C_{3-7} *n*-alkanes and *n*-alk-1-enes.

New techniques employing programmed cryogenic temp gas chromatography (7) with rapid scanning mass spectrometry (8) now permit easy separation and identification of the volatile compounds in complex mixtures. These techniques have already been used to verify and extend the analyses described in this paper. For example, methane, ethane and some branched chain hydrocarbons now have been identified. These methods are also currently being used to extent the investigations to meats other than beef and to several fundamental lipid substances related to meats. The discovery of the widespread occurrence of hydrocarbons in irradiated lipids as well as in oxidized and thermally decomposed lipids suggests that much further detailed investigation is required to understand the various mechanisms involved in the degradation of natural fats and oils.

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Lipid Metabolism in Germinating Flaxseed[1]

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Abstract

Flaxseed was germinated in the dark at 25C for 90 hr and the amt of triglycerides, free fatty acids (FFA) and phospholipids, as well as the fatty acid composition of each, were determined at 18-hr intervals. The amt of FFA increased greatly during germination. There was no preferential metabolism of any particular fatty acid in either the triglyceride or FFA fractions. The percentage of linolenic acid in the phospholipids increased as germination progressed. Behenic, lignoceric and cerotic acids were observed in the FFA fraction after 54 hr of germination. Odd-numbered saturated and unsaturated acids, indicative of an α-oxidation mechanism, were observed in the FFA fraction at 54 hr and in the triglyceride fraction at 72 hr.

Introduction

THE METABOLISM of storage lipid, primarily triglycerides, provides the main source of energy for the germination process in oil-bearing seeds. Although both the glycerol and fatty acid portions of the triglyceride molecule are utilized during germination, the metabolism of the fatty acids was of primary interest in this study. Many workers have studied the changes in the lipids of germinating flaxseed (*Linum usitatissimum* L.) (1). However, most of these reports have dealt with the gross changes in the lipids such as iodine value (I.V.), acid value and oil content. The most extensive study of the changes in germinating flax was made by Desveaux and Kogane-Charles in 1952 (2). They reported the fresh wt, dry wt, total lipid content, FFA content, the amt of reducing, nonreducing and easily hydrolyzable carbohydrates, I.V., neutralization equivalent, non-saponifiable content and the organic acids during 12 days of germination. Most recently, Huber and Zalik (3) reported the changes in oil content, fatty acid composition and amino acid composition in developing and germinating flaxseed. The latter work was the first to use gas chromatography to determine the fatty acid composition during germination.

Although much is known about the changes in lipid content during germination, little is known about the changes in the fatty acid composition of the individual lipid classes. In most of the previous studies, petroleum ether was used as an extraction solvent and consequently the polar lipids were not quantitatively extracted. In addition, observations were not made during the initial 5 days of germination, which Halvorsen has shown to be period of active lipid metabolism (4). Desveaux and Kogane-Charles (2) reported a 23% decrease in the I.V. of the oil while Ermakov and Ivanov (5) and Paatela (6) observed no significant change in the I.V. during germination.

In view of the inadequacies and conflicting results of previous studies on germinating flaxseed, it seemed advisable to reexamine the changes in lipids which occur during the initial germination period.

Experimental

General Comments. All solvents were distilled prior to use. All operations involving the total lipid extract were conducted under nitrogen to reduce the possibilities of oxidation. When not in use, the total lipid extract was stored under nitrogen in the dark at −17C. All of the spectrophotometric analyses were conducted with a Beckman DK-2 spectrophotometer using cells with a 1-cm light path.

Germination. Flaxseed, variety C.I. 1303, was surface sterilized with a 2.67% aqueous sodium hypo-

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[3] This paper is taken in part from the dissertation for the degree of Doctor of Philosophy of D.C.Z., 1964.

chlorite solution, washed with sterilized distilled water and placed in a sterilized 14-cm petri plate containing filter paper wetted with 4 ml of sterilized distilled water. The petri plates were placed in an incubator at 25C ± 0.5C in the dark. Two ml of water were added to each plate after 18 hr and again after 54 hr. Germination was greater than 95% and nonviable seeds were rejected from the sample.

