THE PARTIAL CHARACTERIZATION OF *CUCURBITA FOETIDISSIMA*

(THE BUFFALO GOURD)

by

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INTRODUCTION

Protein deficiency and malnutrition are recognized to occur in many areas throughout the world. The dimensions of these problems are immense and threaten to grow larger, when considering increasing populations and shortages of nutritious foodstuffs. Efforts to develop suitable vegetable protein sources have been investigated as a means of supplementing current dietary protein supplies (1).

The Buffalo gourd, Cucurbita foetidissima, is a wild gourd that is found growing on waste dry lands of Nebraska, Missouri, Kansas, Colorado, Utah, Neveda, Texas, New Mexico, California; and in Mexico as far south as Granajuats (2). Buffalo gourd seeds have been described as protein and oil-rich seeds.

This study was initiated to identify the components of gourd seed lipids by thin-layer chromatography (TLC) and gas-liquid chromatography (GLC). Characterization of seed structure was examined under scanning electron microscope (SEM). The amino acid composition was determined on whole seed, decorticated seed, defatted decorticated seed and seed coat.

REVIEW OF LITERATURE

Growth Habit and Yields of Cucurbita foetidissima

The Buffalo gourd grows in both arid and temperate regions of the earth and requires long periods of warm, dry weather for optimal growth (3). The gourd plant is frost-sensitive, intolerant of wet conditions but drought tolerant. Due to itself adaptibility however it can be found growing in a wide range of climatic conditions.

Yields of Buffalo gourd seeds, growing wild in desert areas, have been estimated from 321 to 899 Kg per acre at the Chillicothe Agricultural Experiment Station, Chillicothe, Texas (4). These yields are comparable to the yield of commercially grown oilseeds.

Gross Composition of Cucurbita foetidissima Seed

Curtis (5) studied C. foetidissima as a source of vegetable fats and protein. He reported that whole seed contained 34.2% protein (N x 6.25), 33.9% crude fat, 17.4% fiber and 4.8% ash.

Shahani et al. (4) analyzed a sample harvested from wild plants growing in Chillicothe, Texas. They gave the seed contents as : Protein 31.7%, crude fat 24.3%, fiber 26.5%, ash 4.8% and moisture 9.6%. They found that both the seed and extracted meal were high in protein, but were also high in ash and fiber and very low in total sugar.

Lipids in Cucurbita foetidissima Seed

Shahani et al. (4) studied the physical and chemical properties of crude oil of C. foetidissima. He reported the oil had the properties listed below:

Iodine value, Wijs	136.1
Refractive index (at 25° C)	1.4735
Saponification value	191.8
Unsaponifiable matter %	1.53
Reichert-Meissl value	0
Polenske value	0.32
Hydroxyl number	9.77
Titer °C	17.1

They found the crude oil very dark in color and exceedingly resistant to bleaching. Refining, bleaching, and deodorization gave a bland oil with good stability and no tendency to revert in flavor.

The fatty acid composition of the C. foetidissima crude oil was also reported (4), and their values were:

Myristic acid	0.17
Palmitic acid	6.13
Stearic acid	2.22
Oleic acid	23.00
Linoleic acid	65.30

Bemis et al. (6) reported the fatty acid composition of cucurbita seeds as palmitic acid 11%, stearic acid 1%, oleic acid 50% and linoleic acid 38%. Weber (7) reported Buffalo gourd oil had a linoleic acid content of 61 percent and he suggested this fatty acid composition indicated it could be a valuable source of edible oil.

Others (2) evaluated the oils of the seed of C. foetidissima, C. digita, and C. palmata for their possible use in paints and varnish. They concluded these three species of wild perennial gourds had potential value. C. digita and C. palmata oils contained conjugated triene glycerides

and similiar to linseed oil, could be used in protective coatings. C. foetidissima lacked the conjugated triene compounds and was a softer drying oil similar to soybean oil.

Protein of Cucurbita foetidissima seed

The essential amino acid content of C. foetidissima was analyzed by Lyman et al. (8), and results were given as: 3.3% lysine, 1.9% histidine, 14.2% arginine, 2.6% threonine, 4.8% valine, 1.9% methionine, 4.4% isoleucine, 6.6% leucine and 4.6% phenylalanine. Weber (9) by microbiological assay of C. foetidissima found it was low in lysine, threonine, phenylalanine and methionine.

In 1973 Hensarling et al. (10) reported that arginine, aspartic acid, and glutamic acid were the most abundant amino acids in C. foetidissima seed while lysine, cysteine, and tryptophane were low and methionine and isoleucine were extremely low. He summarized the amino acid content of C. foetidissima globulin as follows:

Lysine	3.3	Tryptophan	0.6
Methionine	2.5	Arginine	16.8
Cysteine	0.7	Glycine	4.8
Isoleucine	4.1	Histidine	2.5
Leucine	7.8	Alanine	5.0
Phenylalanine	5.9	Aspartic acid	10.9
Tyrosine	3.9	Glutamic acid	21.5
Threonine	3.5	Proline	4.5
Valine	5.0	Serine	5.9

Nutritive Value of the Cucurbita foetidissima Seed

Nutritive value of C. foetidissima was studied by Weber et al. (9) who reported a protein digestion coefficient of 72.39 percent and a protein efficiency ratio (PER) of 3.443. This was compared to whole egg (which had a PER value of 6.901 and protein digestion 82.23 percent) and was of lower protein quality. Autoclaved seeds increased the PER value to 4.695.

Hensarling (10) also studied the nutritive value of storage protein purified from C. foetidissima. The PER value for C. foetidissima globulin was 1.66 while casein gave a PER of 2.50. The nitrogen digestibility of C. foetidissima was as good as that of casein. He concluded that C. foetidissima protein quality and nutritive value was similiar the other oilseeds protein.

MATERIAL AND METHODS

Seeds used in these studies were obtained from Lebanon through the assistance of Dr. L. C. Curtis. Seeds for analysis were ground and stored refrigiration to prevent enzymatic lipolysis and other deleterious effects which might occur at room temperature.

Analysis of Cucurbita foetidissima Whole Seed

The analysis for proximate components were according to AOAC methods (12). Moisture was determined using AOAC method 22.003; protein using method 38.012-38.014; crude fiber using method 22.038; ash using method 22.010; and crude fat using method 22.033.

Lipid Extraction—Differences in Extraction with Various Solvents

Lipids of the seed were extracted by the methods as follows:

1. Petroleum ether and ethyl alcohol extraction

   Samples were extracted by the method of AOAC 22.033(11).

2. Acid hydrolysis method

   The AOAC acid hydrolysis method 22.035 was used to extract the lipids from the sample.

3. Water-saturated n-butanol extraction

   Samples of 5 g were extracted in a Stein Mill (The Fred Stein Laboratories, Atchison, Kansas) with 100, 100, and 50 ml of water-saturated n-butanol for 6, 6, and 2 min respectively as described by Pomeranz et al. (12) with slight modification. Each extract was filtered and the combined extracts were evaporated to dryness using a rotary evaporator under reduced pressure at 40-45^{o} C. The evaporated residues were kept in a vacuum desicator over P_2O_5 for 40 hours at 4^{o} C to remove residual moisture. The

lipids were reextracted three times with petroleum ether and the combined extracts were evaporated to remove solvent residues. The crude lipids were dissolved in chloroform and centrifuged at 39,080 x g at a temperature of 0° C for 10 min to remove insolubles. The lipids obtained were made up to volume and quantitatively transferred into previously weighed vials and dried overnight in a vacuum oven at 50° C. The remaining lipid solution was concentrated and stored at - 15° C for further analysis.

4. Chloroform-methanol extraction

Samples of 0.5 g were extracted four times with 20 ml of chloroform -methanol (2:1 v/v) in a Waring Blender for 3 min each time. The extracts were filtered, combined and washed three times with distilled water to remove any protein (13). The chloroform-methanol solutions were placed in an air oven at 52° C to remove the solvent. Residues were dried in a vacuum oven at 50° C to constant weight.

Thin-Layer Chromatography

Glass plates (20 x 20 x 0.38 cm) were coated with a 250 u layer of silica gel G (E. Merk, A. G., Darmstadt, Germany) with a commercial spreader (Quickfit Instrumentation, England). The plates were activated for 3 hours at 130° C, and stored in a desiccator.

The chromatogrphic solvents used for one dimensional ascending development were a mixture of petroleum ether-diethyl ether-acetic acid (80:20:1) for nonpolar components and a mixture of chloroform-methanol -water (65:25:4) for polar components.

The chromatographic chamber was lined with filter paper to insure saturation of the chamber space with solvent vapor. The solvent vapor was

saturated at least 12 hours as described by Mangold (14). The length of run was 15 cm on the plate and the plates were air dried for 30 minutes before spraying with various detecting reagents.

Spots were initially identified on analytical TLC plates by comparing the Rf values with literature data after using suggested sprays. Tentative identification was confirmed by comparing the Rf values with those of pure standard compounds.

The spots were developed by heating the plates for 25 min at 180° C after spraying them with a solution of $K_2Cr_2O_7$ in 55% by volume of aqueous sulfuric acid.

The specific spray reagents used to detect the polar lipids were as follows:

Ninhydrin reagent (15) -- 0.2% solution of ninhydrin in 99% n-butanol and 1% pyridine was sprayed on the plates. The sprayed plate was heated for a few minutes at 100° C to develope purplish pink spots in the presence of lipids containing free amino group.

Molybdenum blue reagent (16) -- a diluted solution of MoO_3 and powdered Mo in conc. H_2SO_4 was sprayed on the plates to show immediately blue spots on a white or light blue-gray background in the presence of phosphorous containing lipids.

Modified Drangendorff reagent (14) -- spraying with a mixed solution of basic bismuth nitrate ($BiONO_3$) in 20% aqueous acetic acid and aqueous KI solution on the plate results in orange spots on a white or yellow background in the presence of choline-containing phospholipids.

Alpha-naphthol reagent (17) -- spraying with 0.2% alpha-naphthol in ethanol, specific for glycolipids detection, was followed by light

spraying with 95% H_2SO_4 and by heating at 120° C to give dark gray-purple spots.

The nonpolar lipids, 1,3-dipalmitin, 1,2-dipalmitin, tripalmitin, cholesteryl palmitate were used as reference. For polar lipids, DL-α lecithin (synthetic), phosphatidyl ethanolamine, phosphatidyl inositol, and lysolecithin were used as reference.

The plates were photographed after charring with chromic-sulfuric acid.

Preparation of Fatty Acid Esters for Gas-Liquid Chromatographic Analysis

The transesterification procedure for preparation of fatty acid methyl esters of Metcalfe and Schmity (18) was used.

Approximately 150 mg of fatty material was added to a 50 ml volumetric flask; 4 ml of 0.5 N methanolic sodium hydroxide was added to the mixture which was heated on a steam bath until fat globules went into solution. Then 5 ml of BF_3-methanol (10% of BF_3 in methanol) was added to the flask and the mixture was boiled for 2 min. Enough of a saturated sodium chloride solution was added to the flask to float the methyl esters, then the entire mixture was transferred to a separatory funnel. Twenty ml of redistilled petroleum ether (B. P. 30-60° C) was added to extract the methyl esters. The petroleum ether layer was filtered into a small beaker and evaporated on a 60° C water bath. The fatty acid composition was determined by gas-liquid chromatogrphy.

A standard solution of fatty acids was used for qualitative and quantitative analysis. This standard contained lauric, myristic, palmitic, stearic, and arachidic fatty acids.

Gas-Liquid Chromatography

Fatty acid methyl esters were analyzed using a Barber-Coleman 5000 gas chromatograph equipped with a dual flame ionization detector, and a 6 ft. x 1/8 in. o. d. glass U-tube packed with 7.5% diethylene glycol succinate (DEGS) on Chromosorb G (80-100 mesh). Nitrogen was the carrier gas at a flow rate of 70 ml/min; oven temperature, 190° C; injection temperature, 252° C; detector temperature, 250° C (19).

Partial Chemical Properties of Crude Oil of Cucurbita foetidissima

Gourd seed oil extracted with petroleum ether in a Soxhlet extraction apparatus, was used for the following chemical properties determinations.

