extremely important that the variable wavelength UV be used since the RI detector, while a universal detector, is only ca. 10% as sensitive as the UV. Finally, it would be possible to analyze for PE and other phospholipids, although they do elute extremely close to the solvent front using this mobile phase.

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The Properties of *Cucurbita foetidissima* Seed Oil

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ABSTRACT

Oils from the seeds of 15 different selections of the buffalo gourd, *Cucurbita foetidissima*, were characterized in terms of their physical and chemical properties, which indicate that this oil is similar to other common edible oils. Xanthophylls were the predominant carotenoid pigments present in the crude oil, ranging from 51-232 mg/kg oil. Linoleic acid, the predominant fatty acid, ranged from 39-77% with an average level of 61%. Although conjugated unsaturated acids are a significant component in some other xerophytic cucurbit oils, the levels of conjugated dienoic and trienoic fatty acids in this species are only 2.3 and 0.03%, respectively.

INTRODUCTION

The buffalo gourd, *Cucurbita foetidissima* HBK, a feral xerophytic gourd, has been the subject of a number of recent studies (1-5) because of its potential as a source of oil, protein and starch. The domestication of this plant as a crop adapted to arid land agriculture is currently under study (1). Previous investigation of the crude oil from the seed suggested that it could be processed to yield an edible oil (6). Its highly unsaturated nature should make it very attractive for food purposes.

The purpose of this work was to determine the physical and chemical properties of the crude oil from the seeds of this xerophytic plant.

EXPERIMENTAL PROCEDURES

Open-pollinated seed lots from 15 genetically different selections of *C. foetidissima* grown at the University of Arizona Experiment Station were studied. The seeds were obtained by submerging the fruits in water until fermentation disintegrated the placental tissue. They were then washed, air-dried and kept at room environmental conditions. Seed aliquots were taken as needed for different analyses, thus assuring that the determinations were performed on freshly extracted oil.

Seed and Oil Analyses

Seed aliquots were ground in a laboratory Wiley mill to 10-mesh size through a nickel-plated delivery tube. Moisture content was obtained by drying in a vacuum oven for 16 hr at 60 C. Crude oil content was determined with 5 g samples by Soxhlet extraction with hexane; crude protein content of defatted meal was measured by the micro-Kjeldahl method using a conversion factor of 6.25.

Oil for physical and chemical analyses was extracted as already described. The hexane solution was filtered and the oil recovered in a rotary evaporator. Residual hexane was removed by heating the sample to 60 C while flushing the oil with a stream of N_2. Characterization of the oil samples was made using AOCS methods (7).

Total Carotenoids: Visible Spectrophotometry

Carotenoids were determined using matched silica cells of 1-cm path length read in a Perkin-Elmer 202 spectrophotometer. Oil samples were dissolved in cyclohexane (2.5% w/v) and the spectra recorded in the range 350-550 mμ. For

TABLE I

Composition of *Cucurbita foetidissima* Seed

Properties	Range	Mean ± SD
Moisture (%)	4.1 - 8.4	6.2 ± 0.2
Oil (%)	31.8 - 39.4	36.0 ± 2.0
Protein content[a], defatted meal (%)	49.0 - 66.0	54.6 ± 4.6

[a]Crude protein = % N X 6.25.

[1] Arizona Agricultural Experiment Station No. 3081.

TABLE II

Properties of *Cucurbita foetidissima* Oil

Properties	Range	Mean ± SD
Refractive index		
at 25 C	1.4692 - 1.4747	1.472 ± 0.001
at 40 C	1.4652 - 1.4686	1.467 ± 0.001
at 60 C	1.4524 - 1.4603	1.456 ± 0.002
Free fatty acid (%)	0.3 - 0.8	0.5 ± 0.2
Acid value (%)	0.5 - 1.7	1.1 ± 0.4
Peroxide value (mg/kg oil)	8.5 - 44.9	16.9 ± 10.9
Saponification value	190.1 - 194.8	191.5 ± 1.6
Iodine value	123.0 - 138.0	129.9 ± 5.3
Acetyl value	-	7.3 (pooled)
Hydroxyl value	-	7.3 (pooled)
Specific gravity (25°/25 C)	-	0.9172 (pooled)
Carotenoids (mg/kg oil)	51.3 - 231.5	109.7 ± 55.0
Phosphatides (%)	0.3 - 1.2	0.8 ± 0.3
Minerals (ppm)		
P	95.0 - 390.	260.0 ± 100.0
Fe	0.38 - 1.11	0.68 ± 0.22
Cu	0.16 - 0.51	0.31 ± 0.12
Mn	0.09 - 0.37	0.24 ± 0.08

quantitative determination the absorbance was read at 417 mμ and the Zscheile et al. equation was used:

$$\text{mg carotene/kg oil} = \frac{\text{(absorbance at 417 m}\mu\text{) (sample volume, ml)}}{0.204 \times \text{(sample weight, g)}}$$

Conjugated Fatty Acids: Ultraviolet Spectrophotometry

To determine conjugated dienoic acid content, cyclohexane solutions (0.05% w/v) were examined in the region 200-260 mμ; similar solutions were used to detect conjugated trienoic and tetraenoic acids in the region 210-230 mμ.