At 18-hr intervals, duplicate samples of 100 seedlings each were counted, frozen in an acetone-dry ice bath and lyophilized. The dry material was transferred to a tared vial and the dry wt/100 seedlings obtained.

Extraction. Each dried sample was placed in a stainless steel cup with 15 ml chloroform-methanol (2:1 v/v) under nitrogen and homogenzied at 45,000 rpm with a VirTis Model 45 homogenizer for 3 min. The cup was placed in cold water to prevent excessive heating of the solvent mixture. After transfer to a 30-ml Teflon-lined screw cap culture tube, the mixture was placed under nitrogen and allowed to extract at 25C for 18 hr. The mixture was centrifuged to remove cellular debris and the supernatant decanted. Five ml of solvent was added to the tube and centrifuged again. The supernatant solutions were combined, washed via the Folch technique (7) and diluted to 25 ml with chloroform-methanol. This is the total lipid extract referred to in later sections.

Analysis of the Lipid Extract. Two 3-ml aliquots were taken from each lipid extract, evaporated to dryness under a stream of nitrogen and weighed. The wt of total lipid/100 seedlings was thus obtained.

The amt of phosphorous in the total lipid extract was determined by the method of Marinetti (8). The wt of the phospholipids was calculated on the basis of a 3.7% phosphorous content in flax phospholipids (9).

The triglycerides and FFA were separated on thin-layer (250 μ) plates prior to analysis. The thin-layer plates were prepared by mixing 30 g silica gel G, 1 ml 85% phosphoric acid and 64 ml 0.2% aqueous Rhodamine 6G solution and applying this mixture to 5 glass plates (20 cm x 20 cm) with a commercial applicator. The plates were activated at 110C for 1 hr. Aliquots of the lipid extracts, from each of the two samples which were taken at each germination period, were applied in duplicate to thin-layer plates and the latter developed in a petroleum ether-diethyl ether (70:30, v/v) solvent. After chromatography the various lipid components were viewed as fluorescent spots under UV light of 366 mμ wavelength. After marking the location of the triglycerides, equal areas of gel were scraped off the plate into a 12-ml centrifuge tube and the amt of triglycerides in each tube determined colorimetrically by means of the sulfophosphovanillin reaction (10). A standard triglyceride solution was prepared by weighing out a sample of pure linseed oil triglycerides which had been isolated by thick-layer (1 mm) chromatography.

The determination of the FFA in each extract was completed in the same manner as the triglycerides. A standard FFA mixture was prepared from pure (99+%, Hormel Institute) fatty acids in the following composition: stearic acid 10%, oleic acid 40%, linoleic acid 15%, linolenic acid 35%.

For the separation of fatty acid methyl esters with regard to unsaturation, silver nitrate impregnated thin-layer plates were prepared by dissolving 7.5 g of silver nitrate in 75 ml of water and mixing with 30 g of silica gel H.

Fatty Acid Analysis. The total lipid extract was separated into its various lipid classes by chromatography on an acid treated Florisil column (11). Ten ml of the total lipid extract was evaporated under nitrogen to a volume of 0.5 ml and this was placed on the top of a water jacketed Florisil column containing a 1% solution of diethyl ether in petroleum ether (v/v). The column was then eluted with the following amt of solvents: 60 ml of petroleum ether-diethyl ether (99/1); 50 ml of petroleum ether-diethyl ether (96/4); 30 ml of petroleum ether-diethyl ether (50/50); 60 ml of methanol. Ten-ml fractions were collected and assayed by thin-layer chromatography (TLC) to determine the location of the individual lipid classes. Those fractions containing a single class of lipids were combined, coned under nitrogen and examined further by gas chromatography.

