TABLE 2

Fatty Acids Composition of $AgNO_3$/Methanol-Reacted Methyl Ester (% wt) Derived from *Gnetum scandens*, *Sterculia pallens* and *Sterculia foetida* Seed Oils

Fatty acids	*G. scandens*		*S. pallens*		*S. foetida*	
Myristic (14:0)	0.35		3.15		—	
Palmitic (16:0)	15.51		21.14		—	
Palmitoleic (16:1)	—		—		1.0	
Stearic (18:0)	10.20		2.85		3.4	
Oleic (18:1)	16.22		40.20		9.4	
Linoleic (18:2)	15.00		21.79		1.3	
Linolenic (18:3)	2.85		—		—	
Malvalic						
(Ether derivative)	10.36	11.27	3.31	3.87	6.5	7.1
(Ketone derivative)	0.91		0.56		0.6	
Sterculic						
(Ether derivative)	27.25	28.57	6.06	6.97	48.8	51.2
(Ketone derivative)	1.32		0.91		2.4	

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Chemical Evaluation of Egyptian Citrus Seeds as Potential Sources of Vegetable Oils

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Seeds of the citrus fruits orange, mandarin, lime and grapefruit were analyzed. Petroleum ether-extracted oils of such seeds amounted to more than 40% of each. Physical and chemical properties of the extracted oils are presented. Samples of the extracted oils were saponified and the unsaponifiables and fatty acid fractions isolated. The isolated unsaponifiables and fatty acids were analyzed by GLC. GLC analysis of the unsaponifiables revealed compositional patterns different in number, type and relative concentration of fractions according to type of citrus seed oil, depending on the solvent system used for oil extraction and unsaponifiable matter isolation. The compositional patterns of the unsaponifiables were similar to that of cottonseed oil. Mandarin and grapefruit oils are free of cholesterol. The data demonstrate that the fatty acid compositional patterns of the oils differ; Mandarin seed oil contains the largest number of fatty acids, and grapefruit seed oil contains the lowest. The total amounts of volatile fatty acids in these oils are generally higher than those of other edible oils. Lime seed oil is similar, in the degree of unsaturation, to soybean oil. The orange oil pattern is similar to cottonseed oil. The amount of total essential fatty acids in lime seed oil is the highest of the oils studied.

A number of workers have studied the fatty acid composition of citrus seed oils. Mehta et al. (1) studied the fatty acid composition of orange seed oil and found that saturated fatty acids represented 12.08%. French (2) identified the fatty acids palmitic (28%), stearic (5.4%), oleic (22.6%), linoleic (37.2%) and linolenic (6.5%) in citrus seed oils. Braddock and Kesterson (3) stated that the properties and characteristics of citrus seed oils are similar to cottonseed oil. One notable difference is that linolenic acid is present in appreciable amounts in citrus seed oils but only traces are found in cottonseed oil. They added that from the nutritional aspect citrus seed oils are similar to other vegetable oils having a relatively high content of the essential linoleic and linolenic acids.

Abdel-Baki and Hassan (4) reported that in Egyptian orange seed oil oleic acid is the major fatty acid. Abdo (5) studied the fatty acid composition of Egyptian orange seed oil and identified palmitic, stearic, palmitoleic, oleic, linoleic and linolenic acids at 22.46%, 4.73%, 0.73%, 30.3%, 38.88% and 1.86%, respectively.

At present no citrus seed oils are produced commercially in Egypt. Braddock and Kesterson (3) reported that the commercial production of citrus seed oils in Florida (USA) had reached 13 million kg in 1970-1971.

