Regional Differences of Lipid Composition in Morphologically Distinct Fatty Tissues: IV. Safflower and Sunflower Seeds

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ABSTRACT

The lipids were extracted from three different anatomical parts of safflower and sunflower seeds. The neutral lipids and nonsaponifiable components were examined. While the differences in composition of the neutral lipid fractions in the extracts of the various anatomical parts were insignificant, the differences in minor components were noteworthy.

INTRODUCTION

Within the framework of research that we have been conducting on the nature of lipids in the various anatomical parts of oleaginous seeds (1-3) we are now considering two types belonging to the Compositae family, the safflower *(Carthamus tinctorious)* and the sunflower *(Helianthus annuus)*. Previous research (1-8) had shown the existence of qualitative and quantitative differences in the lipid distribution and in particular that of the minor components of anatomical parts of seeds.

These differences, beside being of certain scientific interest (inasmuch as they are a sign of the various biosyntheses of lipids in the seeds), are sometimes of technical interest since they grant the opportunity to separate the cotyledons from the embryos by physical and mechanical processes and thus to extract from them a different type of oil. In this report we are considering the minor components of the nonsaponifiable fraction, and the glyceridic composition of three different anatomical parts—cotyledon, tegument and embryo—of the two seeds studied.

MATERIALS AND METHODS

Materials

The teguments, embryos and cotyledons of safflower and sunflower seeds (high oil type varieties) were separated and extracted separately with chloroform-methanol 2:1 v/v in a homogenizer at 200 rpm. Following extraction

TABLE I

Distribution of the Lipid Content in Different Parts of Safflower and Sunflower Seeds

	Total lipid,[a] %	Fat,[b] %	Unsaponifiable,[c] %
Safflower			
Cotyledon	49.5	45.5	2.17
Embryo	9.0	46.6	1.44
Tegument	41.5	3.9	4.70
Sunflower			
Cotyledon	54.0	56.2	1.5
Embryo	7.4	53.4	3.0
Tegument	37.8	5.0	11.3
Skin	1.3	11.2	---

[a]Weight per cent on total seed.
[b]Per cent on part of seed.
[c]Per cent on fat.

TABLE III

Triglycerides From Different Parts of Sunflower and Safflower Seeds by Gas Liquid Chromatography

	Carbon number				
	48 %	50 %	52 %	54 %	56 %
Sunflower					
Cotyledon	1.9	1.7	24.8	71.6	---
Embryo	0.7	1.8	23.5	74.0	---
Skin	---	1.6	21.2	75.8	1.4
Tegument	---	1.3	23.5	75.2	---
Safflower					
Cotyledon	---	1.7	24.2	74.1	---
Embryo	---	2.3	31.4	66.3	---
Tegument	---	2.0	25.5	72.5	---

TABLE II

Gas Liquid Chromatographic Analysis of Fatty Acids in Different Parts of Sunflower and Safflower Seeds

Fatty acids	Sunflower				Safflower		
	Cotyledon, %	Embryo, %	Skin, %	Tegument, %	Cotyledon, %	Embryo, %	Tegument, %
12:0	---	---	---	---	---	---	0.2
14:0	0.1	0.2	0.2	0.3	0.2	0.2	0.5
16:0	6.2	7.0	7.6	7.2	6.9	8.2	8.8
16:1	0.2	0.1	0.4	0.4	0.2	0.4	0.5
17:0	0.1	---	0.1	0.1	---	Trace	0.2
17:1	Trace	---	0.1	0.1	---	Trace	0.2
18:0	4.6	4.0	4.4	4.9	2.9	2.4	3.2
18:1	26.4	21.5	22.8	24.5	13.2	9.5	15.2
18:2	61.0	65.9	63.0	60.2	75.3	78.2	69.0
20:0	0.5	0.6	0.4	1.0	0.6	0.7	0.6
18:3	0.1	0.2	0.4	0.4	0.2	0.4	1.1
22:0	0.5	Trace	---	Trace	0.2	---	---
22:1	0.3	0.5	0.6	0.9	0.2	---	0.3
IV	134.8	138.8	135.8	132.4	148.5	160.3	141.6