The methyl esters of the fatty acids were prepared from the individual lipid classes obtained from column chromatography. Triglycerides were transesterified with 0.5 *N* sodium methoxide in absolute methanol (12). FFA were esterified with boron trifluoride-methanol reagent (13). Phospholipids were transesterified with boron trifluoride-methanol reagent (14). The preparation of methyl esters was monitored with the use of TLC and the conversion was greater than 95% under the conditions employed for each class. The methyl esters were chromatographed on a 6.1-m diethylene glycol succinate (24% on Anakrom AB, 80/90 mesh) column, 4 mm I.D. and on a 1.5-m SE-30 silicone (5% on Anakrom AB, 80/90 mesh) column, 4 mm I.D. Operating conditions for the DEGS column were oven temp 200C and a flow rate of 6 ml/min of nitrogen. For the SE-30 column, the oven temp was raised to 210C. The two columns were used in conjunction with a hydrogen flame detector mounted in an Aerograph Gas Chromatograph, Model A-90C. Areas of the peaks appearing on the chart were determined by a Disc integrator attached to the 1-mv recorder.

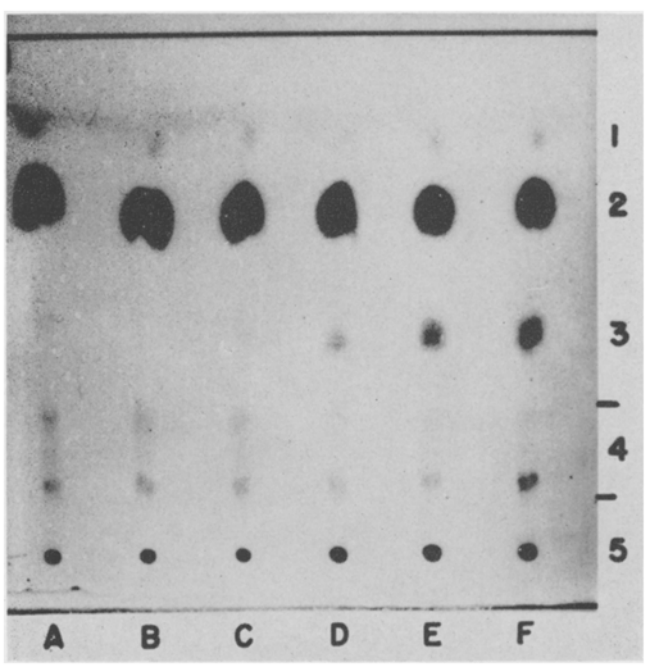

FIG. 1. Chromatoplate of total lipid extracts, 200 μg each, from various germination periods. A, B, C, D, E and F = 0, 18, 36, 54, 72 and 90 hr of germination, respectively. Identification of spots: 1) hydrocarbons and sterol esters, 2) triglycerides, 3) FFA, 4) sterols and triterpenic alcohols, 5) phospholipids. Solvent system, petroleum ether-diethyl ether (70:30, v/v).

TABLE I

Weight of Total Lipid, Triglycerides, FFA and Phospholipids During 90 hr of Germination

Germination hr	Seedling length mm	Per 100 seedlings				
		Dry wt g	Total lipid g	Triglycerides g	Phospholipids g	FFA g
0	0	0.5477	0.1900	0.1735	0.0055	0.0003
18	0–0.5	0.5385	0.1934	0.1699	0.0059	0.0003
36	4–5	0.5197	0.1837	0.1589	0.0076	0.0010
54	12–15	0.5214	0.1650	0.1409	0.0078	0.0058
72	25–40	0.5263	0.1287	0.1021	0.0080	0.0095
90	35–48	0.5489	0.1041	0.0815	0.0069	0.0100
Avg. std. deviation for each germination period		0.0134	0.0049	0.0069	0.0002	0.0003
relative error, 100 $\frac{s}{x}$		2.51%	3.06%	5.01%	3.20%	5.39%

Results

The separation of the various lipid classes is shown in Figure 1.

Diglycerides could not be detected using the thin-layer technique of Rybicka (15) and were adjudged to be absent.

Weight of Lipid Classes. The results of the wt determinations of the lipid classes along with the seedling length and dry wt during 90 hr of germination are listed in Table I.

