LIPIDS OF THE CASHEW (*ANACARDIUM OCCIDENTALE* LINN.)

by

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I hereby recommend that this dissertation prepared under my direction by GERALDO ARRAES MAIA

entitled LIPIDS OF THE CASHEW (ANACARDIUM OCCIDENTALE LINN.)

be accepted as fulfilling the dissertation requirement of the degree of DOCTOR OF PHILOSOPHY

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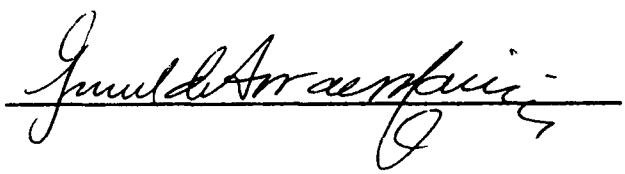
SIGNED: 

DEDICATION

This work is dedicated to my wife, Lucia, and my daughters, Sandra, Patricia, and Valeria.

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ABSTRACT

Cashew nuts (unroasted shelled and unroasted unshelled) received from Fortaleza-Ceara-Brazil, roasted nuts processed in the United States of America, cashew nut testa, cashew apple juice and cashew varieties red and yellow at two stages of maturity were studied. A combination of chromatographic procedures was used to determine the identity, composition and, in some cases, cashew lipid concentration.

Lipids were extracted by a wet extraction method using chloroform-methanol. Fractionation was made by silicic acid column chromatography with subsequent lipid separation into three classes: neutral lipids, glycolipids, and phospholipids.

Phospholipid and glycolipid fractions composition was studied by two-dimensional thin layer chromatography followed by phosphorus analysis of the spots (phospholipids). Cashew nut oil showed nine spots and cashew apple juice lipids showed 11 spots positive to the specific molybdenum spray. Phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE) isolated from cashew nut lipid phospholipids were studied for fatty acid composition. The major fatty acids in PE and PC were palmitic, stearic oleic and linoleic representing 85.3 percent of the total in PE and 73.3 percent in PC.

The following glycolipids were tentatively identified in cashew apple juice and nut: di-galactosyl diglyceride, cerebroside, sterol glycoside, mono-galactosyl diglyceride and esterified steryl glycoside.

Nut shell removal did not significantly affect nut lipids fatty acid composition. Palmitic, stearic, oleic and linoleic acids comprised more than 97 percent of the total fatty acids. Neutral lipids, glycolipids and phospholipids isolated by silicic acid column chromatography were studied for fatty acid composition. Three esterification methods were compared for fatty acid determination in cashew apple juice. Cashew nut testa and pulp and peel from red and yellow varieties of cashew apple at two stages of maturity (mature and immature) were studied for fatty acid composition. Oleic acid increased during maturation whereas linoleic and linolenic acids decreased.

Unsaponifiable matter was analyzed in three nut samples (roasted, unroasted and unroasted in shell). The lipid fraction was saponified and the unsaponifiable matter (0.34 percent) was fractionated on a Florisil column into six fractions. Gas chromatography of the hydrocarbons (fractions 1 + 2) showed that squalene was the major component. A total of 21 peaks were detected being distributed in three homologous series: normal, iso and/or anteiso and branched chain.

Gas chromatography of the third Florisil fraction silyl derivatives indicated the presence of three homologous series of normal, iso and/or anteiso and multiple branched chain alcohols. Beta and/or gamma tocopherol was found to be the major tocopherol and decreased during nut shell removal and roasting. Cycloartenol, amyrin (alpha-beta), 24-methylene cycloartanol were the triterpenoids identified in fraction 3. No significant differences were observed in the values of triterpenoid calculated as percentage of the oil.

Gas chromatography of the sterol fraction silyl derivatives showed that beta-sitosterol was the major sterol followed by campesterol. No significant variation was observed between the three samples.

INTRODUCTION

Cashew nut tree (Anacardium occidentale L.) is native to Brazil (31, 34, 67, 85). During the 16th century, Portuguese navigators and missionaries introduced it into extensive tropical regions, especially East Africa and India (63). It was first planted in Goa to prevent soil erosion (34). Since then, the plant has attained considerable economical importance. It is practically naturalized in the inter tropical region, from 18^{o}-20^{o} parallel north to 18^{o}-20^{o} parallel south (67). Outside of this region, it is found only in areas with favorable microclima such as meridional Mozambique, or in micropopulation in South Africa (67) and South Florida (53). The cashew tree is only suitable for cultivation in frostless regions.

The principal product obtained from cashew tree is the nut. The kernel (about 30 percent of the nut) is enclosed in a hard covering which contains cashew nut shell liquid (CNSL). This material is used to make several types of resins applicable to varnish manufacture (85). The kernel is edible and used as an ingredient in confectionery and baking formulations. It is also consumed salted after roasting.

The cashew apple is processed into several food products for consumption. It has great nutritional importance due to its high ascorbic acid content. The cashew is important in several areas including Brazil where the production is being greatly expanded.

This investigation was conducted to determine the lipid composition components of cashew. Such study is important because the quantity and the composition of lipid material consumed in regular diets is of nutritional and medical importance. A significant portion of dietary fats (60 percent) is invisible fat derived from meat, milk, eggs, and plant sources (35).

The purpose of this investigation was to study the phospholipid and glycolipid composition of cashew apple juice and cashew nut kernel; the fatty acid composition of cashew apple juice, testa, cashew apple at different stages of maturity and the nut at different stages of processing (raw, shelled and roasted). The unsaponifiable matter of cashew nut (raw, shelled and roasted) was also studied. The methods used in this work were based on column chromatography (CC), thin-layer chromatography (TLC) and gas liquid chromatography (GLC). Colorimetric phosphorous determination was used in analysis of the phospholipid fraction.

LITERATURE REVIEW

Botanical Description

The cashew, *Anacardium occidentale* L. is one member of *Anacardiaceae*. The family of some 60 genera, includes other important food species such as the mango (*Mangifera indica* L.) and the pistachio (*Pistacia vera* L.). On the other hand, it includes some notorious poisonous plants such as poison ivy (*Rhus toxicodendron* L.), poison sumac (*R. vernix*) and marking nut (*Semecarpus anacardium* L.) (53). Most of the plants from *Anacardiaceae* are tropical (7). The name *Anacardium* refers to the heart shaped nut (3) and *occidentale* because of its origin in Western America (85).

The cashew tree is an evergreen, of spreading straggly habit, symmetrical and reaching a height of about 12 m when favorably situated. It is easily recognized by its light colored foliage (85). The bark, normally rough and, on old trees deeply fissured, contains an acrid sap which turns black when exposed to air (53). The wood of the tree exudes a yellow gum. Leaves are basically oval or obovate, rounded or even emarginate at the top; flowers are yellowish-pink and fragrant being arranged in clusters terminating the young branches (7). There are both unisexual and bisexual types (53). The nut is kidney or heart shaped with the kernel portion being edible (3). This nut is borne in a fleshy receptacle (the cashew apple) which is about 6-10 cm long when

mature, yellow to red in color sweet and edible. The kernel is enclosed in a coriaceous covering consisting of two layers between which is a thick caustic liquid (CNSL). The trunk and branches of the trees, on being wounded during the ascent of the sap, yield a transparent gum similar in appearance to gum arabic (75).

The cashew tree begins to bear fruit at about three years. There are no definite varieties identified. In tropical America, however, two types may be distinguished based on the color of the peduncle: namely, yellow and red (53).

An interesting aspect of the tree is its "false fruit," the swollen peduncle or hypocarp, commonly known as cashew apple. It is pear shaped or rhomboid to ovate, varying from 6-10 cm in length, bright yellow to red color. It has a waxy skin, is juicy with spongy pulp and musklike fragrance. The "apple" is very astringent until fully ripe when it is sub-acid to acid and edible. This "apple" is actually the receptacle for the true fruit, the cashew nut (53).

Normally the cashew tree is propagated by seeds. Vegetative propagation is rarely used. Studies have been made to determine whether the nuts could be harvested prior to ripening of cashew apples in order to avoid depedrations of wildlife (birds, bats, etc.). It was found that when the nuts changed from green to a full ash color and were dry externally, the kernels were fully developed and mature regardless of the partially ripened condition of the "apples." They could be harvested providing the apples were of no importance to the grower. Nuts intended

for planting will germinate poorly and produce weak plants unless the apples are fully ripened (78).

The cashew tree grows well in a warm, moist climate and can withstand the vagaries of rainfall within certain limits. The tree thrives best in the tropical climates, not far from coastal areas. It has been found that a young tree tolerates transplantation poorly as its long tap root is damaged during the process (31). Cashew trees flower 2-2.5 months before fruit crop maturing. If there are heavy rains at blooming time, little fruit will be set. In India, the main cashew crop is harvested in March and April (85) and in Brazil during September to December. According to Pereira et al. (63) each tree produces an average of 15 kg of nuts and 3,000 "apples." Fruit bearing begins at three years and lasts for 20-25 years (26).

Food Value and Chemical Composition

Cashew Apple

In Brazil, the cashew apple is rated as one of the more important native fruits. Juice from cashew apple is commonly consumed raw, by squeezing directly into the mouth with the fibrous part being discarded. Several utilizations of cashew apple are to be discussed later. Morton (53) gives the chemical composition of cashew apple (Table 1). Maia, Holanda and Martins (46) have reported the composition of cashew apple from Fortaleza (Brazil) in various stages of maturity (Table 2). An important aspect of cashew apple composition is its relatively high ascorbic acid content. Citrus fruit juice is well known for its high ascorbic acid content (50-60 mg/100 ml). Cashew apple has a considerable

Table 1. Components in 100 g Portion of Cashew Apple.

g					mg							
Moisture	Ether Extract	Crude Fiber	Nitrogen	Ash	Minerals Ca	Minerals P	Minerals Fe	Carotene	Thiamine	Riboflavin	Niacin	Ascorbic Acid
					El Salvador							
87.25	0.080	0.50	0.137	0.31	1.65	19.6	0.43	0.131	0.019	0.014	0.479	188.9
					Costa Rica							
85.85	0.200	0.75	0.114	0.33	1.25	16.1	0.46	0.242	0.019	0.020	0.494	183.4
					Guatemala							
84.4	0.50	0.70	0.162	0.34	3.1	21.4	0.33	0.742	0.015	0.016	0.539	347.8
					Cuba							
--	--	--	--	0.2	5.4	11.6	0.71	0.030	0.03	0.04	0.31	312.6

Table 2. Chemical Composition of Mature and Immature Cashew Apple.

Maturity Stage	Soluble Solids	Protein	Ether Extract	Ash	Sugars			Moisture	Starch
					Reducing	Non-reducing	Total		
Immature	7.6	1.28	0.086	0.562	2.73	0.07	2.80	85.02	5.60
Mature	10.7	0.71	0.237	0.331	9.00	0.29	9.29	87.33	1.70

higher amount of this vitamin (213 mg/100 g) (44). Maia and Soares (43) made determinations of ascorbic acid, soluble solids, reducing sugars and total acidity at the extreme ends, the periphery and center of cashew apple. Results indicated that, on the polar extremities of the same hypocarp, the amounts of reducing sugars and soluble solids varied significantly being higher at the point of insertion of the nut. There was also a significant difference in the amount of ascorbic acid between the periphery (243.1 mg/100 ml) and center (204.5 mg/100 ml), the amount on the periphery always being higher than in the center. Maia et al. (46) found that, as the cashew matured, there was an increase in the amount of ascorbic acid from 147.2 mg/100 g for the immature cashew apple to 213.3 mg/100 g for mature cashew. The same authors also reported that the nut comprised 8 percent--juice 77 percent and the fibrous residue 15 percent of the total cashew. Pereira et al. (63) found the following values for cashew apple from Portuguese Guine:

Density	1.0189
pH	4.35
	%
Dry matter	5.14
Total acidity	0.210
Total sugar as glucose	1.98
Reducing sugars	1.60
Tannic substances	1.60
Total N	0.034
Lipids	0.021
Ash	0.160
Water and volatiles	94.79

Ventura and Lima (81) determined the free amino acid composition of cashew apple by paper chromatography and found aspartic acid, asparagine, glutamic acid, serine, glycine, threonine, alanine, valine, proline, tryptophan and leucine besides four unidentified compounds which reacted with ninhydrin.

Cashew Nut

The whole cashew nut which attached to the end of cashew apple, the peduncle or hypocarp, is about 3 cm long and gray-brown in color. It is shaped more like a kidney than a heart as implied in the generic name. The whole cashew nut is comprised of a shell (epicarp, mesocarp and endocarp) (69.14 percent), an edible kernel (27.96 percent) and the testa or membrane of the shell (2.90 percent) (44). The chemical composition of cashew nut from Fortaleza (Brazil) is (45):

Protein	22.10%
Ether extract	46.21%
Ash	2.51%
Moisture	5.26%
Total carbohydrates	23.93%
	(mg/100 g)
Calcium	56.69
P_2O_5	1177.5
Iron	6.34

Oliveira (58) made a comparison of the chemical composition of nuts from several locations (Table 3).

Table 3. Chemical Analysis Comparison of Shell, Kernel, and Testa of Cashew Nuts from Various Areas.

Determinations	Area				
	Guine	Senegal	Cape-Verde	Angola	Mozambique
			(%)		
Shell:					
Ether extract	36.21	35.34	32.01	34.20	38.26
Ash	1.24	1.37	1.12	1.32	1.32
Celulose	16.50	15.28	19.19	17.14	15.48
Protein (N x 6.25)	2.81	3.33	3.00	3.53	2.19
Non-nitrogen extractives	43.24	44.51	44.68	44.85	42.80
Testa:					
Ether extract	3.51	9.5	--	7.96	6.68
Ash	1.42	1.53	--	1.64	1.73
Celulose	11.81	11.19	--	10.67	10.44
Protein (N x 6.25)	11.75	12.98	--	12.29	13.22
Non-nitrogen extractives	71.51	64.69	--	67.45	66.97
Kernel:					
Ether extract	45.32	49.28	48.45	49.55	48.03
Ash	2.63	2.61	2.31	2.78	2.61
Celulose	1.23	0.70	0.80	0.92	1.20
Protein (N x 6.25)	20.25	18.88	21.19	22.28	21.29
Non-nitrogen extractives	30.57	27.02	27.25	24.48	25.33

Subramanian, Rao and Srinivasan (77) determined the amino acid composition of cashew nut globulin by paper chromatography. The composition reported by these authors is:

Amino Acid	(g/100 g of protein)
Cystine	1.02
Lysine	3.32
Histidine	1.81
Arginine	10.30
Aspartic acid	10.78
Serine	5.76
Glycine	5.33
Glutamic acid	28.0
Threonine	2.78
Alanine	3.18
Tyrosine	3.20
Valine	4.53
Methionine	1.30
Phenylalanine	4.35
Isoleucine-Leucine	11.93

The nitrogen content of protein on a moisture and ash free basis was 18.9 percent.

A new globulin protein for which the name "anacardein" is suggested was isolated from cashew nuts in yields amounting 17-18 percent of the fat free seed. The protein has been analyzed by nitrogen distribution and shown to be a biologically complete protein. Cystine was

determined colorimetrically and tyrosine by bromination. The "anacardein" was found to be comprised of carbon (50.41 percent), hydrogen (7.32 percent), nitrogen (19.30 percent), sulfur (0.78 percent) and oxygen (22.19 percent) (9).

Mitchell and Beadles (50) compared the true digestibility and the biological value of protein from beef round and five nuts (Brazil, cashew, almond, filbert and English walnut) using growing albino rats as subjects. Beef protein was superior to all of the nut proteins studied in both particulars, although the cashew nut was not greatly inferior with a digestibility of 96 percent and biological value of 72 compared with a digestibility of 100 percent and biological value of 75.78 for beef. All other nuts exhibited biological values ranging from 50 to 60 percent. Guimaraes and Pechnik (26) in another observation concerning the cashew nut food value, carried out biological experiments with white rats which revealed that the protein of cashew nut was superior in nutritive value to casein. Improvement was noted when cashew nut protein was supplemented with methionine. Addition of lysine and isoleucine had no appreciable effect. These authors concluded that the biological value of "anacardein" the principal cashew nut protein is very close to that of animal origin, thus confirming the work by Mitchell and Beadles (50). Cashew nut protein biological value was also studied by DeMoura Campos (10). He reported that cashew nuts have 20.92 percent total protein with a biological value of 77.2 and digestibility 93.3 percent. Roasted cashew nut (40 percent of the diet)

produced good growth in white rats, and corrected the deficiency due to a diet with poor protein (dry pea 20 percent).

