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.

ACKNOWLEDGMENT

Flaxseed used for germination studies by C. D. Dybing, South Dakota State University, Brookings, S.D.

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[Received July 27, 1964—Accepted October 2, 1964]

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

[1] Presented at AOCS Meeting, Minneapolis, 1963.
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time consuming and only semiquantitative. Several other quantitative approaches have been reported: a) the modified Pensky-Martens closed-cup flashpoint testing apparatus (8); and b) spectrophotometric measurement of benzene in residual hexane extracted with isooctane. Gastrock et al. (4) have listed several more. Some of these various methods were tired, but none yielded reliable results for us.

GLC has been employed in a variety of ways to analyze low-mol wt hydrocarbons (6). Because a direct analysis for hexane in flakes proved extremely difficult, an extraction procedure was devised. Residual hexane was extracted with high purity isooctane and determined chromatographically. At hexane levels below 1%, accuracy of the methods was ±7%. Above that level the accuracy increased to ±2%. Samples of meal containing as little as 0.02% hexane were analyzed with repeated accuracy. Any number of samples can be simultaneously extracted with isooctane and these extracts analyzed by GLC at ca. 5-min intervals. Solvents other than hexane are used in the extraction of flakes (1–3, 5,7). This method may be adapted for analysis of most of these residual solvents.

Apparatus and Procedure

Residual hexane present in extracted flakes was removed for analysis by extraction with Eastman spectrograde 2,2,4-trimethyl pentane (an isooctane isomer). Extraction was carried out in 125-ml Erlenmeyer flasks, equipped with ground-glass stoppers. Approx 50 g of sample was transferred as rapidly as possible into a previously weighed extraction flask. The flask was sealed with a glass stopper previously coated with silicone grease. Fifty g of isooctane were added to the flask and the total wt was recorded. The sample was allowed to shake for one hr at room temp on a laboratory shaker. The flask was left undisturbed while the flakes settled out of suspension. The decanted extract was then analyzed by GLC. Contamination of the column packing due to residual oil in the flakes was negligible since the samples had previously been solvent extracted.

The chromatographed used in the development of this method was a Beckman GC-2A, which is equipped with a thermal conductivity detector. The column contained 10% diethylene glycol succinate (DEGS) adsorbed on 80/100-mesh Chromosorb W. When the hexane level in the isooctane extract was below 5%, the sensitivity of the hexane signal had to be increased. The attenuator was set at not less than five, and a 1.0 μl sample was injected into the chromatograph with a 10-μl Hamilton microsyringe while simultaneously marking zero retention time. A sharp air peak eluted within a few seconds. The air peak was used to calculate retention data. Several seconds after elution of the air peak the hexane isomers eluted. As the hexane response approached the baseline the attenuation setting was changed to 200. This change depressed the signal output of the isooctane in relation to the output of the hexane. The percentage of residual hexane in the flakes was calculated by recording the signal of the hexane and isooctane on a 1-mv full-scale recorder equipped with a Disc integrator, which automatically recorded the area under each peak. The calculation is shown below:

$$R = (100)\ (WI/WS)\ (H/Ir)$$

R = Per cent residual hexane
WI = Weight of isooctane
WS = Weight of sample

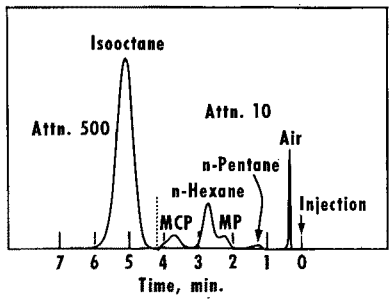

FIG. 1. Typical analysis of an isooctane extract on a 6-ft, ¼-in. column of 10% Apiezon M on 80/100 mesh Celite 545. Column temp was 118C, with a heluim flow rate of 65 cc/min (MP, methyl pentane; MCP, methyl cyclopentane).

H = Hexane peak area
I = Isooctane peak area

$$r = \frac{\text{Isooctane attenuation}}{\text{Hexane attenuation}}$$

Results and Discussion

Extraction Solvent. Although residual hexane in the sample of soybean flakes used was extractable with other solvents, isooctane proved superior. As analyzed by GLC, isooctane had an acceptable purity especially in the range of hexane elution. Even though a trace impurity was observed after elution of the isooctane, it did not interfere with the hexane isomers. One important advantage is that isooctane elutes after the hexane isomers. If the isooctane were eluted before the hexane isomers, it would be necessary to record the isooctane peak at the least sensitive output and to adjust the attenuation before the trace quantities of hexane isomers were eluted. Upon increasing the sensitivity of the signal, the baseline stability would be seriously disrupted owing to the incomplete elution of trace amounts of isooctane. This disruption would interfere with the calculation of the small quantities of hexane isomers.

To investigate the extraction rate of isooctane, extractions were carried out at room temp for 0.5, 1, 2 and 3 hr. Extraction was complete in one hr. When the residual hexane in the flakes was extracted with isooctane at 80C, the heat did not significantly increase the extraction rate.

Commercial Hexane Fractions. Complete analyses of several commercial hexanes were reported by the respective manufacturers. These samples consisted of 65–87% *n*-hexane with the remainder being composed of pentane and hexane isomers. Most of these fractions contained benzene, which was the only component eluted separately. The benzene was eluted after the isooctane and required a separate calculation. Samples of flakes that contained large amt of benzene were analyzed for residual hexane by first determining the content of benzene in the original hexane by GLC. The value obtained for benzene was added to the amount of residual hexane found.

