated by triangulation.

The contents of the various unsaponifiable fractions, expressed in mg/100 g oil, were measured by GLC through the use of cholesterol (pure, Supelco Inc., Bellefonte, PA) as internal standard: cholesterol was added to the oil prior to saponification and the unsaponifiable matter submitted to a TLC development with benzene/acetone (95:5 v/v), employing two chromatoplates. The first was used for sterols quantitation, taking into account the naturally occurring cholesterol. In the second the zone including sterols, 4-methylsterols, triterpene alcohols and aliphatic alcohols (R_f from 0.21 to 0.29) was cut off: the GLC analysis of the resulting chloroform extract and the comparison of the areas of the peaks corresponding to citrostadienol, 24-methylene cycloartanol and C_{26} linear saturated alcohol with that of the peak corresponding to cholesterol allowed the determination of the amounts of these compounds.

The percentages of citrostadienol in the 4-methylsterol fraction, of 24-methylenecycloartanol in the triterpene alcohol fraction and of C_{26} linear saturated alcohol in the linear saturated alcohol fraction, were already separately determined. We could then effect the quantitation of 4-methylsterols, triterpene alcohols, linear saturated alcohols and phytol. One should emphasize that a careful calibration was possible only for the quantitation of linear saturated alcohols and phytol, through the determination of relative weight responses of cholesterol, 1-docosanol (pure, Supelco Inc.) and phytol (for biochemistry, Merck A.G., Darmstadt, Germany) standards. Triterpene alcohol and 4-methylsterol fractions were more roughly quantitated by comparison of cholesterol peak area respectively with citrostadienol and 24-methylene-cycloartanol peaks areas.

RESULTS AND DISCUSSION

Physical and Chemical Characteristics

The physical and chemical characteristics of Iranian olive oils (Table I) were within the usual limits given by the literature, the only exception being a fairly high level of unsaponifiable matter, 1.75% and 1.98% for virgin oils and 5.33% for husk oil.

Lipid Composition

The total fatty acids composition found in Iranian oils (Table II) complies, on the whole, with the limits given by Codex Alimentarius (11) and by Italian (12) and Spanish (13) standards.

The composition of fatty acids in the 2 position found in Iranian olive oils is in agreement with literature data for oils of various origin. The percentages observed for palmitic acid in position 2 (1.0-1.2%) are lower than forensic maximum limits provided for by E.E.C. (4), Italy (12) and Spain (13). These limits are: E.E.C. 2%, Italy 2% for virgin oils and 3% for husk oils, Spain 1.5% for virgin oils and 2.2% for husk oils. When, in an olive oil, the level of palmitic acid in position 2 is higher than these limits, the product analyzed is considered as containing added re-esterified oil.

Proportions also are in fair agreement with values reported by others (5,14-16). Oleic acid proportions (37%) for Iranian oils, were always lower than those of linoleic (45-48%), which is in agreement with data reported in our previous papers (5,15,16), but not with results obtained by Tiscornia and Bertini (14). These authors found proportions of oleic acid always higher than those of linoleic acid.

The triglycerides composition, calculated from Table II according to Vander Wal's 1,3 random – 2 random theory (7), is similar to values reported in the literature for oils of various origin. Eight triglycerides which constitute 87.0-90.5% of this fraction are: OOO (41.8-47.4%), POO (17.7-18.5%), LOO (7.9-9.3%), OLO (6.2-6.5%), SOO (4.7-5.1%), PLO (2.4-2.8%), POP (1,8-1.9%), POL (1.6-2.0%).