Cashew nut fatty acid composition has been studied by several investigators (4, 32, 61) (Table 4). It can be seen that oleic acid is the major fatty acid in cashew nut. It is essential to recognize that this acid is undoubtedly the most widespread of all natural fatty acids in many fats and oils. It forms more than 30 percent of the total fatty acids and has been found in all natural fats or phosphatides (29).

Jacqmain (32) gives results found by Patel, Sudborough and Cruz in 1923. The results found by Jacqmain (32) are based on the quantitative determination by fractional distillation of methyl esters and by spectrophotometry. The results presented by Barroso et al. (4) were based on GLC of methyl esters. The results found by Pereira and Pereira (61) are also based on GLC of the methyl esters using diethylene glycol succinate and chromosorb W column. These authors (61) were the first to report the presence of palmitoleic acid in cashew nut.

Jacqmain (32) studied the cashew nut from Portuguese East Africa. He reported that it contained 0.32 percent unsaponifiable matter. He also studied the fatty acid composition, some aspects of unsaponifiable matter and the CNSL. The major sterol in the unsaponifiable matter was beta-sitosterol. Total tocopherols comprised 5.3 percent, hydrocarbons 11.0 percent (21.2 percent squalene) and 2.3 percent unidentified compounds.

Table 4. Fatty Acid Composition of Cashew Nut Oil Reported by Several Authors.

Fatty Acid	Reference (32)	(32)	(4)	(61)
		(%)		
Saturated:				
Myristic	0.2	--	--	--
Palmitic	11.5	6.4	7.5	14.0
Stearic	4.7	11.2	4.5	9.0
Arachidic	4.6	--	Tr	1.0
Lignoceric	--	0.5	--	--
Unsaturated:				
Palmitoleic	--	--	--	2.0
Oleic	59.7	73.8	73.7	59.0
Linoleic	18.1	7.7	14.3	15.0
Linolenic	1.2	--	Tr	--

Cashew Kernel Testa

Cashew nut kernel is covered with a thin reddish-brown skin or testa. The testa comprises 2.9 percent of the whole cashew nut (45), and has been reported to be a good source of catechol-type tannins (42). In normal cashew nut processing, a small percentage of the nuts develop bluish-black patches on the surface during the moisture conditioning step. Such nuts are not marketed although their taste is unaffected. Because testa are in close contact with the kernel, the water soluble testa constituents may reach the nut. Using two-dimensional paper chromatography, Mathew and Parpia (48) determined the polyphenols of cashew nut testa. Presence of (+) catechin and (-) epicatechin as the major polyphenols has been confirmed by co-chromatography with authentic samples. The characteristic bluish-black discoloration noticed in cashew nuts has been identified as an iron-polyphenol complex formed during processing. The polyphenols were found to be derived from the testa (48).

Cashew Nut Processing

The following operations are generally accomplished in the cashew nut processing.

Harvesting

Cashews are harvested manually, the nut removed and sold to processing plants. The nuts are sun dried to a moisture content of 6 to 8 percent and then stored.

Selection

The nuts are selected according to small, medium and large sizes. This selection is very important due to the variation in nut size. The main purpose of the selection is to facilitate shelling.

Shelling

The nuts are autoclaved at 0.703 kg/cm^2 pressure for 10 to 20 minutes depending on the nut size. They are then cooled to room temperature (26^oC) for 24 hours. Following this process, the nuts become rigid and friable and are ready for cutting. This operation is quite difficult since cutting is accomplished one at a time with a great amount of manual operation. In shelling, it is important to minimize breaking the kernel or contaminating it with CNSL. Broken kernels command a lower price (15-30 percent) than do whole nuts (18). The whole nut is placed between knives and the shell opened longitudinally in two halves. Since the cutting is done by manual machines, work has been directed toward completely mechanizing this operation. Esteves (17) reported tests carried out on a cashew nut shelling machine. The machine yielded 30 cashew nuts per minute or about 72 kg per day (8 hours) while manual shelling had a yield of 19-21 and 28-35 kg in the same period of time. The advantages and disadvantages of the machine are outlined by the author (17). Esteves (19) gives consideration to the problem of cashew in Mozambique including shelling procedures, statements of the conditions for industrialization and mechanical solutions. Esteves (18) gives a description based upon the available information about the cashew nut

shelling machine, "Oltremare," and gives an analysis of its mechanical and technological behavior during experiments.

Peeling

After shell removal, the kernels are placed in ovens at temperatures between 70-100°C. Kernel drying helps in easy removal of the outer skin or testa. Dry heat is avoided. The testa or peel is removed in giratory cylinders.

Classification

Classification is based on the removal of broken kernels or kernels with adhering peel and separation by size and color. Color separation is done electronically resulting in a significant savings in labor. Namburidi and Lakshminarayana (56) in studying improvement of cashew nut processing, found that grading cashew nuts before processing reduced broken kernel. Easy shelling, high recovery of shell liquid and low kernel moisture were achieved by steam conditioning. Blue discoloration was totally eliminated. Properly controlled dryer use can therefore increase whole, white kernel production. Grading determines product market value. Processed kernels are usually graded into "wholes" which are uniform in color and size; halves; broken consisting of broken kernels, and "spoilts" or rejects consisting of charred, discolored and broken kernels. The first two grades are usually exported (31). Graded nuts are packed under nitrogen or carbon dioxide. Kernels may also be roasted in vegetable oil, salted and packed.

Cashew nut processing varies between India, Brazil and Africa with the major variation being in the shelling operation.

Storage

Cashew nuts can be stored at room temperature (26°C) for a considerable period of time. Kernels from which the reddish-brown skin have not been removed can be stored longer (34). Cashew nuts packed under an inert atmosphere have a storage life greater than one year. Prasad, Kapur and Mathur (64, 65) suggest that rancidity development in the oil used for roasting causes initial deterioration in roasted cashew kernel quality. They also found that the optimum amount of oil for 200 g kernel was 7.5 g with the roasting medium being added at the commencement of the roasting operation. Another conclusion was that roasted and salted kernels could be stored without any deterioration for six months when refined groundnut oil was used as the roasting medium.

The incidence and types of cashew nut microflora during various processing stages has been enumerated (38). A feature of practical significance was complete absence of coliforms, putrefactives and pathogens at any stage of the labor intensive processing procedures. Mold infestation of foods may result in the production of certain metabolites termed "mycotoxins." There are wide species differences in the pathological disorders caused by mycotoxin ingestion. Aflatoxin production in cashew nut by Aspergillus flavus and other types of molds is of concern to exporters since some countries have instituted limits for aflatoxin content (39).

Oil-Bath Process

This is a process used in India. Graded nuts are soaked in water and steamed for 8-10 minutes. After steaming or soaking, the nuts are passed through a bath of CNSL maintained at temperature about 160°C at uniform rate. Because of the high temperature and the presence of moisture the liquid bearing cells burst and the extruded CNSL passes into the bath and at the same time the nuts are ready for shelling. After passing through the bath, the nuts are centrifuged to remove excess CNSL and then passed to the shelling section where the kernels are removed (31, 34, 56). It is claimed that this process gives higher yields of CNSL (53).

Cashew Nut Shell Liquid (CNSL)

CNSL is one of the few major economic natural sources of phenols (53). It occurs in the hard nut shell which is about 0.3 cm thick. The shell has a honeycomb-like structure on the inside. It consists of two layers with an oily liquid between them. The outer layer, which is smooth surfaced, is thin and tough. The inner layer is hard. CNSL is a viscous vesicant liquid rich in natural phenols. Its principal constituent is anacardic acid an ortho-hydroxybenzoic acid with an unsaturated side chain and appears as a brown crystalline substance. It also contains cardol, a dark brown phenolic oil. Both are toxic and irritating producing blisters similar to those caused by poison ivy (85). Cardol, the irritating principle in CNSL, is a chemical relative of uroshiol, which is the irritating principal in poison ivy (85).

According to Jacqmain (32), the first work on CNSL was by Staedeler in 1847 who isolated two principal constituents. One possessing acidic characteristics, was called anacardic acid and represented 90 percent of the total liquid. The other, representing 10 percent, was a phenolic compound called cardol. The composition varies according to the heat treatment given to the liquid. Marketed CNSL is rich in anacardol originated from decarboxylation of anacardic acid. This decarboxylation is promoted by heating the product during industrialization. Heating also changes the degree of unsaturation (82). Anacardic acid is easily decarboxylated to anacardol by heating (83).

The phenolic liquid of cashew shell has been utilized by Africans and Indians for a considerable period of time in their pharmacopea for treatment of skin diseases (82). The interest of CNSL by industrialized countries is due to its several applications. Because of its high molecular weight phenolic compounds and its great capacity for polymerization, it has found many applications in industry (82). Distillation and polymerization of the oil yields materials highly resistant to the action of acids, alkalis, solvents and oils. Resins obtained by condensation of the shell liquid with formalin in linseed or tung oil, are used in the preparation of laminating varnishes and baking enamels. They also have insulating properties of special value in electrical machinery, transformers, etc. Many processes have been patented for the extraction, polymerization and distillation of CNSL to obtain purified cardanol free of darkening compounds and more readily soluble in drying oils (53).

In order to have an assured market, it is necessary that CNSL present uniform characteristics. Vilar (82) presents characteristic values of good acceptability regarding CNSL.

Maia, Holanda and Martins (45) analyzed cashew nuts from different localities in the coastal zone of Ceara (Brazil). Percentages of CNSL, refractive index, iodine number, moisture and volatiles were determined in the shell liquid.

Shell liquid extraction can be done in the following way: shells from the shelling operation are subjected to pressure expelling. The shells are then extracted with a solvent such as hexane. The CNSL extracted is then decarboxylated, filtered or decanted and packaged for shipment. The final product must be within the standards required by the international market. Another way to extract the oil is by the oil-bath process previously described. In an industrial unit, CNSL comprises 7.5-10 percent of the total nut revenue (82).

Cashew Apple

The cashew apple, peduncle, false fruit or hypocarp has several utilizations. It may be eaten fresh or preserved in several ways such as: in syrup, as puree, juice (cloudy or clear), jam, wine, crystallized, dried, etc. Pereira et al. (63) gives a description of several products made from cashew apple. In Costa Rica, Brazil, the Philippine Islands and India, cashew wine is bottled commercially (53). The most important product made from cashew apple is the cloudy juice which may be preserved by heat or by use of chemical additives (79). Another important product is cashew puree.

Studies on cold storage of cashew apple have been reported by Singh and Mathur (73). They found that optimum conditions were storage at 0-2°C and a relative humidity of 85-90 percent. The approximate storage life is five weeks. Pereira and Graca (62) found that when liquid or freeze dried cashew apple juice was stored at 0-2°C for one year, a marked decrease in ascorbic acid was detected. There was a slight decrease in ascorbic acid during a freeze-drying process.

Economic Importance

Cashew kernels rank second only to almonds among nine nuts that are of importance in world trade (53). In recent years, cashew nut production has attained an annual world market valued at 65 million dollars (67). Cashew nut processing is an agro-based, labor intensive industry (38). During the past 40 to 50 years, Indian cashew industry has had no competition in the world market. This situation is changing, however, with competition from Africa (Mozambique and Tanzania) where mechanization is being introduced (56, 67) and from Brazil.

In 1966, the offering of kernels in the international market was: India 47,957,000 kg; Mozambique 5,400,000 kg; Brazil 1,790,000 kg; others 1,668,000 kg (5, 68). Although the current contribution of Brazil is small, its potentialities are great.

African countries supply a large amount of raw nuts to India. It is interesting to note that 60-70 percent of the nuts processed in India are imported from Mozambique, Tanzania and Quenia. Mozambique contributes 55 percent and Tanzania 43.1 percent (68). In Africa,

cashew nuts are produced mainly in Tanzania, Kenya and Portuguese East Africa (53). In 1967, India produced 81,720,000 kg; Mozambique 169,600,000 kg; Tanzania 80,300,000 kg; and Quenia 12,000,000 kg (68).

According to Barroso (5), cashew nut production (raw nut) in Northeast Brazil represented more than 90 percent of Brazilian production. The cashew tree is found in almost all states in Brazil with the exception of Parana, Santa Catarina and Rio Grande do Sul (79). It is estimated that annual cashew production in Northeastern Brazil is 400,000,000 kg with the nut comprising ten percent and the false fruit 90 percent of the total (79). In 1966, the cultivated area in Brazil was 73,472 hectares with 46,148 hectares being in the state of Ceara (68). The principal producers of cashew nut in Brazil are the states of Ceara and Pernambuco. The 1967 production of 24,180,000 kg was derived from these two states at 16,750,000 kg and 3,783,000 kg, respectively (5).

The principal cashew nut importers from Brazil are the United States and Argentine. Importers from India are United States, Russia and England (68).

When the cashew nut was first introduced, it was promoted as a substitute for the more costly almond (85). Confectionery and baking industry incorporated the kernel in numerous varieties of candies, chocolates, mixed nut meats, cakes, biscuits and cereals. Roasted and salted kernels proved to be a favorite for the palate (85).

Cashew nut production from the largest producers in 1966 was (68):

Brazil	13,700,000 kg
India (produced)	77,200,000 kg
(imported)	140,800,000 kg
Mozambique (produced)	99,600,000 kg
(exported)	69,900,000 kg
Tanzania (produced)	80,700,000 kg
(exported)	71,100,000 kg
Kenya (produced)	6,100,000 kg
(exported)	5,600,000 kg

The other important cashew product, CNSL, had the following 1966 exportation values:

India	11,600,000 kg
Mozambique	2,100,000 kg
Brazil	1,100,000 kg

Demand for CNSL is mainly from the United States, England and Japan (68).

MATERIALS AND METHODS

Cashew Nut

In this work, unroasted cashew nuts samples (shelled and unshelled) were obtained from a nut processing plant in Fortaleza-Ceara, Brazil. The dry roasted samples were purchased from food markets in Tucson, Arizona.

The following sequence of events were involved in the preparation of the unroasted nuts:

1. Harvesting. The nuts were received from cashew plantation areas or from processing plants (1972-1973 harvest).
2. Drying. After pedunculum removal, the nuts were placed in layers about 5 cm deep for sun drying to a moisture content of 6-8 percent.
3. Storage. After drying, the nuts were placed in storage rooms at room temperature.
4. Classification. The nuts were selected according to size (large 4 cm, medium 3 cm, small 2 cm) in order to facilitate the future cutting operation.
5. Shelling. Following classification, the nuts were autoclaved (0.703 Kg/cm^2 for ten minutes). After this treatment, the nuts were cooled to room temperature (26°C) for 24 hours, followed by cutting with manual machines to separate the shells from the

kernels. The nuts prepared in this way have a thin reddish-brown testa on the outside which protects the kernel. They were sent to Tucson via air mail. The other group of nuts (unshelled) was sent after the sun drying process.

Cashew Apple Juice

The cashew apple juice used in this work was prepared in a food processing plant in Ceara-Brazil. The following sequence of events were involved in cashew juice preparation:

1. Washing. Cashews were washed with water at room temperature. After washing, the nuts were removed.
2. Selection. Peduncles were selected according to proper appearance and absence of defects.
3. Extraction. Cashews were passed through a desintegrator, and the juice extracted in an extractor with helicoidal spindles.
4. Pre-heating. The extracted juice was pre-heated in a tube heat exchanger (75^{o}C for two minutes) coupled with a deaerator (600 mm Hg of vacuum).
5. Homogenization. Juice was homogenized at a piston pressure of 100 atm.
6. Heat treatment. Heat treatment was conducted in a plate heat exchanger at 98^{o}C for 96 seconds. The hot juice was filled in 160 ml glass bottles followed by closing and cooling in the bottles with water to 26^{o}C. Several bottles of cashew apple juice prepared in this way were sent to Tucson via air mail.