Fatty Acid Composition of the Lipid Classes. In the remainder of this report, the shorthand method of Farquhar et al (16) will be used in place of the common names of the fatty acids. The method of Landowne and Lipsky (17) was used to test for the presence of branched-chain fatty acids and none were detected. No attempt was made to determine the presence of fatty acid methyl esters shorter than 14 carbon atoms. Quantitative results with National Heart Institute Fatty Acid Standard Mixture C agreed with the stated composition data with a relative error less than 4.1% for major components (>10% of total mixture) and less than 9.8% for minor components (<10% of total mixture). The percentage composition of the fatty acids in the triglyceride, FFA and phospholipid fractions is shown in Tables II, III and IV, respectively.

The results of gas chromatography of the methyl esters of the FFA at 54 hr and the fatty acid methyl esters of the triglyceride fraction at 72 hr are shown in Tables V and VI, respectively.

In Tables II, and III and IV it was noted that 18:3 might contain a small amt of 20:1. Analysis of the triglyceride fraction at 54 hr on the SE-30 silicone column indicated the presence of an unsaturated 20-carbon fatty acid. This particular component was collected from the silicone column and then rechromatographed on the DEGS column. Its retention time corresponded to that of a monounsaturated 20-carbon fatty acid, 20:1. The location of the double bond was not determined. Since this acid has the same retention time on the DEGS column as 18:3, it was not observed as a separate component on that column. The amt of 20:1 in each of the individual fractions was not determined; however, in those fractions in which it was observed, it amounted to ca. double the 20:0 percentage in the phospholipids and triglycerides and ca. one-half of the 20:0 percentage in the FFA.

The equivalent chain length (ECL) and the retention volume relative to that of 18:0 for the minor constituent fatty acids observed in this study are given in Table VII.

TABLE II

Composition of the Fatty Acids in the Triglyceride Fractions

Acid	Hours of germination					
	0 %	18 %	36 %	54 %	72 %	90 %
14:0	tr.[a]	tr.	tr.	tr.	0.1	tr.
15:0	.....	tr.	tr.	tr.	tr.	.....
15:1	.....	.....	.....	.....	tr.	.....
16:0	7.3	9.2	7.6	6.2	7.6	6.6
16:1	tr.	.....	.....	tr.	0.3	0.2
17:0	.....	.....	.....	tr.	0.1	tr.
17:1	.....	.....	.....	tr.	0.1	tr.
18:0	1.9	2.2	2.0	2.2	4.6	3.2
18:1	35.2	38.3	36.4	37.8	31.2	36.6
18:2	10.9	12.5	15.5	12.5	16.2	15.2
18:3[b]	44.7	37.8	39.5	40.9	39.2	38.0
20:0	.....	.....	.....	4.0	0.4	0.1
22:0	.....	.....	.....	tr.	tr.	tr.
24:0	.....	.....	.....	.....	tr.	.....

[a] tr. = less than 0.1%.
[b] May contain a small amt of 20:1, see text.

TABLE III

Fatty Acid Composition FFA Fractions

Acid	Hours of germination					
	0 %	18 %	36 %	54 %	72 %	90 %
14:0	0.8	tr.[a]	0.6	0.1	0.5	0.2
15:0	tr.	tr.	0.4	tr.	0.2	tr.
15:1	.....	.....	.....	.....	0.1	.....
16:0	9.5	5.3	13.3	10.4	9.4	12.3
16:1	1.2	0.3	1.0	0.6	0.9	0.8
17:0	.....	.....	0.2	0.2	0.4	0.1
17:1	.....	tr.	0.2	tr.	0.1	tr.
18:0	1.9	0.9	3.5	1.6	3.9	3.3
18:1	37.3	23.8	20.5	33.2	29.6	28.5
18:2	23.2	15.7	13.8	13.4	17.9	14.6
18:3[b]	26.1	54.0	28.7	31.2	33.2	19.9
20:0	tr.	tr.	3.6	0.6	0.8	0.7
22:0	.....	.....	2.3	0.9	0.5	1.7
24:0	.....	.....	11.8	7.2	2.5	13.8
26:0	.....	.....	.....	0.5	.....	3.9

[a] tr. = trace, less than 0.1%.
[b] May contain a small amt of 20:1, see text.