Sterols

The amounts of sterols found in the two Iranian virgin olive oils (104 and 111 mg/100 g oil) were low but within

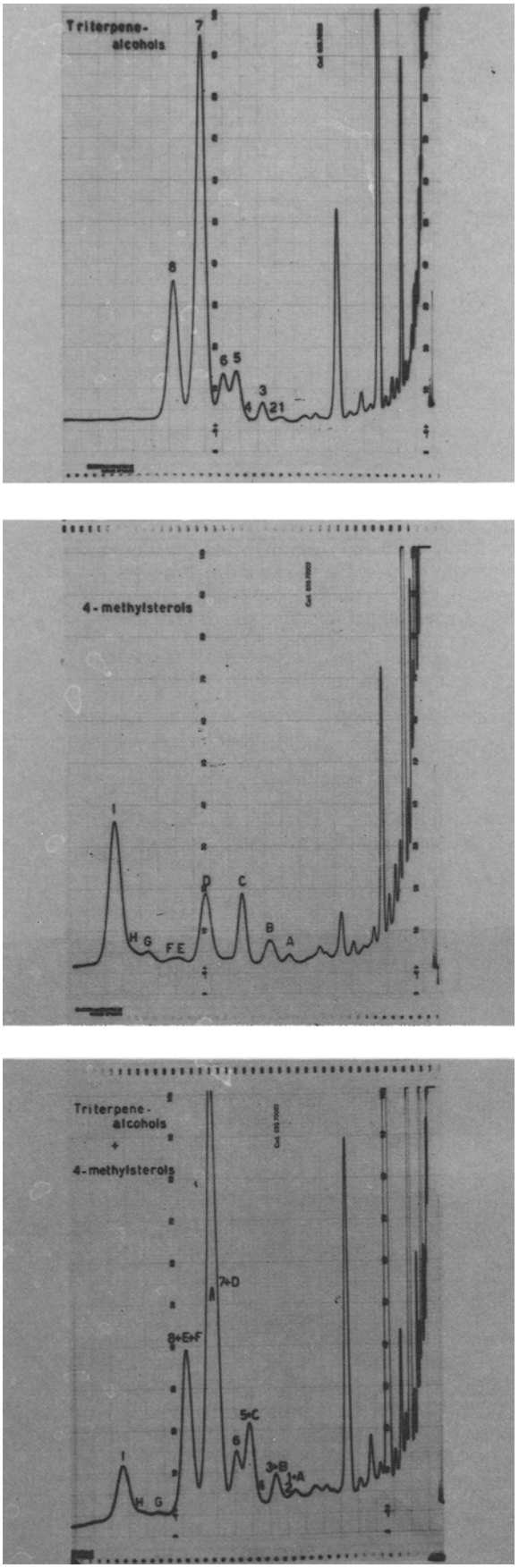

FIG. 1. GLC chromatograms of triterpene alcohol and 4-methylsterol fractions of an Iranian olive oil. 5: βamyrin; 6:butyrospermol; 7:cycloartenol; 8: 24-methylenecycloartanol; B: lophenol (?); C: obtusifoliol; D: cycloeucalenol and/or gramisterol; I: citrostadienol; 1, 2, 3, 4, A, E, F, G, H: unidentified peaks.

the range of values reported in the literature (9,17-21). On the other hand, the sterol content in the husk oil sample was 562 mg/100 g oil, which is higher than the 110-380 mg/100 g oil reported by others for this kind of olive oil (9,17,18,22).

For all the olive oils, including the Iranian ones, the predominating sterol is β-sitosterol (Table III). For the two Iranian virgin olive oils examined, the forensic limits for the apparent β-sitosterol (sum β-sitosterol + $\Delta_5$avenasterol) content are complied with. The laboratory husk oil examined was not within the legal limits.

It has to be emphasized that, according to E.E.C. (4), Italian (23) and Spanish (13) official methods, the gas chromatography of sterols has to be performed with SE 30. With this stationary phase, Δ_5 avenasterol has about the same retention time as β-sitosterol and shall be counted as though it was β-sitosterol.

The forensic limits are: for E.E.C., 93% considering all the peaks but the Δ7avensterol one; for Italy and Spain, 94% considering only the main sterols (campesterol, stigmasterol, SE 30 apparent β-sitosterol). When the SE 30 apparent β-sitosterol content in an olive oil is higher than these limits, the product analyzed is considered as containing other oils.