Saponification value (20) -- Four g of seed oil was placed in a 250 ml round bottom flask. Fifty ml of 0.5 N ethanolic potassium hydroxide solution was added and the reflux condenser was attached to flask. The solution was boiled gently until the sample was completely saponified (about 1 hour). One ml of 1% phenolphthalein indicator solution in ethanol was added. The mixture was slowly titrated while hot with 0.5 N hydrochloric acid until the pink color disappeared. Saponification value was calculated using the formula;

Saponification value = 51.6 x Normality of Hydrochloric Acid x (titration of blank - titration of sample) / weight of oil

Unsaponification value (21) -- To 5 g of seed oil in 250 ml round bottom flask was added 50 ml of 1.0 N ethanolic pottassium hydroxide solution. The mixture was refluxed for 1 hour, and then transferred to a 500 ml separatory funnel. The flask was rinsed with 100 ml water

then with 100 ml diethyl ether. The mixture was shaken vigourously. The ethanol-water layer was extracted with 100 ml portions of diethyl ether three times. The combined ether extracts which contained the unsaponifiable fraction was washed with 40 ml of 0.5 N potassium hydroxide. The ether layer was then washed with 40 ml portions of water until the water layer no longer become pink upon addition of phenolphthalein. The ether extract was evaporated to dryness and weighed.

Calculation; P - sample weight; p - residue weight

Unsaponifiable matter% = p/10P %

Iodine value (22) -- One tenth of a g of seed oil was dissolved in 15 ml carbon tetrachloride. Twenty five ml of Wijs solution (9 g of iodine trichloride in mixture of 700 ml glacial acetic acid and 300 ml carbon tetrachloride) was added. The flask was stoppered, contents were mixed and allowed to stand in dark at about 20° C for 2 hours. After standing, 20 ml of 10% potassium iodide solution and 150 ml of water were added. The mixture was titrated with 0.1 N sodium thiosulfate solution using a starch solution as the indicator. Calculation;

W = weight of sample

V_1 = titration of sample

V_2 = titration of blank

N = normality of thiosulfate solution

Iodine value = $12.69 \times N \times (V_2 - V_1) / W$

Amino Acid Composition Analysis

Amino acids were performed on whole seed, decorticated gourd seed, decorticated defatted gourd seed and seed coat material following

acid hydrolysis. A sample of 30 to 50 mg was placed in a 15 x 150 mm test tube and 10 ml of 6 N hydrochloric acid was added. The top of the tube was necked down to facilitate sealing. The contents were frozen in a dry ice-methanol bath, evacuated to 71 cm of mercury, and sealed. Hydrolysis of the samples was done at 110° C for 22 hours. Following hydrolysis samples were filtered through a sintered glass filter (medium porosity) to remove insoluble matter. Hydrochloric acid was removed from hydrolyzates by evaporating to dryness three times using a rotary evaporator. After final drying, the residue was made to a specific volume with 0.2 N sodium citrate buffer, pH 2.2. Buffer was added so that 1 ml of sample contained approximately 1 mg of protein. Prepared samples were stored at - 20° C until used. Amino acid analyses were made following the procedure of Spackman et al. (23) and Moore et al. (24) using a Beckman automatic amino acid analyzer (Beckman Instrument, Inc., Fullerton, California).

Scanning Electrn Microscopic (SEM) Examination of the Seed Structure of Cucurbita foetidissima

The seed was sliced with a razor blade and attached to a circular (9 mm diameter) specimen stub with plastic cement. The mounted specimen was coated with a 100-200 A° layer of gold with a high vacuum, electron-evaporation apparatus (kinney Corporation, Boston, Mass.). The sample was examined by an Autoscan SEM (ETEC Corporation, Hayward, California) at an accelerating potential of 20 KV and photographed using Polaroid film.

RESULTS AND DISCUSSION

Proximate Analysis of Cucurbita foetidissima

The results of proximate analysis of C. foetidissima seed is shown in Table 1 together with values previously reported.

The protein, lipid, and fiber contents were in good agreement with reported valued. The high fiber content was due to the large portion of seed coat which accounts for about 31% of the whole seed weight.

For comparative purposes the gross composition of soybean, cotton-seed, seasame, and sunflower is listed in Table II. Only soybean has a higher protein content than that of Buffalo gourd seeds. That cotton-seed, sunflower and seasame have been developed into usable products is an established fact. Since the high oil and protein content of the Buffalo gourd are comparable to other oilseeds, it appears it could be used in both human and animal feeds.

Lipids Extraction--Differences in Extraction with Various Solvents

The results of lipid extraction with five different methods is given in Table III. Water-saturated n-butanol was the most effective solvent system for the extraction of Buffalo gourd seed oil. A mixture of chloroform-methanol (2:1 v/v) was also a good solvent system. They were more polar solvent systems, thus more lipids could be dissociated from the complex. When compared with other methods, acid hydrolysis method also gave high percent of lipids. This method however results in hydrolysis of a part of lipids. Petroleum ether and ethyl

TABLE I. ANALYSIS OF CUCURBITA FOETIDISSIMA WHOLE SEED[a]

	%	PREVIOUSLY REPORTED (4,5)
Moisture	6.6	9.61
Fiber	27.0	17.4-26.5
Protein (N x 6.25)	33.7	34.2-31.7
Lipid	27.8	24.3-31.4
Ash	2.5	4.8

[a]Values are expressed as percent of the ground seed sample.

TABLE II. ANALYSIS OF THE WHOLE OILSEEDS

TYPE OF SEED	MOISTURE %	ASH %	FAT %	FIBER %	PROTEIN %
C. foetidissima	6.6	2.5	27.8	27.0	33.7
Soybean[a]	9.1	4.9	17.4	5.3	37.9
Cottonseed[a]	7.3	3.5	22.9	16.9	23.1
Seasame[a]	8.0	5.6	42.9	10.3	22.3
Sunflower[a]	6.4	3.1	25.9	29.0	16.8

[a]Atlas of Nutritional Data on United States and Canadian Feeds by the National Academy of Sciences. Washington, D. C. 1971 (25).

TABLE III. LIPIDS EXTRACTED FROM C. FOETIDISSIMA

EXTRACTION METHODS	LIPID, %
Petroleum ether extraction	27.80
Ethyl alcohol extraction	27.91
Acid hydrolysis methos	30.60
Water-saturated n-butanol extraction	31.20
Chloroform-methanol extraction	31.00

alcohol extraction were less effective in removing total lipids.

Thin-Layer Chromatography

Thin-layer chromatograms of the lipids of C. foetidissima are shown in Fig 1 and 2. The development of neutral lipids showed that triglycerides were the major component, with small amounts of 1,3-diglycerides, 1,2-diglycerides, and free fatty acids. A trace of sterol was also shown. The polar lipids separated by TLC were tentatively identified and listed in the order of decreasing Rf value as:

A. Neutral lipid

B. Sterol

C. Phosphatidyl ethanolamine

D. Phosphatidyl choline

E. Phosphatidyl inositol

F. Lysolecithin

Fatty Acid Composition

The fatty acid composition of Buffalo gourd seed oil determined by gas-liquid chromatography (GLC) is listed in Table IV. The data obtained indicate that linoleic acid at 71.08 percent was the major component followed by 8.45 percent palmitic acid, 3.31 percent stearic acid, 17.11 percent oleic acid, and traces of myristic acid and arachidic acid. The ratio of saturated fatty acids to unsaturated fatty acids was 11.76 to 88.19.

A semi-log plot of retention time versus equivalent chain length (Fig 3) prepared from five known saturated fatty acid esters (Fig 4) was used to identify the components present in the gas chromatogram

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Figure 1. Thin layer chromatogram of lipids from Cucurbita foetidissima. Developed with petroleum ether-diethyl ether -acetic acid (80:20:1) to separate the neutral components. From left to right: 1. Tripalmitin, 2. 1,3-dipalmitin, 3. 1,2-dipalmitin, 4. palmitic acid, 5. cholesteryl palmitate, 6. sample. Spots were identified as:

A. Sterol ester

B. Triglycerides

C. Fatty acid

D. Diglycerides

E. Polar lipids

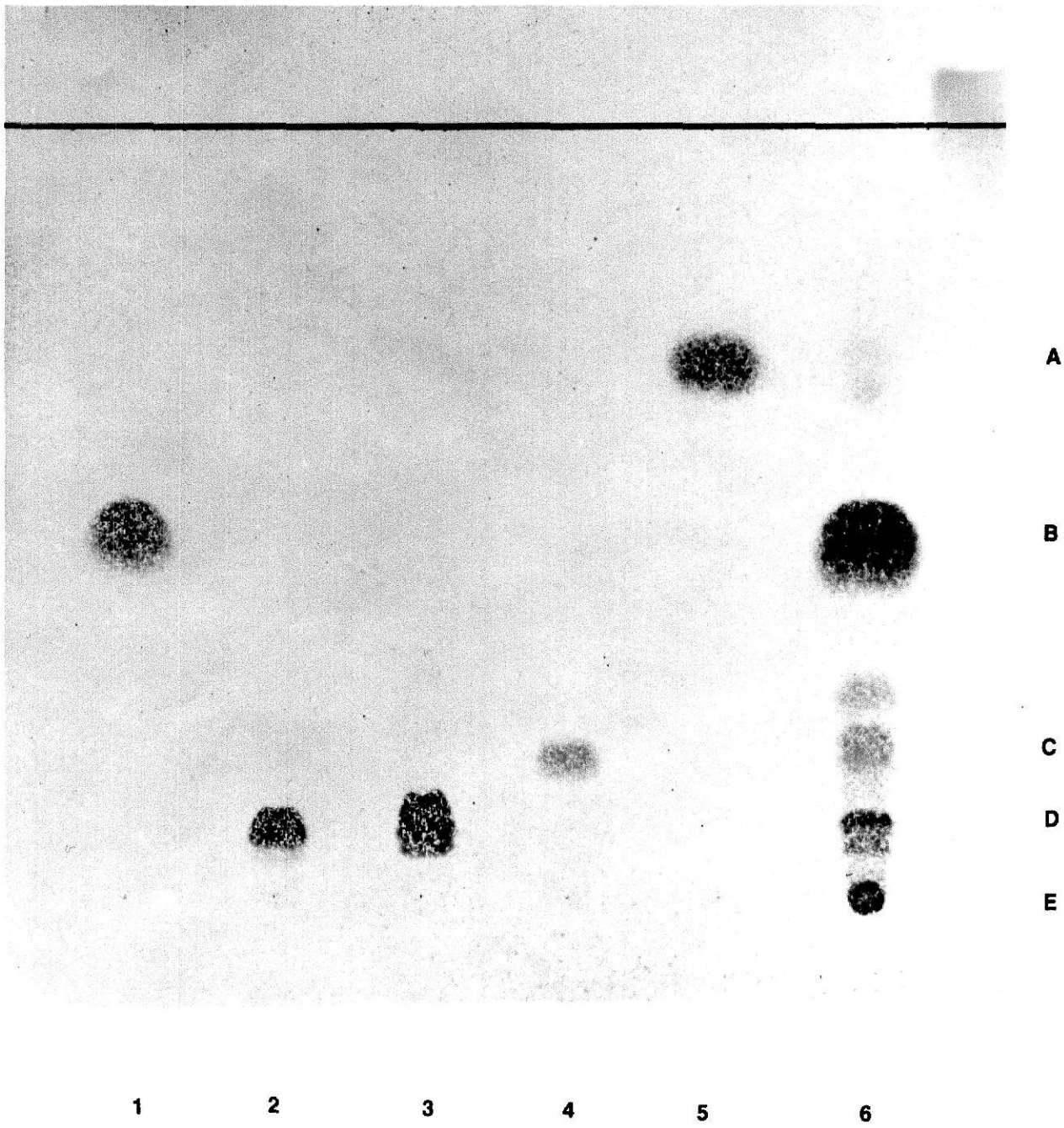

A
B
C
D
E
1
2
3
4
5
6

Figure 2. Thin layer chromatogram of lipids from Cucurbita foetidissima. Developed with chloroform-methanol-water (65:25:4) to separate the polar components. From left to right: 1. Phosphatidyl ethanolamine, 2. Phosphatidyl inositol, 3. DL- Lecithin, 4. Phosphatidyl serine, 5. Lysolecithin, 6. Sample. Spots were identified as:

A. Neutral lipids

B. Steryl esters

C. Phosphatidyl ethanolamine

D. Phosphatidyl choline (Lecithin)

E. Phosphatidyl inositol

F. Lysolecithin

G. Original spot

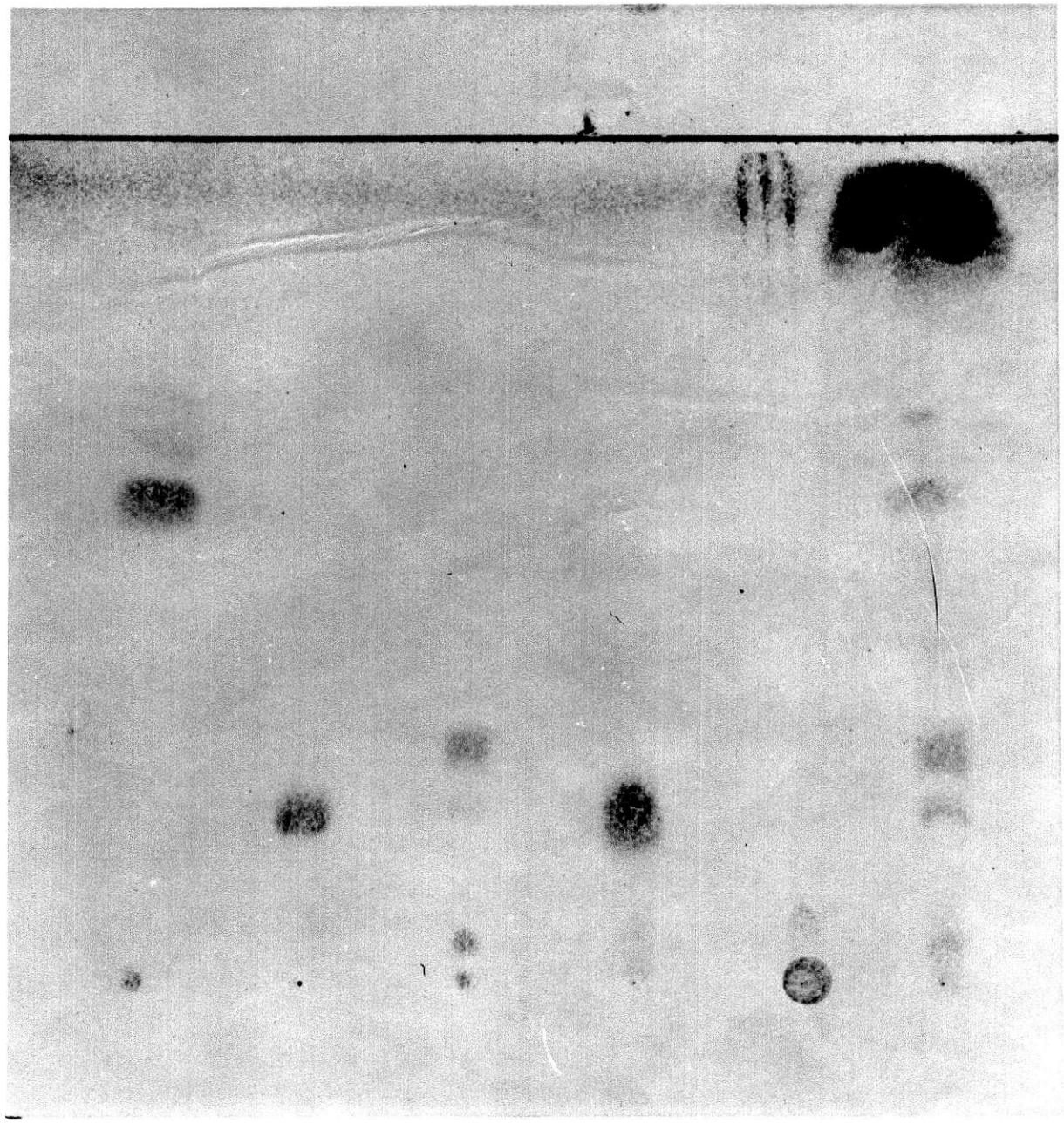


Figure 3. Semi-log plot of retention time of methyl esters of saturated fatty acids versus equivalent chain length (methyl laurate, methyl myristate, methyl palmitate, methyl stearate, and methyl arachidate).

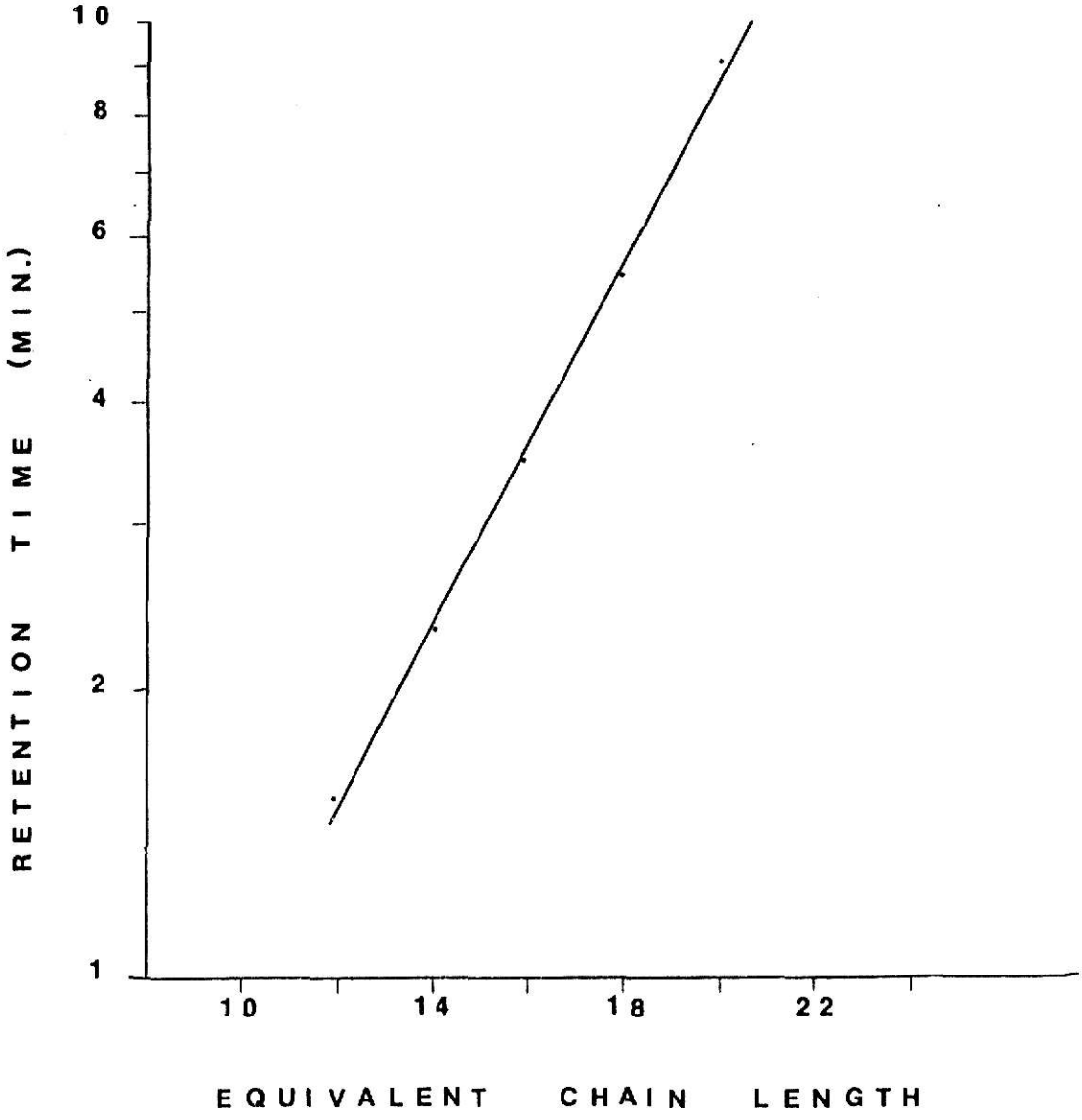

10
8
6
4
2
1
RETENTION TIME (MIN.)
10
14
18
22
EQUIVALENT CHAIN LENGTH

Figure 4. Gas-liquid chromatogram of standard saturated fatty acid methyl esters.

Designation:

A. Methyl laurate

B. Methyl myristate

C. Methyl palmitate

D. Methyl stearate

G. Methyl arachidate

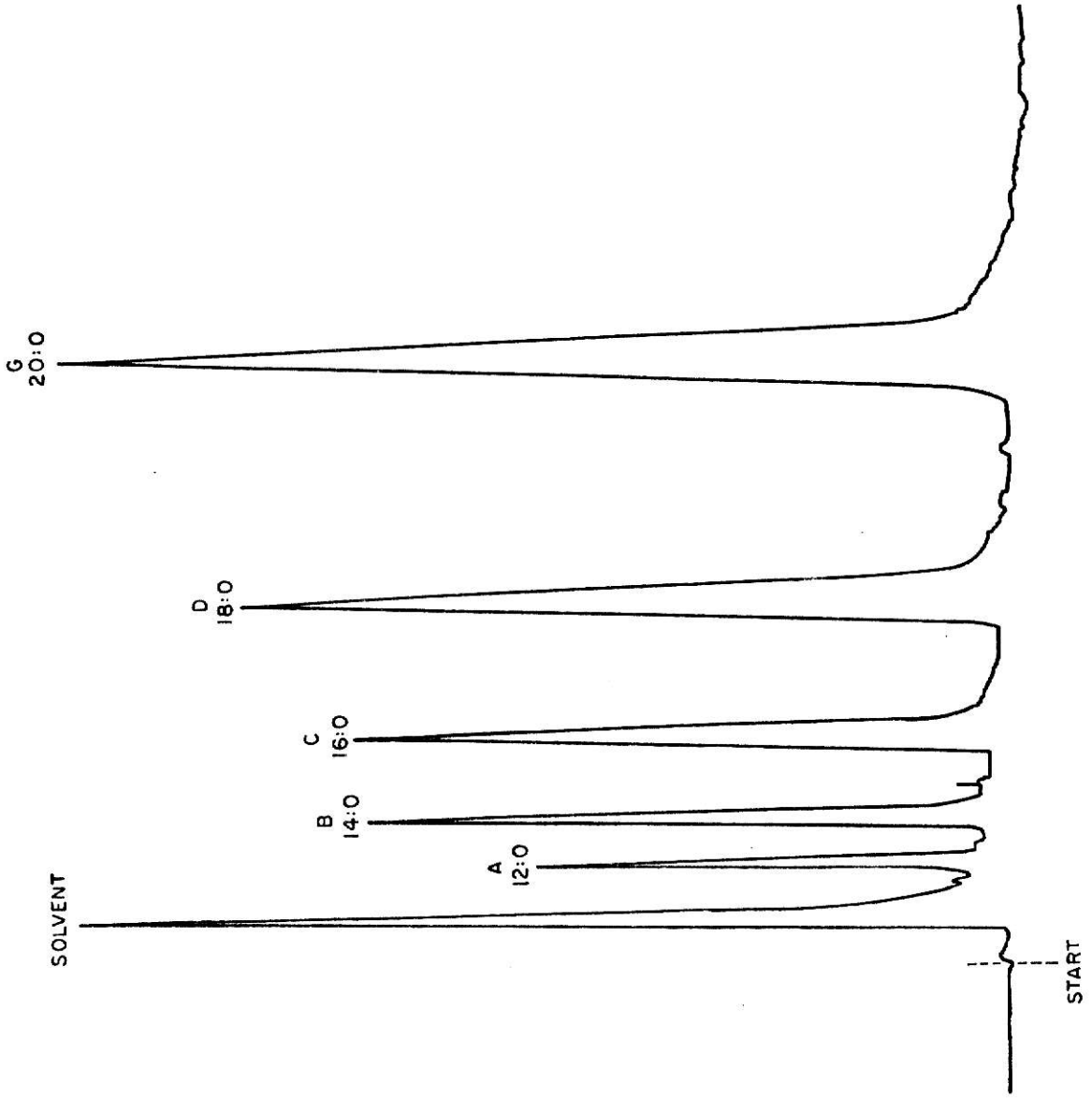

SOLVENT
A
12:0
B
14:0
C
16:0
D
18:0
G
20:0
START

Figure 5. Gas-liquid chromatogram of fatty acid methyl esters of Cucurbita foetidissima lipids.

