

# ✿ Detection of Adulterated and Misbranded Olive Oil Products

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## ABSTRACT

The Food and Drug Administration has been examining bulk and packaged olive oil products in a continuing program to detect adulteration of olive oil products. Thirteen of 20 products collected in 1983-84 labeled as olive oil contained undeclared esterified (synthetic) olive oil and four contained undeclared olive-residue oil (derived from olive pomace and pits). Seven of 13 brands of imported olive oil contained undeclared esterified oil, suggesting that considerable quantities of esterified oil have been shipped to the United States identified as olive oil.

## INTRODUCTION

Italian government and industry officials notified the Food and Drug Administration (FDA) several years ago that considerable adulteration and misbranding of olive oil products existed in the U.S. A survey by the Italian Experiment Station for the Fat and Oil Industry in Milan of olive oils purchased in the U.S. in 1982 demonstrated that various brands of olive oil contained undeclared esterified oil and olive-residue oil as well as undeclared seed oils (E. Fedeli, private communication, 1982). Accordingly, the FDA instituted an olive oil sampling program in 1982 to take action against adulterated and misbranded olive oil products. Although the FDA has not established standards of identity for olive oil products (1), the agency recognizes that consumers want to know whether an olive oil product or one derived from olive oil has been expressed by physical means from sound olives (virgin olive oil), obtained from olive residues (pits and pomace remaining after physical expression of the oil) by solvent extraction and refining (refined olive-residue oil), or is a synthetic product with altered glyceride structure produced by reaction of low grade olive oil or olive oil by-product with glycerol (synthetic or "esterified" oil). Accurate labeling of olive oil products can be enforced under general provisions of the Federal Food, Drug and Cosmetic Act which state that a food is deemed to be adulterated "if any valuable constituent has been in whole or in part omitted or abstracted therefrom, or if any substance has been substituted wholly or in part thereof [section 402(b) (1) and (2)]; and is judged to be misbranded "if its labeling is false or misleading in any particular" [section 403(a)(1)], or if the label does not bear the common or usual names of each ingredient with certain exceptions pertaining to spices, flavorings and colorings [section 403(i)].

To determine the extent of adulteration and misbranding of olive oil products, the FDA initiated examination of bulk lots of imported olive oil as well as packaged olive oil products. A set of analytical methods suitable for detecting olive oil adulteration was used to analyze samples collected from commercial sources by FDA inspectors. The methods included procedures useful for detecting the presence of esterified oil, olive-residue oil and seed oils in olive oil. This report deals with the analysis of various olive oils and olive oil brands in a continuing program to control the adulteration and misbranding of olive oil in the U.S.

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## EXPERIMENTAL PROCEDURES

Olive oils were collected by FDA inspectors at points of import and from repackers, dealers and market shelves. The products, with several exceptions, were analyzed in FDA's Boston District Laboratory.

### Fatty Acid Composition

A 500-mg portion of sample was analyzed by AOAC method 28.060-28.068 (2) after preparation of methyl esters by AOAC method 28.056-28.059 (2). Gas chromatography (GC) was performed on a Hewlett-Packard HP-5890A gas chromatograph equipped with a flame ionization detector (FID) and a 1.8 mm × 2 mm glass column packed with 3% SP-2130/2% SP-2300 on 100/120 mesh Chromosorb WAW. Injections were made under a timed protocol with the initial oven temperature at 175 C for 2 min, then programmed at a rate of 2 C/min and held at 220 C for 10 min to elute all of the fatty acids.

### Sterol and Triterpene Diol Analysis

Sterols and triterpene diols (erythrodiol and uvaol) were determined by a modification of Italian method NGD C 51 (3) and IUPAC method 2.403 (4). A 10-g portion of sample was saponified and extracted with ether. Combined extracts were water washed, passed through a column of anhydrous sodium sulfate, shaken with 25 g of neutral alumina and then filtered and dried under nitrogen. The residue, in chloroform, was streaked on a silica gel thin layer chromatographic (TLC) plate and developed in petroleum ether-ether (1:1), dried and sprayed with a solution of 2,7-dichlorofluorescein, and the sterol and triterpene diol fractions were visualized under ultraviolet (UV) light. The combined sterol and triterpene diol bands, scraped from the plate, were eluted with hot chloroform, dried under a stream of nitrogen, dissolved in chloroform and rechromatographed by TLC as above. The combined sterol and triterpene diol residue, dissolved in ethyl acetate, was analyzed by GC (HP-5890A) using a 1.8 mm × 2 mm or 1.2 mm × 4 mm o.d. glass column packed with 1.5% OV-17 on 230/270 mesh Gas-Chrom Q. Injections were made under a timed protocol with an initial oven temperature of 254 C for 45 min, then programmed at a rate of 2 C/min to 280 C and held at 280 C for 20 min to elute the triterpene diols. A standard mixture of plant sterols and erythrodiol was chromatographed before analysis of the residue.