Discussion

Weight of Lipid Classes. The decrease in dry wt/100 seedlings during the early stages of germination was partially due to a diffusion of mucilage out of the seed or seedling. After 36 hr the conversion of lipid to carbohydrate resulted in a net fixation of oxygen with a small increase in the dry wt of the seedling. A similar increase in the dry wt of the flax seedlings was observed by Desveaux and Kogane-Charles (2) and of soybean seedlings by Brown et al. (18). The change in total lipid content was in general agreement with that noted by other workers (2,3) with the exception that the changes occurred more rapidly due to the higher temp used in this study. During the first 18 hr there was only a slight decrease in the amt of triglycerides; thereafter they were metabolized more rapidly, until at 90 hr only 47% of the original amount remained. It should be emphasized that the changes reported here are the net changes in the various lipid classes in the whole seedling. It is quite plausable that triglycerides were catabolized in one tissue of the seedling and resynthesized in another tissue simultaneously.

An increase in the amt of phospholipids was observed up to 72 hr of germination. However, the amt at 90 hr had decreased. A similar decrease in the phospholipids was observed by Hardman and Crombie (19) in watermelon seed that was germinated in the dark, but which did not occur when germination was conducted in the presence of light.

The FFA content remained unchanged during the initial 18 hr of germination but increased greatly thereafter, comprising almost 10% of the total lipid in the seedling at 90 hr. The constant increase after 18 hr indicated that the activity of the lipase enzyme was greater than at least one of the enzymes involved in the oxidative breakdown of the fatty acids and that

TABLE IV

Composition of the Fatty Acids in the Phospholipid Fractions

Acid	Hours of germination					
	0 %	18 %	36 %	54 %	72 %	90 %
14:0	2.5	0.5	0.7	0.6	0.4	0.7
15:0	tr.[a]	tr.	0.4	0.3	tr.	.....
15:1	.....	.....	.....	.....	.....	.....
16:0	26.0	28.3	21.3	23.8	18.3	18.5
16:1	5.5	0.9	0.3	1.0	1.4	1.9
17:0	tr.	.....	0.3	0.2	0.4	0.5
17:1	1.0	.....	tr.	0.3	0.1	0.4
18:0	6.6	2.1	3.0	4.6	3.5	3.4
18:1	22.5	30.1	22.4	15.7	13.3	12.8
18:2	18.4	22.1	28.4	24.4	32.4	21.9
18:3[b]	17.5	16.1	23.2	27.4	27.4	37.2
20:0	tr.	.....	tr.	0.3	0.2	0.4
22:0	tr.	.....	tr.	0.2	0.8	1.2
24:0	.....	.....	.....	1.0	1.5	0.6

[a] tr. = trace, less than 0.1%.
[b] May contain a small amt of 20:1, see text.

the rate of hydrolysis of the triglycerides by lipase is not limiting the rate of fatty acid oxidation.

Minor Constituent Fatty Acids. Tables II and IV show that the triglycerides and phospholipids contained varying amt of 14:0, 15:0, 15:1, 16:1, 17:0, 17:1, 20:0, 20:1, 22:0 and 24:0 at different times during the germination. Table III shows that these same acids as well as 26:0 were present in the FFA fractions.

The most interesting feature about these minor constituent fatty acids is their time of appearance in the various lipid classes. At 36 hr significant amt of 17:0, 17:1, 20:0, 22:0 and 24:0 appear in the FFA fractions. Eighteen hr later these same acids appear in larger concn in the triglyceride and phospholipid fractions. Small amt of 16:1, 17:0, 17:1, 20:0 and 22:0 were observed in most of the germination periods. The appearance of these minor acids in the triglyceride fraction was possibly due to the fact that the triglycerides were in a dynamic state and were continually being hydrolyzed to glycerol and FFA and resynthesized into triglycerides. An alternate explanation was that the catabolism and anabolism of the triglycerides were occurring simultaneously in two separate tissues within the seedling, for example, oxidation in the cotyledons and synthesis in the root (hypocotyl). Since the different tissues were not separated prior to analysis, it cannot be stated definitely which of the explanations above is correct.