Cashew Apple Juice Lipid Extraction

Lipid extraction was accomplished according to the method of Bligh and Dyer (6) with certain modifications. One hundred grams of juice was blended with 100 ml chloroform and 200 ml methanol for two minutes at room temperature. To this mixture, 100 ml chloroform was added and the blending continued for another 30 seconds. To this mixture, 100 ml water was added and blended for 30 seconds. The homogenate was filtered through Whatman paper Number 1 on a Buchner funnel under suction. The filtrate was transferred to a separatory funnel, allowed to separate into layers and the chloroform layer removed. Chloroform was evaporated to a small volume (about 5 ml) under vacuum at $45^{\circ}C$, and the lipid stored in sealed containers under nitrogen atmosphere at $0^{\circ}C$ until analyzed. The juice lipids extracted were analyzed for phospholipids, glycolipids and fatty acid composition.

Cashew Nut Lipid Extraction

Cashew nut lipid extraction was performed by an adaptation of the Bligh and Dyer method (6). A 10 g sample was homogenized in a blender for two minutes with 50 ml chloroform, 100 ml methanol and 40 ml water. To this mixture was added 50 ml chloroform and after blending for 30 seconds, 50 ml water was added and blending continued for another 30 seconds. The homogenate was filtered through Whatman filter paper Number 1 on a Buchner funnel under suction. The filtrate was transferred to a separatory funnel and the phases allowed to separate. The chloroform layer contains the purified lipids. For total lipid quantitative estimation, the filtrate was transferred to a 250 ml graduated cylinder

and after allowing the complete separation and clarification, the volume of chloroform layer was recorded and the other layer removed. A lipid extract aliquot was evaporated to dryness under vacuum using a rotatory evaporator and the residual lipid weight determined. Sample lipid weight was calculated as follows:

$$\text{Total lipid} = \frac{\text{Lipid aliquot weight x Chloroform layer volume}}{\text{Aliquot volume}}$$

In this extraction method, it is important that the volumes of chloroform, methanol and water before and after dilution be kept in the proportions 1:2:0.8 and 2:2:1.8, respectively. These ratios represent the total volumes present in the ternary systems, including the water present in the samples.

Lipids obtained from cashew nuts were analyzed for fatty acid composition, phospholipids, glycolipids and unsaponifiable matter.

Cashew Kernel Testa

Cashew nut kernel is covered with a thin reddish-brown skin or testa which is removed during processing.

Cashew Kernel Testa Lipid Extraction

Lipids were extracted in the same way as for cashew nut previously described. Testa lipids were analyzed for fatty acid composition.

Cashew Apple

Pedunculum material was obtained from two different trees at the School of Agronomy in Fortaleza-Ceara-Brazil. These trees, one giving

red cashew and the other yellow, have a history of producing sweet tasting rather than rancid or sour cashew apples. The immature cashew apples chosen had not quite attained full size. After picking, the peduncles were frozen (-10°C) with the nuts still attached. Peeling was accomplished by submerging the completely frozen cashew apple in water (26°C) for 2-3 seconds to thaw the peel. The peel was removed with a knife and tweezers to prevent hand oil contamination.

Cashew Apple Lipid Extraction

Lipid extraction was accomplished by an adaptation of the Bligh and Dyer method (6).

Varying material amounts were available for lipid extraction. The same proportional solvent volume was used for extraction of each batch with the exception that for red mature peduncle in which double the amount of solvent was used. The following procedure was used: the material was homogenized in a blender, and weights were obtained. The homogenized material was blended with 100 ml chloroform and 200 ml methanol for two minutes. After adding another 100 ml chloroform, the mixture was blended for 30 seconds. Following the addition of 100 ml water and blending for 30 seconds, the total mixture was poured directly into a 500 ml separatory funnel. After separation and clarification (1-2 hours), the chloroform layer was removed. One additional extraction of the residue with 100 ml chloroform was made which removed the bulk of the yellow pigment. Before evaporating, approximately 0.1 g hydroquinone was added in 0.5 ml hexane solution. Chloroform and hexane were removed in a rotatory evaporator at 50°C. The samples prepared in

this way were sealed and sent to Tucson by air mail. After receiving the samples, they were evaporated to small volume (1 ml) and dried with anhydrous sodium sulfate. Lipids were extracted from various types or portions of cashew apples. They were red mature peel and pulp, red immature peel and pulp, yellow mature peel and pulp, yellow immature pulp. These samples were studied for fatty acid composition.

Lipid Fractionation by Column Chromatography

Extracted lipids from cashew nuts (unroasted), cashew apple juice were separated by silicic acid column chromatography according to the method of Rouser et al. (71).

Silicic acid (100 mesh, Malinkrodt) was activated overnight at 110°C and cooled in a dessicator to room temperature. The silicic acid was transferred in redistilled chloroform to chromatographic tubes (2.5 internal diameter, 26 cm long) equipped with a teflon stopcock and glass wool plugs for adsorbent retention. After filling the column to a depth 5 cm, a 100-200 mg sample was applied in 5 ml chloroform. The column volume was 20 ml. The eluting solvents were: (a) chloroform (8-column volumes) for neutral lipids, (b) acetone (40-column volumes) for glycolipids, and (c) methanol (10-column volumes) for phospholipids. These solvents were removed by rotatory vacuum evaporator at 45°C. The fractions were transferred to tared vials, dried under nitrogen and under vacuum and weighed to estimate the total lipid extract gross composition. After evaporation, the phospholipid fraction was taken up in a small volume of methanol-chloroform (1:2). The glycolipid fraction was taken up in small volume of acetone. Because of the small amounts

available, the phospholipid fraction from cashew apple juice was studied only at the qualitative level (phospholipids and glycolipids). Each of the three lipid fractions from the nuts were analyzed for fatty acid composition. No detectable levels of phosphorus were found in the neutral lipid and glycolipid fractions based on the reaction with molybdenum blue spray which is specific for phospholipids (11).

Phospholipids

Phospholipids were separated by a two dimensional TLC procedure (59, 60) using a solvent system consisting of chloroform-methanol-water-28 percent aqueous ammonium (65:35:4:0.5 v/v) in the first dimension and chloroform-acetone-methanol-acetic acid-water (50:20:10:10:5 v/v) in the second dimension. Several other solvent systems for two dimensional TLC were compared but the one adopted was found to give the best resolution.

For TLC separation, the phospholipid fraction was applied to silica gel HF precoated plates (250 microns thick), (Uniplate, Analtech, Inc.). The plates were activated one hour at 110^{o}C before sample application and subjected to ascending chromatography in a chamber previously saturated by using a piece of Whatman Number 1 filter paper around the developing tank. The solvent was allowed to rise to within 2 cm of the top of the adsorbent. The average running time was one hour for each direction.

After removal from the chamber, the plates were air dried. For phospholipid phosphorous analysis, the plates were exposed to iodine vapor and the spots immediately outlined by a needle point. Other detection methods included spraying with the following reagents:

1. Ninhydrin spray (Applied Science Lab., Inc.)--0.2 percent in n-butanol-aqueous acetic acid 10 percent 95:5 for phospholipids containing free amino groups such as phosphatidyl ethanolamine and phosphatidyl serine. After spraying, the plates were heated for five minutes at 100°C, and the spots which showed positive reaction to this spray gave a purple color.
2. Rhodamine 6 G (Applied Science Lab., Inc.)--this spray is prepared by dissolving 1 mg rhodamine 6 G in 100 ml acetone (76). After chromatogram spraying was observed under long wave UV light, the lipids gave fluorescence.
3. 2', 7'-dichlorofluorescein (Applied Science Lab., Inc.)--this is a fluorescent indicator for saturated and unsaturated lipids (76). This spray consisted of 0.2 percent ethanolic solution of 2', 7'-dichlorofluorescein. The plates were sprayed with this reagent and inspected under long wave UV light.
4. Molybdenum blue reagent (Applied Science Lab., Inc.)--this is a specific spray for phospholipids (11). This spray is prepared in the following way: Solution I--to one liter of 25N H_2SO_4, 40.11 g of MoO_3 is added and the mixture is boiled gently until the MoO_3 is dissolved. Solution II--to 500 ml of solution I, 1.78 g of powdered molybdenum is added and the mixture boiled gently for 15 minutes. The solution is cooled and decanted from any residue that may be present. Molybdenum spray--equal volumes of solutions I and II are mixed and the combination is mixed with two volumes of water. The final solution is greenish-yellow

in color. If too little water is used, it will be blue; if too much, yellow. The spray is stable for months.

The plates were sprayed lightly until the adsorbent was uniformly damp. Compounds containing phosphate ester show up immediately as blue spots on a white or light blue-grey background. The intensity of the color increases on standing. After several hours, the background darkens to a deep blue and the spots became obscured.

For the detection of phospholipids whose fatty acids were to be analyzed by GLC, the plates were sprayed with rhodamine 6 G or with 2', 7'-dichlorofluorescein and inspected under UV light. The observed spots were then outlined with a needle point.

Successive sprays were used in some plates in the following way: the plates were first sprayed with rhodamine 6 G and the spots marked. After the plate was completely dried it was sprayed with ninhydrin and the spots which gave positive reaction were noted. Finally the plate was treated with the molybdenum spray. Individual phospholipids were identified by means of these selective spray reagents and by comparing Rf values with those of pure reference compounds. All reference phospholipids were obtained from Applied Science Lab., Inc.

Analysis of Phospholipid Phosphorus

Phosphorus was determined by an adaptation of the method by Rouser, Siakotos and Fleischer (70). After the iodine had evaporated from the plate, an outlined spot was scraped directly into a 100 ml micro Kjeldahl digestion flask. The spots were first digested with

one ml nitric acid: sulfuric acid (3:1) for two hours at low temperature until frothing ceased. Next, one ml perchloric acid 70 percent was added and heat increased. Ashing was completed when only H_2SO_4 remains and the solution is clear. After digestion, the sample was cooled and deionized water was added to make a 10 ml volume. The tubes containing silica gel were centrifuged for 20 minutes. Phosphorous was determined in this fraction.

The relative amount of phosphorous in each spot was determined by using reagents supplied in a kit (Pierce Chemical Co.). The kit includes: phosphorous reagent A (reducing agent), reagent B (a buffer), reagent C (phosphorous standard--5 mg/100 ml). Components were added in the following exact order: 0.5 ml of sample was added in a clean spectrophotometer tube, 1.50 ml of reagent A was added and mixed, 0.50 ml of reagent C was then added. The mixture was allowed to stand for five minutes and the absorbance was determined at 690 nm in a Spectronic 20 spectrophotometer. Deionized water was used as a blank.

Analysis of Phospholipid Fatty Acids

After the conclusion of each TLC run, the plates were sprayed with rhodamine 6G. The two major spots were then scraped from the plate and eluted with chloroform: methanol (1:1) 50 ml. The solvent was evaporated to a small volume under a stream of nitrogen and kept in small vials at $-10^{o}C$.

Glycolipids

Glycolipids were separated by two-dimensional TLC in a manner similar to that described by Nichols (57). TLC plates (silica gel G)

were developed in the first direction with chloroform-methanol-7N ammonium hydroxide (65:30:4), followed by chloroform-methanol-acetic-acid-water (170:25:25:6) in the second direction. The detection methods included spraying with (a) Diphenylamine: 10 percent diphenylamine in 96 percent ethanol plus 100 ml concentrated HCl and 80 ml acetic acid. Blue grey spots on a light background indicate a positive test for glycolipids. The spray is sensitive to approximately five micrograms (47); (b) chromic-sulfuric acid solution (76) 5 g potassium dichromate dissolved in 100 ml 40 percent sulfuric acid; (c) exposure to iodine vapors.

Glycolipid components, separated by TLC, were identified by co-chromatography with pure standards (Supelco, Inc.) and from published information (23, 24, 57).

Preparation of Fatty Acid Methyl Esters

Fatty acid methyl esters were prepared from lipid extracted from the following material: nuts (dry roasted, unroasted shelled, and unroasted with shell), apple juice, apple peel and pulp from red and from yellow apple both mature and immature, testa. Fatty acids were also determined in the lipid fractions separated by silicic acid column chromatography, previously described as being obtained from samples of unroasted nuts in the two major phospholipids from cashew nut isolated by TLC.

Fatty acid methyl esters suitable for gas chromatography analysis were prepared in the following way (49): four ml of 0.5 N methanolic potassium hydroxide was added to approximately 100 mg lipid

material in a screw capped, graduated centrifuge tube. 0.5 ml benzene was added to improve lipid material solubility (52). The mixture was then heated for five minutes in a boiling water bath. Five ml BF_3-methanol 14 percent was then added to the reaction mixture and heated under the same conditions for an additional two minutes. Two volumes of saturated sodium chloride solution was added and the mixture transferred to a separatory funnel. Methyl esters were extracted from the water-saturated salt esterification mixture with ten ml hexane. The hexane layer was concentrated under a nitrogen stream and analyzed by GLC.

For fatty acid methyl ester preparation from cashew apple juice lipids two additional methods were used. In the first, a 0.2 ml portion of oil was dried under vacuum at 100°C for ten minutes. Five ml freshly prepared sodium methoxide was added to each dried sample to prepare the fatty acid methyl esters of the glycerides (41). The methyl esters were extracted with one ml hexane and stored at -10°C until analysis by GLC.

The other method used was acid catalyzed esterification using ten ml absolute methanol containing four percent concentrated sulfuric acid. A 100 mg lipid portion was heated with 10 ml methanolic solution in a water bath at 100°C for one hour (41). After cooling, the esters were extracted with 5 ml hexane and stored at -10°C until analysis by GLC.

Samples were injected into a Micro Tek (Micro Tek Instruments, Inc.) gas chromatograph model DSS 170 equipped with a dual flame ionization detector, two glass columns, (dimensions 0.4 cm internal diameter

x 1.65 m long) packed with 15 percent diethyl glycol succinate (DEGS) on 60-80 mesh Chromosorb W. Carrier gas used was Argon at 0.281 kg/cm^2 (flow rate--60 ml minute). Column temperature was 175°C and sample size was approximately 0.5 microliter. Ester identification was made by comparison with standard compounds injected under the same conditions. Relative amounts of each ester were determined by peak areas comparisons as calculated by an Infrotonic Model CRS-108 integrator (Infrotonic Corp., Houston, Texas).

Unsaponifiable Matter

All solvents used in this work were redistilled. For saponification and extraction a method was used based on section 26.071 of Official Methods of Analysis (2) and Eisner and Firestone (13).

Fifteen grams of cashew nut oil extracted as previously described was weighed and the following reagents were added: 150 ml absolute ethyl alcohol, 5 ml hydroquinone solution (30 mg hydroquinone in 1 ml absolute ethyl alcohol) and 10 ml potassium hydroxide solution (3 g KOH:2 ml H_2O). The mixture was refluxed for 90 minutes on a water bath under a stream of nitrogen.

The alcoholic soap mixture was transferred while stil warm to a 1000 ml separatory funnel containing 300 ml distilled water. The saponification flask was rinsed with 300 ml ethyl ether, the ethyl ether rinses transferred to the separatory funnel, the funnel shaken vigorously and the phases allowed to separate and clarify. The lower aqueous layer was removed and the ether layer was transferred into another separatory funnel containing 120 ml distilled water. Two more

aqueous layer extractions were made using 200 ml ethyl ether each. The combined ethyl ether extracts were gently rotated with 120 ml water (violent shaking at this stage may cause troublesome emulsions). The layers were allowed to separate and the aqueous layer drained. Two more washings using 120 ml water each were made.

The ether solution was washed three times with alternate 120 ml portions of 0.5 N aqueous potassium hydroxide and water followed by shaking vigorously each time. After the third potassium hydroxide treatment, the ether solution was successively washed with 120 ml portions of water until the washings were no longer alkaline to phenolphtalein (1 percent alcoholic).

