Regional Differences of Lipid Composition in Morphologically Distinct Fatty Tissues: III. Peanut Seeds

E. FEDELI, G. FAVINI, F. CAMURATI, and G. JACINI,
Centro Nazionale per la Lipochimica del Consiglio Nazionale delle Ricerche, Milano, Italia [1]

Abstract

Lipids extracted from three different parts of peanut seeds (cotyledons, germs, and hulls) have been examined for glyceride, fatty acid, and unsaponifiable composition. Notable differences have been shown, as expected, the unsaponifiable material being more abundant in germs than other parts of the seed. Germ oil, in turn, appears as more unsaturated.

The presence of minor components, not detected when studying the unsaponifiable material from the whole oil, has been demonstrated. Besides qualitative distribution, the quantity of components of the unsaponifiable material has been shown to vary over wide limits in morphologically distinct parts of the seeds.

Introduction

A NUMBER OF PAPERS have reported that morphologically distinct fatty tissues both from plants and animals bear differences in fatty acid and triglyceride composition (1–5).

The composition of minor component mixtures from fatty tissues of different regions of seeds has been explored in cocoa and soybean seeds (6,7) in our laboratory. Quantitatively significant differences have been found; for instance, in germs from soybean seeds the unsaponifiable matter was about 100 times higher than in cotyledons, and the relative proportions of unsaponifiable components were shown to differ according to the selected region (7).

The existence of regional differences in the composition of fats opens new perspectives related to the biosynthesis of fatty acids and minor components, to the specialization of fatty tissues, and to the role played by minor components during the germination of seeds.

In this study, the lipid compositions of three different parts of peanut seeds have been examined; namely germs, cotyledons, and hulls. Unsaponifiable and glyceride composition as well as bound fatty acids has been studied.

Experimental Procedures

Materials

Peanut seeds were subdivided into hulls, germs, and cotyledons by mechanical means. Each of the morphologically distinct parts was ground and homogenized at 200 rpm in the presence of chloroform-methanol 2:1, repeatedly. The extracts were combined, dried over anhydrous sodium sulfate, filtered, and evaporated under vacuum. The residue was taken up with chloroform, filtered, and brought to constant weight. The results, taken as average values from 3 determinations on 250 seeds (96.06 g), are shown in Table I.

[1] Center for the Chemistry of Lipids of the National Council of Research, Milan, Italy.

TABLE I
Distribution of the Lipid Content Between Different Parts of Peanut Seeds

Part of seeds	Weight g	Weight %	Lipid weight g	Lipid[b] %	Lipid[a] %	Unsaponifiable[b] %
Cotyledons	89.20	93.0	39.88	44.6	41.5	0.76
Germs	3.37	3.7	1.74	51.6	1.8	3.18
Hulls	2.80	2.9	0.52	18.6	0.54	3.80

[a] = Per cent on the total seed.
[b] = Per cent on part of seed.

Methods

Preparation of the Unsaponifiables

The lipids prepared as above were treated with methanolic KOH 2 N (20 cc each gram of oil) and kept overnight under nitrogen at room temperature. Water (20 ml each gram of oil) and fresh distilled ether in three portions of 40 ml were then added. The combined extracts were washed first with water, then with bicarbonate, with water again, and finally were dried over anhydrous sodium sulfate. By evaporating the filtered solution under vacuum a residue was obtained and brought to constant weight. The results are shown in Table I and must be corrected for the presence of free fatty acids (8) as shown in Table II.

Fractionation of Unsaponifiables by TLC

Unsaponifiables were fractionated by TLC on silicagel G (E. Merck), Darmstadt, Germany), impregnated with phosphoric acid (2% of 85% phosphoric acid) and Rhodamine 6G. (B.D.H., Poole, England). Plates of 20 by 20 cm, 0.25 cm thick, dried at 105C for 90 min were used. Fifty milligrams of the unsaponifiable material were loaded on each plate and eluted with hexane–ethyl ether 70:30. Bands were isolated by use of UV light, and then scraped and eluted with chloroform–anhydrous ethyl ether 2:1.