### Determination of Saturated Fatty Acids in the 2-Position of the Triglycerides

A 5-g portion of sample was analyzed by IUPAC method 2.210 (4). The test portion was dissolved in 25 ml of hexane and purified through a 15-g column of neutral alumina. The solvent was evaporated in a stream of nitrogen, and 0.1 g of fat was partially hydrolyzed with pancreatic lipase. The hydrolyzed test portion was extracted with ether and fractionated on a silica gel TLC plate. The monoglyceride band was removed from the plate and the monoglycerides were converted to methyl esters and extracted from the silica gel mixture with petroleum ether. The extract was then evaporated to dryness under nitrogen, dissolved in heptane and analyzed by GC as described above for determination of fatty acid composition.

### UV Determination of Purity and Deterioration

Specific extinction (K), that is, the absorption of a 1% solution of the oil in isoctane (1-cm cell), was measured according to Italian method NGD C 40 (5). Measurements were made in the regions of conjugated diene (232 nm) and conjugated triene (268 nm) to calculate  $K_{232}$ ,  $K_{268}$ , and  $\Delta K = K_{268} - (K_{262} + K_{274})/2$  (see also Codex Alimentarius Recommended International Standard CAC/RS 33-1970, procedure 8.15.4 [6]).

### Additional Analyses

Acidity (expressed as percent oleic acid) was determined by Italian method NGD C 10-1976 (5). Chlorophyll and artificial dyes were detected by spectrophotometric measurement of a 1:1 mixture of the sample in hexane, scanning between 350 and 800 nm.

## RESULTS AND DISCUSSION

International concern about adulteration of olive oil products has led to the development of draft standards for olive oil including the Codex Alimentarius Recommended International Standard for Olive Oil, CAC/RS 33-1970 (6). The Codex standard includes the following definitions for olive oil:

(i) Olive oil is the oil obtained from the fruit of the olive tree (*Olea europaea* L.) without any manipulation or treatment not specified in definitions (ii) and (iii).

(ii) Virgin olive oil is the oil from the fruit of the olive tree obtained by mechanical or other physical means under conditions, particularly thermal, which do not lead to alteration of the oil. Virgin olive oil is suitable for consumption in the natural state.

(iii) Refined olive oil is the oil obtained from virgin olive oil with high acid content and/or undesirable organoleptic characteristics by refining methods which do not lead to alterations in the initial glyceride structure.

(iv) Refined olive-residue oil is the oil obtained from olive residues (pomace and pits remaining after extraction of the oil from olives by physical means) by solvent extraction and made edible by refining methods which do not lead to alteration in the initial glyceride structure.

The standard does not mention esterified oil (also called grade B glycerol oil), which is banned for sale as an edible food in Italy and other Mediterranean countries and presumably not considered by Codex as an olive oil product. The Codex Recommended International Standard also applies to blends of olive oils, and notes that refined olive oil and refined olive-residue oil may be marketed alone or blended with virgin olive oil. FDA's definitions of olive oil products are essentially in agreement with the Codex definitions. In addition, FDA has stated that a blend of virgin olive oil and refined olive oil may be represented as such or simply called "olive oil"; however, "refined olive-residue oil" should be identified as such, and blends of olive oil and refined olive-residue oil should be clearly labeled as, for example, "a blend of refined olive-residue oil and refined olive oil."

Approximately 35 olive oils, comprising 13 brands, were collected and analyzed by FDA in 1983-84. Fatty acid and sterol analyses were used to distinguish between olive oil and seed oils. Spectrophotometric (UV and visible) measurements were useful for confirming identification of virgin (pressed) oils vs. refined oils, and to discover the presence of artificial dyes. Acidity measurements were also useful for quality evaluation. Quantitation of the triterpene diols, erythrodiol and uvaol, relative to the sterols, provided a means of distinguishing between pressed olive oil and refined olive-residue oil. Paganuzzi (7-9) confirmed the validity

of the requirement of the Italian law for pressed oil (10), which requires that the content of erythrodiol and uvaol relative to the sum of the sterols + erythrodiol and uvaol shall be <5%, and Choukroun et al. (11) demonstrated more recently the utility of the TLC-GC method for detecting adulteration of pressed oils with refined olive-residue oils.