The percentages of dienoic, trienoic and tetraenoic conjugated fatty acids were calculated according to AOAC Methods of Analysis (9).

Trans Fatty Acids: Infrared Spectrophotometry

Absorption spectra were obtained with a Perkin-Elmer 337 Grating Infrared spectrophotometer using NaCl cells with a film thickness of 0.5 mm. Samples were prepared as 2% solutions (w/v) in carbon disulfide. Concentration of the *trans* fatty acids was expressed as the percentage of trielaidin.

RESULTS AND DISCUSSION

The seeds upon extraction with hexane yielded ca. 36% oil, whereas the protein content of the remaining defatted meal was ca. 55%. The recovered oil had a bland taste and odor and the color varied from a dark reddish-brown to a light greenish-yellow in the seed lots examined.

Physical and chemical properties of the oil are summarized in Tables I and II. Certain properties, along with visible spectral characteristics, are compared with those of some common edible oils in Table III and Figure 1, respectively. These data show that *C. foetidissima* oil closely resembles other common oils which are processed for food use.

The level of carotenoids found in *C. foetidissima* oil was similar to that in cottonseed oil, ranging from 51.3-231.5 mg/kg, with a mean of 110 (Table II). Xanthophylls apparently are the predominant pigments present, as shown by absorption in the 400-420 mμ region (Fig. 1). In contrast, α- and β-carotenes predominate in red palm and soybean oils (10), and the spectrum of the β-carotenes is much less intense in the region indicated.

The mean and range for each fatty acid constituent measured in the 15 selections examined are shown in Table IV. Linoleic acid averaged 61% of the total fatty acids.

TABLE III

Characteristics of Various Crude Vegetable Oils[a]

	Soybean	Cottonseed	Corn	Safflower	*Cucurbita foetidissima*
Oil in seed (%)	21.0	22.9	4.5	30.0	36.0
Free fatty acid (%)	0.5	0.7	1.5	0.4	0.5
Acid value (%)	1.0	1.4	3.0	0.8	1.1
Iodine value	126.0	105.0	128.0	145.0	129.9
Sapon. value	193.0	195.0	190.6	191.0	191.5
Phosphatides (%)	2	1	2		0.8
Carotenoids (mg/kg oil)	40.0	167.0			109.7
Conjugated fatty acids (%)					
Dienoic	0.3	0.8			2.3
Trienoic	0.1	0.3			3.3 N 10^{-2}
Tetraenoic					0.0
Refractive index (25 C)	1.4730	1.4700	1.4720	1.4750	1.4720
Specific gravity (25°/25 C)	0.919	0.917	0.9175	0.921	0.9172

[a]Data for soybean, cottonseed, corn and safflower oils from Ref. 14.

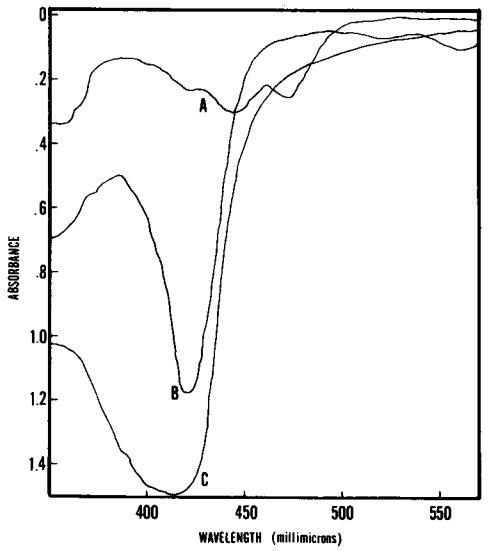

FIG. 1. Visible spectra of the crude oils from soybean, cottonseed and *C. foetidissima* in cyclohexane: A—soybean oil (2.9% w/v); B—*C. foetidissima* oil (2.507% w/v); C—cottonseed oil (2.528% w/v).