TABLE IV

Distribution of Fatty Acids in the 2-Position of the Triglycerides

Fatty acids	Sunflower Seed				Safflower Seed		
	Cotyledon, %	Embryo, %	Skin, %	Tegument, %	Cotyledon, %	Embryo, %	Tegument, %
16:0	1.2	1.4	2.8	0.9	1.8	1.2	2.2
16:1	0.81	1.0	1.2	0.6	0.9	1.7	0.7
18:0	1.0	0.6	0.9	0.5	0.7	0.5	1.3
18:1	23.2	23.6	26.5	26.9	13.2	12.9	14.2
18:2	73.1	72.7	67.1	71.1	82.8	82.6	81.6
18:3	0.7	1.3	1.0	Trace	0.6	1.1	Trace

TABLE V

Analysis of Triglycerides by Thin Layer Chromatography and Densitometry

Glycerides	Sunflower Seed				Safflower Seed		
	Cotyledon, %	Embryo, %	Skin, %	Tegument, %	Cotyledon, %	Embryo, %	Tegument, %
Double bonds							
2	7.2	7.5	4.4	9.7	5.5	2.1	1.4
3	12.8	8.1	8.1	12.6	5.9	1.9	1.5
4	18.6	19.5	10.5	16.8	19.0	19.6	21.4
5	32.4	31.7	39.5	34.8	24.5	18.7	25.8
6 or more	29.0	33.1	37.4	25.0	45.5	57.4	49.9

TABLE VI

Quantitation of Unsaponifiable Substances by Densitometry and Gas Liquid Chromatography

	Densitometry		Gas liquid chromatography (GLC)		
	Sterols, %	Terpenes, %	Sterols, %	Terpenes, %	Ratio[a]
Sunflower					
Cotyledon	7.9	1.35	6.7	1.13	5:9
Enbryo	18.2	2.42	16.6	1.17	14:2
Tegument	15.7	3.6	16.0	3.5	4:3
Safflower					
Cotyledon	8.6	1.2	5.4	1.0	4:9
Embryo	20.2	8.6	15.9	2.2	7:2
Tegument	14.9	5.1	14.5	5.0	2:9

[a]Ratio of sterols to terpenes by GLC.

TABLE VII

Gas Liquid Chromatographic Analysis of Sterols and Triterpenes

Unsaponifiable	Sunflower			Safflower		
	Cotyledon, %	Embryo, %	Tegument, %	Cotyledon, %	Embryo, %	Tegument, %
Stigmasterol	12.2	13.2	14.9	12.5	21.7	26.4
Campesterol	11.4	11.6	11.6	10.1	6.6	8.6
β-Sitosterol	76.4	65.6	73.4	67.3	63.4	54.3
X_1[a]	---	9.6	---	10.1	8.3	6.3
α-Amyrin	4.2	1.9	4.0	17.0	14.8	15.0
β-Amyrin	8.6	5.9	8.5	59.9	53.6	55.0
Cycloartenol	53.0	43.9	54.0	11.7	14.9	14.0
24-Methylenecycloartanol	25.6	48.3	23.5	11.4	16.7	16.0
X_2[b]	8.6	---	---	---	---	---

[a]Retention time vs. cholesterol 1.47.

[b]Retention time vs. cholesterol 1.73.