The ether extract was evaporated under a nitrogen stream on a water bath at 45°C. After cooling, the extract was passed through a funnel containing glass wool and 15 g anhydrous sodium sulfate. The extract was then evaporated to dryness on a water bath under a nitrogen stream. The unsaponifiable matter was dried in a vacuum oven at 45°C to constant weight. The dried unsaponifiable matter was stored in sealed vials under nitrogen at -10°C.

Unsaponifiable Matter Fractionation

Unsaponifiable matter fractionation was accomplished by the method of Eisner and Firestone (13). Florisil (60-100 mesh, Floridin Co) was activated at 140°C for four hours and then cooled to room temperature. A one hundred gram portion was weighed into a round bottom flask, 10.5 ml water added and the mixture swirled so that water did not collect in one area (12). The flask was shaken vigorously for about 15

minutes until it felt cool (exothermic reaction stops). The Florisil was allowed to equilibrate overnight before use. For unsaponifiable matter separation, a glass column (2.5 cm x 30 cm long) was filled three-fourths with hexane, 30 g of Florisil was added using a vibrator to insure even packing. After all of the Florisil has been added, the excess hexane was drained through the stopcock until the hexane level was about 4 cm above the packing surface. A 2 cm layer of anhydrous sodium sulfate was added, the inner tube side washed down with hexane and solvent excess was drained until it reached the sodium sulfate layer. The samples (30 mg) were dissolved in 0.5 ml chloroform and transferred to the column top. The flask was rinsed with two 5 ml hexane portions and the elution carried out. The following elution sequence was used: fraction 1 (hydrocarbons) was eluted with 40 ml hexane; fraction 2 (hydrocarbons) was eluted with 120 ml hexane-ethyl ether (95:5); fraction 3 (aliphatic alcohols, tocopherols and triterpenoid alcohols) was eluted with 120 ml hexane-ethyl ether (85:15); fraction 4 (sterols) was eluted with 175 ml hexane-ethyl ether (70:30); fraction 5 (sterols) was eluted with 175 ml hexane-ethyl ether (50:50); fraction 6 (unidentified compounds) was eluted with 150 ml ethyl ether-methanol (98:2). For further analysis fractions 1 and 2 and fractions 4 and 5 were combined into one portion each.

Gas Liquid Chromatography (GLC)

A gas chromatograph Micro Tek (Micro Tek Instruments, Inc.) Model DSS 170, equipped with a dual flame ionization detector, two glass columns (with the dimensions 0.4 cm x 1.65 m) was used in this work.

The gas chromatographic columns were each packed with 3 percent SE 52 on chromosorb W, 60-80 mesh (Analabs) in small amounts using a vibrator and suction. The ends of the columns were filled with glass wool. Column conditioning was by heating for 24 hours at 295°C with 40 ml per minute argon flow. The columns were then silanized with hexamethyldisilazene (HMDS) (Pierce Chemical Inc.) by injecting three 0.5 microliter portions at one minute intervals. The gas flow was turned off and the temperature maintained at 295°C for 24 hours.

Each Florisil fraction was weighed and diluted in 0.5 ml chloroform. Trimethylsilyl derivatives preparation was done in the following way: 0.5 ml of each fraction (except hydrocarbons) was transferred to a vial and 0.5 ml N,O-Bis-Trimethylsilylacetamide (BSA) (Supelco Co., Inc.) and 20 microliters trimethylchlorosilane (TMCS) (Supelco, Inc.), were added, the vials tightly closed and the mixture allowed to stand for 30 minutes at room temperature. Aliquots of the samples were then injected directly into the gas chromatograph. Relative component amounts were determined by peak areas comparison calculated by an Infrotonic Model CRS-108 integrator (Infrotonic Corp., Houston, Texas).

The following conditions were used for programmed temperature runs: column temperature 180-280°C for alcohols and sterols and 200-280°C for hydrocarbons. The temperature of the detector and injection port was 295°C. The program rate was 5°C per minute. The carrier gas was argon at a flow rate of 60 ml per minute.

Standard Curves Preparation

A standard solution was prepared (5 mg per ml chloroform) for the following hydrocarbons: eicosane, tetracosane, octacosane, triacontane, and dotriacontane. A standard solution (7 mg per ml chloroform) was prepared for the following aliphatic alcohols: eicosyl, tetracosyl and hexacosyl (Analabs). The following tocopherols were used: d-alpha-tocopherol, d-gama-tocopherol, d-delta-tocopherol and d-beta-tocopherol (Supelco, Inc.). For sterol identification, campesterol, stigmasterol and beta-sitosterol solutions (25 mg per ml chloroform) were used. These standards were supplied by Dr. W. H. Kircher, Professor of Agricultural Biochemistry, The University of Arizona. Preparation of the TMSE derivatives for alcohols, tocopherols and sterols was done in the way as previously described.

These pure standards were gas chromatographed to obtain the respective retention times. Separate retention time plots versus carbon number were prepared on arithmetic graph paper for hydrocarbons and aliphatic alcohols. Unknown compounds were identified by extrapolation from the respective arithmetic plots and by comparison with other similar published works. Sterol identification was based on comparison with the chromatographic behavior of pure compounds. Triterpenoid alcohols and squalene were identified by comparison with similar published information.

Thin-Layer Chromatography (TLC)

An additional analysis of fraction 3 cashew nut tocopherol was performed by TLC. Silica gel G precoated plates 20 x 20 cm, 250 microns

thickness were used. Fraction 3 was applied at the bottom of the plates with solutions of alpha-tocopherol, beta-tocopherol, gamma-tocopherol and delta-tocopherol being spotted along the plate side as reference standards. The plates were developed in benzene-methanol (98:2 v/v) (16). After development, the plates were sprayed with rhodamine 6 G and viewed under UV light.

RESULTS AND DISCUSSION

Lipid Extraction

The method used for cashew (nut, juice, apple and testa) lipid extraction gave excellent results. Average lipid content values were 49 percent and 0.25 percent for cashew nut and apple, respectively.

Chloroform/methanol mixtures have been used widely as lipid extractants. The method of Bligh and Dyer (6) is based on the following hypothesis: on the basis of a chloroform-methanol-water phase diagram, optimum lipid extraction should result when tissue is homogenized with a chloroform and methanol mixture which when mixed with the water in the tissue would yield a monophasic solution. The resulting homogenate could then be diluted with water and/or chloroform to produce a biphasic system, the chloroform layer of which should contain the lipids and the methanol-water layer the non-lipids. Hence a purified lipid extract should then be obtained when the chloroform layer is isolated. The results obtained by the authors (6) confirmed their initial hypothesis that more lipids were extracted by monophasic layer mixtures than by those of the biphasic layer. They also compared this extraction method with that of Folch, Lees and Stanley (21) and AOAC (2). They found that the average lipid content was higher with the present method (6). The Folch et al. (21) method and the present method gave yields which were not significantly different. The extract from the former method, however, usually contained small amounts of non-lipid material.

Silicic Acid Column Chromatography

The most generally useful procedure for separation of the less polar or neutral lipids (eluted with chloroform) from the polar lipids was separation on silicic acid.

With lipid mixtures from some sources, the elution from a silicic acid column with a sequence of chloroform, acetone and methanol provides an essentially quantitative separation into three groups: less polar (so-called neutral lipids) lipids, glycolipids and phosphatides (72).

Silicic acid, sometimes called silica gel, has been the most widely used substance for column chromatography of lipids (69). Larger particle sizes are usually preferred since proper flow rates are difficult to obtain with fine particles.

By using silicic acid, it was possible to separate cashew lipids into three classes based on the work of Rouser et al. (71). These authors utilized silicic acid column chromatography for separation of brain and spinach lipids into neutral lipids, glycolipids and phospholipids.

The following values were obtained for cashew nut lipids after separation by silicic acid column: neutral lipids 95.5 percent, glycolipids 1.9 percent and phospholipids 1.7 percent. It can be observed that the polar lipid fraction constituted 3.6 percent of the total lipids. Recoveries from the column averaged 98.7 percent for cashew nut oil.

The values obtained for cashew apple juice lipids separation by silicic acid column chromatography were as follows: neutral lipids

72 percent, glycolipids 10.5 percent and phospholipids 2.4 percent. Total recovery was 85 percent.

Neutral or less polar lipid elution from silicic acid with chloroform is useful and very popular for the separation of the components (hydrocarbons, glycerides, sterols, sterols esters, etc.) from the polar lipids. The neutral lipid and glycolipid elutes showed no reaction to molybdenum reagent of Dittmer and Lester (11) which is specific for phospholipids.

In the majority of mature seeds, the quantitatively major lipid class is triglyceride, while phospholipids and glycolipids normally represent less than two percent of the total (30). Parsons and Patton (60) found that, cocoa bean lipids separated by silicic acid column chromatography, contained 98 percent neutral lipids and 1-2 percent polar lipids of which approximately 70 percent was glycolipids.

The isolation of phospholipids was also accomplished by TLC. The same number of spots positive to molybdenum spray of Dittmer and Lester (11) was detected by isolation by column or TLC. After spraying with 2,7-dichlorofluorescein, however, it was observed that there were more spots in the fraction isolated by TLC which were negative to the molybdenum reagent. These results indicate that the separation by column chromatography was more selective for the isolation of phospholipids in cashew nut oil.

Reagents such as 2',7'-dichlorofluorescein and rhodamine 6 G permit detection without chemical alteration of the lipids which may then be recovered by eluting relevant areas from the chromatograms.

Iodine is also a non-destructive reagent except that it can alter some fatty acids and is therefore not suitable for the location of lipids whose fatty acid composition is to be subsequently determined. Other reagents, including ninhydrin for detection of primary amines such as phosphatidyl ethanolamine (PE) and phosphatidyl serine (PS), and molybdenum spray for phospholipids are more specific and may give some information regarding lipid structure. These reagents are of variable sensitivity, however, and do not permit the recovery of unaltered lipids. Various reagents based on sulfuric acid are non-specific but are extremely sensitive, detecting as little as one microgram of lipid. These reagents may also give some information regarding the nature of the lipids (30).

Phospholipids

A representative two dimensional chromatogram of phospholipid separation from unroasted nuts gives nine components (Figure 1). Unknown phospholipids represented by X_1, X_2, X_3, X_4, and X_5 were positive to the specific molybdenum spray (11). Four phospholipids were identified as lysophosphatidyl choline (LPC), phosphatidylcholine (PC), phosphatidyl ethanolamine (PE) and phosphatidyl serine (PS). These spots showed similar behavior as the standards. PS and PE were also positive to ninhydrin spray. On the basis of other similar work, the spots 5, 6, and 8 could be tentatively identified as phosphatidyl glycerols (59). A similar distribution of phospholipids was found by Parsons and Patton (60) in cocoa bean phospholipids. These authors reported that PC contributed 36-40 percent of the phospholipids.

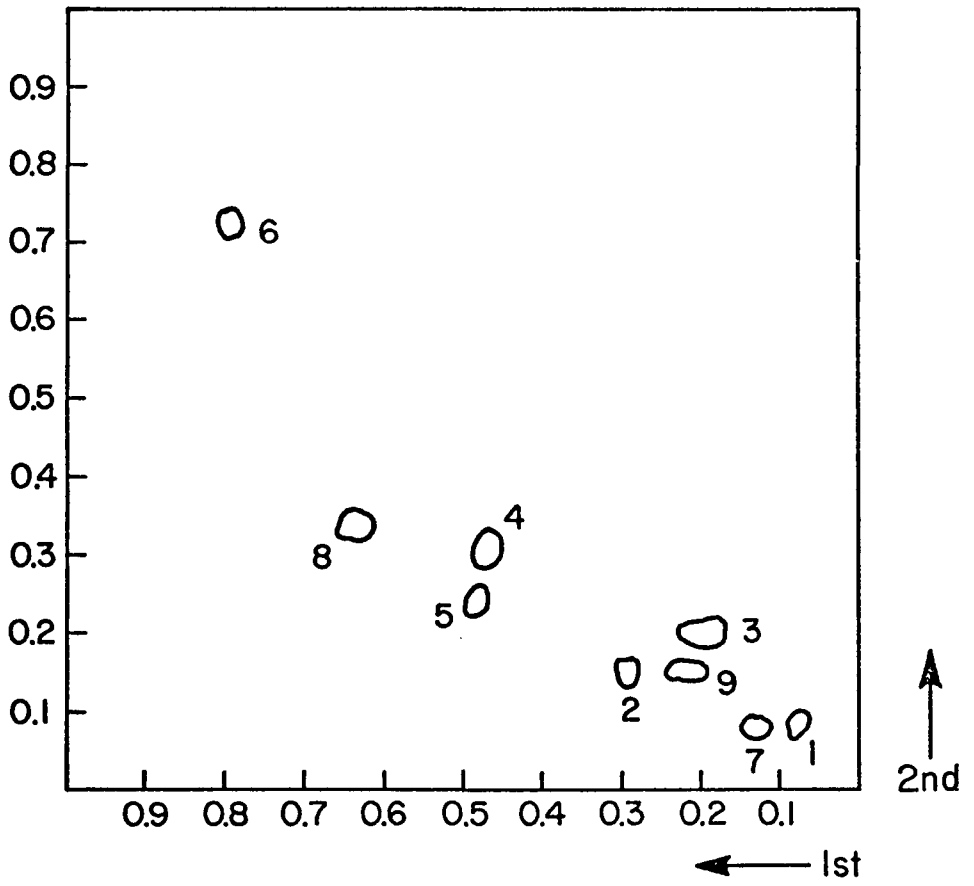

Figure 1. Typical Two-dimensional Chromatogram of Cashew Nut Oil Phospholipids. -- Silica gel HF. Development in horizontal direction from right to left with chloroform-methanol-water-28% aqueous ammonium (130:70:8:0.5) and in vertical direction with chloroform-acetone-methanol-acetic acid-water (100:40:20:20:10).

The major phospholipids in unroasted cashew nut are PC and PE which together contributed over 60 percent of the total (Table 5). The identified phospholipids accounted for 79.2 percent of the total.

Table 5. Relative Percentages of Phospholipids Isolated from Unroasted Cashew Nut.

Spot Number	Component	Proportion of Total (%)
1	LPC	5.1
2	PS	6.5
3	PC	53.6
4	PE	14.0
5	X_1	3.0
6	X_2	3.8
7	X_3	5.0
8	X_4	4.0
9	X_5	5.0

For further identification, spot number 3 was scraped from the plate and eluted with chloroform-methanol (1:1) and evaporated to small volume. An aliquot was spotted on a plate and developed in one direction with chloroform-methanol-7N NH_4OH. On the same plate, a phosphatidyl

ethanolamine standard was applied. Spot number 3 and the standard displayed similar behavior.

PC and PE have been found to be the major phospholipids in potato tubers and apples (23, 24), in cucumber and peppers (35), in orange, lemon and grapefruit (80).

A typical two-dimensional chromatogram of cashew apple juice phospholipids shows eleven spots positive to molybdenum spray (Figure 2). The following spots were identified: No. 3 LPC, No. 5 PC, No. 7 PS, No. 8 PE. Numbers 9, 10, and 11 were identified as phosphatidyl glycerols (23, 24, 60).

Eleven phospholipid types were established for cashew apple juice and nine for unroasted cashew nut. The phospholipids from cashew apple juice were not quantified.

A two-dimensional TLC obtained with standards, shows good separation with the solvent system chloroform-methanol-water-28 percent aqueous ammonium (130:70:8:0.5) in one direction followed by chloroform-acetone-methanol-acetic acid-water (100:40:20:20:10) in the second direction (Figure 3).