Designation:

B. Methyl myristate

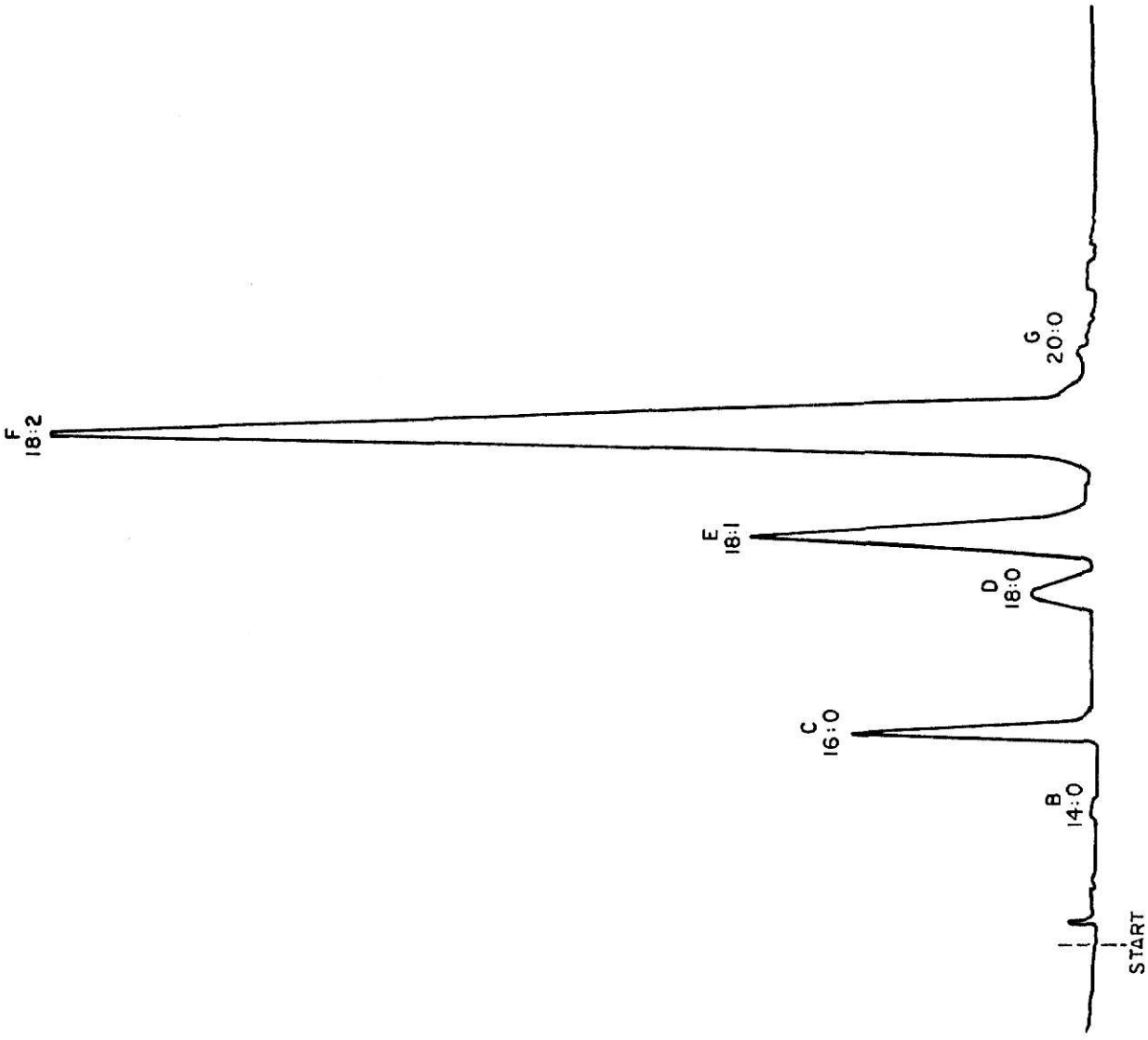
C. Methyl palmitate

D. Methyl stearate

E. Methyl oleate

F. Methyl linoleate

G. Methyl arachidate


F
18:2
E
18:1
D
18:0
C
16:0
B
14:0
START
G
20:0

of the Buffalo gourd seed fatty acid esters (Fig 5). Although there is a good agreement with the two studies (4, 7) relative to palmitic, stearic and oleic acid, there is some value differences for oleic and linoleic acid. These data give higher values for linoleic acid and lower values for oleic acid. These seeds were from a different source and may indicate species varation.

Chemical Properties of Lipids of Cucurbita foetidissima

The partial chemical properties of the C. foetidissima seed oil are given in Table V. There is good agreement on iodine value and saponifiable matter between this study and that of Shahani et al. (4).

Amino Acid Composition of Cucurbita foetidissima

The amino acid composition of whole seed, seed coat, decorticated seed and decorticated defatted seed of C. foetidissima is given in Table VI. The protein of Buffalo gourd seed has highest contents of arginine 12.2%, aspartic acid 10.0%, and glutamic acid 15.1%. It also appears to be a good lysine source (5.3% of crude protein). It is low in threonine, phenylalanine, and methionine. The amino acid composition of the seed coat showns high lysine (8.39%), aspartic acid (14.0%), glutamic acid (16.39 %) and glycine (17.0%) levels in the crude protein. But phenylalanine and methionine contents were very low. In spite of the high fiber content (69.7%), and poorer amino acids balance, it could be used as a protein possible energy source in feed for ruminant animals. Due to separation problems, there was still some seed coat remaining in the decorticated seed flour. The amino acid composition of decorticated seed flour and whole seed was very

TABLE V. PARTIAL CHEMICAL PROPERTIES OF LIPIDS OF C. FOETIDISSIMA

Saponifiable value[a]	182.30
Unsaponifiable matter[b]	1.39
Iodine value[c]	138.00

[a]Saponifiable value is expressed as mg KOH per gram of sample.

[b]Unsaponifiable matter is expressed as percentage of sample.

[c]Iodine value is expressed as number of grams of iodine absorber per 100 grams of sample.

TABLE VI. AMINO ACID COMPOSITION OF CUCURBITA FOETIDISSIMA

	WHOLE SEED	SEED COAT[a]	DECORTICATED SEED[b]	DECORTICATED DEFATTED SEED MEAL[c]
Crude Protein %	33.70	19.80	34.60	55.70
Lysine	5.30	8.39	5.05	3.54
Histidine	2.16	1.82	2.44	2.21
Arginine	12.21	8.73	14.55	14.99
Aspartic acid	10.07	14.03	10.19	8.97
Threonine	1.85	1.04	2.74	2.72
Serine	4.08	4.69	4.71	4.93
Glutamic acid	15.14	16.39	17.58	16.94
Proline	2.61	0.57	3.03	2.79
Glycine	7.57	17.01	6.31	5.94
Alanine	2.92	1.27	3.85	3.93
1/2 cystine	1.27	0.23	1.09	1.29
Valine	2.64	1.11	3.55	4.25
Methionine	0.50	0.42	1.48	1.66
Isoleucine	2.42	0.86	3.28	3.74
Leucine	4.64	1.10	5.97	6.55
Tyrosine	3.76	8.32	3.67	3.27
Phenylalanine	2.64	0.47	4.29	4.10

a, b, c The gross composition are given in Table IX.

TABLE VII. COMPARISON OF ESSENTIAL AMINO ACID COMPOSITION OF OILSEEDS WITH CUCURBITA FOETIDISSIMA

	C. FOETIDISSIMA SEED MEAL	SOYBEAN MEAL[a]	COTTONSEED MEAL[a]	SUNFLOWE MEAL[a]
Crude protein %	55.70	45.29	39.61	21.02
Lysine	3.54	6.17	4.20	3.81
Leucine	6.55	7.69	6.20	5.95
Isoleucine	3.74	5.50	4.01	4.52
Histidine	2.21	2.49	2.70	2.19
Arginine	14.99	7.46	11.02	7.76
Methionine	1.66	1.39	1.49	2.19
Phenylalanine	4.10	4.86	5.25	5.12
Threonine	2.72	4.03	3.47	3.43
Tryptophan	-	1.69	1.59	1.38
Valine	4.25	5.40	4.98	4.90

[a]Data was according to Lyman et al. (8).

TABLE VIII. A:E RATIO[a] OF CUCURBITA FOETIDISSIMA SEED MEAL

	SEED MEAL		FAO PATTERN[b]	
AMINO ACID	CONTENT	A:E RATIO	CONTENT	A:E RATIO
Lysine	3.54	113	4.2	134
Methionine	1.66	53	2.2	71
Cysteine	1.29	41	2.0	62
Isoleucine	3.74	120	4.2	134
Leucine	6.55	210	4.8	153
Phenylalanine	4.10	131	2.8	89
Tyrosine	3.27	105	2.8	89
Threonine	2.72	87	2.8	89
Valine	4.25	136	4.2	134
Tryptophan	-	-	1.4	45

[a]A:E ratio values are mg of amino acid per g of total essential amino acid.
[b]From FAO/WHO. 1956 (26).

TABLE IX. THE GROSS COMPOSITION OF C. FOETIDISSIMA SEED COAT, DECORTICATED SEED, AND DECORTICATED DEFATTED SEED MEAL[a]

TYPE OF SAMPLE	MOISTURE %	ASH %	FAT %	FIBER %	PROTEIN %
Seed coat	6.7	1.0	5.1	69.7	19.8
Decorticated seed	5.0	3.6	39.5	12.0	34.6
Decorticated defatted seed meal	8.1	6.1	17.4	13.0	55.7

[a]Values are expressed as percent of the sample.

similar. Lysine content of decorticated seed flour was lower than that of whole seed. This would appear to be due to the removal of the seed coat which has a higher lysine content than the whole seed. The increase in phenylalanine would also appear to be associated with the removed seed coat which was very low in this amino acid.

The amino acid composition of decorticated defatted gourd seed meal was similiar to that of decorticated gourd seed flour, except that lysine and aspartic acid contents were lower. This might have been a result of the lipid extraction process. Through the action of lecithin and other solubilizers, some amino acids would be carried out into extracts.

For comparative purposes, the amino acid composition of soybean, cottonseed, sunflower meals is given in Table VII. The data indicates that C. foetidissima is generally comparable to the other oilseed meals but varies from individual protein sources. The A:E ratio of C. foetidissima is presented in Table VIII.

Structural Studies of Cucurbita foetidissima Seed

Macroscopic structure;

The bottle-shaped seed was about 0.8 cm long and of pale-cream color. The seed coat was markedly rigid and, internally, there were two prominent cotyledons (Fig 6, 7 and 8).

Scanning electron microscopic studies of the seed;

The outer epidermis consisted of palisade cells, pitted parenchyma cells and epidermal ribs. The epidermal ribs were attached on the epidermis wall, and branched toward the outside (Fig 9). Within

the epidermis there was a region of reticulated parenchyma cells with intercellular air spaces and several layer of pitted parenchyma cells (Fig 9, 10 and 11).

Next to the testa, there was a perisperm layer which consisted of a few layers of small cells (Fig 10 and 12). Followed by the perisperm, there was a single layer of endosperm cells which contained oil drops and aleurone grains (Fig 12). The cotyledon cells were mainly parenchyma with oil drops (Fig 13). Near the edge of the cotyledon, oil drops were embedded within the protein matrix as shown in Fig 14.