The fractions recovered by evaporation under nitrogen were weighted, giving the results shown in Table II. Bands containing sterols and triterpenic alcohols were separately examined in GLC with procedures already described (7,9).

Quantitative TLC Analysis of Sterols and Triterpenes

Silicagel G plates impregnated with phosphoric acid but without Rhodamine, previously described, were used. As reference compounds, cholesterol (for sterols) and cycloartenol (for triterpenes) were used in known amounts in chloroform solutions. The spots were made visible by charring in the usual way (10),

TABLE II
Analysis of Crude Unsaponifiables by TLC

Fraction %	Cotyledons	Germs	Hulls
Hydrocarbons[a]	10.4	11.5	38.0
Not identified[a]	15.0	14.2	12.9
Free fatty acids[a]	48.0	21.2	28.8
Fatty alcohols[a]	6.2	24.3	28.8
Terpenes + sterols[a]	20.4	28.8	9.5

[a] Per cent on the unsaponifiable.

TABLE III
Analysis by Densitometry of the Sterols and Triterpenes Fractions of the Unsaponifiables

Part of the seeds	A = sterols[a] %	B = triterp.[a] %	A/B Ratio
Cotyledons	17.5	2.9	6
Germs	22.0	6.8	3.2
Hulls	7.1	2.4	3

[a] Per cent on the fraction.

then scanned in a photodensitometer, (Chromoscan, Joyce, Loebl & Co. Ltd., Galeshead, England). Data are shown in Table III. Triglycerides as recovered from TLC plates on silicagel and freed from other lipids were fractionated on silver nitrate–impregnated plates.

These plates were prepared according to Morris (11) and developed with benzene-cyclohexene 9:1. The amount of each fraction was calculated by quantitative GLC analysis of the methyl esters, after addition of a known amount of methyl heptadecanoate. See Table IV for data.

GLC Analysis of Sterols and Triterpenes

Analysis of the sterol and triterpene fractions was made with a Model D gas chromatograph (C. Erba, Milan) equipped with a hydrogen flame ionization detector containing a glass column (2 m long, 4 mm inner diameter), packed with SE 30, 1% on gas-chrom P, 100–200 mesh, silanized. The temperature of the column was 220C and nitrogen flow rate 30 ml/min.

GLC Analysis of Hydrocarbons

The analysis of hydrocarbons was made with a Model C gas chromatograph (C. Erba, Milano) equipped with a hydrogen flame ionization detector, containing an aluminum column (2 m long, 4 mm inner diameter) packed with SE 30, 1% on gas-chrom P, 100–120 mesh. The temperature of the column was programmed from 100C to 300C at 5C/min.

Fatty Acid Composition by GLC

Triglycerides were converted to their methyl esters by methanolysis and were catalyzed by hydrochloric acid. Analyses were made on a Fractovap Model C (C. Erba, Milan) flame ionization detector. Column (2 m by 4 mm) was packed with DEGS 30% (w/w) on acid-washed 60/89 mesh Chromosorb W. The data are presented in Table V.

Triglycerides Composition by GLC

GLC of triglycerides was accomplished using the general procedure described by Kuksis and McCarthy (12). Analyses were run on a Fractovap Model D (C. Erba, Milan) equipped with a flame ionization detector and a 60-cm by 4-mm column packed with 3% (w/w) SE 30 on silanized 60–80 mesh Chromosorb W. Temperature programming from 200C to 320C at 5C/min was employed with nitrogen as the carrier gas at 100 ml/min.

TABLE IV
Weight Per Cent Distribution of Triglycerides

Part of seeds	1 Double bond	2 Double bonds	3 Double bonds	4 Double bonds	5 Double bonds
Cotyledons	11.5	32.2	38.4	17.2	0.7
Germs	7.0	22.1	31.4	23.2	16.3
Hulls	9.3	39.7	41.5	9.5	.....