The present investigation was therefore performed to

TABLE 1

Chemical Composition of Citrus Seeds (on dry weight basis)

	Percentage composition				mg/100 g			
Citrus type	Lipid	Protein	Carbo-hydrate	Ash	P	Ca	K	Na
Orange	45.46	17.40	34.13	2.95	40	45	66	18
Mandarin	40.20	16.50	39.75	3.53	53	30	50	12
Lime	42.65	13.75	40.56	2.19	51	46	30	22
Grapefruit	41.36	16.60	39.20	2.60	58	32	52	32

TABLE 2

Physical and Chemical Characteristics of Citrus Seed Oils

	Oil properties					
Source of oil	UNS %	Density $d^{26}C$	Index $n^{26}C$	Iodine value	Acid value	Sap. value
Cottonseed	1.65	0.921	1.4641	108.3	0.74	196.0
Orange	1.28	0.933	1.4681	99.2	0.21	196.8
Mandarin	1.18	0.912	1.4650	82.5	0.65	186.2
Lime	1.15	0.922	1.4671	89.3	1.20	191.3
Grapefruit	0.95	0.913	1.4662	91.4	0.90	189.6

TABLE 3

Unsaponifiable Matter Composition of Orange Oil as Affected by Solvent Systems

		Relative concentration (%)[b]			
Fraction	RRT[a]	I	II	III	*Cottonseed* (UNS)
Unknown	0.08	2.26	—	8.73	3.77
Unknown	0.11	1.28	—	—	—
C_{18}	0.14	2.05	1.90	9.14	0.72
Unknown	0.20	1.28	4.93	1.87	10.38
C_{20}	0.25	25.81	1.90	8.31	1.88
Unknown	0.30	—	—	2.07	13.31
C_{22}	0.35	0.64	5.30	8.81	3.14
Unknown	0.40	—	—	—	13.21
C_{23}	0.53	1.92	—	—	2.36
C_{24}	0.53	34.57	41.71	29.09	12.26
C_{26}	0.57	—	—	1.62	12.26
C_{28}	0.63	—	0.95	—	3.77
Squalene	0.73	1.28	37.16	29.92	20.29
C_{32}	0.82	2.69	—	0.25	0.88
Cholesterol	0.87	—	0.26	0.16	0.38
Campesterol	0.91	—	—	0.50	0.56
β-sitosterol	1.00	17.92	0.19	—	0.88
Number of fraction		12	10	12	16
Total sterols %		17.92	0.45	0.66	1.82
Unknowns %		7.08	4.93	12.24	40.67

[a]RRT, Retention time of fractions relative to β-sitosterol (16 min).

[b]Solvent systems. I, Chloroform-MeOH, 2:1 for oil ext., diethyl ether for UNS ext; II, Pet. ether for UNS ext; III, *n*-Hexane for oil ext., pet. ether for UNS ext.

chemically evaluate the Egyptian citrus seeds, namely orange, mandarin, lime and grapefruit, with comparison to cottonseed and soybean oils as common edible oils, for oil production.

The recovery of edible citrus seed oil and meal would result in increasing the world supply of food and upgrading the product utility and economic value, and also serves as a means of reducing problems of waste control and pollution.

MATERIALS AND METHODS

Orange, mandarin, lime and grapefruit seeds were obtained from "Al-Mohands National Food Products" (Schweppes) Ismailia, Egypt. Cottonseeds and soybeans were obtained from the local market.

The seeds were dried at about 70 C for 12 hr and then well ground. The crude fat, protein, carbohydrate and ash contents were determined according to AOAC methods (6). Phosphorus was determined according to the Chapman and Pratt procedure (7). Sodium and potassium were determined by a DU Flame spectrophotometer as described by Chapman and Pratt (7). Calcium and magnesium were determined by titration with standard versene solution according to Jackson (8).

The crude oils were extracted with petroleum ether, and the ordinary oil constants, i.e. acid value, saponification value, iodine value, refractive index and specific gravity, were estimated according to AOAC methods (6).

The crude oil samples were saponified overnight with methanolic KOH at room temperature. The unsaponifiable matters were isolated by extraction with diethyl ether or petroleum ether three times. The fatty acids were freed from their potassium slats by acidification with sulfuric acid (5N), followed by extraction with diethyl ether. The ether solutions were washed with distilled water and dried over anhydrous sodium sulfate.