Methods developed for two-dimensional TLC separation of various animal tissue phospholipids give unsatisfactory results when applied to plant lipid separation. This is attributed to the differing nature and great complexity of plant lipids (57). One problem in phospholipid separation is the lack of sensitivity in one-dimensional TLC. Good separation of phospholipids using one-dimensional TLC was obtained by Skipski, Peterson and Barclay (74); however, one disadvantage of this

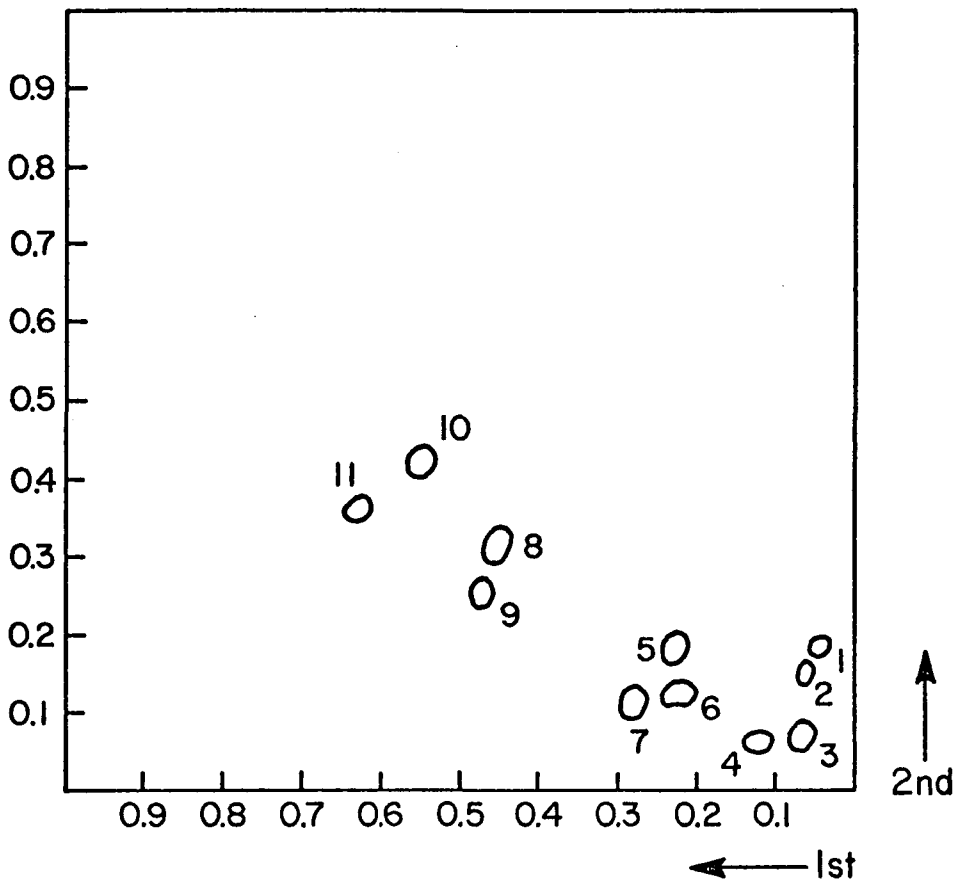

Figure 2. Typical Two-dimensional Chromatogram of Cashew Apple Juice Phospholipids. -- Silica gel HF. Development in horizontal direction from right to left with chloroform-methanol-water-28% aqueous ammonium (130:70:8:0.5 v/v) and in vertical direction with chloroform-acetone-methanol-acetic acid-water (100:40:20:20:10).

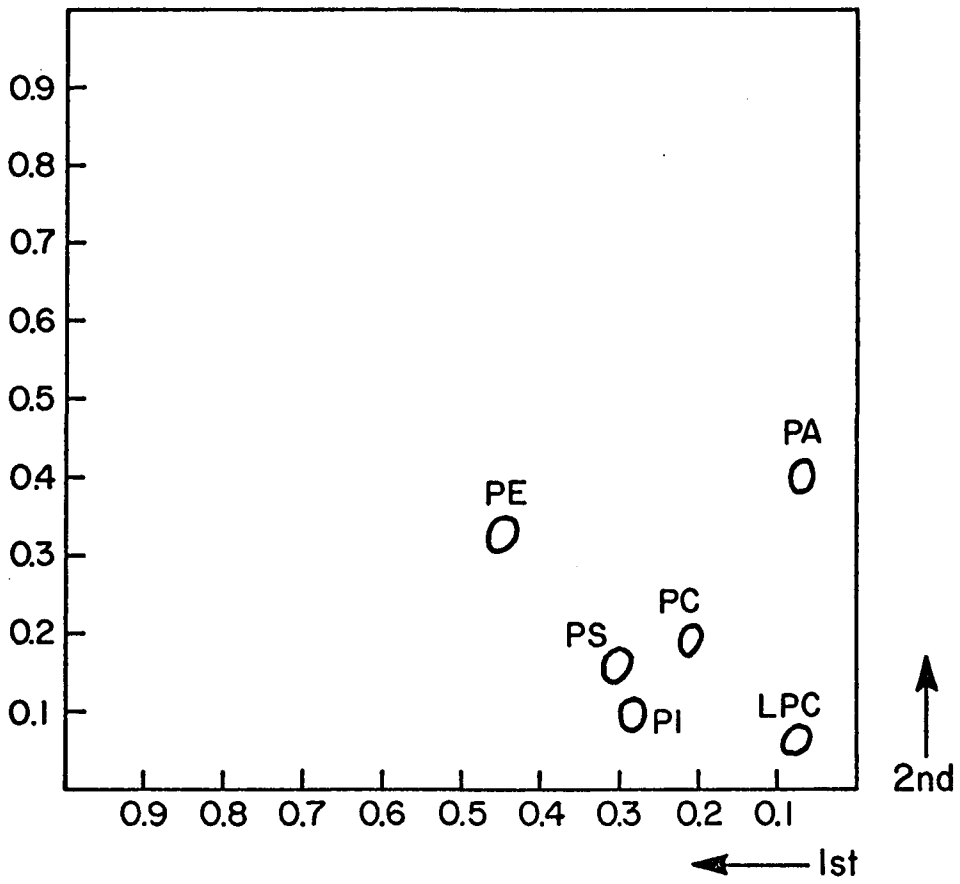

Figure 3. Typical Two-dimensional Chromatogram of Standard Phospholipids. -- (Conditions are the same as in Figure 1,)

method was that certain nitrogen free phospholipids (phosphatidic acid and cardiolipin) could not be differentiated. In two-dimensional TLC, some problems have been found in separating phosphatidyl inositol from phosphatidyl serine. A two-dimensional TLC system was developed by Parsons and Patton (59) which overcomes these difficulties.

Two-dimensional TLC with the solvent system used in this work was useful for quantitative study. With this solvent system, spread occurs during chromatography in the first direction but the spots are made compact in the second dimension or acidic system.

Phosphorus Analysis

Phosphorus analysis of the spots separated by TLC is based on the following principle: inorganic phosphate reacts with molybdic acid to form phosphomolybdate in acidic solution only. In alkaline solution, the Mo_6 of phosphomolybdate is reduced to Mo_5, molybdenum blue, by a mixture of sulfite and para-methylaminophenol. The developed color is stable for at least 30 minutes.

Fatty Acid Composition of Phospholipids

The major fatty acids in cashew PE and PC are palmitic, oleic and linoleic (Table 6). These fatty acids together represent 85.3 percent of the total in PE and 73.3 percent of the total in PC. Some differences in fatty acid distribution of these two phospholipids can be observed. PC shows a greater percentage of $C_{12:0}$, $C_{14:0}$ and $C_{18:0}$. PE shows a greater value for $C_{16:0}$, $C_{18:1}$ and $C_{18:2}$. PE shows 73.5 percent unsaturation and PC 62.6 percent. Comparable results were found in orange juice phospholipids (55).

Table 6. Major Component Fatty Acids in Phosphatidyl Ethanolamine and Phosphatidyl Choline Isolated from Unroasted Cashew Nut Oil.

Fatty Acid	PE	PC
	(%)	(%)
$C_{12:0}$	0.6	2.1
$C_{14:0}$	1.7	6.2
Unknown	1.4	4.6
$C_{16:0}$	16.7	15.8
$C_{16:1}$	2.9	2.4
$C_{16:2}$	0.2	0.4
$C_{18:0\ iso}$	2.6	2.3
$C_{18:0}$	3.5	5.5
$C_{18:1}$	57.0	49.5
$C_{18:2}$	11.5	8.0
$C_{18:3}$	1.8	2.3

Glycolipids

Glycolipid fraction components from cashew nut and cashew apple juice were separated by TLC (Figures 4 and 5). Spots were visualized and tentatively identified as follows: 1 = unknown; 2 = unknown; 3 = di-galactosyl diglyceride; 4 = cerebroside; 5 = sterol glycosides; 6 = mono-galactosyl diglyceride (MGDG); 7 = esterified steryl glycoside; 8 = unknown; 9 = unknown. Spots 3, 4 and 5 were identified by co-chromatography with pure standards. The other spots were identified by

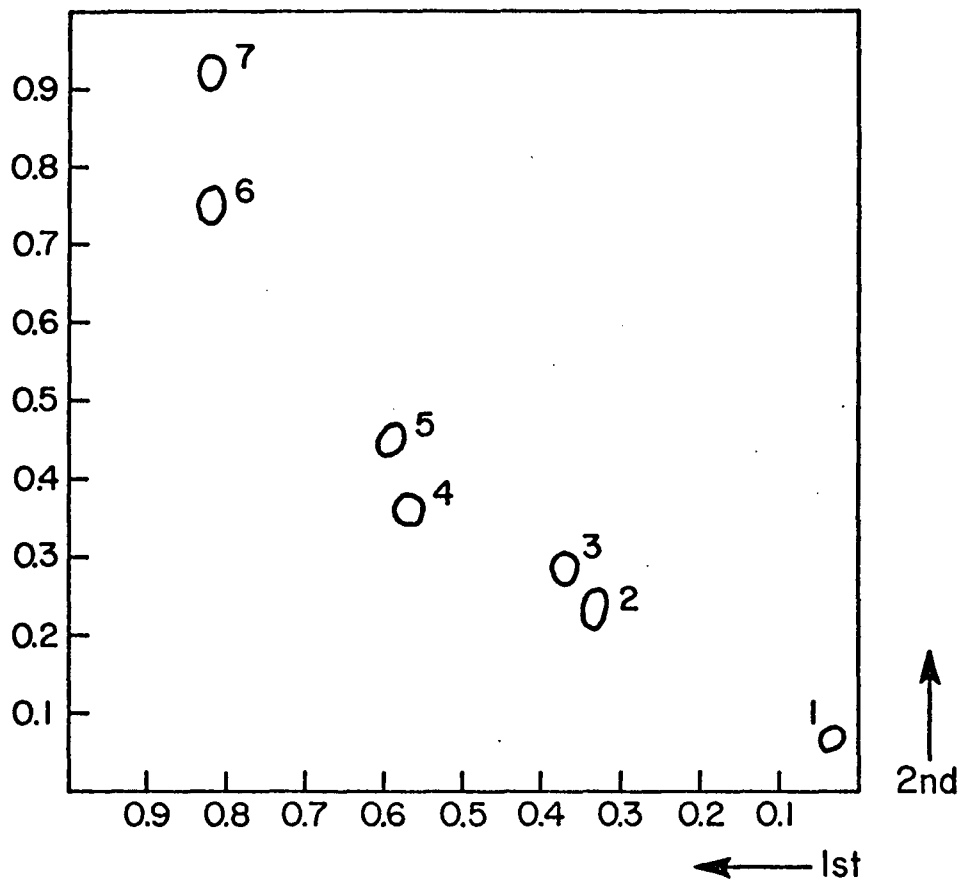

Figure 4. Typical Two-dimensional Chromatogram of Cashew Nut Oil Glycolipids. -- Silica gel G. Development in the horizontal direction from right to left with chloroform-methanol-7N ammonium hydroxide (65:30:4) and in vertical direction with chloroform-methanol-acetic acid-water (170:25:25:6).

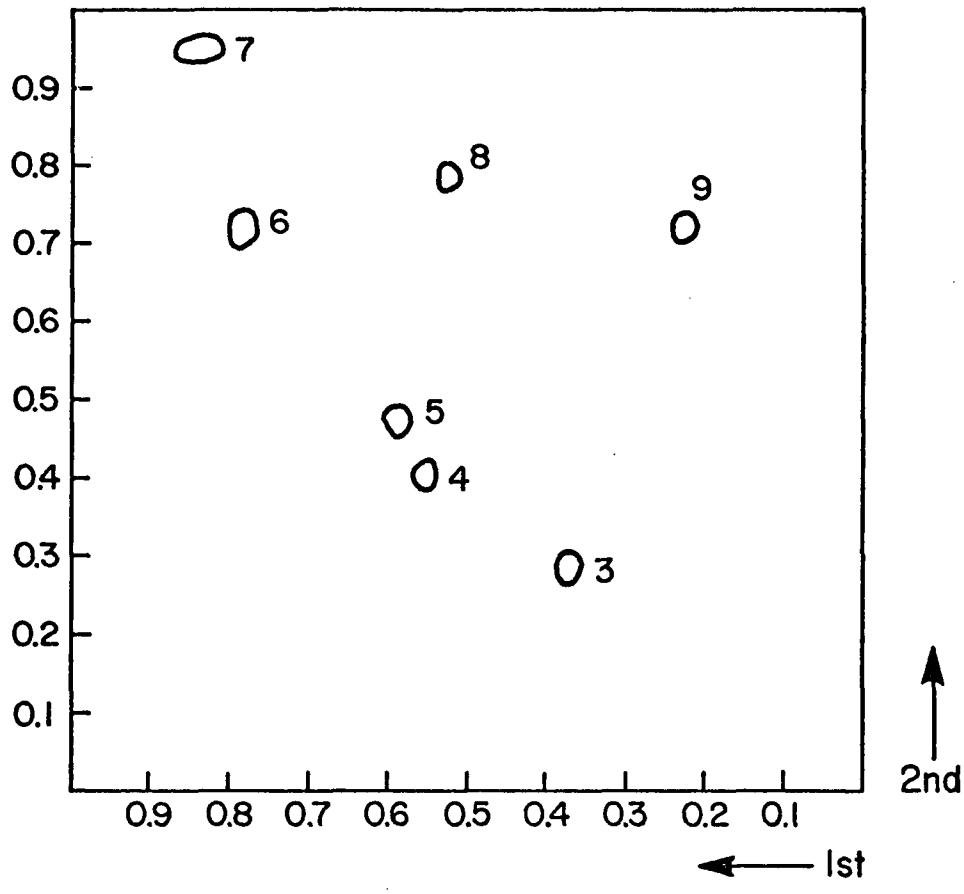

Figure 5. Typical Two-dimensional Chromatogram of Cashew Apple Juice Glycolipids. -- Silical gel G. Development in horizontal direction from right to left with chloroform-methanol-7N ammonium hydroxide (65:30:4) and in vertical direction with chloroform-methanol-acetic acid-water (170:25:25:6).

comparison with published results for plant lipids (57, 30, 35, 23). Spot 1 is similar to that found in peanut glycolipids characterized as a phytoglycolipid precursor (57).

The major glycolipid components of cashew apple juice and nut were well separated by two-dimensional TLC, developing the chromatogram with chloroform-methanol-7N ammonium hydroxide (65:30:4) in one dimension and then with chloroform-methanol-acetic acid-water (170:25:25:6) in the other (Figures 4 and 5).

Glycolipids are involved as energy reserve, aging and sugar transport in plants (30). Many publications have established that the mono- and di-galactosyl diglycerides are present in a wide variety of plant tissues (30).

Fatty Acids

The major fatty acids of cashew nut lipid from roasted, unroasted (shelled), and unroasted (unshelled) are: palmitic ($C_{16:0}$), stearic ($C_{18:0}$), oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) (Table 7). Collectively these comprise more than 97 percent of cashew nut fatty acids. Palmitoleic acid ($C_{16:1}$) was found in detectable amounts. This confirms the findings of Pereira and Pereira (61) who reported the presence of this acid in cashew nut as detected by GLC. These authors (61) reported six fatty acids in cashew nut lipids namely palmitic, stearic, arachidic, palmitoleic, oleic and linoleic. Barroso et al. (4) found palmitic 7.5 percent, stearic 4.5 percent, oleic 73.7 percent, linoleic 14.3 percent, and trace amounts of arachidic and linolenic fatty acids in Brazilian cashew nuts. Jacqmain (32) studied fatty acid

Table 7. Component Fatty Acids of Oil from Roasted, Unroasted (Shelled) and Unroasted (Unshelled) Cashew Nuts.