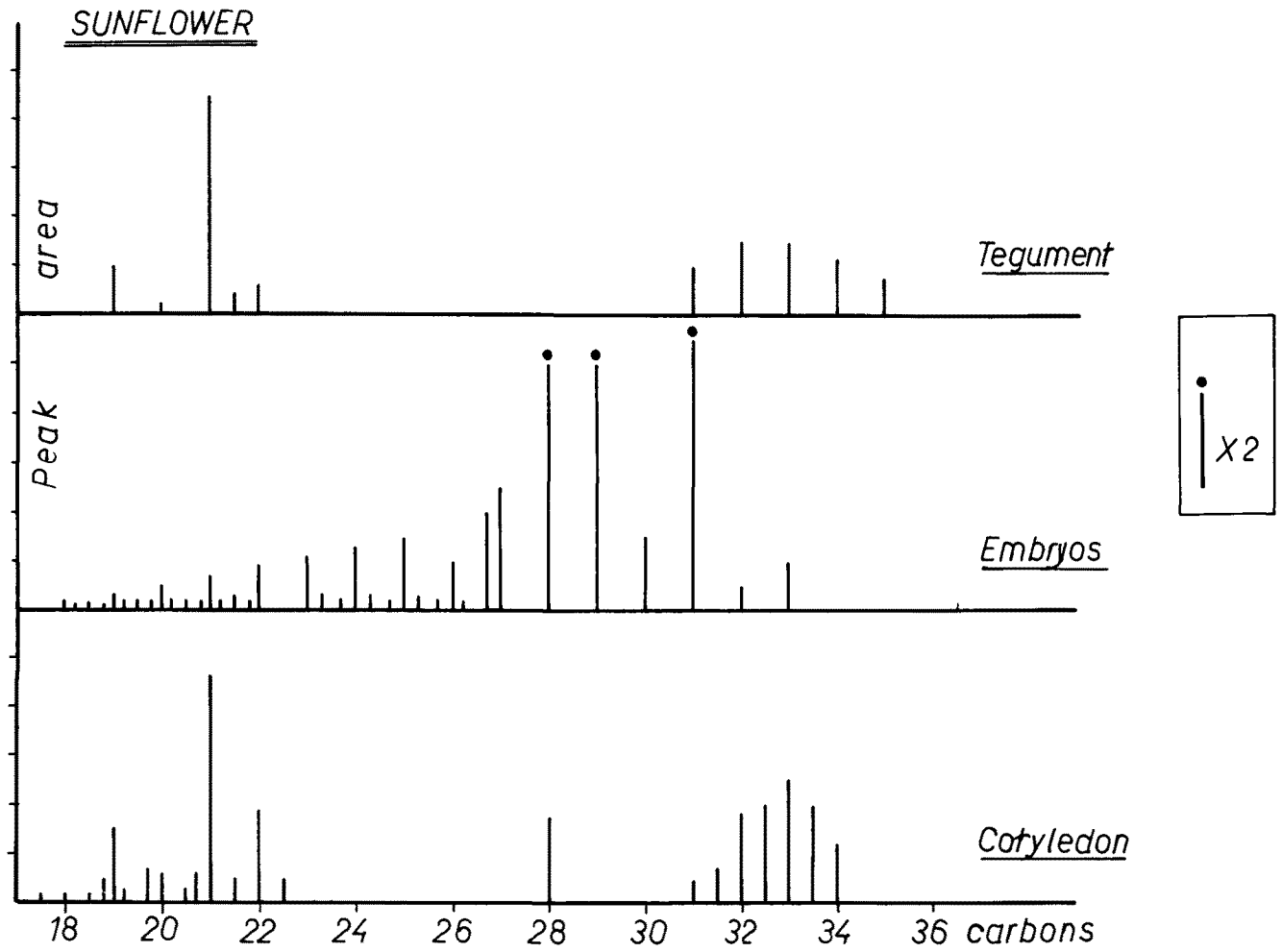

FIG. 1. Hydrocarbons of sunflower seed.

the solutions were dried over sodium sulphate, reduced to a small volume and reextracted with hexane. The hexane fraction was then evaporated, weighed and stored. The results are average values obtained from three different experiments.

Preparation of Nonsaponifiable Substances

The lipids were treated with a 2 N solution of potassium hydroxide in methanol (20 ml/g of oil) and kept under nitrogen at room temperature for 12 hr. Water was added (20 ml/g of oil) and the solution extracted with three 40 ml portions of ethyl ether, freshly distilled. The combined extracts were washed with water and dried over sodium sulphate. The solvent was removed and the residue was brought, under vacuum, to a constant weight.

Fractionation of Nonsaponifiable Substances

The nonsaponifiables were separated by thin layer chromatography (TLC) on Silica Gel G (E. Merck, Darmstadt, Germany), impregnated with 2% phosphoric acid on plates 20 x 20 cm (2). The nonsaponifiable products (50 mg) were placed on each plate and developed with hexane-ether 2:3 (v/v). The bands were revealed with 2,7 dichloro-fluoresceine under UV light. Chloroform-ether 2:1 v/v was used to recover the products from the plates. The fractions were weighed and used for the gas liquid chromatography (GLC) analyses by using published procedures (1,9,10).