At the present time, α-oxidation is the only mechanism known to account for the occurrence of odd-numbered long-chain fatty acids in higher plants. Alpha-oxidation of 18:0 and 18:1 could account for the appearance of 17:0 and 17:1 in the FFA fraction at 36 and 54 hr. If α-oxidation was occurring at 54 hr, 17:3 should also have been detected. In order to achieve the complete separation of the expected esters, it was necessary to separate the monoenes, dienes and trienes prior to analysis by gas chromatography. This was accomplished by chromatography of the fatty acid methyl esters on thin-layer plates of silica gel H impregnated with silver nitrate. From the data shown in Tables V and VI it was concluded that the significant amt of 15:0, 17:0, 17:1 and 17:3 present in the FFA fraction at 54 hr, and in the triglyceride fraction at 72 hr, provided strong evidence for the existance of an α-oxidation process in germinating flaxseed. The 15:0, 17:0 and 17:1 acids present in most of the phospholipid fractions (Table VI), indicated that α-oxidation was proceeding prior to as well as coincident with, formation of the phospholipids during germination. Additional investigations on the nature of the α-oxidation process are in progress.

TABLE V

Fatty Acid Methyl Esters from the FFA Fraction at 54 hr, as Observed on the DEGS Column after Separation by Silver Nitrate TLC

Class	Designation
Saturates	11:0, 12:0, 13:0, 14:0, 15:0, 16:0, 17:0 20:0, 22:0, 24:0, 26:0
Monoenes	16:1, 17:1, 18:1, 20:1
Dienes	18:2
Trienes	17:3, 18:3

TABLE VI

Fatty Acid Methyl Esters from the Triglyceride Fraction at 72 Hr, as Observed on the SE-30 Column after prior Separation by Silver Nitrate TLC

Class	Designation
Saturates	14:0, 15:0, 16:0, 17:0, 20:0, 22:0, 24:0
Monoenes	13:u,[a] 15:u, 17:u, 18:u
Dienes	18:u
Trienes	17:u, 18:u

[a] u = unsaturated, see text.

The significant amt of 20:0, 22:0, 24:0 and 26:0 which were observed in the FFA at 54 hr (Table III) were due to synthesis occurring at this time, since they were not detected in earlier fractions. The reason that the 22:0, 24:0 and 26:0 acids have not been reported previously is that the lipids in the seedling tissue have not been examined with the sensitive techniques used in the present study. Huber and Zalik (3) made no mention of any acids other than five major acids in their fatty acid composition data. Since they were using the comparatively insensitive thermal conductivity detector in their gas chromatograph, it is understandable why these compounds were not observed. It is felt that all of the minor constituent fatty acids would have been detected in this study during the early germination periods had an even more sensitive detector been available. When a very large sample of fatty acid methyl esters, prepared by transesterification of the oil from variety B-5128, was analyzed using the hydrogen flame detector, small amt of 14:0, 15:0, 15:1, 16:1, 17:0, 17:1 and 20:0 were observed. It would thus appear that although the absolute amt of these minor constituent fatty acids may vary, they are present in all flax varieties.

Major Constituent Fatty Acids. Table II shows that the fatty acid composition of the triglycerides with regard to the five major acids, 16:0, 18:0, 18:1, 18:2 and 18:3, did not vary significantly during the course of the germination with but one exception. The 18:3 content dropped from 44.7 to 37.8% during the first 18 hr of germination. This was accompanied by a large increase in the 18:3 content of the FFA (26.1% to 54.0%). It therefore appears that the initial action

TABLE VII

Equivalent Chain Lengths (ECL) and Relative Retention Volumes (r_{18}) of the Minor Constituent Fatty Acids on DEGS and SE-30 Columns