Scanning electron microscopic studies of the sovent extracted seed;

Seeds were compared under the scanning electron microscope (SEM) after the following treatments: 1. petroleum ether (P. E.) extraction for 24 hours, 2. petroleum ether (P. E.) extraction for 72 hours, 3. water-saturated n-butanol (WSB) extraction for 24 hours, 4. water -saturated n-butanol (WSB) extraction for 72 hours.

Changes after petroleum ether extraction for 24 hours:

There was no change in the seed coat (Fig 15). Most of the changes observed were in the cotyledon cells (Fig 16, 17 and 18). The structures identified as oil drops were extracted to a small extent.

Changes after water-saturated n-butanol extraction for 24 hours:

The higher polarity of water-saturated n-butanol, resulted in more lipids being extracted than (Fig 20, 21 and 22) by petroleum ether extraction. There was no change in the seed coat structure.

Changes after petroleum extraction for 72 hours:

Changes were observed in the cotyledon cells. Some of the oil drops in the parenchyma cells were completely removed (Fig 24). Generally

there did not appear to be a quantitative difference (Fig 23 to 28) when compared to that of petroleum ether extraction for 24 hours (Fig 17 and 18). This was apparently due to the nonpolar character of petroleum ether. Many of the bound lipids did not appear to be liberated even by the longer periods of extraction.

Changes after water-saturated n-butanol extraction for 72 hours:

Large quantities of the oil drops in the parenchyma cells were removed under this treatment (Fig 29 to 34). Broken parenchyma cells were also observed (Fig 33 and 34). The high magnitude of changes was thought to be due to the high polarity of the water-saturated n-butanol solvent which extracted both free and bound lipids, altered the permeability of parenchyma cell walls and loosened the structural cells.

Figure 6 and 7. Macroscopic views of Cucurbita foetidissima. (x 8)

Figure 8. Longisection of the seed showing testa (T), and cotyledon area (COTS). (x 9)

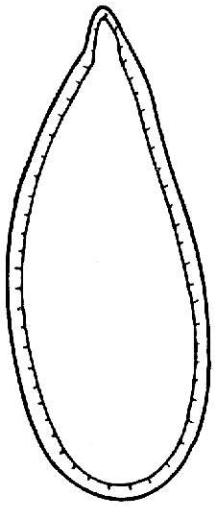


FIG. 6

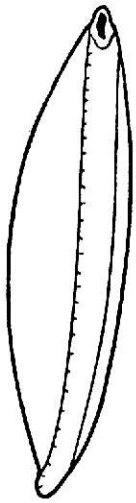


FIG. 7

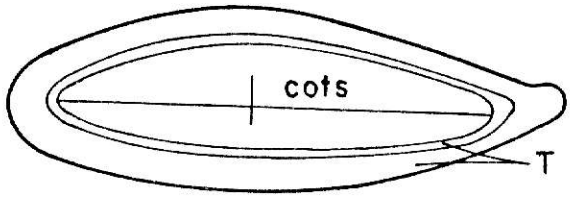
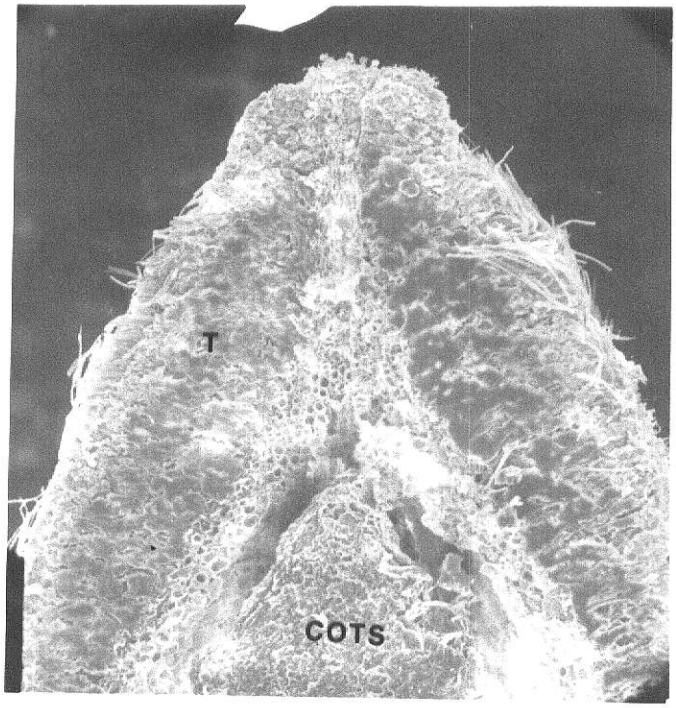


FIG. 8

Figure 9. SEM of a longisection view of C. foetidissima seed showing testa (T), and cotyledon area (COTS). (x 84)

Figure 10. SEM of a longisection of the cotyledon area (COTS). (x 306)


T
COTS

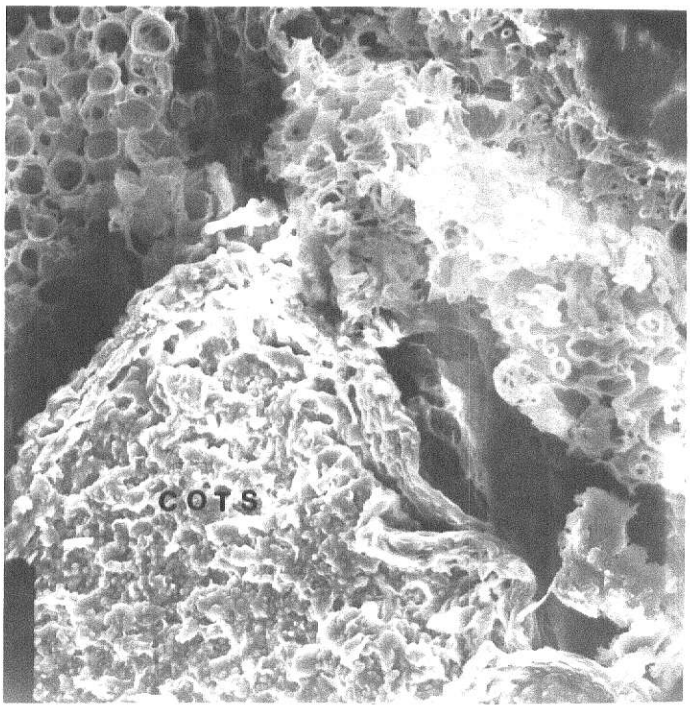
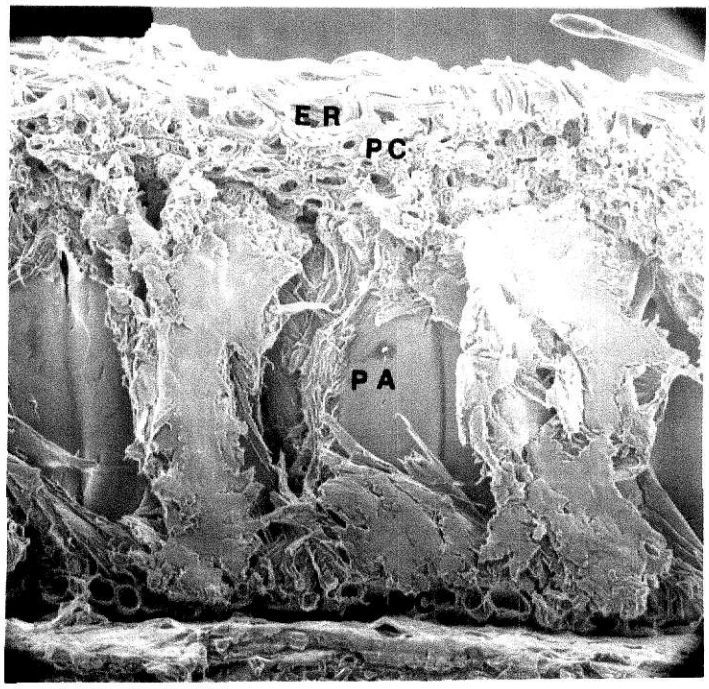

COTS

Figure 11. SEM of the testa of the seed showing epidermal ribs (ER), parenchyma cells (PC), and palisade cells (PA). (x 317)

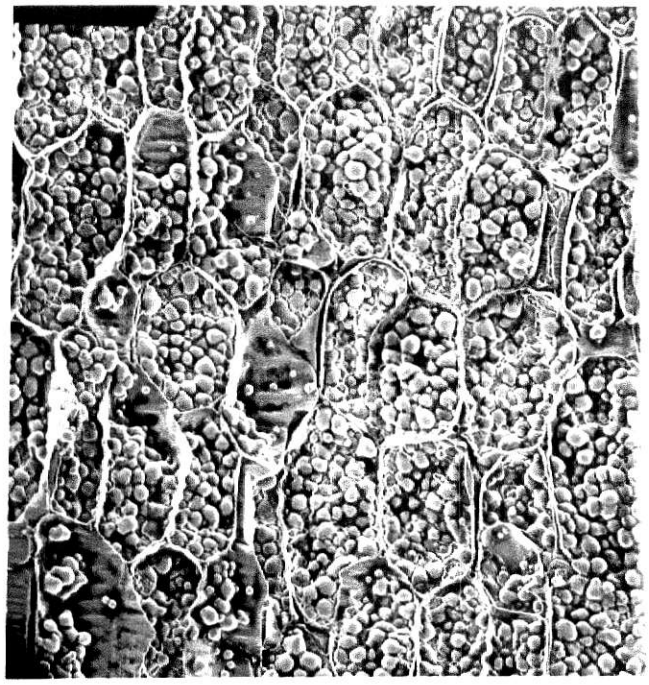
Figure 12. SEM of a cross section seed showing perisperm (PS), endosperm (ES), and cotyledon cells (COTS). (x 1202)


ER
PC
PA


PS

Figure 13. A cross section view of the cotyledon cells showing the regular arrangement of parenchyma cells with oil drops. (x 334)

Figure 14. A longisection view of the edge area of the cotyledon cells showing oil drops embedded within the protein matrix. (x 1202)



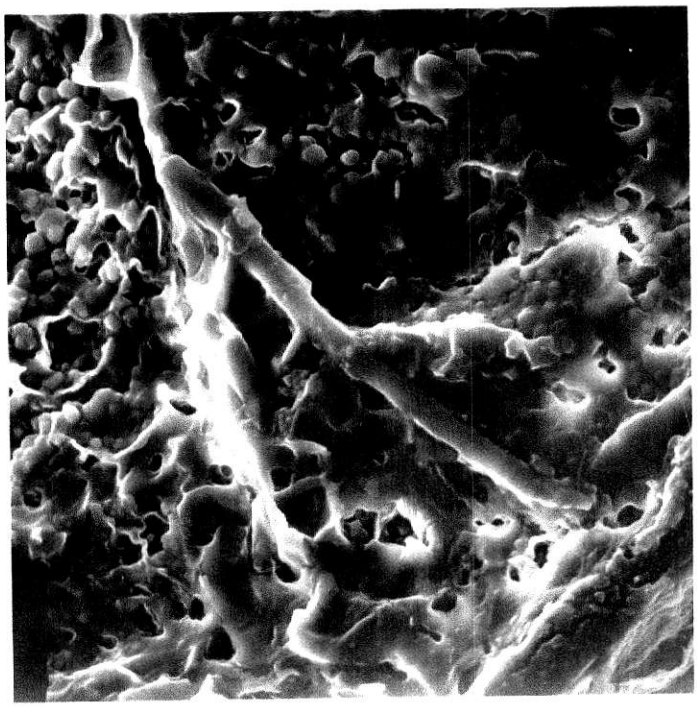
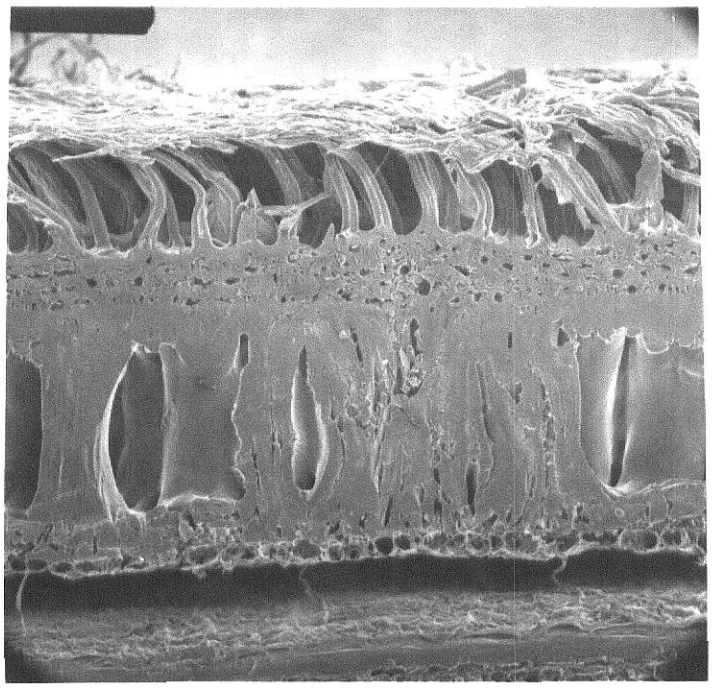