TABLE V
Fatty Acid Composition of Morphologically Distinct Parts of the Peanut Seeds

Fatty acids	Cotyledons	Germs	Hulls
C 14:0	Tr	Tr	Tr
C 16:0	9.2	16.2	11.9
C 16:1	0.3	0.4	0.4
C 17:0	0.1	0.2	0.2
C 17:1	.....	.....	0.2
C 18:0	3.7	2.7	3.5
C 18:1	62.7	45.5	55.1
C 18:2	16.2	26.6	20.0
C 18:3	1.6	2.0	1.9
C 20:0	2.0	1.6	1.8
C 22:0	2.7	2.8	3.2
C 24:0	1.5	2.0	1.8

Triglyceride fractions, previously obtained from TLC according to their degree of unsaturation, were catalytically hydrogenated (Pd/C) and then analyzed in programmed GLC. Results are shown in Table VI.

Results and Discussion

As described in the experimental section the morphologically distinct parts of peanut seeds are extracted after grinding with chloroform methanol 2:1 to determine the amount of fatty material present. Germs have been shown, by this procedure, to be richer in total fats than cotyledons and hulls; hulls, as expected, contained far less fatty material than the other two (Table I). In regard to the chloroform-methanol extracts, two main aspects have been taken under examination, the composition of the unsaponifiable, both from a qualitative and quantitative standpoint, and the fatty acid composition of the neutral fat. The distribution of fatty acids in the triglycerides has also been taken into account by utilizing a simplified procedure. Table I shows that germs and hulls have comparable amounts of unsaponifiable matter, and that the same classes of compounds are shown present in both the unsaponifiables (Table II). Upon further analysis, however, several differences are noted in the distribution of the components. The main components of unsaponifiable material from the hulls are the hydrocarbons which constitute about 33% of the neutral unsaponifiables. Similar amounts of fatty alcohols, sterols, and triterpenes are, however, present. Germs are richer in alcohols (triterpenes + sterols + fatty alcohols) than hulls and have lower hydrocarbon contents. Cotyledons, which contain far less unsaponifiables than the other two parts (0.76%), show, as main components, cyclic alcohols (triterpenes and sterols).

Unsaponifiables from the three extracts were further analyzed by fractionation on TLC plates impregnated with phosphoric acid and each fraction was submitted to a GLC examination. Saturated hydrocarbons ranging from C18 to C35 were present in all of the fractions examined and only the relative amount of each individual compound was variable (Fig. 1).

Squalene is the most important hydrocarbon; it

TABLE VI
GLC Analysis of Hydrogenated Triglycerides

TGC	Cotyledons	Germs	Hulls
50	3.0	8.8	3.3
52	23.8	31.9	24.8
54	58.4	39.5	53.4
56	8.0	9.7	9.5
58	5.2	7.1	6.7
60	1.6	3.0	2.3