Distribution of Fatty Acids in the 2 Position

The method utilized was the same as that described by Jurriens et al (13).

Quantitative Analysis of Sterols and Terpenes

Quantitative analyses of sterols and terpenes present in the nonsaponifiable substances were carried out by densitometry using a Chromoscan photodensitometer (Joyce and Co., Ltd., Galeshead, England) by the procedure previously described (1).

Analysis of Sterols and Terpenes

Terpenes and sterols were analyzed by GLC using a dual column gas chromatograph model D (C. Erba, Milan, Italy) equipped with a flame ionization detector. A 2 m x 4 mm glass column packed with 1% SE 30 on 100-200 mesh silanized Gas Chrom P was used. The column temperature was 220 C, nitrogen flow 30 ml/min.

Analysis of Hydrocarbons

Analysis of the hydrocarbons was carried out with a Fractovap model C gas chromatograph (C. Erba, Milan) on a 2 m x 4 mm column filled with 1% SE 30 on Gas Chrom P (100-200 mesh). The instrument contained a flame ionization detector. The column temperature was programmed from 100 to 300 C at 5 C/min.

Fatty Acid Composition

The methyl esters were prepared by methanolysis (catalyst: hydrochloric acid in a closed vial). The GLC analyses were obtained with the model C Fractovap gas chromatograph, employing a 2 m x 4 mm column filled with 20% DEGS w/w on Chromosorb W 60-90 mesh.

Mass Spectrometry

A Perkin-Elmer model 270 instrument was utilized.