Figure 15. A longisection view of the seed coat after P. E. extraction for 24 hours. (x 255)

Figure 16. A longisection view of the cotyledon cells after P. E. extraction for 24 hours. (x 510)



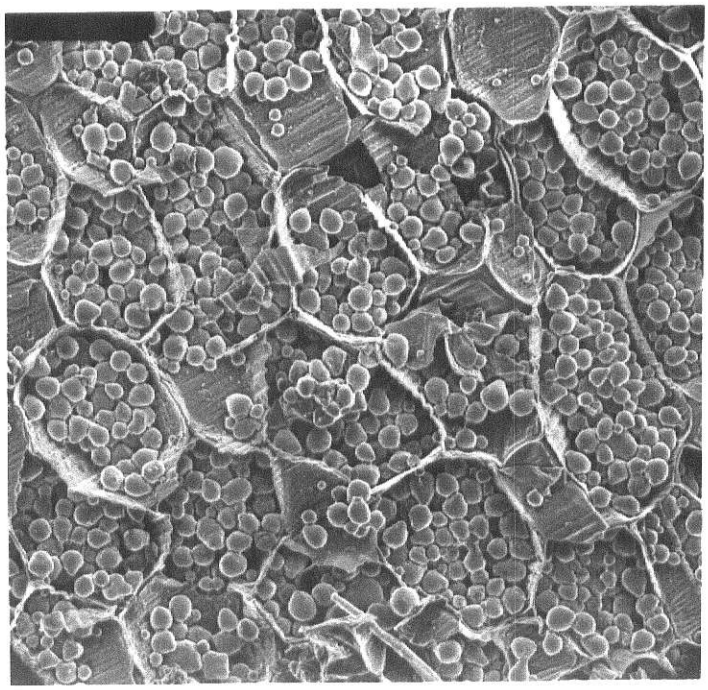
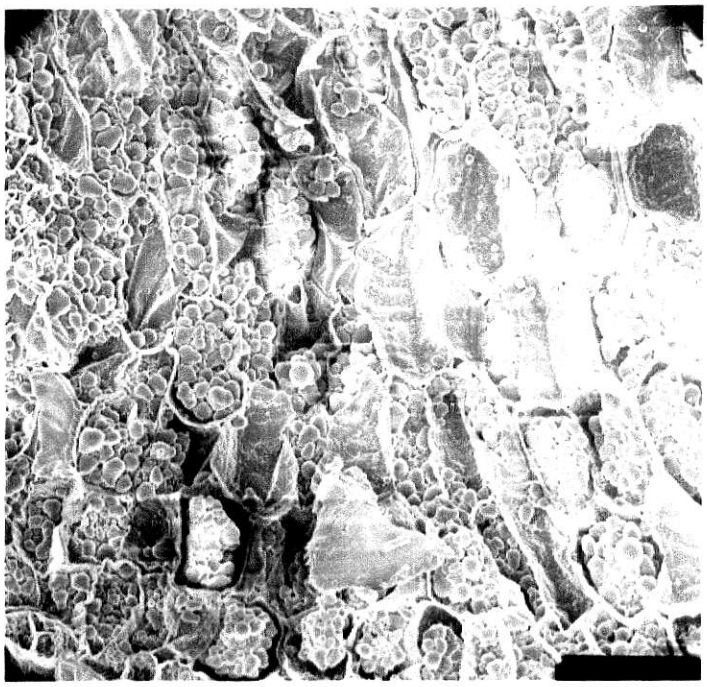


Figure 17. A cross section view of the cotyledon cells after P. E. extraction for 24 hours. (x 490)

Figure 18. An enlarged view of the cotyledon cells after P. E. extraction for 24 hours. (x 1400)



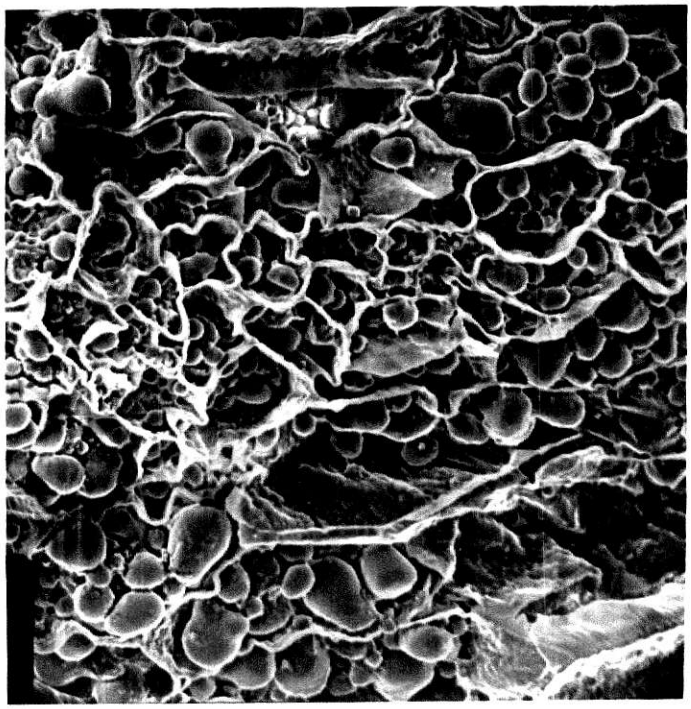
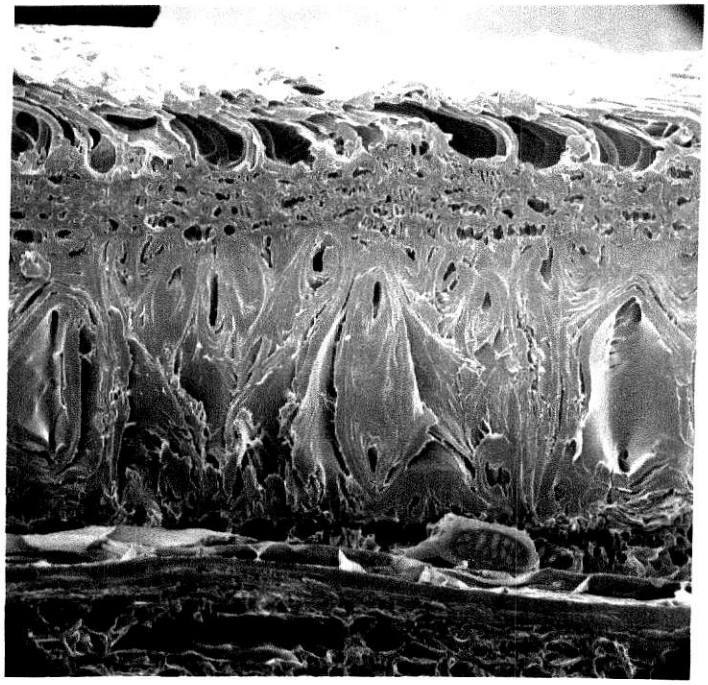


Figure 19. A longisection view of the seed coat after WSB extraction for 24 hours. (x 306)

Figure 20. A cross section view of the cotyledon cells after WSB extraction for 24 hours. (x 547)



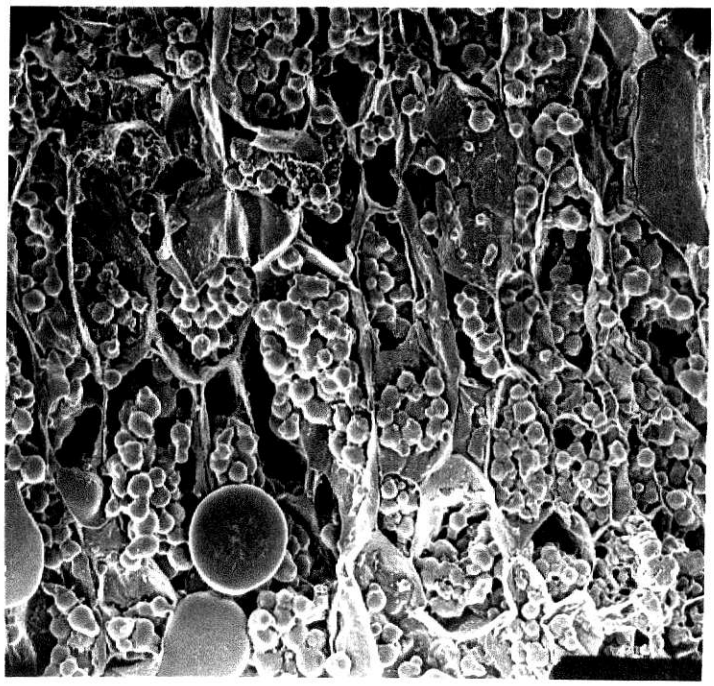
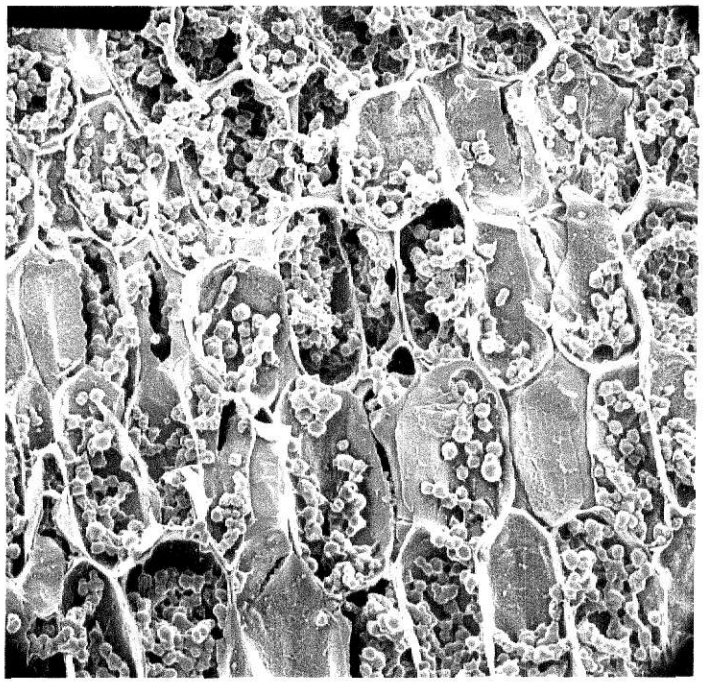


Figure 21. A longisection of the cotyledon cells after WSB extraction for 24 hours. (x 378)

Figure 22. An enlarged longisection view of the cotyledon cells after WSB extraction for 24 hours. (x 700)