Fatty Acid	Roasted	Unroasted (Shelled)	Unroasted (Unshelled)
	(%)	(%)	(%)
$C_{12:0}$	tr	tr	tr
$C_{14:0}$	0.1	tr	tr
$C_{16:0}$	9.3	8.8	8.9
$C_{16:1}$	1.0	1.0	1.1
$C_{16:2}$	0.2	0.2	0.3
$C_{18:0}$ (iso)	0.2	0.2	0.1
$C_{18:0}$	8.0	7.3	7.0
$C_{18:1}$	61.5	64.8	63.6
$C_{18:2}$	18.2	16.5	17.1
$C_{20:0}$	1.3	0.9	1.5
$C_{18:3}$	0.2	0.3	0.4

composition using fractional distillation of methyl esters and spectrophotometric techniques. He found the following saturated fatty acids values: myristic 0.2 percent, palmitic 11.5 percent, stearic 4.7 percent, arachidic 4.6 percent, and the unsaturates oleic 59.7 percent, linoleic 18.1 percent, and linolenic 1.2 percent. The value found for arachidic acid is high when compared with the present work.

Since the fatty acid compositions of unroasted nuts and unshelled unroasted nuts are very similar, it is apparent that the processing steps involved in shell removal (such as autoclaving) did not significantly alter the fatty acid distribution. Unroasted nuts were subjected only to sun drying. Roasted nuts used in this work were subjected to a dry roasting process without oil immersion. Deep frying of nuts in vegetable oils is a common practice for nut preparation.

It is common to refer to fatty acids as major, minor and unusual. Major fatty acids are those responsible for a large proportion of fatty acid present in most plant lipids (30). Other fatty acids are ubiquitous but usually present in small quantities and are therefore referred as minor fatty acids; unusual fatty acids are found in few sources. According to Hitchcock and Nichols (30) the major fatty acids in plants are saturated or unsaturated monocarboxylic acids with a straight, even-numbered carbon chain. The reason for this structure rests on the biosynthesis mechanism. The saturated homologes, lauric (dodecanoic), myristic (tetradecanoic), palmitic (hexadecanoic) and stearic (octadecanoic) acids all occur in plants, but even more abundant are the unsaturated analoges, oleic (cis-9-octadecenoic), linoleic (cis-9-, cis-12-octadecadienoic) and linolenic (cis-9-, cis-12-, cis-15-octadecatrienoic) acids. These seven acids alone accounted for 94 percent of those in the world's commercial vegetable fats in 1969 (30). In general, they are also widely distributed in lipids throughout all parts of all plants, often with palmitate, oleate and linoleate predominating. The minor and unusual acids sometimes are disregarded in analytical reports and their

detection often depends on the sensitivity of the methods employed. Small quantities of hexanoic, octanoic, and decanoic acids are probably ubiquitous (30). The minor class is taken to include all saturated acids and unsaturated analoges which are directly related to the major unsaturated acids. All other natural fatty acids are classed as unusual. These unusual acids are often found only in the seeds of related plants, and may be restricted to a few individual species a genus or a whole family; however, within this narrow distribution, they may represent the principal acid in the oil.

Oleic acid has been found to be the major fatty acid in peanut (73,22), rapeseed "erucic acid free"(30). The fatty acid composition of peanut and cashew nut are more or less similar up to the C_{20} acids. Cashew nut oil consists of up to 18.2 percent of linoleic acid which is a polyunsaturated fatty acid known to be essential to growth and health in humans and animals. Linoleic acid reserves in humans are high, the half-life in adults being on the order of two years (8) and deficiency symptoms are difficult to induce even in controlled experiments where prolonged periods of fat free diet are necessary to reduce the linoleate reserves to a critical level. It does not appear necessary therefore to maintain high linoleic acid diets to prevent essential fatty acid deficiency. While the relationship between the nature of dietary fats and the incidence of various cardiovascular ailments is still largely unresolved, it is generally felt that fats which maintain relatively low blood serum cholesterol levels will be effective in preventing artheriosclerosis resulting from abnormal deposition of

cholesterol in the arteries (8). It is reasonably well established that fats containing predominantly polyunsaturated fatty acids reduces serum cholesterol levels, while fats containing predominantly saturated acids shows the opposite effect. Monounsaturated oleic acid appears to be neutral in this respect (8). Certain fatty acids have also been shown to have an influence on blood clotting. In experiments with rats maintained on a diet designed to induce thrombosis, mortality was reduced by supplementing the diet with oleic, linoleic and linolenic acids the latter being more effective in reversing or preventing certain blood clotting phenomena (37). "In vitro" studies on the influence of fatty acids on blood clotting have shown that saturated acids of long chain length (from stearic) promoted certain clotting phenomena, while short chain saturated acids (palmitic and below) did not. In this respect, linoleic acid showed behavior similar to that of short chain, saturated acids and oleic was intermediate between stearic and palmitic (8). From these nutritional considerations concerning fatty acids, it can be concluded that cashew nut oil presents a fatty acid composition which can be considered nutritionally satisfactory.

Esterification of Fatty Acids

The reason for using methyl esters in GLC analysis is based on their greater volatility. James and Martin (33) were the first to use fatty acids methyl esters in GLC. They found that the higher free fatty acids were very difficult to chromatograph as free acids.

Boron-fluoride is the most electropolar of the boron halides and is extremely reactive toward many types of organic compounds. When

coordinated with methanol, it becomes a useful reagent for fatty acids methyl esters preparation. Morrison and Smith (52) compared the effects of equimolar concentrations of boron chloride and boron fluoride for methyl ester preparation and the results showed that boron fluoride was a better catalyst. They concluded that boron fluoride-methanol was a satisfactory, generally applicable methanolysis reagent. The presence of a free acid is not required for esterification to take place since BF_3-alcohol complexes will react directly with triglycerides, phospholipids, etc., to form esters of the combined acids. Some compounds are difficult to esterify in this manner so they must be subjected to a preliminary saponification in order to free the fatty acids.

The esterification method used for cashew nut lipids is relatively rapid and applicable for commercial fat and oil laboratories. A rapid saponification (heating the lipids for five minutes with 0.5N KOH) was followed by boiling the soaps with BF_3-methanol for two minutes. This procedure results in quantitative fatty acid conversion to methyl esters in a 10-minute operation. The methyl esters were floated out of the mixture using saturated NaCl solution. This salting out technique almost completely overcomes objectionable loss of lower molecular weight fatty acids in the water layer. Metcalfe, Schmitz and Pelka (49) found that triglyceride fatty acids conversion to methyl esters appeared to be quantitative by TLC analysis. The amount of conversion was far more complete than with any other short period, interesterification procedure tried. It was also found that saturated salt solution added to the esterification mixture resulted in a quantitative recovery of acids

down to the C_4 range. Using this method, the authors have esterified fatty acids, soaps, triglycerides, polyesters, lipoproteins, phospholipids and other fatty acids esters. The BF_3-methanol esterification method gave very good results in the work with cashew.

Fatty Acid Composition of Lipid Classes

The fatty acid composition of the neutral lipids, phospholipids, and glycolipids fractions previously separated by column chromatography (silicic acid) was determined (Table 8). Comparing the three classes, it can be seen that palmitic acid had the highest value in the phospholipid fraction. The only fraction that showed detectable amounts of arachidic acid was the neutral lipid fraction. Linoleic acid was predominant in the glycolipid fraction. Oleic acid showed a higher value for phospholipid fraction. The values found for most of these fatty acids compares well with those found by Kinsela (35) for cucumber and peppers. According to Hitchcock and Nichols (30) few data are available regarding the distribution of individual fatty acids between the different classes of polar lipids.

Fatty Acid Composition of Cashew Apple Juice

Fatty acid composition of cashew apple juice determined by three different esterification methods shows that with BF_3-methanol 14 percent and sulfuric-acid-methanol (4 percent sulfuric acid) a significant peak with the same retention time as lauric acid was produced (Table 9).

Table 8. Component Fatty Acids of Unroasted Cashew Nut Oil Present in Neutral, Phospholipid, and Glycolipid Fractions.

Fatty Acid	Neutral Lipid	Phospholipid	Glycolipid
	(%)	(%)	(%)
$C_{10:0}$	0.5	0.4	--
$C_{12:0}$	0.1	1.0	0.2
$C_{14:0}$	0.1	1.0	0.5
$C_{16:0}$	9.0	14.5	10.4
$C_{16:1}$	0.9	1.2	1.2
$C_{16:2}$	0.2	0.6	2.0
$C_{18:0}$ (iso)	0.1	--	--
$C_{18:0}$	7.4	6.3	7.9
$C_{18:1}$	63.9	64.0	57.8
$C_{18:2}$	16.4	11.0	20.0
$C_{20:0}$	1.0	--	--
$C_{18:3}$	0.4	tr	tr

Table 9. Compound Fatty Acids of the Lipids from Cashew Apple Juice Esterified by Three Different Methods.

Fatty Acid	Esterification Method		
	Sodium Metoxide	BF_3-methanol	H_2SO_4-methanol
	(%)	(%)	(%)
$C_{12:0}$	0.6	23.7	31.5
$C_{14:0}$	0.2	0.4	0.3
$C_{16:0}$	18.9	14.9	14.6
$C_{16:1}$	1.3	0.7	0.7
$C_{16:2}$	0.2	0.4	0.4
$C_{18:0}$	1.5	0.9	1.0
$C_{18:1}$	67.3	50.6	46.2
$C_{18:2}$	2.6	1.7	1.7
$C_{18:3}$	4.4	4.7	2.5
$C_{20:1}$	3.0	2.0	1.1

When the esterification of the cashew apple juice lipids was done with sodium methoxide, this peak was detected but only as a minor component in the order of 0.6 percent. Since this peak was not detected in large amounts by the sodium methoxide method, it seems that it occurs as a free fatty acid in the juice lipids. When the triglyceride fraction was removed from TLC plates and esterified with BF_3-methanol, it was found that this component occurred only as a small peak (about 2 percent). The presence of large amounts of lauric acid could be explained as being due to a possible lipase action during the preparation of the juice prior to heat treatment.

Transesterification with methanol and catalytic amounts of sodium methylate has proved to be a rapid and effective method of converting glycerides to methyl esters and has been employed for many years (56). Since boron fluoride alcoholates behave like strong acids, they would be expected to promote methanolysis of lipids in a manner similar to HCl or H_2SO_4 added to methanol, with the added advantages conferred by the extreme electropolarity of the boron fluoride (55). It can be seen that similar results were obtained for cashew apple juice lipids using boron fluoride-methanol and sulfuric acid-methanol (Table 9).

Fatty Acid Composition of Cashew Kernel Testa

The major fatty acids present in cashew nut testa are: palmitic, stearic, oleic, linoleic and linolenic (Table 10). It is interesting to note that oleic and linoleic acids occur in almost identical amounts which is different than their distribution in the kernel where oleic acid predominates.

Table 10. Component Fatty Acids of Cashew Kernel Testa Lipids.

Fatty Acid	(%)
$C_{12:0}$	0.2
$C_{14:0}$	0.3
$C_{14:1}$	0.4
$C_{16:0}$	16.4
$C_{16:1}$	1.1
$C_{16:2}$	1.4
$C_{18:0}$	6.4
$C_{18:1}$	35.3
$C_{18:2}$	30.4
$C_{18:3}$	5.8
$C_{20:1}$	1.6
$C_{20:2}$	0.8

Fatty Acid Composition of Cashew Apple

The fatty acid composition of cashew apple peel and pulp from yellow and red cashew at two stages of maturity shows varying trends (Table 11). Comparing red mature pulp (RMF) with yellow pulp (YMF) the major difference is in the amount of $C_{10:0}$ acid which occurs in higher concentration in the yellow pulp (YMF) and only in trace amounts in red pulp. Red mature pulp (RMF) showed a higher value for $C_{18:3}$ acid

Table 11. Component Fatty Acids of Yellow and Red Cashew Apple at Different Stages of Maturity.

Fatty Acid	RMF[a]	RGF	RMP	RGP	YMF	YGF	YMP
	(%)	(%)	(%)	(%)	(%)	(%)	(%)
$C_{10:0}$	tr[b]	--	tr	tr	6.6	tr	1.2
$C_{12:0}$	0.8	tr	0.1	0.2	0.2	0.2	0.5
$C_{14:0}$	0.5	0.2	0.4	0.3	0.3	0.2	0.5
$C_{14:1}$	0.2	--	0.4	--	0.1	tr	1.3
$C_{16:0}$	18.5	26.5	21.3	23.9	15.0	19.3	17.6
$C_{16:1}$	1.3	1.5	1.5	1.0	1.5	1.2	2.0
$C_{16:2}$	0.3	0.2	0.2	tr	0.2	0.1	0.2
$C_{18:0}$ (iso)	0.3	tr	0.1	tr	tr	tr	tr
$C_{18:0}$	1.2	0.5	3.0	3.0	0.8	0.5	1.5
$C_{18:1}$	64.4	51.2	65.4	49.3	65.8	64.3	68.3
$C_{18:2}$	2.5	11.6	3.2	10.8	2.2	5.9	3.2
$C_{20:0}$	tr	--	tr	--	0.6	--	0.5
$C_{18:3}$	6.0	8.3	3.3	9.5	3.8	6.5	2.4
$C_{20:1}$	4.0	--	1.0	--	2.9	2.0	1.2

[a] RMF = red mature pulp; RGF = red immature pulp; RMP = red mature peel; RGP = red immature peel; YMF = yellow mature pulp; YGF = yellow immature pulp; YMP = yellow mature peel.

[b] tr = traces.

and $C_{20:1}$ acid than the yellow mature pulp (YMP). Comparing the red mature peel (RMP) with yellow mature peel it can be seen that the latter showed a greater value for $C_{10:0}$ acid than the former where this acid appeared only in trace amounts. Red mature peel (RMP) showed a greater amount of $C_{16:0}$ and $C_{18:0}$ acid than that in yellow mature peel (YMP). The amount of $C_{16:0}$ was found to be higher in both red cashew pulp and peel. Differences in fatty acid composition in the red mature peel (RMP) and red immature peel (RGP) can be compared. The most significant changes found were that $C_{18:1}$ was higher in the mature peel than in the immature peel. The red immature peel showed a higher percentage of $C_{18:2}$ and $C_{18:3}$ than that in red mature peel. Comparing the yellow mature pulp (YMF) with yellow immature pulp (YGF), it can be seen that there was a decrease in $C_{18:2}$ and $C_{18:3}$ from the immature to the mature pulp. These results agree with those found by Goldstein and Wick (25) in a study of the lipids in ripening banana fruit. These authors found that the ripe banana pulp showed higher amounts of $C_{18:1}$ (14.4 percent) than the unripe (11.35 percent). $C_{18:2}$ acid was found to be higher in the unripe banana pulp (33.88 percent) decreasing to 16.10 percent in the ripe pulp. There was a threefold decrease in linoleate as the banana matured.

A small decrease in $C_{16:0}$ acid from the immature to the mature both for the red and yellow cashews was also observed. The immature cashews showed higher unsaturation than the correspondent mature parts. It can also be seen that the $C_{18:2}$ and $C_{18:3}$ decreased from immature to mature in the samples tested in this experiment.

Table 12. Total Unsaponifiable Matter and Recoveries Obtained from Florisil Chromatography Separation of Cashew Nut Oil Unsaponifiable Matter Fractions.

Sample	Unsaponifiable Matter	Proportion of Total Unsaponifiable Matter				
		Fraction 1+2	Fraction 3	Fraction 4+5	Fraction 6	Total
	(%)	(%)	(%)	(%)	(%)	(%)
Roasted	0.32	19.4	14.6	55.1	4.0	93.1
Unroasted	0.36	21.0	19.0	50.0	5.1	95.1
Unroasted in shell	0.34	18.5	15.0	51.5	6.0	90.5
Average:	0.34	19.6	16.2	52.2	5.0	92.9

the sterol fraction comprising more than half of the total unsaponifiable matter.