GLC analysis of citrus seed oil unsaponifiable matters. The isolated unsaponifiables (UNS) were analyzed by a Perkin Elmer Sigma 3B gas chromatograph under the following operating conditions: coiled glass column 3% 2100, program 180-300 C, 5 C/min; gas flow rate, 30 ml/min N_2, 33 ml/min H_2, and 200 ml/min air.

Analysis of fatty acid methyl esters by GLC. The total fatty acids and standard fatty acids were methylated by using an ether solution of diazomethane, as described by Vogel (9).

The methyl esters prepared from oil samples and standard materials were analyzed by a Pye Unicum gas chromatograph equipped with a dual flame ionization

TABLE 4

Composition of Unsaponifiable Matter Isolated by Extraction with Petroleum Ether from Oil Extracted with n-Hexane from Orange, Mandarin, Lime and Grapefruit, Compared to Cottonseed

		Relative concentration (%)				
Fraction	RRT[a]	Cotton-seed	Orange	Man-darin	Lime	Grape-fruit
Unknown	0.08	3.77	8.73	4.40	0.62	—
Unknown	0.11	—	—	1.22	1.92	5.60
C_{18}	0.14	0.72	9.14	0.73	4.94	0.56
Unknown	0.20	10.38	1.87	10.27	8.99	16.79
C_{20}	0.25	1.88	8.31	0.73	2.52	0.63
Unknown	0.30	13.21	2.07	9.78	11.99	—
C_{22}	0.35	3.14	8.81	9.29	8.99	1.12
Unknown	0.40	13.21	—	13.20	10.31	—
C_{23}	0.45	2.36	—	8.31	—	16.79
Unknown	0.50	—	—	4.40	—	—
C_{24}	0.53	12.26	29.09	4.40	35.37	7.46
C_{26}	0.57	12.26	1.62	—	1.56	—
C_{28}	0.63	3.77	—	9.48	4.08	3.73
Unknown	0.70	—	—	—	5.93	—
Squalene	0.73	20.29	29.92	19.56	0.60	28.74
Unknown	0.79	—	—	0.77	—	0.74
C_{32}	0.82	0.88	0.25	2.20	0.24	0.29
Cholesterol	0.87	0.38	0.16	—	0.35	—
Campesterol	0.91	0.56	0.50	0.29	—	0.19
Unknown	0.96	—	—	—	—	—
β-sitosterol	1.00	0.88	—	0.73	0.48	0.52
Number of fractions		16	12	17	16	13
Total sterols %		1.82	0.66	1.02	1.02	0.71
Unknowns %		40.55	12.67	43.64	43.64	23.13
Cholesterol %		0.38	0.16	—	—	—

[a]RRT, retention time of fraction relative to β-sitosterol (16 min).

TABLE 5

Fatty Acid Compositions of Oils Obtained from Seeds of Orange, Mandarin, Lime and Grapefruit in Comparison to Those of Cottonseed and Soybean Oils

Fatty acid	RRT[a]	Relative percent of fatty acid					
		Cottonseed[b]	Soybean[b]	Orange	Mandarin	Lime	Grapefruit
8:0	0.05	1.24	—	—	2.19	—	—
10:0	0.09	1.10	—	5.42	3.23	0.85	8.43
Unknown	0.19	—	—	1.52	3.72	—	—
12:0	0.24	0.73	—	—	3.09	0.99	—
Unknown	0.28	—	—	—	2.44	—	—
Unknown	0.32	—	—	0.43	5.15	—	—
Unknown	0.36	0.93	—	—	3.14	—	—
Unknown	0.43	—	—	—	2.22	—	—
14:0	0.50	1.33	—	—	3.46	—	—
Unknown	0.61	—	—	—	5.98	—	—
15:0	0.69	—	—	—	2.41	—	—
15:1	0.79	0.53	—	—	3.18	—	—
16:0	1.00	18.98	13.52	28.81	18.08	19.08	42.64
16:1	1.12	0.88	—	0.65	2.09	0.28	—
18:0	1.95	7.08	9.81	2.60	7.79	0.84	0.83
18:1	2.14	26.03	26.65	23.72	16.76	17.07	12.18
18:2	2.62	41.17	46.01	30.98	13.53	18.62	34.49
18:3	3.33	—	3.00	6.50	—	42.27	1.41

[a]RRT, retention time of fraction relative to palmitic acid (8 min).
[b]Cottonseed and soybean oils were GLC analyzed under conditions similar to those used for citrus oils.

detector. The separation of fatty acid methyl esters was conducted in a coiled glass column (1.5 × 4 m) packed with diatomite C (100-120 mesh) and coated with 10% PEGA. The column was operated isothermally at 190 C with N_2 at 300 ml/min; detector and injector temperatures were 220 C and 200 C, respectively.