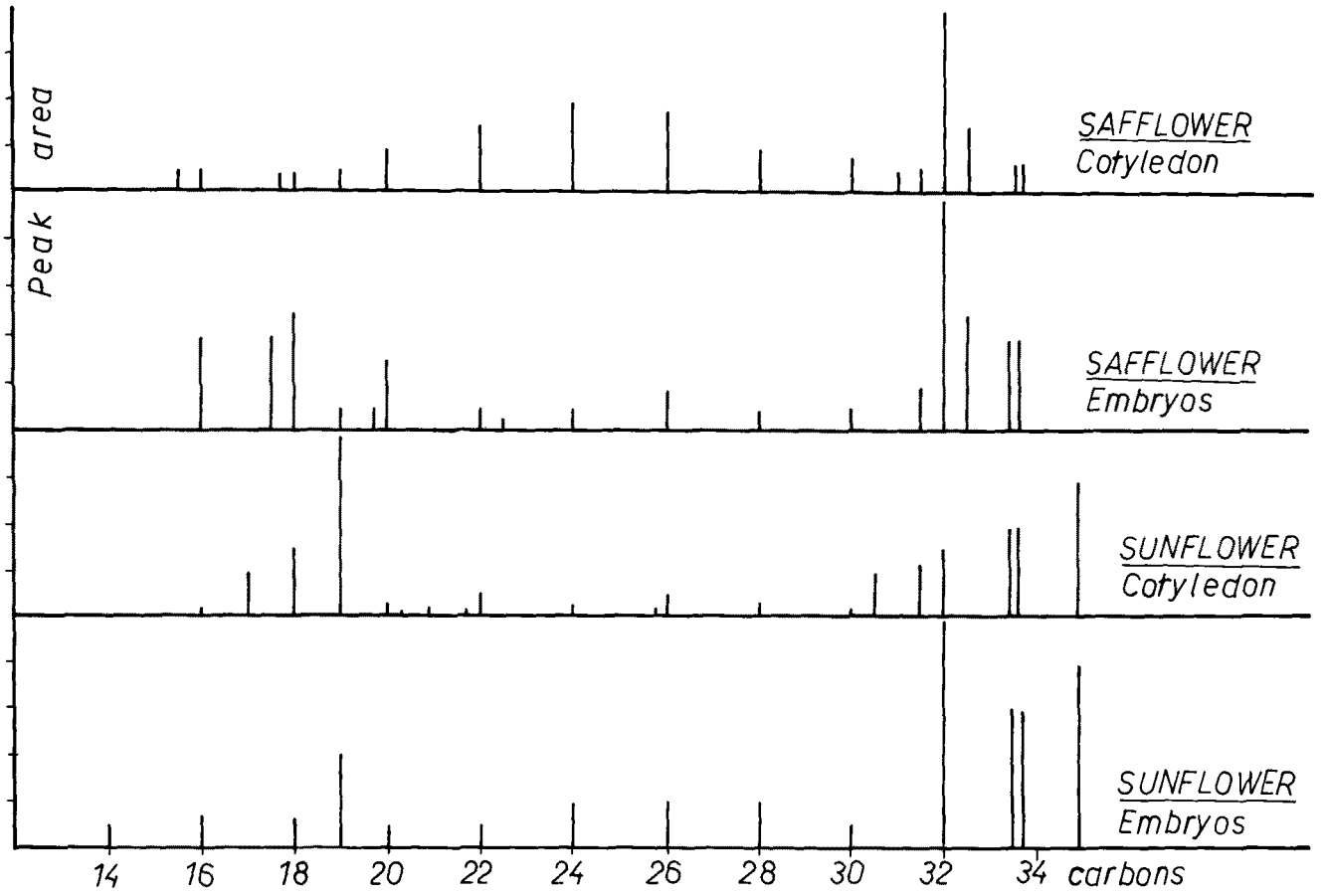

FIG. 2. Fatty alcohols of sunflower and safflower seeds.

Working conditions were: GLC 1% SE 30 on Gas Chrom P column, 2 m x 4 mm; column temperature, 220 C; helium flow, 30 ml/min; mass spectrometry, accelerating voltage 70 ev.

Triglyceride Composition by GLC

Before GLC analysis the triglycerides were hydrogenated in presence of palladium on carbon black (5%). The procedure described by Kuksis and McCarthy (12) was followed for the GLC using the model D Fractovap gas chromatograph. The column 60 cm x 4 mm was packed with 3% SE 30 on Chromosorb W, 60-80 mesh; temperature was programmed from 200 to 320 C at 5 C/min.

Triglyceride Composition by TLC

Silica Gel G 20 x 20 cm plates impregnated with 20% silver nitrate were used. Benzene-ether-methanol mixture 85:15:15 v/v/v was used for the development. The unsaturation of the different fractions was determined by GLC of the methyl esters. Quantitation of the glycerides was determined with the Chromoscan photodensitometer.

RESULTS AND DISCUSSION

Sunflower

The four anatomical parts of the seed (cotyledon, embryo, skin and tegument) contain different quantities of lipids (Table I). Cotyledons and embryos are richer in lipids than are teguments with the skin having a lesser amount. Contrary to what was noted in peanuts (1,3) the fatty acid composition (Table II) of sunflower seed parts is almost always the same even with respect to the less important fatty acids. The small differences noted are reflected in correspondingly modest variations of the iodine value (IV); the lipids of the skin have IV = 135.8 while the lipids in the cotyledons have 134.8.

The GLC analysis of the triglycerides indicate some differences in composition of the different parts of the seed; the most marked differences again are observed between cotyledons and skin (Table III).

Results of the fatty acid distribution in the glycerides indicated a preferential distribution of the more unsaturated fatty acids in the 2 position—a rule more or less valid, with slight differences, for all the glycerides obtained from the four parts of the seed (Table IV).

The preceding data accompanied by the results of the densitometer analysis of the lipid components of various unsaturation (Table V) lead us to conclude that over and above the differences due to experimental errors the glyceride composition of the four extracts is very similar.

The differences in content of the unsaponifiable substances in the lipids are noteworthy. The tegument has the greatest content of unsaponifiable substances compared with the other anatomical parts while the embryo has twice the content of the cotyledons (Table I). The correlation between the main components of the unsaponifiable substances (Tables VI, VII), the triterpenic alcohols and the sterols, is variable in the various extracts as determined by GLC; cotyledons and tegument have ratios of sterols-triterpenes that are very similar (r 5:9 and 4:3 respectively) while embryos show a very high sterol content in relation to triterpene (r 14:2). Differences of this type have been noted in other cases showing that the embryos have a consistently high sterol content.