Gas-Liquid Chromatography

The various fractions obtained by Florisil column chromatography were examined by gas chromatography in order to identify individual components. Satisfactory gas chromatographic separation must be based on reasonably volatile constituents. One of the most practical methods of producing increased volatility in chemical compounds is to convert them to silyl ether derivatives. These silyl ethers can be prepared from any compound that contains a reactive hydrogen. They have shorter retention times than the parent compounds, allowing analysis to be completed in less time and the columns to be operated at lower temperatures. While silyl ethers are easy to prepare in an anhydrous medium, the presence of moisture may cause some difficulties.

Hydrocarbons (Fractions 1 + 2)

Chromatograms of the hydrocarbons from three cashew nut oil samples (roasted, unroasted and unroasted unshelled) were similar. A typical chromatogram shows 21 peaks (Figure 6 and Table 13). By the use of an arithmetic plot constructed with pure standards (Analabs) these 21 peaks were found to be distributed in three homologous series such as: first series comprised of straight chain hydrocarbons in the range C-19 to C-31; second series comprised of iso and/or anteiso-hydrocarbons from C-20 to C-31; and a third series comprised of branched chain hydrocarbons from C-20 to C-31. The procedure for the identification of the

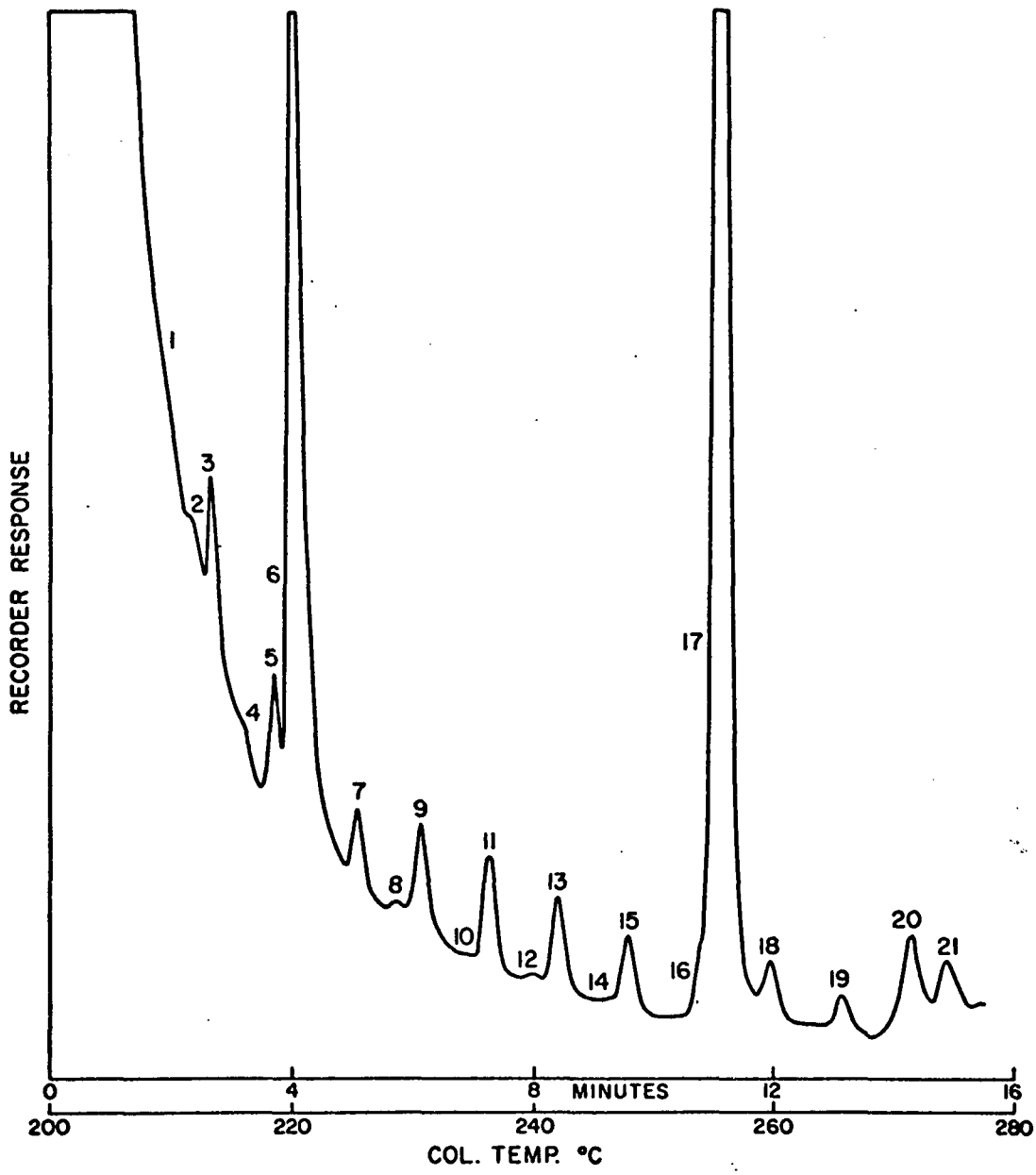

Figure 6. Programmed Temperature Gas Chromatogram of Cashew Nut Oil Hydrocarbons: Program Rate 5°C/min.

Table 13. Hydrocarbons (Fractions 1 + 2) in Cashew Nut Unsaponifiable Matter Expressed in Terms of Total Peak Area.

Peak Number	RT (Min.)	Samples			Identification
		Roasted	Unroasted	Unroasted in Shell	
		(%)	(%)	(%)	
1	1.8	tr*	0.2	tr	C-19
2	2.2	tr	0.2	0.2	C-20 br
3	2.5	2.0	1.5	1.4	C-20
4	3.1	tr	tr	tr	C-21 br
5	3.5	2.9	5.5	7.0	C-21 iso
6	3.8	28.5	29.0	25.0	C-21
7	4.9	1.0	1.3	2.0	C-22
8	5.6	0.2	1.0	0.9	C-23 iso
9	5.9	2.2	1.0	2.3	C-23
10	6.7	tr	tr	tr	C-24 iso
11	7.0	2.7	1.8	3.0	C-24
12	7.8	tr	0.3	0.3	C-25 iso
13	8.2	tr	tr	tr	C-25
14	9.0	tr	tr	tr	C-26 iso
15	9.4	3.0	2.7	3.0	C-26
16	10.6	0.3	0.3	0.4	C-28
17	10.8	49.7	48.0	47.2	Squalene
18	11.7	1.0	1.2	1.2	C-29 br
19	12.9	0.8	1.0	0.8	C-30 br
20	14.0	3.9	4.0	4.3	C-31 br
21	14.6	1.0	0.4	0.5	C-31

* Trace amounts.

hydrocarbons in cashew nut oil was similar to that used by Eisner et al. (14). These authors found normal straight chain hydrocarbons, iso and/or anteiso hydrocarbons, and multiple branched chain hydrocarbons using a plot of retention time of standards versus carbon number, in olive oil.

From the chromatogram (Figure 6) it can be seen that the major hydrocarbon fraction components are peak 17 tentatively identified as squalene and peak 6 identified as C-21. Squalene was found to occur in the unsaponifiable matter of cashew nut oil, by Jacqmain (32). This compound was also found to occur in a number of vegetable oils such as coconut (54) and olive oil (14).

In this work, squalene was found to comprise 47.2 percent of the hydrocarbon fraction calculated as peak area by an integrator. The variation of squalene was found to be very small between the three samples tested.

The most significant variation in the hydrocarbon fraction was found to be for peak number 5 which was identified as C-21 iso. This peak showed the smallest value for the roasted nut and the largest for the unroasted in shell. It seems evident that the process of removal of the shell promoted a decrease in this component. Peak 6 (C-21) showed a small variation between the samples. The most interesting finding here is that peak 17 is the predominant hydrocarbon in the three types of nut tested. The branched hydrocarbons (except C-31 br) and the iso and/or anteiso except (C-21-iso) appeared in small amounts.

The amounts of hydrocarbons calculated as percent of the oil shows small variations, the highest value being found in the unroasted nut (Table 14).

Table 14. Comparison of Total Unsaponifiable Matter and Hydrocarbons of Cashew Nut Oil.

Sample	Unsaponifiable Matter	Hydrocarbons
	(%)	(%)
Roasted	0.32	0.062
Unroasted	0.36	0.075
Unroasted (in shell)	0.34	0.063

Aliphatic Alcohols, Tocopherols and Triterpenoid Alcohols

A typical temperature programmed chromatogram of fraction 3 shows aliphatic alcohols, tocopherols and triterpenoid alcohols (Figure 7). A comparison of fraction 3 chromatographed before and after conversion to trimethyl silyl derivatives shows much better separation of the derivatives.

Aliphatic Alcohols

Three homologous series of aliphatic alcohols were found in cashew nut oil samples: (a) normal aliphatic alcohols in the range C-15 to C-31, (b) probably iso and/or anteiso alcohols in the range C-15 to C-30, (c) probably multiple branched chain alcohols in the range C-16 to C-31 (Table 15). Reference standards for the latter two series of alcohols were not available and tentative identifications were made after examination of arithmetic plots of the gas chromatographic data. Similar series of alcohols were previously found in olive oil (14) using

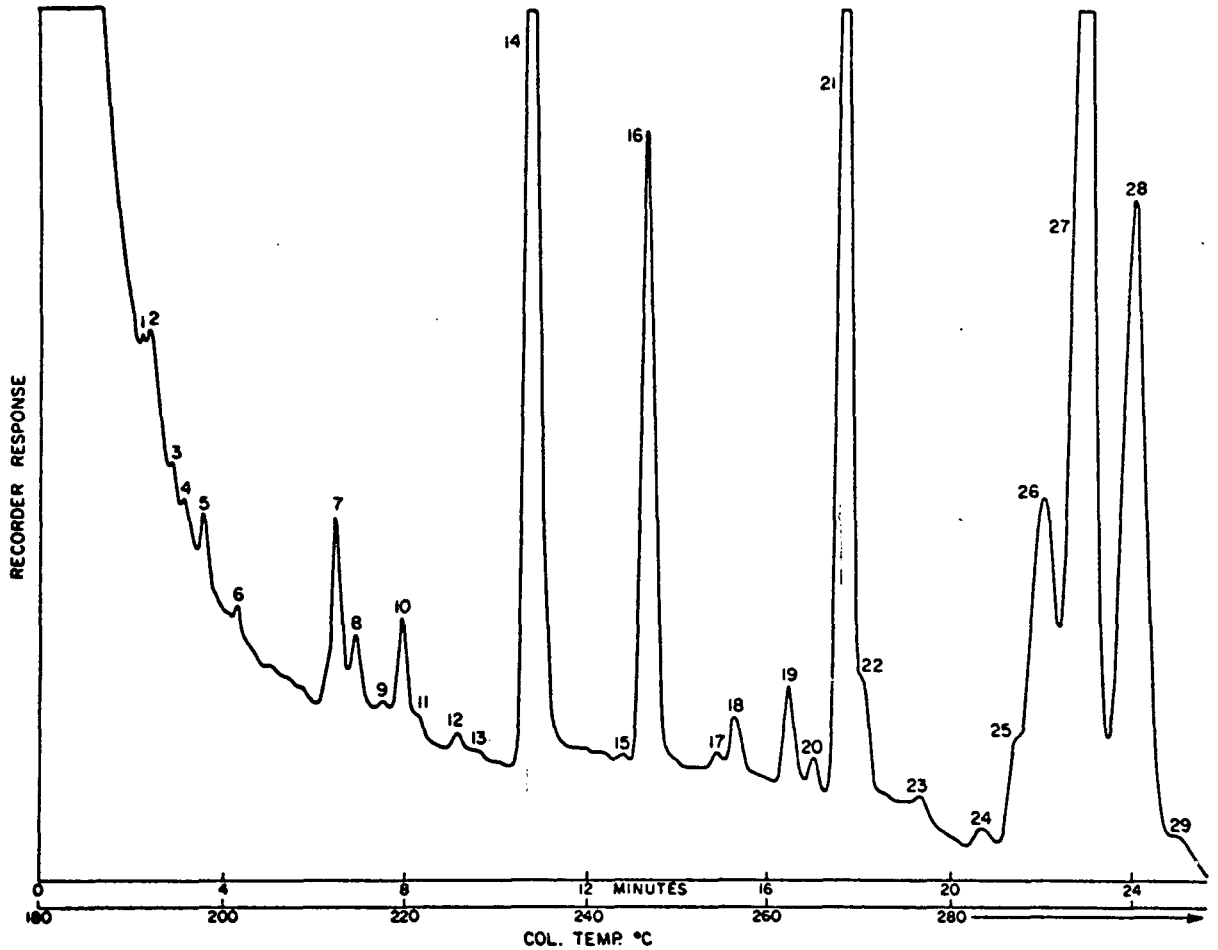

Figure 7. Programmed Temperature Gas Chromatogram of Cashew Nut Oil Aliphatic Alcohols, Tocopherols and Triterpenoid Alcohols: Program Rate 5°C/min.

Table 15. Aliphatic Alcohols, Tocopherols and Triterpenoid Alcohols (Fraction 3) Present in the Unsaponifiable Matter of Cashew Nut Oil, Expressed in Terms of Total Peak Areas.

Peak N.	RT (min.)	Samples			Identification
		Roasted	Unroasted	Unroasted in Shell	
		(%)	(%)	(%)	
1	2.1	0.2	0.2	0.2	C-15 iso OL
2	2.3	0.5	0.5	0.3	C-15 OL
3	2.8	0.1	0.1	0.1	C-16 br OL
4	3.0	0.1	0.1	0.1	C-16 iso OL
5	3.4	0.2	0.2	0.2	C-16 OL
6	4.2	0.1	0.3	0.2	C-17 br OL
7	6.3	1.9	1.3	1.0	C-18 OL
8	6.8	0.5	0.9	0.8	C-19 br OL
9	7.4	0.1	0.3	0.3	C-19 iso OL
10	7.7	1.0	1.5	1.0	C-19 OL
11	8.2	tr	tr	tr	C-20 br OL
12	9.0	0.2	0.2	0.2	C-20 OL
13	9.4	tr	tr	tr	C-21 br OL
14	10.2	16.0	16.0	12.0	C-21 OL
15	12.6	0.1	0.4	0.3	C-23 iso OL
16	13.0	8.5	10.5	8.0	C-23 OL
17	14.7	0.2	0.3	0.4	C-25 br OL
18	15.1	0.6	0.2	0.2	C-25 iso OL
19	16.3	1.2	1.2	1.0	C-26 iso OL and/or delta tocopherol
20	16.8	0.4	0.4	0.4	C-26 OL
21	17.3	16.9	21.0	26.5	gamma and/or beta tocopherol
22	18.1	0.2	0.2	0.2	C-27 OL
23	19.2	0.2	0.5	0.7	C-28 iso OL and/or alpha tocopherol
24	20.5	0.5	0.7	1.0	C-29 iso OL
25	21.2	1.0	1.0	1.0	C-30 br OL
26	21.6	10.0	8.5	9.0	Amyrin (alpha/beta)
27	22.5	25.0	23.0	25.0	Cycloartenol
28	23.5	14.0	10.0	9.5	24-Me-Cyclo-artanol
29	24.9	tr	tr	tr	Unknown

Total Aliphatic Alcohols 32.4; 34.9; 28.0. Total Tocopherols 18.3; 22.7; 28.2. Total Triterpenoids 49.0; 41.6; 43.5.
OL = aliphatic alcohols.

similar procedures, and in several other oils (15) such as butter fat, corn, cottonseed, linseed, milo maize, peanut, rice bran, safflower, soybean and tung oil. By fractionation of the unsaponifiable matter on a Florisil column, these authors showed that this fraction consisted of three homologous series tentatively identified as normal, iso and/or anteiso and multiple branched chain alcohols.

The major normal aliphatic alcohols found in fraction 3 were C-21 OL and C-23 OL. These two alcohols showed minor differences between the three types of nuts, being present in the largest amount in the unroasted sample.