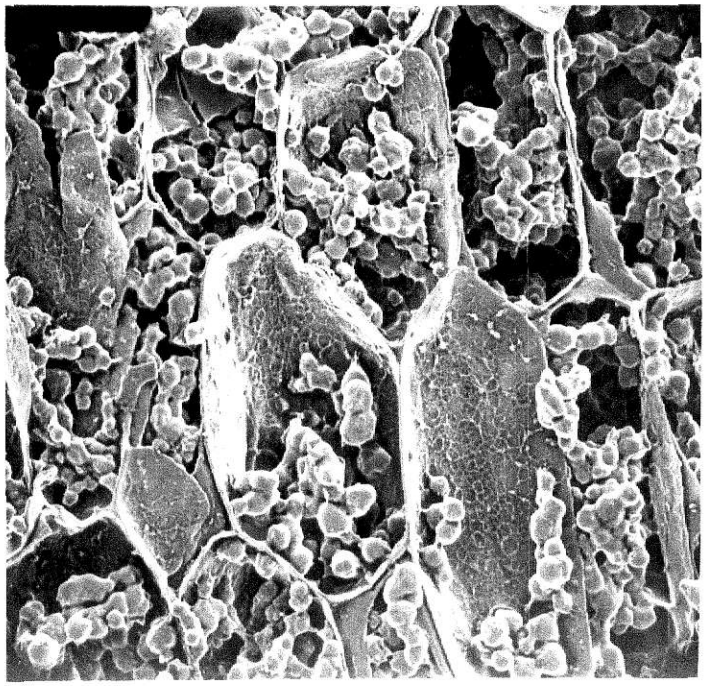
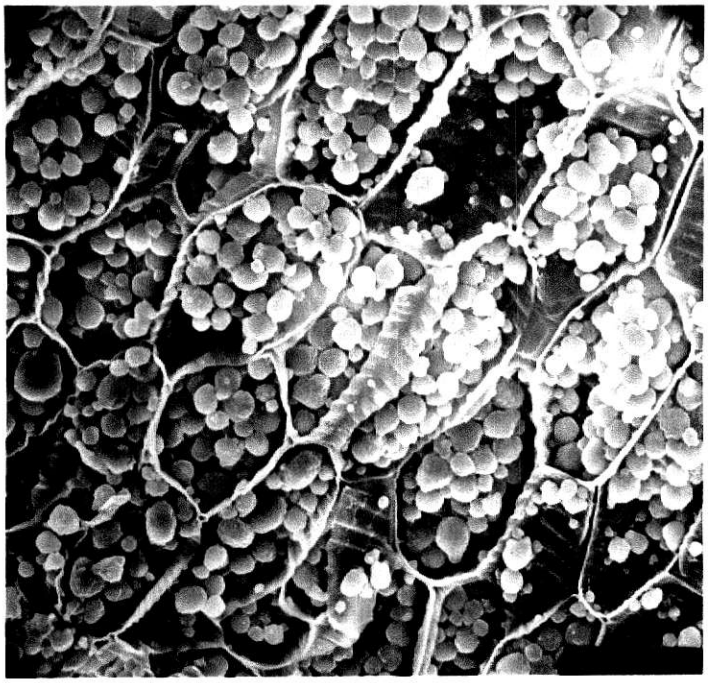


Figure 23. A cross section view of the cotyledon cells after P. E. extraction for 72 hours. (x 680)

Figure 24. An enlarged cross section view of the cotyledon cells after P. E. extraction for 72 hours. (x 1360)



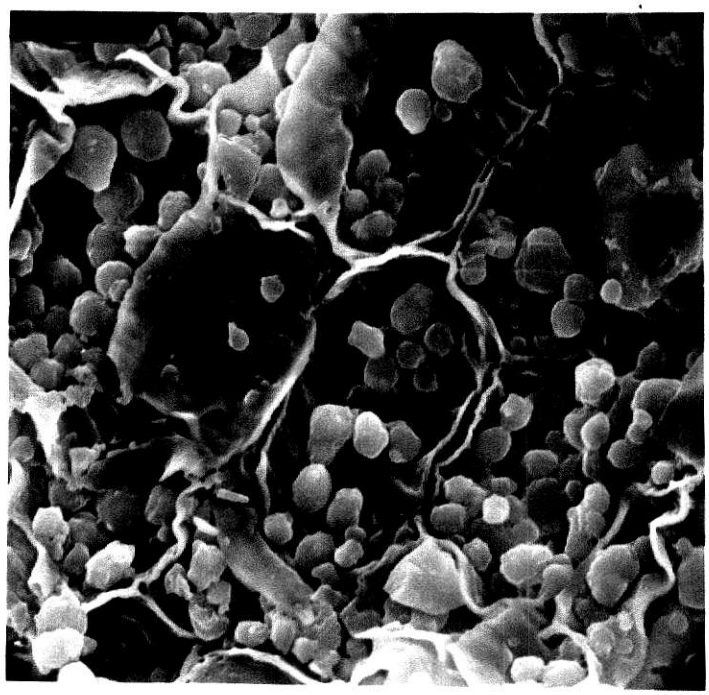
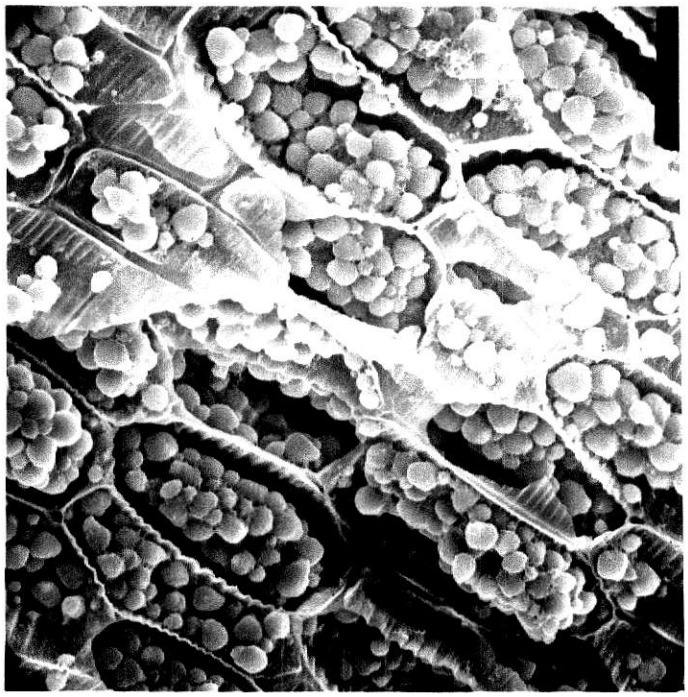


Figure 25. A longisection view of the cotyledon cells after P. E. extraction for 72 hours. (x 668)

Figure 26. An enlarged longisection view of the edge area of the cotyledons after P. E. extraction for 72 hours. (x 1328)



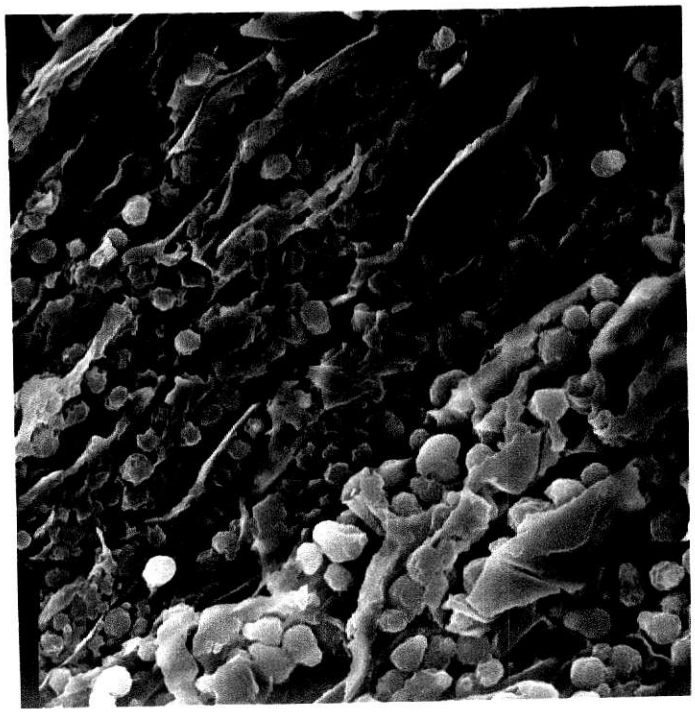


Figure 27. An enlarged cross section view of the cotyledon cells after P. E. extraction for 72 hours. (x 946)

Figure 28. An enlarged longisection view of the cotyledon cells after P. E. extraction for 72 hours. (x 1344)

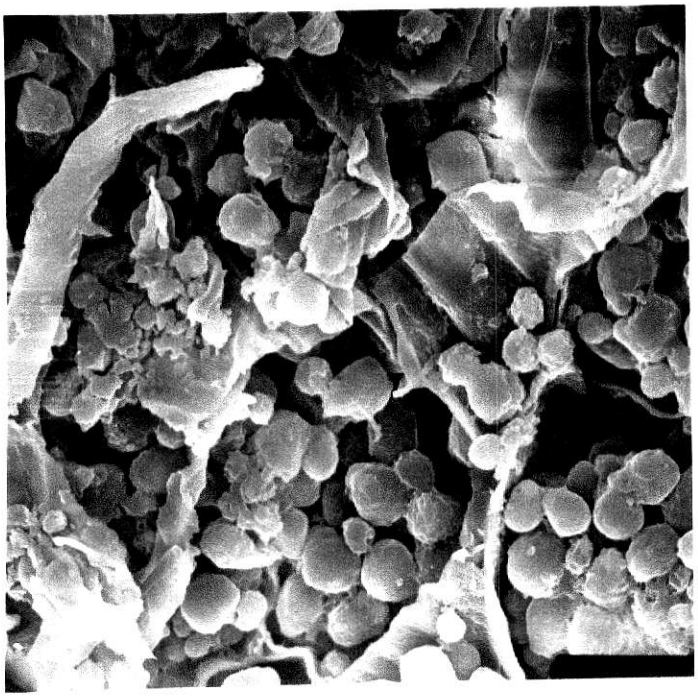
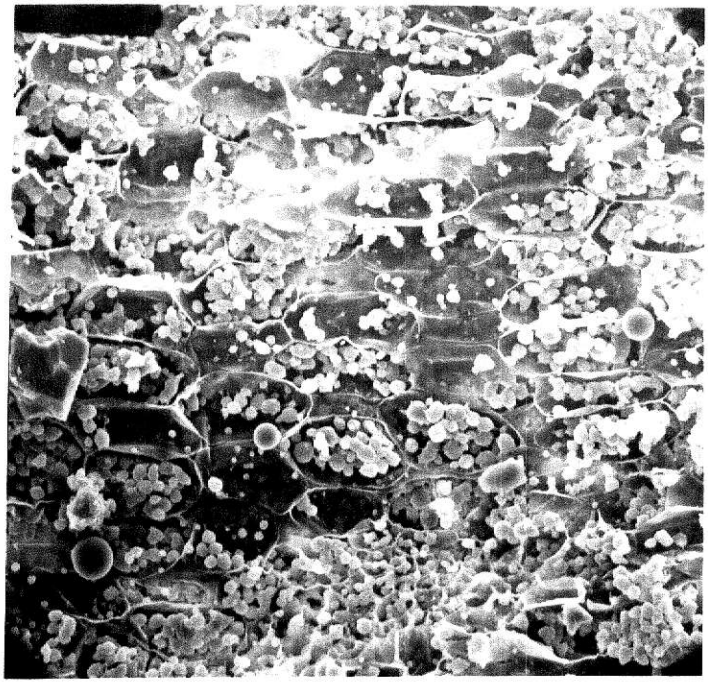


Figure 29. A cross section view of the cotyledon cells after WSB extraction for 72 hours. (x 344)

Figure 30. An enlarged cross section view of the cotyledon cells after WSB extraction for 72 hours. (x 688)