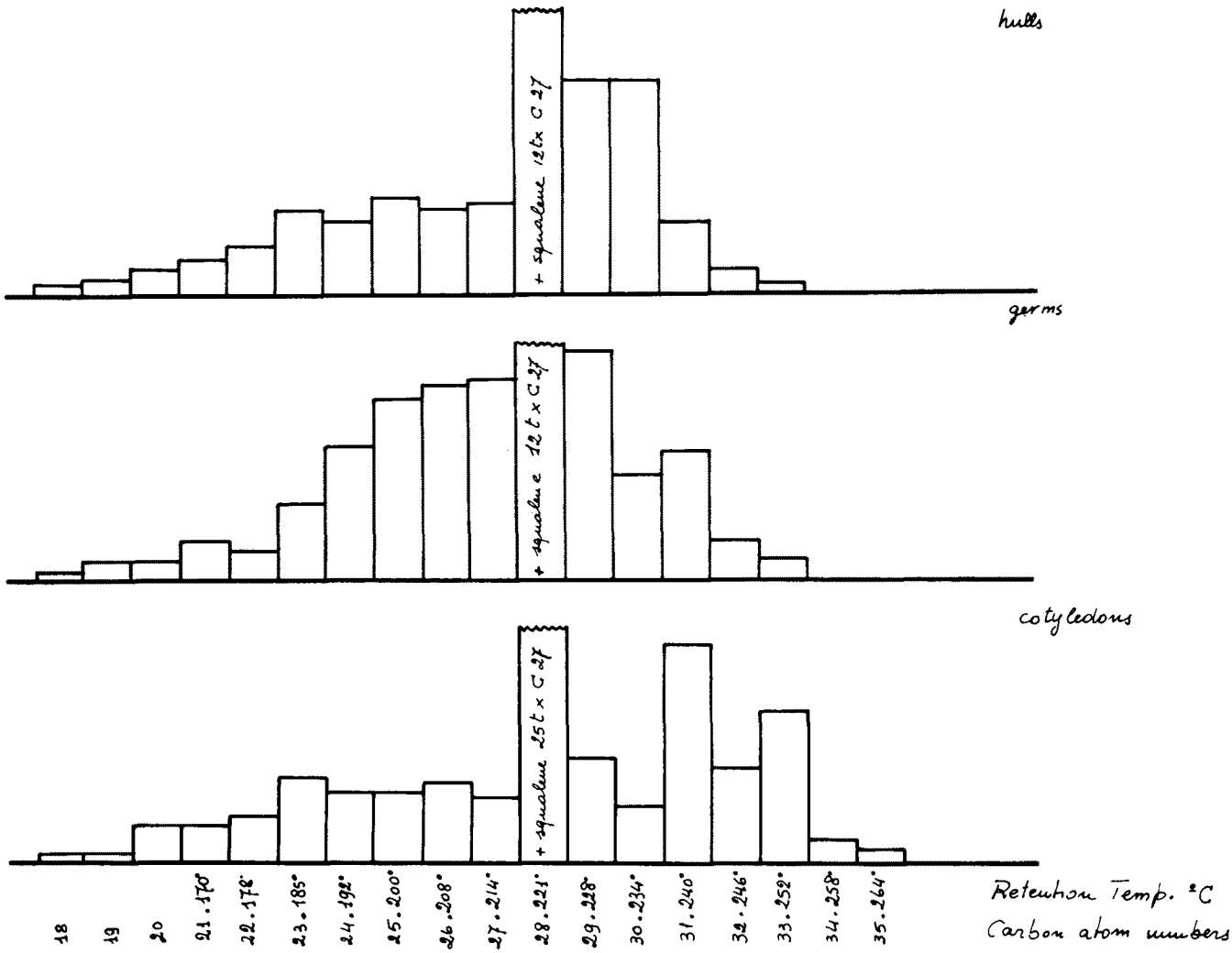

FIG. 1. Quantitative distribution, by GLC, of the hydrocarbons from parts of peanut seeds. The squalene and the C28 peaks, not separated, are given as their sum and are expressed as a multiple of the C27 peak.