Determination of the percent palmitic and stearic acid in the 2-position of the triglycerides was used to identify esterified oil. Tiscornia and Bertini (12) reviewed earlier work on the determination of specific distribution of fatty acids in vegetable oil triglycerides and presented results of analyses of various vegetable oils, including olive oils, from Italy and other countries. Fedeli (13) pointed out that genuine olive oil never contains more than 2% palmitic acid in the 2-position. The Italian government has issued specifications for olive oils (14), and the Codex Committee on Fats and Oils has issued recommended fatty acid (15) and sterol (16) ranges for oils and fats including olive oil. Important identity characteristics (6,14,15) are shown in Table I.

Results of analysis of 28 of the 35 olive oils collected by FDA inspectors in 1983-84 are shown in Table II. (The samples do not necessarily represent a cross section of the olive oil products and brands available during those years.) One of five samples labeled as virgin olive oil contained esterified olive oil (sample 3:5.5% palmitic acid in the 2-position of the triglycerides). Of 20 samples labeled olive oil, 13 contained undeclared esterified olive oil and four contained undeclared olive-residue oil as indicated by high

TABLE I  
Identity Characteristics for Olive Oil

Characteristic	Virgin olive oil	Refined olive oil	Refined olive-residue oil
Palmitic acid in the 2-position of the triglycerides, % <sup>a</sup>	Max 1.8	Max 1.8	Max 2.2
Specific extinction in UV: <sup>a</sup>			
max $K_{232}$	—	3.50	6.00
max $K_{268}$	0.25	1.10	2.00
max $\Delta K$	0.010	0.16	0.20
Erythrodiol + uvaol, % of total sterols + erythrodiol and uvaol <sup>a</sup>	Max 5.0	Max 5.0	—
Acidity, expressed as % oleic acid <sup>a</sup>	4.0	0.3	0.3
Fatty acid composition <sup>b</sup>			
% of total fatty acids:			
14:0	<0.05	<0.05	<0.05
16:0	7.5-20	7.5-20	7.5-20
16:1	0.3-3.5	0.3-3.5	0.3-3.5
18:0	0.5-3.5	0.5-3.5	0.5-3.5
18:1	56-83	56-83	56-83
18:2	3.5-20	3.5-20	3.5-20
18:3	<1.5	<1.5	<1.5
20:0	Trace	Trace	Trace
22:0	Trace	Trace	Trace
Sterol composition, GC: <sup>a</sup>			
% of total sterols:			
Cholesterol, max	0.5	0.5	0.5
Campesterol, max	4.0	4.0	4.0
Stigmasterol	c	c	c
$\beta$ -Sitosterol + $\Delta^5$ -avenasterol, min <sup>d</sup>	93.0	93.0	93.0
$\Delta^7$ -Stigmasterol, max	0.5	0.5	0.5

<sup>a</sup>Reference 14.

<sup>b</sup>References 6 and 15.

<sup>c</sup>Must be less than campesterol.

<sup>d</sup>Data on sterol ranges for olive oil (GC, OV-17 stationary phase, percent of total sterols) are as follows: campesterol, 2.4-5.6; stigmasterol, 0.3-3.9;  $\beta$ -sitosterol, 82.4-91.4;  $\Delta^5$ -avenasterol, 4.1-13.6;  $\Delta^7$ -stigmasterol, 0-0.3;  $\Delta^7$ -avenasterol, 0-0.1 (see ref. 16).