Identification and determination of GLC fractions. Peak identification was performed by comparing the relative retention time of each fraction with those of standard materials. The relative retention time (RRT) of β-sitosterol and palmitic acid was given a value of 1.00. The peak area was measured by triangulation; the relative proportion of the individual compound was, therefore, obtained by determining the partial areas in relation to total areas.

RESULTS AND DISCUSSION

Data recorded in Table 1 indicate that Egyptian orange seeds contain 45.5% oil, which is within the average amounts reported by Diedrichs (10), Loba et al. (11), Mehta et al. (1), Zaganiaries (12) and Abdo (5). Seeds of Egyptian mandarin, lime and grapefruit contain similar percentages of oil to orange seeds. It is noted that the oil content is relatively higher in comparison with other oil seeds traditionally used as sources of edible oils. Therefore, citrus seeds which are considered as waste materials, may be used as good and new additive sources of oil. These seeds also contain considerable amounts of protein, i.e., from 13.7% in lime seed to 17.4% in orange seeds. Citrus seeds also have relatively high mineral contents.

Physical and chemical properties of citrus seed oils. The oils isolated from citrus seeds were pale yellow in color. They had a slight odor and a characteristic taste. Table 2 indicates that physical and chemical properties of orange, mandarin, lime and grapefruit seed oils are quite similar to those of cottonseed oil. However, the acid value of lime seed oil is quite high in comparison with the other citrus seed oils and cottonseed oil.

GLC analysis of unsaponifiables of citrus seed oils: Effect of extraction solvents on composition. Three solvent systems were used in extracting oil samples and their unsaponifiable matter contents. They were: System I, chloroform-methanol (2:1, v/v) for oil extraction and diethyl ether for unsaponifiable extraction; System II, petroleum ether for oil extraction and diethyl ether for unsaponifiable extraction; System III, n-hexane for oil extraction and petroleum ether for unsaponifiable extraction.

The isolated unsaponifiables were analyzed by GLC, and the results are presented in Tables 3 and 4.

It is shown in Table 3 that the composition, in terms of number, type and concentration of fractions isolated from orange oil unsaponifiables, varied markedly with the three solvent systems. Unsaponifiables isolated with System I contained 12 fractions with higher relative content of sterols (17.9%); System II extracted the lowest number of fractions, 10, with the lowest total sterol content (0.45%). System III extracted 12 fractions with the highest total sterol content (0.66%). The effect of solvent systems also was obvious in the presence and concentration of cholesterol. Because the presence of cholesterol, which showed by GLC analysis, has not been confirmed by other means such as NMR and MS, it could be something else.

TABLE 6

Characteristic Fatty Acids Groups in Oil Extracted from Seeds of Orange, Mandarin, Lime and Grapefruit in Comparison with Those of Cottonseed and Soybean Oils

FA groups[a]	Relative percent of FA					
	Cotton-seed	Soybean	Orange	Man-darin	Lime	Grape-fruit
Total No. of FA	11	5	9	17	8	6
TVFA	2.34	—	5.42	5.41	0.85	8.43
TUFA	68.61	75.66	61.42	32.38	78.30	48.08
TSFA	30.46	24.34	36.83	46.79	21.70	51.92
TEFA	41.17	49.01	37.48	13.53	60.89	35.90
Major fatty acids						
Palmitic	18.98	13.52	28.81	18.08	19.08	42.64
Oleic	26.03	26.65	23.72	16.76	13.07	12.18
Linoleic	41.17	46.01	30.98	13.53	18.62	34.49
Linolenic	—	—	—	—	42.22	—

[a]FA, fatty acid, TVFA, total volatile fatty acids ($<C_{10}$); TUFA, total unsaturated fatty acids; TSFA, total saturated fatty acid; TEFA, total essential fatty acid ($C_{18:2} + C_{18:3}$).