TABLE IV

Fatty Acid Content of *Cucurbita foetidissima* Oil

Fatty acids	Range	Mean ± SD
Major fatty acids (%)		
Palmitic	6.6 - 24.4	11.8 ± 4.8
Stearic	1.2 - 10.2	3.5 ± 2.2
Oleic	10.0 - 31.6	2.19 ± 6.2
Linoleic	39.3 - 77.2	60.6 ± 11.0
Conjugated fatty acids (%)		
Dienoics	1.9 - 2.8	2.3 ± 0.3
Trienoics	0.0 - 0.10	0.033 ± 0.049
Tetraenoics	0	0

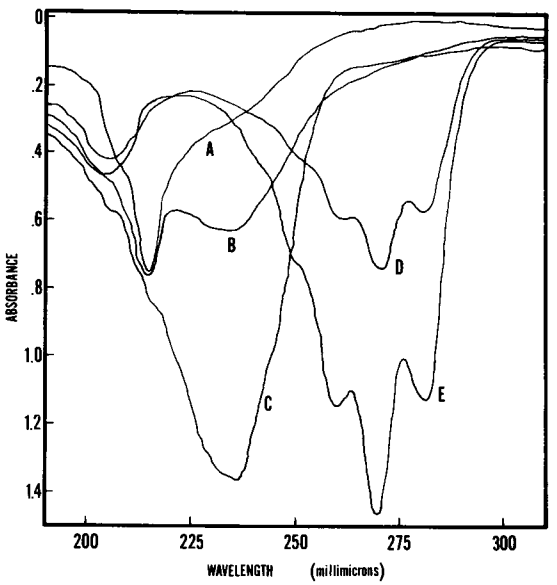

FIG. 2 Ultraviolet spectra of the crude oils from soybean, cottonseed, *C. foetidissima*, *C. digitata* and *A. undulata* in cyclohexane: A—soybean oil (0.051% w/v); B—cottonseed oil (0.054% w/v); C—*C. foetidissima* oil (0.051% w/v); D—*C. digitata* oil (0.0026% w/v); E—*A. undulata* oil (0.0025% w/v).

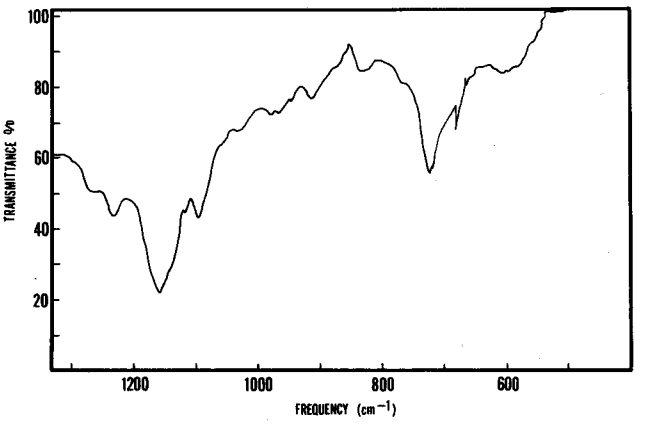

FIG. 3. Infrared spectrum of crude *C. foetidissima* oil in carbon disulfide (2.01% w/v).

Oleic, palmitic and stearic acids were present at levels of 22, 12 and 3.5%, respectively. The wide range of variation found for these fatty acids suggests the possibility of modification through plant breeding.

A comparison of the UV spectra of *C. foetidissima*, cottonseed and soybean oils for their content of conjugated dienoic fatty acids is shown in Figure 2. The level found in *C. foetidissima* oil was 2.3%, ca. 3 times that (0.8%) found in the sample of cottonseed oil and ca. 8 times the level (0.3%) found in soybean oil. This level, however, is similar to amounts found in some crude edible oils.

A comparison with oils of other xerophytic cucurbits for the quantity of conjugated trienoic fatty acids is also presented in Figure 2. The average level of these acids was found to be 0.03% in *C. foetidissima* whereas the levels in *Cucurbita digitata* and *Apodanthera undulata* were 8.6 and 17% of the total fatty acid content. Punicic acid, 9,11, 13-c,t,c-octadecatrienoic acid, has been reported in these 2 species at much higher levels (17 and 30%, respectively), suggesting wide variation of this fatty acid in *Cucurbita* species (11,12).

The levels of conjugated fatty acids found in *C. foetidissima* oil have no important effect on its possible use as an edible oil. They are not only low but equivalent to levels found in other oils of edible quality. Smith et al. (13), for example, report levels of total conjugated fatty acids ranging from 0.31 to 1.39% for 5 different samples of margarines and from 0.62 to 0.74% for 5 different samples of butter.