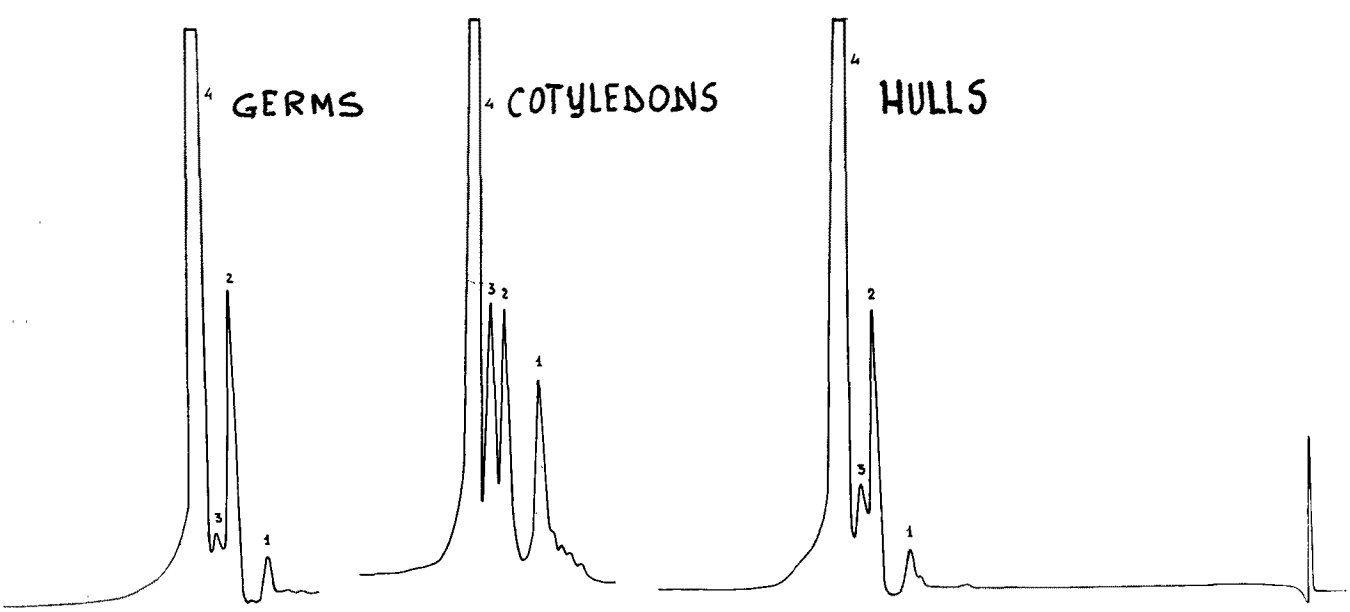

FIG. 2. GLC of the sterols fraction in the three parts of the seed. (1) Unknown, (2) stigmasterol, (3) campesterol, (4) β-sitosterol.

is of interest to note that saturated hydrocarbons tend to have maximum intensity at range C25–C30 for hulls and germs, while cotyledons have maximum intensity between C29 and C33. This fact has probably some relation to different biosynthetic paths.

From the analysis of triterpenic alcohols by GLC some observations can be reported. Cycloartenol (CA), 24-methylencycloartanol (24-MCA), and β-amyrin are always present, but in hulls a peak emerging after 24-MCA is also present. This peak is shown to represent the main component of the triterpenic alcohols mixture. In the other extracts 24-MCA is no longer dominating over the other triterpenes and is present in amount similar to that in CA. No differences have been noted between triterpenes present in cotyledons and in germs, both from a qualitative and a quantitative standpoint, except in the region of diterpenic alcohols.

It has been shown that phytol (13) is an artifact produced during saponification from chlorophyll; therefore, relating the quantity of phytol to chlorophyll, it is to be noted that hulls have, as expected, the highest chlorophyll content and germs the lowest.

Sterols found in all three extracts confirm previous reports (9) that β-sitosterol, campesterol, and stigmasterol are the main components of the sterol fraction, even if their relative proportions vary within the three parts of the seed. In effect, since β-sitosterol is always predominant, stigmasterol and campesterol show appreciable variations (Fig. 2).

Unknown components of the sterol fraction are also shown in the chromatogram (Fig. 2); it has not as yet been determined whether they are natural components or artifacts from saponification. The relative proportions among sterols and triterpenes are shown in Table III and have been calculated from densitometry on TLC plates, as indicated in the experimental part.

Even when the absolute quantities are very different, germs and hulls have a similar sterol-to-triterpene ratio. This ratio is higher for cotyledons and is comparable to the data found in soybean, showing that germs contain higher quantities of sterols than any other part of the seed (7). The presence of higher amounts of sterols in germs can be related to the germination mechanism. It has been demonstrated (14) in fact that sterols are transformed during the germination of seeds.

Remarkable differences regarding the distribution of fatty acids in the morphologically distinct parts of the peanut seeds are presented in Table V. Iodine values calculated from the GLC analysis are very close for hulls and cotyledons, while germs have a higher value (90.6, 89.8, and 94.5, respectively).

Germs are richer in palmitic and linoleic acids, while cotyledons and hulls have more oleic acid than germs. Differences in the distribution of fatty acids correspond to the data obtained for the distribution of triglycerides according to unsaturation. In fact, germs have higher values for the more unsaturated glycerides (four and five double bonds) than either hulls or cotyledons.

The compositions of the glycerides (Table VI) obtained from GLC analysis also confirm the differences found in the composition of fatty acids. Cotyledons and hulls have similar distribution of the triglycerides according to MW, while germs are richer in palmitic acid and contain more low molecular weight triglycerides. These differences in the distribution of glycerides must be taken into account when dealing with distribution theories of fatty acids in fats and oils.

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[Received February 16, 1968]