Iso and/or anteiso and multiple branched chain alcohols were present in small amounts. These results are comparable to those found by Eisner, Iverson and Firestone (15) with several vegetable oils.

Aliphatic alcohols relative peak areas were: 32.4 percent for roasted nuts and 34.9 percent for unroasted and 28 percent for unroasted in shell.

Tocopherols

GLC of pure tocopherol standards showed the following elution order: delta-tocopherol, beta-tocopherol and gamma-tocopherol as one peak; and alpha-tocopherol. Under the conditions of this experiment gamma and beta-tocopherols were not separated.

On the basis of standards retentions times, it was observed that peaks 19, 21 and 23 showed the same values as those for delta, gamma-beta and alpha tocopherols, respectively. The largest peak area corresponded to that identified as gamma and/or beta tocopherol. This peak showed a

value of 26.5 percent for unroasted nut in shell, 21.0 percent for unroasted nut and 16. percent for roasted nut. This variation can be explained by the fact that the tocopherols were protected against oxidation in the nut containing a shell covering. The process of heating the nut to remove the shell was found to promote a decrease in the levels of gamma and/or beta tocopherol and a decrease in the amount of alpha tocopherol. An additional evidence for the presence of gamma and/or beta tocopherol as being the major tocopherol in cashew nut was supplied by TLC. When chromatographed with pure standards a spot with the same Rf as gamma tocopherol was detected.

Tocopherols relative peak areas were: 28.2 percent of unroasted nuts in shell, 22.7 percent of unroasted nuts and 18.9 percent of roasted nuts. Eisner et al. (15) found that beta and/or gamma was the major component of the tocopherol fraction in the oils of corn, peanut, tung, linseed, soybean and cottonseed. Erickson, Weissberger and Keeney (16) reported that the beta-gamma isomers comprised the major tocopherol fraction component of cocoa lipids analyzed by TLC and GLC.

According to Ames (1) alpha tocopherol (5,7,8-trimethyl tocol) is biologically the most active member of the naturally occurring tocopherols and tocotrienols. The other have biological activities ranging from 0 to 50 percent that of alpha tocopherol. Some work has been done to study the effect of processing on the tocopherol levels in foods (28, 51). Herting and Drury (28) studied the tocopherol content of refined and unrefined vegetable oils. Rao, Rao and Achaya (66) separated alpha, beta, gamma and delta tocopherols by TLC with subsequent

colorimetric estimation. They found that gamma tocopherol was the major tocopherol in castor oil, groundnut, neem, sesame and soybean. Alpha tocopherol was the major component in cottonseed and safflower. Lamberstein, Myklestad and Braekkan (40) studied tocopherols in eight different nuts using spectrophotometry and TLC. Jacqmain (32) determined the amount of total tocopherol in cashew nut. He found that it comprised 5.3 percent of the unsaponifiable matter. This author did not identify individual tocopherol isomers.

Triterpenoid Alcohols

The peaks found in the region corresponding to triterpenoid alcohols were identified tentatively as: peak 26 as amyrin (alpha-beta), peak 27 as cycloartenol and peak 28 as 24-methylene cycloartanol (Table 15). The identification was based on work done by other investigators (14, 15, 20). Fedeli et al. (20) studied 18 vegetable oils such as linseed, peanut, olive, rice bran, palm kernel, corn, sesame oiticica, palm, coconut, rapeseed, grape seed, sunflower, poppy seed, castor, tea seed, cocoa butter, and soybean. Two triterpenoid alcohols, cycloartenol, and 24-methylene cycloartanol, were found to be present in all oils except soybean oil which contained only cycloartenol. These authors also indicated that beta amyrin eluted before alpha amyrin. Eisner et al. (15) cited that Shimizu and others studied the gas chromatographic behavior of a number of triterpenoid alcohols.

In this work, the triterpenoid alcohols relative peak areas varied from 49.0 percent for roasted and 41.6 percent for unroasted to 43.5 percent for the unroasted nuts in shell. Cycloartenol was the major

triterpenoid alcohol in cashew nut oil (Table 16). The highest value for this component was found in unroasted nut in shell. The highest value for unsaponifiable recovered as Florisil fraction 3 was found in unroasted nuts (Table 17). No significant variations was observed in the values of triterpenoid calculated as percentages of the oil (Table 18).

Sterols (Fraction 4 + 5)

GLC of the sterol fraction indicated the presence of nine peaks (Figure 8). Of these, peak 5 was identified as campesterol and peak 6 as beta-sitosterol. These compounds were identified by comparison with the retention time of pure standards which eluted in the following order: cholesterol, campesterol, stigmasterol, and beta-sitosterol.

Beta-sitosterol was found to be the most abundant sterol comprising a relative peak area of 91.4 percent for roasted nut, 90.7 percent for unroasted and 91.8 percent for unroasted in shell (Table 19). Jacqmain (32) reported that 89.4 percent of sterol fraction in cashew nut oil was beta-sitosterol. He did not identify the other sterols.

Small amounts of aliphatic alcohols were eluted in the sterol fraction.

Vegetable oils contain variable amounts of phytosterols (sitosterols and stigmasterol are the common phytosterols) which differ from cholesterol, the characteristic sterol of animal fats.

Eisner and Firestone (13) studied the sterols of several oils such as corn, cottonseed, olive, milomaize, rice bran, safflower, peanut,

Table 16. Distribution of Total Triterpenoid Alcohols Expressed in Terms of Total Peak Area.

Samples	beta Amyrin	Cycloartenol	24-Me-Cycloartanol
	(%)	(%)	(%)
Roasted	20.4	51.0	28.5
Unroasted	20.4	55.2	24.0
Unroasted in shell	20.5	57.4	21.8

Table 17. Distribution of Triterpenoids as Found in Florisil Fraction 3 and by GLC Peak Area.

Samples	Fraction 3	GLC Triterpenoid Portion
	(%)	(%)
Roasted	14.6	7.2
Unroasted	19.0	7.9
Unroasted in shell	15.6	6.8

Table 18. Total Triterpenoids Expressed as a Percentage of the Oil.

Samples	Unsaponifiable Matter	Triterpenoid Portion
	(%)	(%)
Roasted	0.32	0.023
Unroasted	0.36	0.028
Unroasted in shell	0.34	0.023

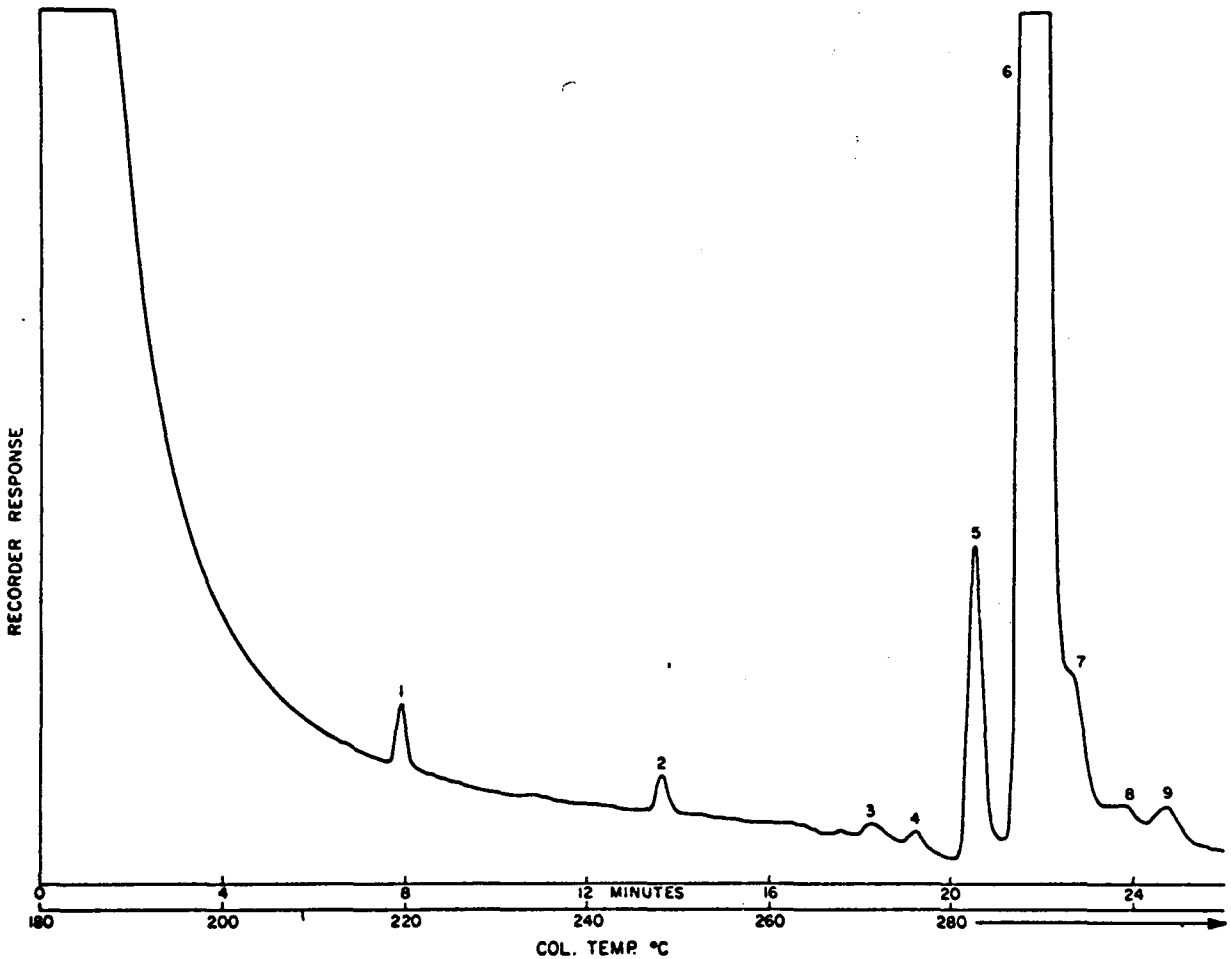

Figure 8. Programmed Temperature Gas Chromatogram of Cashew Nut Oil Sterols: Program Rate 5°C/min.

Table 19. Sterols (Fractions 4 + 5) in the Unsaponifiable Matter of Cashew Nut Oil Expressed in Terms of Total Peak Areas.

Peak N.	RT (min.)	Samples			Identification
		Roasted	Unroasted	Unroasted in shell	
1	7.7	0.6	0.6	0.6	C-19 OL
2	13.5	tr	tr	0.4	C-23 OL
3	18.0	tr	tr	0.3	Unknown
4	18.9	0.1	0.4	0.2	Unknown
5	20.1	6.1	5.6	5.8	Campesterol
6	21.2	91.4	90.7	91.8	beta-sitosterol
7	22.6	1.0	0.8	0.6	Unknown
8	23.4	0.2	1.5	1.0	Unknown
9	24.5	0.3	0.4	0.3	Unknown

soybean and cocoa butter. They found that beta-sitosterol was the major sterol found in each of the oils investigated.

Fedeli et al. (20) showed that the following order of elution was observed for sterols on GLC (glass column 2 m x 2 mm I.D. packed with silanized Gas Chrom. P, 100-200 mesh and coated with one percent SE-30. The column was operated at 230°C with nitrogen at 20 ml per minute as carrier gas. Evaporator temperature was 280°C) cholesterol, brassicasterol, campesterol, stigmasterol, and beta-sitosterol.

Rapeseed oil (20) showed a GLC pattern for the sterol fraction somewhat similar to cashew nut. In this oil, beta sitosterol, campesterol and brassicasterol were identified. Since peak 5 showed a retention time similar to that of cholesterol and the retention time of brassicasterol was indicated by Fedeli et al. (20) to be very close to cholesterol, this would suggest that peak 5 could be brassicasterol (Figure 8).

It can be seen that there was practically no variation in the sterol fraction between the three nut samples studied in the present work (Tables 20 and 21).

Table 20. Distribution of Sterols as Found in Florisil Fraction 4 + 5 and by GLC Peak Area.

Samples	Fraction 4 + 5	GLC Sterol Portion
	(%)	(%)
Roasted	55.1	53.6
Unroasted	50.0	48.2
Unroasted in shell	51.5	50.0

Table 21. Comparison of the Total Unsaponifiable Matter and Sterols in Cashew Nut Oil.

Samples	Unsaponifiable Matter	Sterol Portion
	(%)	(%)
Roasted	0.32	0.17
Unroasted	0.36	0.17
Unroasted in shell	0.34	0.17

CONCLUSIONS

1. The chloroform methanol method used for lipid extraction gave very good results. Average lipid content for cashew nut was 49 percent.
2. Silicic acid was found to be very good for separation of lipids into three classes: neutral lipids, glycolipids, and phospholipids.
3. Phospholipids from cashew nut and apple were separated by two-dimensional TLC and nine components positive to the specific molybdenum spray were detected. Four phospholipids were identified in nut such as lysophosphatidyl choline (LPC), 5.1 percent (LPC), phosphatidyl serine 6.5 percent (PS), phosphatidyl choline 53.6 percent (PC), phosphatidyl ethanolamine 14 percent (PE). Eleven spots positive to molybdenum spray were detected for cashew apple phospholipids. The following were identified: LPC, PC, PE, PS and probably phosphatidyl glycerols. The major fatty acids in cashew nut PC and PE were palmitic, oleic, and linoleic representing 85.3 percent of the total in PE and 73.3 percent in PC.
4. Glycolipids from cashew nut and apple were separated by two-dimensional TLC. Seven spots were detected and the following

were tentatively identified: di-galactosyl diglyceride, cerebroside, sterol glycoside, sterified steryl glycoside.

5. The major fatty acids in cashew nut lipids from roasted, unroasted (shelled) and unroasted in shell are: palmitic, stearic, oleic and linoleic. Collectively they comprise more than 97 percent of the total acids. It was found that the operations for nut removal did not significantly change the fatty acid composition of cashew nut oil. The fatty acid composition present in the neutral lipid, glycolipid and phospholipid fractions was determined and they showed qualitatively similar results. Fatty acid composition was also determined in cashew apple juice using three different methods of esterification, and in cashew nut testa using BF_3-methanol. The major testa oil components were palmitic, stearic, oleic, linoleic, and linolenic. Fatty acid composition of cashew apple peel and pulp from yellow and red cashews at two stages of maturity showed varying trends. Oleic acid increased from unripe to ripe. Linoleic and linolenic acids decreased from unripe to ripe.

6. Unsaponifiable matter percentages in cashew nut oil averaged 0.34 percent. The unsaponifiable matter was fractionated by Florisil column chromatography as: (a) Hydrocarbons (fraction 1 + 2) 19.6 percent. The major peak in this fraction was squalene. Three homologous series of hydrocarbons were found in the cashew nut oil unsaponifiable matter, namely: normal, iso and/or anteiso and branched chain. (b) Aliphatic alcohols,

tocopherols and triterpenoid alcohols (fraction 3) comprised 16.2 percent of unsaponifiable matter. Aliphatic alcohols were present in three homologous series, normal, iso and/or anteiso and branched chain. Aliphatic alcohols relative peak areas were: 32.4 percent for roasted nuts, 34.9 percent for unroasted and 28 percent for unroasted in shell nut.

A peak identified as gamma-beta tocopherol was found to decrease with shell removal kernel roasting. Tocopherols relative peak areas were: 28.2 percent of unroasted nut in shell, 22.7 percent of unroasted nut and 18.9 percent of roasted nut. Peaks found in the region corresponding to triterpenoid alcohols were tentatively identified as amyrin (alpha-beta), cycloartenol and 24-methylene cycloartanol. No significant variation was observed in the values for triterpenoids calculated as percentages of the oil. Sterols (fraction 4 + 5) comprised 52.2 percent of unsaponifiable matter and beta-sitosterol was found to be the most abundant sterol comprising a relative peak area of 91.4 percent for roasted nut, 90.7 percent for unroasted and 91.8 percent for unroasted in shell nut. There was practically no variation in the sterol fraction between the three types of samples studied.

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