TABLE II

Selected Analytical Results: Olive Oil Samples Collected by FDA in U.S. in 1983-84

Sample	Label declaration	% of total fatty acids		% at position 2 of triglycerides		E + U <sup>a</sup> , % of sterols + triterpene diols	K <sub>232</sub>	K <sub>268</sub>	ΔK	Finding <sup>b</sup>
		16:0	18:0	16:0	18:0					
1	Virgin olive oil	14.0	3.0	1.0	0.7	—	0	0	0	A
2	Virgin olive oil	10.6	3.0	1.8	0.9	—	—	0.18	0.004	A
3	Virgin olive oil	11.2	5.5	5.5	1.7	0.6	1.90	2.51	0.26	B
4	Virgin olive oil	11.0	3.0	2.1	0.6	1.3	—	0.10	0.002	A
5	Virgin olive oil	8.8	3.4	0.8	0.2	1.0	—	0	0	A
6	Olive oil	14.7	3.0	14.5	2.8	0.3	5.76	2.50	0.50	B
7	Olive oil	14.0	2.8	13.0	2.5	0.4	5.63	2.56	0.53	B
8	Olive oil	12.0	4.6	4.6	1.1	—	3.63	0.84	0.14	B
9	Olive oil	14.8	2.9	11.8	3.5	—	3.48	1.86	0.23	B
10	Olive oil	14.1	2.9	9.0	3.1	—	2.97	1.51	0.19	B
11	Olive oil	11.1	3.3	1.8	0.8	—	2.36	1.00	0.12	A
12	Olive oil	13.6	3.0	13.2	3.5	—	5.69	2.83	0.64	B
13	Olive oil	10.0	2.8	10.8	3.3	—	5.98	2.18	0.41	B
14	Olive oil	14.9	2.8	15.7	3.0	—	7.21	2.25	0.40	B
15	Olive oil	9.9	3.3	0.8	0.6	—	2.23	0.36	0.03	A
16	Olive oil	13.4	2.9	5.5	2.8	0.4	5.13	1.80	0.27	B
17	Olive oil	9.6	2.6	8.9	2.4	—	6.56	2.19	0.40	B
18	Olive oil	14.0	2.6	15.6	2.9	0.6	6.01	2.79	0.42	B
19	Olive oil	14.9	2.7	15.5	3.1	0.6	5.77	2.76	0.44	B
20	Olive oil	10.8	3.6	0.9	0.2	0.9	1.05	0.38	0.05	A
21	Olive oil	9.3	2.5	9.0	2.6	2.5	6.09	2.21	0.47	B
22	Olive oil	10.1	2.9	1.6	0.6	5.8	2.42	0.90	0.16	C
23	Olive oil	12.3	Tr	3.0	0.7	10.7	5.24	1.11	0.12	C
24	Olive oil	12.3	Tr	2.4	1.1	11.9	3.99	1.32	0.22	C
25	Olive oil	11.0	2.7	1.6	0.5	13.5	4.53	1.19	0.14	C
26	Type B (olive-residue oil)	10.0	4.6	4.6	1.2	9.3	4.74	1.81	0.32	D
27	Type B (olive-residue oil)	10.1	2.9	9.4	3.0	4.0	5.08	2.05	0.37	D
28	Type B (olive-residue oil)	10.9	2.7	2.0	0.6	8.5	4.39	1.26	0.14	A

<sup>a</sup>Erythrodiol + uvaol (triterpene diols).<sup>b</sup>A, sample appears to be properly labeled; B, sample contains or consists entirely of esterified olive oil; C, sample contains olive-residue (pits and pomace) oil; D, sample contains esterified olive oil as well as olive-residue oil.

(>5%) levels of the triterpene diols. Two of three bulk samples declared to be type B (olive-residue) oil (samples 25 and 26) also contained esterified olive oil. Seven of 13 brands of packaged olive oil contained undeclared esterified oil, suggesting that considerable quantities of esterified oil are shipped to the United States identified as olive oil.

A domestic brand collected in 1982 and labeled "Pure Imported Olive Oil" consisted entirely of esterified olive oil. The oil contained 15.5% palmitic acid, whereas the fatty acids in the 2-position of the triglycerides contained 17.6% palmitic acid. Spectrophotometric measurement (425-750 nm) of the sample indicated that artificial color was present (peak maxima at 585 and 630 nm) in addition to a trace of chlorophyll. A blue band was observed with R<sub>f</sub> near the front during TLC isolation of the sterols. A spectrum of the material in the blue band, eluted with chloroform, was characteristic of an anthraquinone dye. Additional artificial color was isolated from the oil by silica gel and neutral alumina column chromatography and was stable to treatment with strong alkali. The undeclared artificial color was judged to be a dye similar to Ext. D&C Blue No. 5.