DEGS			SE-30		
Fatty acid	ECL	r_{18}[a]	Fatty acid	ECL	r_{18}[a]
14:0	14.00	0.34	(13:1)	12.80	0.18
15:0	15.00	0.45	15:1	14.75	0.34
15:1	15.50	0.51	17:1	16.72	0.66
16:1	16.60	0.69	17:3	16.67	0.65
17:0	17.00	0.75	17:0	17.00	0.57
Unknown	17.20	0.79	Unknown	18.30	1.12
17:1	17.60	0.90	(19:u)[b, c]	18.65	1.24
17:3	19.67	1.54	Unknown	20.45	2.23
20:0	20.00	1.68	(22:u)[b, c]	21.35	3.07
20:1	20.70	1.94	22:0	22.00	3.76
(21:0)[b]	21.10	2.27	24:0	24.00	7.06
(20:2)[b]	21.40	2.49	26:0	26.00	11.27
22:0	22.00	2.84			
24:0	24.00	4.83			
26:0	26.00	8.40			

[a] Retention volume relative to that of 18:0, at 197° on DEGS and 202° on SE-30.
[b] Designations in parenthesis are tentative since they appeared on only one column.
[c] u = usaturated, number of double bonds not known.

of lipase was to produce a somewhat larger amt of 18:3 in the free form. This was due to the fact that 18:3 was the most abundant acid in the triglycerides of the ungerminated seed and therefore showed a larger change. The reason for the sharp drop in the 18:1 content at 72 hr is not known. It is not likely that oxidation could account for the drop since 18:3 and 18:2 are much more susceptible to oxidation than 18:1. The minor variations in the compositions may have been partly due to the minor constituent acids which were discussed previously. It was concluded that there was no preferential metabolism of the fatty acids of the triglycerides during the germination period.

The larger variation in the composition of the FFA fraction (Table III) was due to the much smaller amt of material present in this fraction. The large increase in 18:3 percentage at the 18-hr period, reflected the drop of this same acid in the triglyceride fraction.

The fatty acid composition of the phospholipids showed a marked change during the germination period, whereas the fatty acid composition of the triglycerides and FFA were similar. Table VI shows that this was not the case with the phospholipids. Compared to the two other classes, the amt of 16:0 was much greater and the amt of 18:3 was much lower in the phospholipids. During the course of germination, the 18:3 increased from 17.5–37.2% and the 18:1 decreased from 22.5–12.8%. These changes reflect a significant difference in the composition of the phospholipids in the mature ungerminated seed and that in the young seedling.

These findings with the phospholipids constitute the first report of the fatty acid composition in the phospholipids in germinating flaxseed. It is understandable that the fatty acid composition of this class showed a pronounced change as germination proceeded, since the role of the cells produced during the growth of the root (hypocotyl) was different from those associated with the embryo. The correlation between fatty acid composition of the phospholipids and metabolic function of the tissue involved is difficult to make since little is known about the participation of phospholipids in metabolic reactions.

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Gas-Chromatographic Determination of Residual Hexane in Hexane-Extracted Soybean Flakes[1]

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Abstract

A method was developed to determine residual hexane in hexane-extracted soybean flakes by gas-liquid chromatography (GLC). After residual hexane was extracted with pure isooctane, the quantity extracted was determined by GLC. Analyses were run on three different columns. Column efficiency was varied to obtain maximum speed and accuracy. An attenuator was used to amplify the low-signal output of the extracted residual hexane.

Accuracy of the method was established by analyzing soybean flakes containing 0.01–7.5% added hexane. The lower limit of accuracy is approx 0.02%, and the time required for analysis is ca. one hr.

Residual hexane present in other kinds of hexane-extracted oilseed flakes might also be analyzed by this method. It is probable that extraction solvents other than hexane which are infinitely soluble in isooctane can also be determined by the method proposed.

Introduction

INDUSTRIAL EXTRACTION of soybean flakes with commercial hexane is in large-scale operation. An accurate and rapid determination of residual hexane The need for a satisfactory method of analysis is present in extracted meal has long been a problem. threefold: a) residual hexane in meal in quantities sufficient to produce explosive mixtures with air must be avoided; b) fat-free meals derived from extraction processes and intended for animal feed must be free of hexane; and c) solvent loss is of considerable economic importance to this industry.

In our laboratory, residual hexane in flakes was previously determined by the combination of two methods. A Karl Fischer Aquameter (9) was used for water analysis, and total volatiles were determined by heating the sample in a vacuum oven. The difference was reported as hexane. This method was

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