The compositional pattern of unsaponifiable matter of mandarin oil also was influenced by the different solvents used. Whereas System III-extracted unsaponifiables contained 1.02% total sterols, the unsaponifiables extracted with system II contained 14 fractions with 2.16% total sterols. The unsaponifiables extracted with System I contained nine fractions and 3.8% total sterols. The unsaponifiables extracted with the three different solvent systems were completely free of cholesterol.

The data obtained indicate that unsaponifiables isolated from line oil also varied in their compositions according to the solvent system used for extraction. Unsaponifiables characterized with the lowest number of fractions, 13, and highest sterol contents (1.99%) with zero cholesterolwas obtained by using System I. Solvent Systems II and III extracted cholesterol and 0.30% and 0.35% concentrations, respectively.

Quite similar observations were made in unsaponifiables extracted from grapefruit seed oil, but the importance was that cholesterol could not be detected with the three solvent systems used in this study.

Comparative studies on citrus seed unsaponifiables. From the previous data it can be concluded that the preferable solvent system is System III. The compositional data for all oils with System III are listed in Table 4. These data indicate that the compositional pattern of unsaponifiable matters extracted from citrus seed oils is varied in number, type and relative concentration of fractions. Cholesterol content in cottonseed unsaponifiables was higher than that found in all citrus seed unsaponifiables. The unsaponifiables of mandarin seed oil and grapefruit seed oil are completely free of cholesterol. The major components of unsaponifiables varied according to citrus variety; while the hydrocarbons C_{20} and C_{24} represent the major fraction in orange unsaponifiables, squalene represents the major fraction in mandarin unsaponifiables. C_{22}, C_{26} and squalene represent the major components of lime unsaponifiables. Grapefruit unsaponifiables contain C_{23} and squalene as major fractions, while cottonseed unsaponifiables contain C_{24},C_{26} and squalene as major components.

Fatty acid composition of citrus seed oils. Tables 5 and 6 show the fatty compositional patterns found in oils extracted from the seeds of orange, mandarin, lime and grapefruit in comparison with those of cottonseed and soybean oils.

It is clear from these data that the fatty acid composition varies significantly with the source of oil. Such differences can be grouped as follows:

- The total number of fatty acids was 9, 17, 8 and 6 in orange, mandarin, lime and grapefruit oils, respectively, in comparison with 11 in cottonseed oil and 5 in soybean oil.
- The relative concentration of total volatile fatty acids (TVFA) was 5.42%, 5.41%, 0.85% and 8.43% in oils of orange, mandarin, lime and grapefruit, respectively, in comparison with 2.34% in cottonseed oil and zero in soybean oil.
- The relative amount of total unsaturated fatty acids (TUFA) was 61.42%, 32.38%, 78.30% and 48.08% in oils of orange, mandarin, lime and grapefruit. The concentration of TUFA in oils of cottonseed and soybean was 68.1% and 75.66%, respectively.
- Total essential fatty acids (TEFA) content was 37.48%, 13.53%, 60.89% and 35.90% in oils of orange, mandarin, lime and grapefruit, respectively; it was 41.17% and 49.01% in cottonseed and soybean oils, respectively.
- Palmitic, oleic and linoleic acids represent the major fatty acids in all oils; lime oil contains, in addition, a high content of linolenic acid (42.22%).

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Number of Double Bonds in Fatty Acids from Fats and Oils by HPLC Using Pentafluorobenzyl Esters

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A simple, one-pot procedure for the saponification of fats and oils and the subsequent esterification of the resulting fatty acid salts with pentafluorobenzyl bromide is presented. A normal phase high pressure liquid chromatographic procedure for the separation of these pentafluorobenzyl esters, primarily on the degree of unsaturation, is also given. Chromatographic detection of the esters can be carried out conveniently at 254 nm, and the procedure should therefore find application in the routine determination of the number of double bonds in fatty acids from fats and oils.