The results of analysis of a set of samples collected in 1984 from one domestic repacker of imported olive oil can be cited as typical of current violative practices. Samples were obtained from the firm's bulk stock of soybean oil, virgin olive oil and olive-residue oil as well as packaged products identified on the label as "Pure Imported Olive Oil" and "75% Pure Vegetable Oil, 25% Pure Imported Olive Oil." Results of determination of fatty acid composition, fatty

acids in the 2-position of the triglycerides and sterol/triterpene diol composition are shown in Table III. Chromatograms of the sterol/triterpene diol fractions are shown in Figures 1 and 2. The erythrodiol/uvaol levels in the sterol/triterpene diol fraction of sample D, labeled "Pure Imported Olive Oil," indicate that the sample consists substantially or entirely of residue olive oil. Also, the erythrodiol/uvaol content in sample E, the blend labeled "25% Pure Imported Olive Oil," indicates that the olive oil constituent is a residue olive oil. Sample F, another packaged product labeled "Pure Imported Olive Oil," was substantially or entirely esterified olive oil, as demonstrated by the stearic/palmitic content of the fatty acids in the 2-position of the triglycerides, approximately equal to the content of these acids in the whole oil.

Adulteration of olive oil with seed oils is a problem that has existed for centuries, and more than 150 years ago Poutet (17) reported a procedure for determining adulteration with seed oils such as poppyseed oil or rapeseed oil, which were common adulterants at the time. Today, mixtures of seed oils with olive oils generally can be identified by analysis of the fatty acids and sterol fraction. A brand labeled "Pure Imported Olive Oil," collected during 1983, contained about 30% soybean oil as determined by fatty acid analysis (2.8% linolenic acid) and sterol composition (16% campesterol and 15% stigmaterol in the sterol fraction). Another brand, sampled during 1984, labeled as a blend containing 50% olive oil, was mostly soybean oil (6.3% linolenic acid in the sample vs. 7.1% linolenic acid in the soybean oil ingredient used to prepare the blend; 20% cam-

## OLIVE OIL ADULTERATION

TABLE III

Results of Analysis of Samples Collected in 1984 from Firm Importing and Packaging Olive Oil Products

Component	Label declaration on consumer package or bulk container					
	(A) <sup>a</sup> Soybean oil	(B) <sup>b</sup> Residue (Grade B) olive oil	(C) <sup>c</sup> Virgin olive oil	(D) <sup>d</sup> Pure imported olive oil	(E) <sup>e</sup> 25% Pure imported olive oil	(F) <sup>f</sup> Pure imported olive oil
Fatty acid composition, %:						
16:0	10.3	10.9	8.8	11.0	10.3	9.3
16:1	0.1	1.0	0.6	1.0	0.3	0.9
18:0	4.1	2.7	3.4	2.7	4.0	2.5
18:1	23.2	73.2	77.4	72.9	33.1	73.2
18:2	52.9	9.1	6.7	9.2	44.3	10.6
18:3	7.2	0.6	0.7	0.6	6.0	0.7
20:0	0.5	0.6	0.5	0.6	0.5	0.5
22:0	0.4	0.2	0.1	0.2	0.3	0.1
Sterol/triterpene diol composition, %:						
Cholesterol	0.2	0.0	0.0	0.0	0.1	0.0
Campesterol	19.1	3.0	2.2	2.5	15.8	2.2
Stigmasterol	18.4	2.1	0.3	1.9	14.3	1.5
$\beta$ -Sitosterol	55.1	80.5	87.9	76.1	62.0	64.3
$\Delta^5$ -Avenasterol	1.7	2.4	8.6	2.5	2.2	29.5
$\Delta^7$ -Stigmasterol	3.8	3.2	0.0	2.9	2.4	0.0
$\Delta^7$ -Avenasterol	1.6	0.0	0.0	0.0	0.6	—
Erythrodiol	0.1	6.6	1.0	11.4	2.2	2.5
Uvaol	0.0	1.9	0.0	2.0	0.3	0.0
Fatty acids in the 2-position of the triglycerides, %:						
16:0	—	2.0	0.8	1.6	—	9.0
16:1	—	0.9	0.5	0.7	—	1.1
18:0	—	0.6	0.2	0.5	—	2.6
18:1	—	85.2	89.1	85.9	—	75.6
18:2	—	10.9	9.1	11.1	—	11.4

<sup>a</sup>Sample (bulk product) is a soybean oil.<sup>b</sup>Sample (bulk product) is a residue olive oil.<sup>c</sup>Sample (bulk product) is a pressed (virgin) olive oil.<sup>d</sup>Sample (consumer package) is a residue olive oil.<sup>e</sup>Sample (consumer package) contains residue olive oil and soybean oil.<sup>f</sup>Sample (consumer package) is an esterified olive oil.

pesterol and 18% stigmasterol in the sterol fraction). On the other hand, a third brand sampled in 1984, labeled as a 25% blend of olive oil plus soybean, cottonseed and corn oils, was pure soybean oil (7.2% linolenic acid; 18% each of campesterol and stigmasterol in the sterol fraction).