The chief sterol is always β-sitosterol together with approximately equal quantities of campesterol and stigmasterol (Table VII). An unknown component is also present whose retention time compared with cholesterol is 1.47. The mass spectrum of the trimethylsilyl (TMS) derivative of the component shows that the derivative has an M^+ of 486, confirmed by M-15 at m/e 471. The peaks

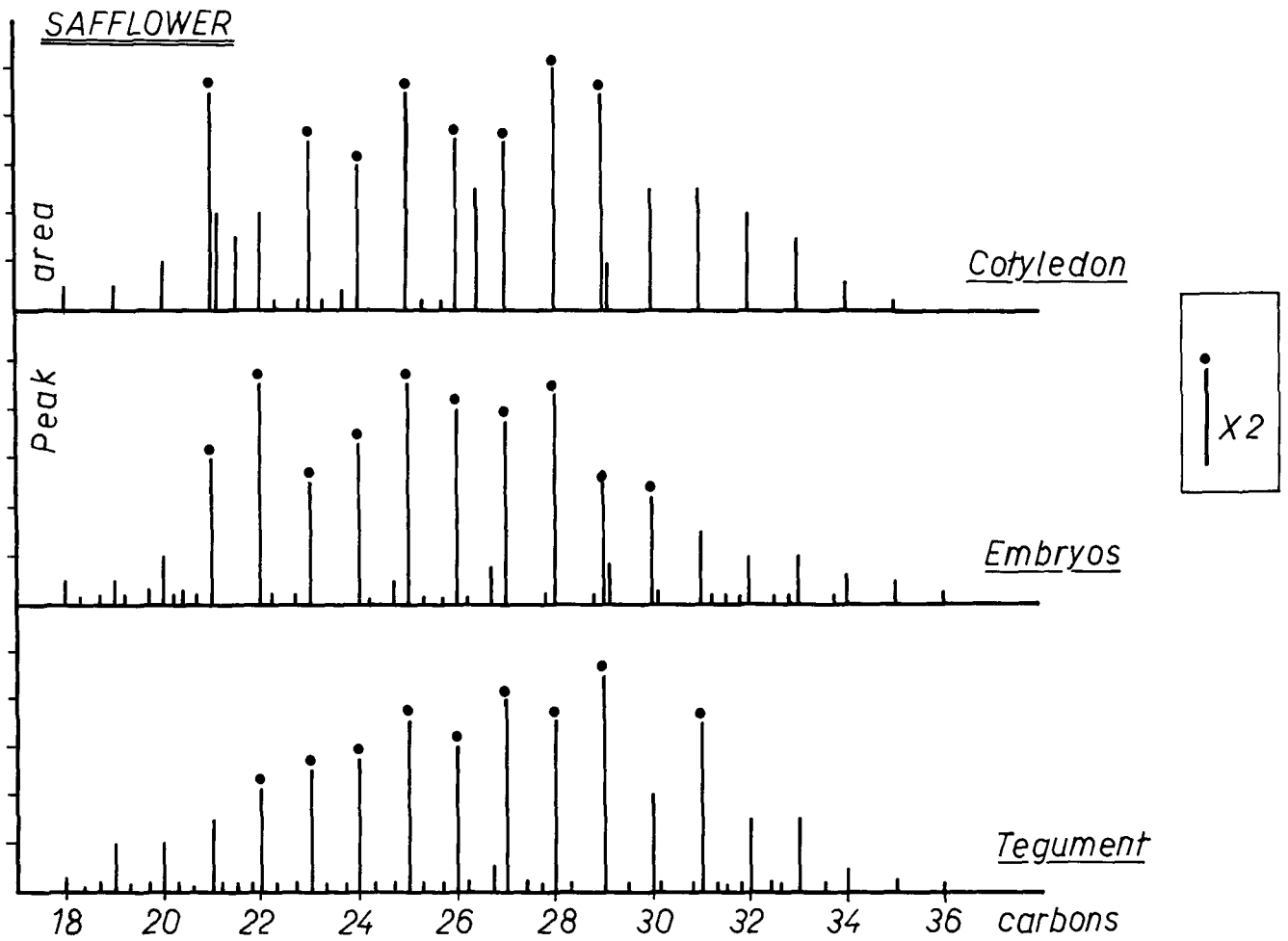

FIG. 3. Hydrocarbons of safflower seed.