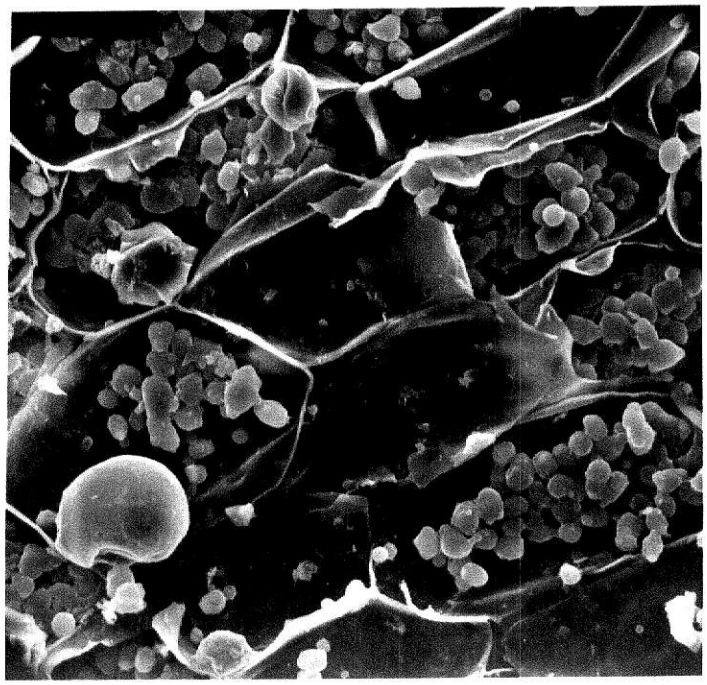
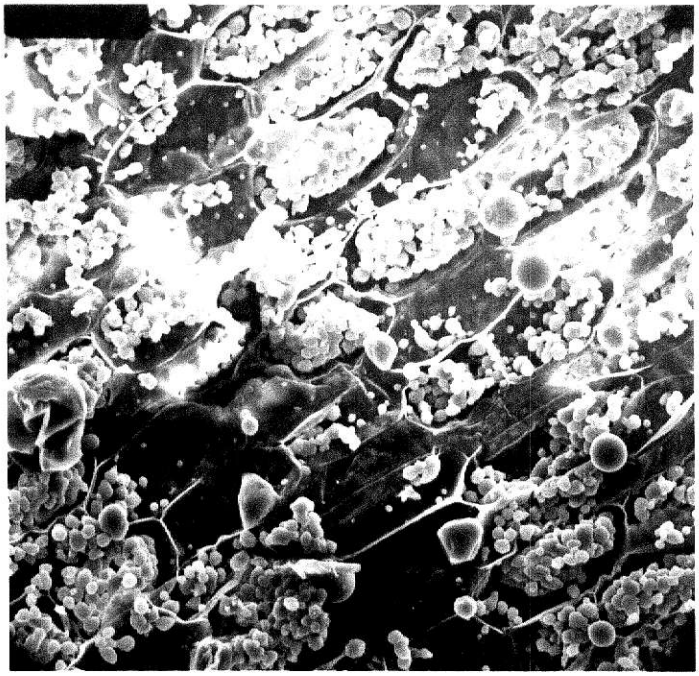


Figure 31. A longisection view of the cotyledon cells after WSB extraction for 72 hours.
(x 344)

Figure 32. An enlarged longisection view of the cotyledon cells after WSB extraction for 72 hours.
(x 1032)



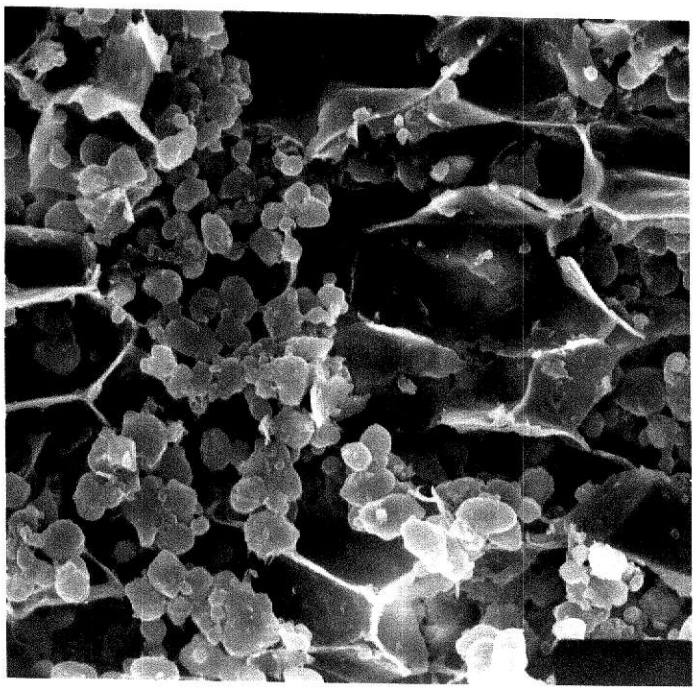
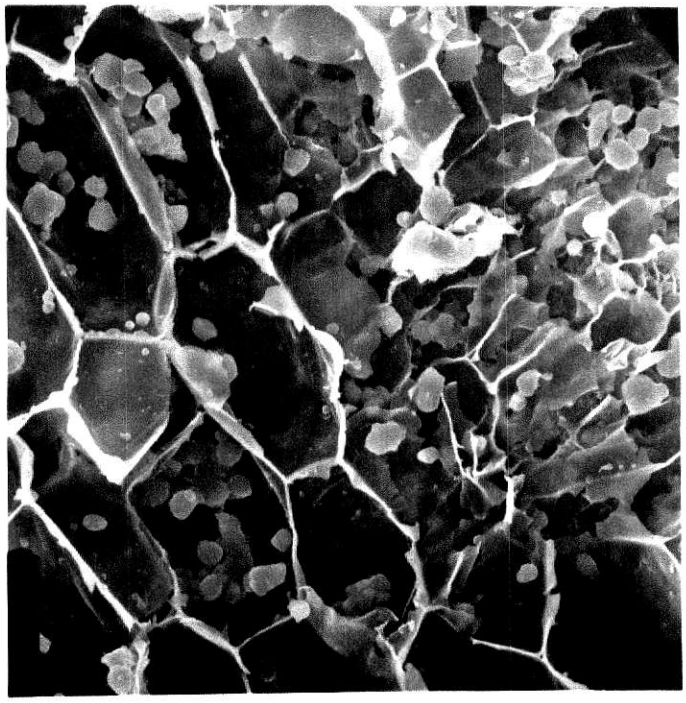
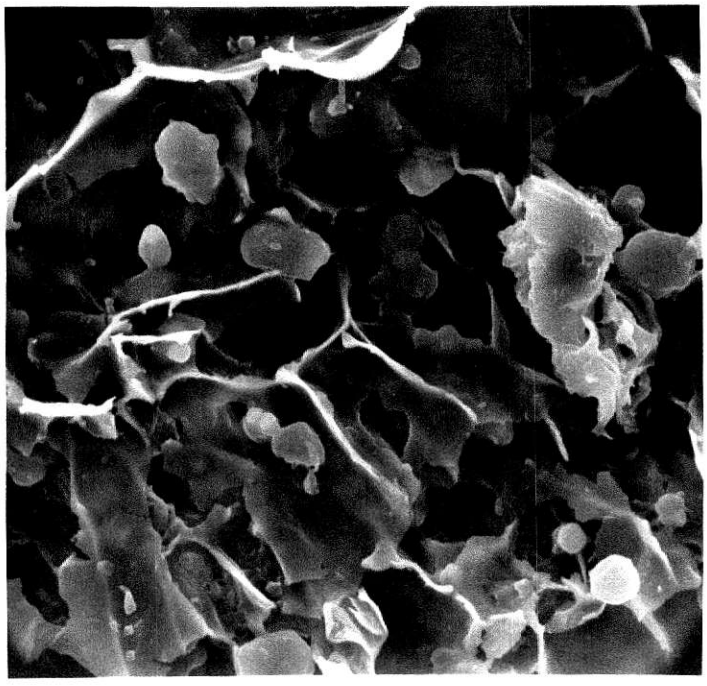


Figure 33 and 34. An enlarged cross section view of the cotyledon cells after WSB extraction for 72 hours. (x 1376)





SUMMARY

The seeds of Buffalo gourd, Cucurbita foetidissima, a wild gourd native to Southern United States and Northern Mexico, has been studied as a potential source of oil and protein. Seeds contained 6.6% moisture, 27.0% fiber, 33.7% protein, 26.8% lipids and 2.5% ash. The protein and lipid analysis of C. foetidissima seed studies here agree with literature values.

Five methods were used to extract lipids from the seed sample; petroleum ether extraction, ethyl alcohol extraction, an acid hydrolysis method, water-saturated n-butanol extraction, and chloroform-methanol (2:1 v/v) extraction. The water-saturated n-butanol was found to be the most effective method.

The characterization of lipids of C. foetidissima seed was studied by thin-layer chromatography. Triglycerides were found to be the predominant compound in the neutral lipids whereas phosphatidyl ethanolamine and lecithin were the major components of the polar lipids. The fatty acid composition determined by gas-liquid chromatography on a DEGS column revealed the composition as palmitic acid 8.45%, stearic acid 3.31%, oleic acid 17.11%, linoleic acid 71.08%, and traces of myristic and arachidic acids.

It was also found that lipids of C. foetidissima had a saponification value of 182.30 (mg KOH per gram of sample), unsaponifiable matter 1.39%, and a iodine value of 138.10 (number of grams of iodine absorbed per 100 grams of sample).

The amino acid composition of the whole seed, seed coat, decorticated seed and decorticated defatted seed meal was analyzed using an automatic amino acid analyzer. Arginine, aspartic acid, and glutamic acid were the most abundant amino acids, and there was a lower content of threonine, phenylalanine and sulfur containing amino acids. The highest lysine content was found in the seed coat, but the seed coat was low in phenylalanine and sulfur containing amino acids. The decorticated seed sample had a pattern of amino acids similar to whole seed, except for the lysine content which decreased from 5.30 to 5.05 percent of the protein. In the decorticated defatted seed meal, lysine content was reduced to 3.54 percent of the protein.

The seed structure of C. foetidissima was examined by scanning electron microscopy (SEM). It showed that testa consisted of palisade cells, pitted parenchyma cells, epidermal ribs and reticulated parenchyma cells with intercellular air spaces. Next to testa, there was a periaperm layer followed by a single layer of endosperm cells which contained oil drops and aleurone grains. The cotyledon cells were mainly parenchyma with oil drops. Structural changes of the solvent extracted seeds were also studied. Petroleum ether extraction for 24 and 72 hours did not show significant changes from the untreated seed structure. Only a small amount of the oil drops were extracted. The most noticeable changes were observed after water-saturated n-butanol extraction for 72 hours. Large quantities of the oil frops were removed. Broken parenchyma cells were also observed.

ACKNOWLEDGEMENTS

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THE PARTIAL CHARACTERIZATION OF *CUCURBITA FOETIDISSIMA*

(THE BUFFALO GOURD)

BY

MEIFAN CHEN TU

B. S., National Chung Hsiung University (TAIWAN), 1971

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

in

FOOD SCIENCE

Department of Grain Science and Industry

KANSAS STATE UNIVERSITY
Manhatan, Kansas

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The gross composition, lipid characterization, amino acid pattern and structural examination of Cucurbita foetidissima seeds were studied.

It was found the seeds contained 33.7% protein and 26.8% lipids.

The lipids were extracted with five different methods. Petroleum ether extraction, ethyl alcohol extraction, an acid hydrolysis method, water-saturated n-butanol extraction, and chloroform-methanol (2:1 v/v) extraction. The water-saturated n-butanol method was found to be the most effective method for removing lipids from the seed.

The lipids of C. foetidissima seed were identified by thin-layer chromatography. Triglycerides were found to be the predominant compound in neutral lipids whereas phosphatidyl ethanolamine and lecithin were the major components in polar lipids. The fatty acid composition determined by gas-liquid chromatography revealed that linoleic acid was the major component. The saponification value (mg KOH per gram of sample), unsaponifiable matter (%), and iodine value (number of grams of iodine absorbed per 100 grams of sample) of the lipids were 182.30, 1.39, and 138.10 respectively.

The amino acid composition of the whole seed, decorticated seed and decorticated defatted seed meal showed that arginine, aspartic acid, and glutamic acid were the most abundant and there was a lower content of threonine, phenylalanine and sulfur containing amino acids. The seed coat was highest in lysine content, but was low in phenylalanine and sulfur containing amino acids.

Structural studies showed the cotyledon cells were mainly parenchyma with oil drops. There were no observable structural changes in

the cotyledon cells. The most noticeable structural changes were a loss of oil drops from parenchyma cells. The highest magnitude of changes was observed after WSB extraction for 72 hours. Large quantities of the oil droplets were removed and broken parenchyma cells were observed.