The problem of deliberate adulteration of olive oil products will continue, so that vigilance is required to protect the consumer and the responsible olive oil trade. As violative practices change, it will be necessary to update and improve available analytical methods as well as to devise new procedures. International cooperation in developing composition data, promulgating standards and testing and validating analytical procedures is an important aspect of any program to eliminate contamination and adulteration of olive oil products.

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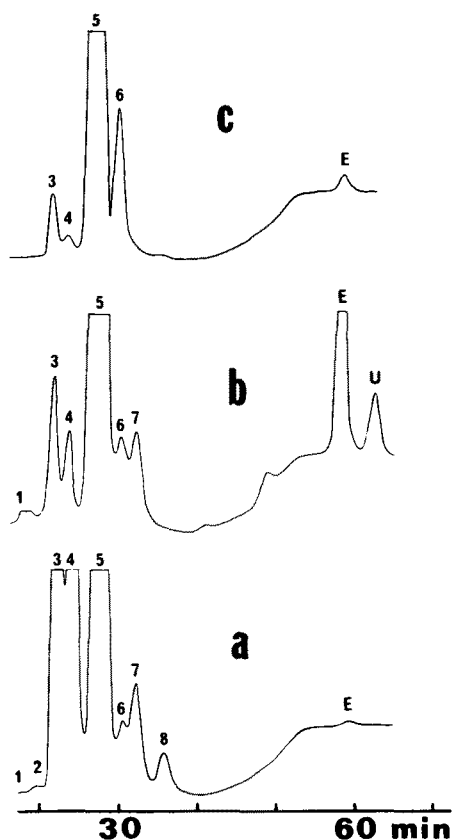


FIG. 1. Gas chromatograms of sterol/triterpene diol fraction from (a) soybean oil; (b) residue (grade B) olive oil, and (c) virgin olive oil collected from firm importing and packaging olive oil products (see Table III). Peaks are: (1) cholesterol; (2) brassicasterol, (3) campesterol; (4) stigmasterol; (5)  $\beta$ -sitosterol; (6)  $\Delta^5$ -avenasterol; (7)  $\Delta^7$ -stigmasterol; (8)  $\Delta^7$ -avenasterol; (E) erythrodiol and (U) uvaol. Note the large E peak in chromatogram (b), which is characteristic of residue olive oils.

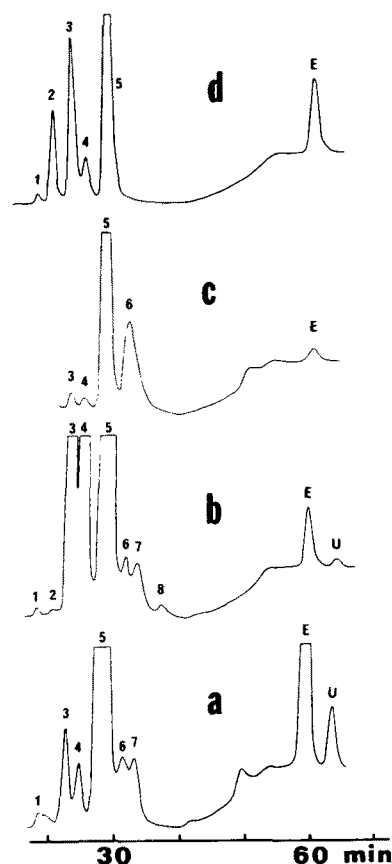


FIG. 2. Gas chromatograms of sterol/triterpene diol fraction from (a) sample labeled "Pure Imported Olive Oil" (Table III, sample D); (b) sample labeled "25% Pure Imported Olive Oil"; (c) another sample labeled "Pure Imported Olive Oil" (Table III, sample F), and (d) standard mixture of sterols and erythrodiol. See Figure 1 for source of samples and peak identities.

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