The infrared spectrum of *C. foetidissima* oil (Fig. 3) resembles closely those of cottonseed, corn and soybean oils. The absence of bands at 933 and 984 cm^{-1}, which are characteristic of *cis, trans, cis* conjugated linkages, reflects the negligible level of punicic acid (0.03%) already reported.

The oil properties which have been discussed indicate that this plant could become an important oilseed crop for arid land agriculture. Additional technical and agronomic studies are being made to determine the potential for domestication of this species.

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Detection of Reesterified Oils: Determination of Fatty Acids at Position-2 in the Glycerides of Oils

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ABSTRACT

The IUPAC method II.D.27 for detection of reesterified oils by analysis of fatty acids at position-2 in fat and oil glyceride is reinvestigated and the effect of storage and different refining processes on the glyceride structure of genuine olive oil and olive-residue oil is studied. The method is unsuitable for oils neutralized by steam distillation because of changes in the triglyceride structure. An improved procedure is proposed in order to minimize erroneous conclusions for several other analyses.

INTRODUCTION

Reesterified oils are produced by esterification of glycerol and distilled fatty acids or by direct reesterification of oils with high percentages of free fatty acids. For the production of reesterified oils, olive oil or olive-residue oil often is used. The products thus obtained have an identical fatty acid composition to olive oil (fatty acid composition of olive oil and olive-residue oil is practically identical). Since reesterified oils are inexpensive, adulteration of the expensive olive oil or even the less expensive olive-residue oil with these oils is attractive.

Olive oil or olive-residue oil and reesterified oils can be characterized by their triglyceride structures. Saturated fatty acids, i.e., palmitic and stearic acids, are esterified almost exclusively at the combined 1,3-positions of olive oil and olive-residue oil triglycerides, as shown with many other triglycerides of vegetable origin (1,2). However, in reesterified oils, fatty acids have a statistical distribution on the 3-positions of glycerol. Determination of palmitic acid at position-2 of the glycerides of olive oil and olive-residue oil has been used for detecting adulteration by reesterified oils in these products. Published procedures include selective enzymatic hydrolysis by mammalian pancreatic lipase, separation of hydrolytic products by thin layer chromatography (TLC), conversion of isolated 2-monoglycerides to methyl esters, and their fatty acid analyses by gas liquid chromatography (GLC) (3,4).

In this study, a reinvestigation of IUPAC method II.D.27 (5) for the determination of fatty acids at position-2 in the glycerides of oils and fats is undertaken. The method has been provisionally agreed upon by the Codex Alimentarius Committee on Fats and Oils to be forwarded for assessment to the Codex Committee on Methods of Analysis and Sampling (6), and has been adopted by the EEC (7). Furthermore, the effect of storage and different refining processes on the glyceride structure of genuine olive oil and olive-residue oil and, consequently, on the results obtained by this method has been studied and an improved procedure is proposed in order to minimize erroneous conclusions for several analyses.

EXPERIMENTAL PROCEDURES

Samples

Samples of genuine virgin olive oil and olive-residue oil were collected from the main oil producing areas of Greece. Industrially processed samples of these oils were supplied by Elais Co., Athens, Greece, and by Avea Co., Crete, Greece.

MATERIALS AND METHODS

The determination of fatty acids at position-2 of glycerides was performed according to IUPAC method II.D.27 (5), except where indicated. Fatty acid methyl esters were prepared according to AOCS method (8) (BF_3 + methanol). Pancreatic lipase was from Sigma Chemical Co., USA. The chromatographic plates were from Macherey-Nagel & Co. (SIL G 50,UV 254). Agitation during hydrolysis was performed either with a magnetic stirrer or with a dental amalgamator. GLC: Varian 2800 or Tracor 550 with FID detectors. Columns: DEGS 15% on Chromosorb AW, 100-120 mesh, and EGGS 10% on Gas Chrom Q, 100-120 mesh, both 1.80 × 3 mm id of stainless steel.

Passage over kieselgel: A chloroform solution of oil (4 g in 66 ml) was percolated through a column (33 × 1.8 cm) with kieselgel 60, 70-230 mesh, (Merck, 30 g) at a rate of ca. 60 drops per min. The triglycerides were eluted with benzene (25 ml) at the same rate. The solvent was removed in a rotatory evaporator under nitrogen.

RESULTS AND DISCUSSION

The percentage of palmitic acid at position-2 in the glycerides of 62 samples of genuine virgin olive oil from the main