m/e 396 (M-90) and 381 (M-90-15) which correspond to the loss of $(CH_3)_3$-SiOH from M^+ and M-15 are found in TMS derivatives of sterols. The free sterol must have molecular weight 414 and according to the fragmentation a 3-β-hydroxy structure (M-129, M-143). The fragments at m/e 255 and 213 are indicative for a structure similar to that of β-sitosterol. The sterol could be Δ^{-7} stigmasterol but further work is needed to clarify the structure.

The fraction of triterpenic alcohols in the unsaponifiable substances of various extracts of sunflower seed parts consists mainly of cycloartenol and 24-methylene cycloartanol; α-amyrin and β-amyrin (Table VII) are present in minor proportions. In the unsaponifiable substances of the cotyledon there is also a substance of unknown nature with a retention time of 1.73 in respect to cholesterol taken as a standard. The fraction of hydrocarbons derived from the unsaponifiable substances of the different extracts shows a marked complexity (Fig. 1).

Besides the known components (hydrocarbons from C-18 to C-35), numerous other hydrocarbons of unknown structure also appear in cotyledons and embryos. The distribution of the various components is very different in the three cases listed in Figure 1. For example, in the tegument, squalene which constitutes the chief component of the hydrocarbons of the embryo is missing, whereas a hydrocarbon at C-21 present as one of the most important components in the cotyledons, predominates.

The alcohols, too, show differences among the four parts of the seed and their composition is decidedly complex. The profile for two of these parts is shown in Figure 2. In cotyledons and embryos the same linear alcohols are present from C-14 to C-32 but in different proportions: C-32 and unknown components, eluted between C-32 and C-36, predominate in the embryos. In the cotyledons the most important component is an alcohol with a retention time close to that of a n-C-19.

Safflower

The cotyledons and embryos contain almost equal amounts of lipids (Table I); the tegument also contains lipids but to a much lesser degree. The fatty acid compositions of all the tissues (Table II) are quite similar both from a quantitative and qualitative point of view. However, the embryos present a greater over-all unsaturation (IV=160.3) than do the other two seed components.

The triglyceride composition of the neutral lipids reveals that the difference between cotyledon and tegument is minimal whereas it is more marked between these two components and the embryo (Table III).

The distribution of fatty acids in the 2-position, preferential as far as the unsaturation in this position is concerned, is not very different in the three cases considered (Table IV). The polyunsaturated glycerides (Table V) are predominant in the extracts of all three anatomical tissues; the embryos contain the highest percentage of polyunsaturated fractions as compared with cotyledons and tegument.

The unsaponifiable content of the tegument is markedly higher than that of the cotyledons and embryos (Table I). The latter show the highest sterol content (Table VI); the quantities of triterpenes vary in the three extracts with the tegument having the highest percentage.

The predominant sterol in all cases is β-sitosterol, followed by stigmasterol and campesterol (Table VII). However, the relative quantities of the three components vary, as does that of the fourth compound with retention

time of 1.47 relative to cholesterol. The β-amyrin in the triterpenic alcohol fraction of all the unsaponifiable substances of the three anatomical parts represents 53-60% of the entire mixture with relatively small variations. Cycloartenol, 24-methylenecycloartanol and α-amyrin are present in approximately equal proportions (Table VII). The composition of the hydrocarbon fraction (Fig. 3) is very complex because of the presence of numerous unknown substances; the identified hydrocarbons, of similar quantity in all three cases, are within the range of C-18 to C-36. Hydrocarbons of unknown structure are present in the cotyledons with retention times close to those of the hydrocarbons of the normal series at C-21, C-26, and C-29. In the cotyledons and embryos the chief alcohol is C-32 (Fig. 2) but there are also moderate quantities of normal alcohols from C-16 to C-32 together with unknown products of which the most abundant appears in GLC close to the C-32 alcohol.

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