In a recent publication (1), we examined the chromatographic properties of fatty acid pentafluorobenzyl (PFB) esters in both normal and reversed phase systems. We found a normal phase system that essentially separates the fatty acid PFB esters on the number of double bonds in the fatty acid moiety. Although there is a small separation based on the number of carbon atoms, we felt this technique offered considerable promise for rapidly monitoring the degree of unsaturation of various commercial fats and oils. This note, then, presents a simple method for achieving this aim and gives the results of its application to some domestically used fats and oils.

EXPERIMENTAL

The following fats and oils were obtained from local retail outlets: Tandaco suet mix, 40% rendered beef suet, 60% aerated flour, Cerebos (Aust.) Ltd., Seven Hills, New South Wales Australia; Allowrie Lard, PDS Rural Products Ltd., Sydney, New South Wales, Australia; No Frills Butter, Franklins P/L, Chullora, New South Wales, Australia; San Giorgio olive oil, San Giorgio Sez. Agric SpA, Pomezia, Italy; No Frills Sunflower Oil, Polyunsaturated, Franklins P/L, Chullora, New South Wales, Australia; Meadow Lea polyunsaturated margarine (P/S>2:1), Vegetable Oils P/L, Mascot, New South Wales, Australia, and Glendale raw linseed oil, Glendale Chemical Products P/L, Alexandria, New South Wales, Australia. Approximately 20 mg (54 mg of suet, to compensate for flour content) of each fat or oil was added to a small screwcap tube followed by 1 ml of 0.2 M KOH in methanol. The tubes were then incubated at 90 C for 25 min. As Kihara et al. (2) have pointed out, derivatization procedures that utilize fatty acid salts can be performed in the same reaction vessel as the saponification. Accordingly, the methanol was evaporated from each tube under a stream of N_2. In the case of suet only, the white solid material, which was presumably the flour, was removed by filtration using a Gelman Sciences Inc. (Ann Arbor, Michigan, USA) Acro LC13 0.45 micron disposable filter. The addition of 1 ml of 0.1 M tetrabutylammonium hydrogen sulphate (Fluka, Buchs, Switzerland), 1 ml dichloromethane and 20 µl (ca. 2-fold XS) pentafluorobenzyl bromide (Fluka) followed. The mixtures were then incubated at room temperature with vigorous shaking for 40 min. These conditions essentially reproduce those given by Ehrsson (3), and therefore all

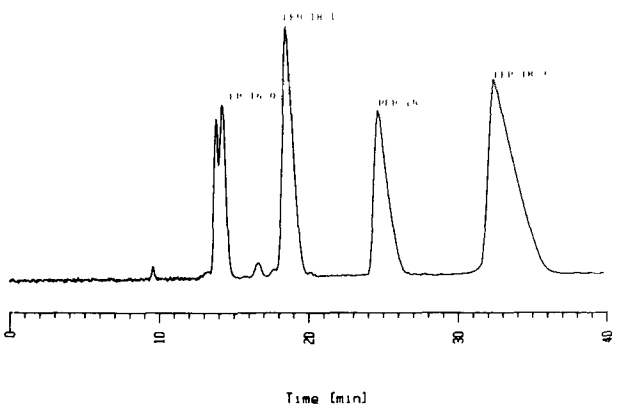

FIG. 1. PFB esters from linseed oil. Contains PFB 16:0, PFB 18:0, PFB 18:1 (0.069 AU), PFB 18:2, PFB 18:3. Chromatograms (Figs. 1 and 3-8) and log retention data (Fig. 2) for the PFB esters derived from various fats and oils. In all cases the column was 5 µm silica eluted with 10% dry dichloromethane, 90% half water saturated hexane. The chromatograms were monitored at 263 nm and are normalized to the largest peak (absolute absorbance given for each chromatogram).