Triterpene Dialcohols

The amount of triterpene dialcohols observed in Iranian virgin oils was 1 mg/100 g oil, corresponding to 0.05-0.06% of the unsaponifiable matter, which is much lower than 0.45-0.56% of the unsaponifiable reported by Tiscornia and Bertini (24) for oils of various origins and 2.7-21.8 mg/100 g oil, reported by Leone et al. for Italian olive oils.

The relative contents of triterpene dialcohols (Table III) for virgin oils Nos. 1 and 2 were on the same level as the lowest data mentioned in literature and far from the 5% maximum limit imposed by the Italian law (25) for pressed oils.

The content found for the husk oil sample (5%) was much lower than literature values for industrial husk olive oils, but this oil was solvent-extracted from husks obtained in laboratory and probably contained a higher quantity of pulp oil which is lower in erythrodiol and uvaol.

Triterpene Alcohols

The contents of triterpene alcohols in the two Iranian virgin oils (78 and 91 mg/100 g oil) were lower than the 100-315 mg/100 g oil reported in the literature (9,17,18, 20-22,26). On the other hand, the amount of triterpene alcohols observed in the solvent-extracted husk oil sample (329 mg/100 g oil) is high but within the range (56-370 mg/100 g oil) of values mentioned in the literature (9,17,18,21).

The identification of the single components, when possible, has been performed through comparison with RRT of TMS derivatives mentioned in literature. The main triterpene alcohols found in Iranian olive oils (Fig. 1 and Table IV) have already been observed in olive oils of various origins (9,18,27-30).

From a quantitative point of view, it is worth mentioning that in the Iranian olive oils examined, the cycloartenol content (60.2-66.9%) was higher than the 24-methylenecycloartanol one (22.8-26.6%). This is in agreement only with results reported by Camera and Angerosa (30) for the oil obtained from the Castiglionese variety olives. In all the other olive oils mentioned in the literature, the percentage of cycloartenol was lower than the 24-methylene cycloartanol.

Comparing virgin and husk oils coming from the same olives (Nos. 2 and 3), a slightly lower cycloartenol and 24-methylenecycloartanol contents and a corresponding higher percentage of β-amyrin, butyrospermol and an

TABLE IV

Composition (%) of Triterpene Alcohol Fractions of Iranian Olive Oils

	Triterpene alcohols	1 Commercial virgin oil	2 Laboratory virgin oil	3 Laboratory-extracted husk oil
1	Unidentified	0.3	0.2	0.4
2	Unidentified	0.3	tr.	tr.
3	Unidentified	0.1	0.2	2.2
4	Unidentified	0.1	0.2	tr.
5	β-amyrin	6.2	2.1	7.3
6	Butyrospermol	3.5	3.8	7.1
7	Cycloartenol	63.7	66.9	60.2
8	24-methylenecycloartanol	25.8	26.6	22.8

TABLE V

Composition (%) of 4-Methylsterol Fractions of Iranian Olive Oils[a]

	4-Methylsterols	1 Commercial virgin oil	2 Laboratory virgin oil	3 Laboratory-extracted husk oil
A	Unidentified	0.2	1.2	1.3
B	Lophenol (?)	2.4	3.3	6.0
C	Obtusifoliol	20.2	18.4	15.3
D	Cycloeucalenol and/or gramisterol	17.1	23.0	20.6
E	Unidentified	2.2	0.9	1.3
F	Unidentified	1.6	1.1	1.5
G	Unidentified	4.9	4.5	4.8
H	Unidentified	n.d.	n.d.	n.d.
I	Citrostadienol	51.4	47.6	49.2

[a]n.d. = non determinable.

unidentified compound with RRT 0.73 (triterpene alcohol No. 3) were found in the hexane-extracted oil.

4-Methylsterols

The amounts of 4-methylsterols found in the two Iranian virgin olive oils (18 and 20 mg/100 g oil) were in line with those (8-20 mg/100 g oil) reported by Itoh et al. (20) and Boskou (26). The 4-methylsterols content (70 mg/100 g oil) was much higher in the husk oil sample. No data is available from the literature for solvent-extracted husk oils.

As for triterpene alcohols, the identification of the individual 4-methylsterols was made by comparison with RRT of TMS derivatives given by the literature. The main peaks observed for Iranian olive oils (Fig. 1 and Table V) may be attributed to obtusifoliol (15-20%), cycloeucalenol and/or gramisterol (17-23%) and citrostadienol (48-51%). These percent data are in line with those reported by other authors (10,28,31,32) for five samples: obtusifoliol 10-17%, cycloeucalenol and/or gramisterol 15.5-27% and citrostadienol 36-69%.

No particular difference was observed in the 4-methylsterols contents of virgin and husk Iranian olive oils.

Aliphatic Alcohols

The linear saturated alcohol contents observed in the two Iranian olive oils were 16 and 38 mg/100 g oil for virgin oils and 593 mg/100 g oil for husk oil. This is in fair agreement with data reported in literature for pressed oils (10-36 mg/100 g oil) and for husk oils (81-530 mg/100 g oil) (9,17,18,21).

As reported here and in earlier papers (9,30,33), the main components of the aliphatic alcohol fraction in olive oils were found to be the linear saturated alcohols with an even number of carbon atoms and phytol (Fig. 2 and Table VI).

The percentages of C_{18} (0.6-2.7%) and C_{20} (0.2-0.4%) linear saturated alcohols found in Iranian olive oils were very low if compared with data reported by Camera and Angerosa (30) for oils extracted from ripe olives from Abruzzi (Italy): C_{18} 5.7-8.1%, C_{20} 9.7-26.1%.

The ratio C_{26}/C_{24} in Iranian olive oils was found to be lower than 1, unlike the results mentioned by Fabbrini et al. (33) for Italian olive oils.

The amount of phytol, related to the chlorophyll contained in the oil, is higher in the husk oil sample (18 mg/100 g oil) than in the virgin oil ones (6 and 12 mg/100 g

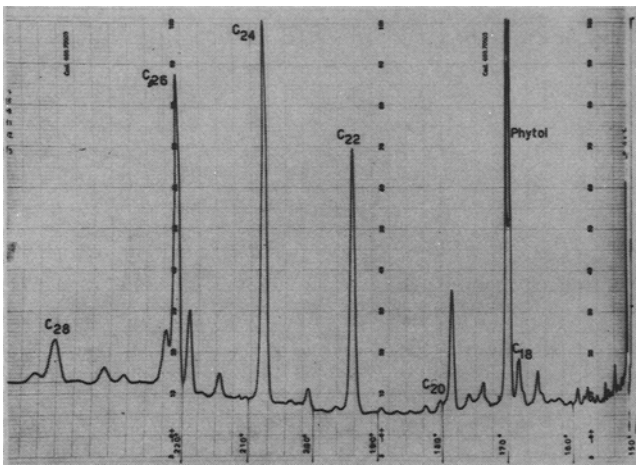

FIG. 2. GLC chromatogram of aliphatic alcohol fraction of an Iranian olive oil.

TABLE VI

Composition (%) of Linear Saturated Alcohol Fractions and Phytol Relative Content (Parts Per 100 Parts of Linear Saturated Alcohols) of Iranian Olive Oils

Aliphatic alcohols	1 Commercial virgin oil	2 Laboratory virgin oil	3 Laboratory-extracted husk oil
C_{18}	0.6	2.7	0.7
C_{20}	0.2	0.4	0.4
C_{22}	23.9	24.1	21.5
C_{24}	38.0	33.9	34.8
C_{26}	29.3	30.2	32.8
C_{28}	8.0	8.7	9.8
Phytol	30.1	35.5	3.0

oil), but the difference is not so great as for linear saturated alcohols. Therefore, the ratio linear saturated alcohols/phytol, which is about 3 for virgin oils, becomes 27 when the hexane-extracted husk oil is considered. No data is given by earlier works. In addition, the phytol relative content (Table VI) was, roughly, ten times lower for the husk oil than for the virgin oils examined.

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