Column Selection. Known mixtures of isooctane and a commercially available hexane fraction (Skellysolve B) were prepared. These calibration mixtures were used to compare responses of three different columns. The first column (Fig. 1) contained Apiezon, a nonpolar substrate. This column yielded a height equivalent to a theoretical plate (HETP) of 0.37 cm. Most of the isomers of the commercial hexane fraction were partitioned by the Apiezon column. This column possessed two definite disadvantages: a) separation of

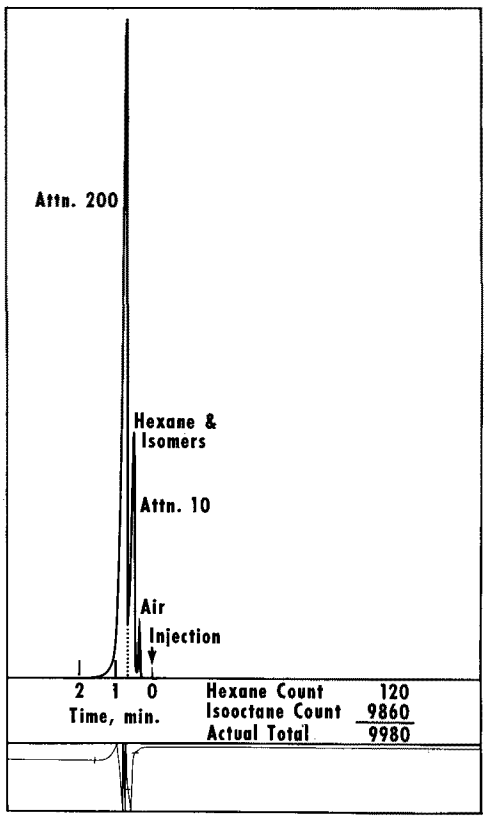

FIG. 2. Typical analysis of an isooctane extract on a 6-ft, ¼-in. column of 10% DEGS on 80/100 mesh Chromosorb W (acid washed). Column temp was 25C with a helium flow rate of 78 cc/min. This column was selected for the collection of all analytical data.

small quantities of residual hexane into its isomers yielded several broad peaks that could not be easily distinguished from baseline drift and that caused considerable difficulty in calculation b) a discrepancy in column response between isooctane and hexane yielding a relative correction factor of 0.815 for the hexane.

The second column contained the more polar stationary phase DEGS polyester. The HETP for this column was 0.77 cm. This disadvantage of partitioning the hexane isomers was nearly overcome by the 11-ft DEGS column; however, the differences in column responses were still evident. Because the hexane correction factor varied at different percentages, it was difficult to obtain a valid factor.

The third column (Fig. 2), which was 6 ft in length, also contained DEGS and yielded an HETP of 0.89 cm. As shown by the data in Table I, a 6-ft column produced nearly equal responses for isooctane and hexane. The single hexane peak produced by the 6-ft DEGS column increased the accuracy of the calculation. Also the accuracy of the calculation shown in Figure 2 can be improved by increasing the chart speed. This speedup will increase the area under each peak and spread the integrator lines apart. Known

TABLE I

Comparative Column Responses Between *n*-Hexane and Isooctane

Hexane added to isooctane, wt %	Ave. hexane,[a] wt %	Standard deviation	Relative deviation
39.10	39.26	0.0495	0.00126
8.35	8.43	0.1627	0.01930
3.53	3.57	0.0570	0.01597
1.19	1.20	0.0200	0.01667
0.28	0.24	0.0212	0.08833
0.080	0.073	0.0081	0.11096
0.0002	0.008	Below recovery range	

[a] Three determinations.

TABLE II

Accuracy of the Isooctane Extraction of Hexane Added to Soybean Flakes

Hexane[a] added to meal, wt %	Hexane found, wt %	Error, %
0.034	0.032	5.9
0.060	0.054	6.0
0.13	0.12	7.7
0.17	0.16	5.9
0.49	0.45	8.2
0.55	0.51	7.3
0.97	0.98	1.0
1.74	1.69	2.9
4.24	4.16	1.9
7.56	7.46	1.3

[a] Benzene free.

samples of solvent-free soybean flakes and commercial hexane at various concentrations were prepared and allowed to equilibrate for 48 hr. The isooctane extract of each of these samples was analyzed on the 6-ft DEGS column (Table II).

Determination of Maximum Sensitivity. The size of the sample of isooctane extract used for analysis was limited to 1.0 μl maximum. The sample injected into the chromatograph was instantaneously flashed by the preheater operated at 75C. This vaporization of the sample caused an additional column pressure which on samples larger than 1.0 μl produced a baseline shift. The lower attenuation limit was five, since more signal output caused excessive baseline instability. The time required for each GLC analysis was only two min; however, at least three min was necessary at a high signal attenuation to obtain a stable baseline for the next analysis.

A flow rate greater than was indicated as optimum by a Van Deempter plot yielded a decrease in column response differences. To obtain the greatest sensitivity the detector was operated at maximum (allowable) current, which was 450 ma. Maximum resolution of the hexane and isooctane was obtained by use of a small amt of stationary phase. Calculation of very small peaks was achieved more accurately by cutting these peaks out of the graph paper and weighing them.

Conclusion

Based on these studies, a sensitive analytical method for determination of residual hexane has been developed. In addition to hexane the foregoing method might also be applicable to ethanol, methanol, isopropanol, nitroethane and several other solvents useful as oilseed extraction materials. Good miscibility in isooctane of the solvent to be determined is of primary importance. Also retention data and column responses would have to be determined for each of these solvents. In addition to soybean flakes, the determination of residual solvent might also be performed on other kinds of oilseed flakes, such as cottonseed or linseed; however, in this evaluation no tests were made using solvents other than hexane or oilseed flakes other than soybean.

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[Received May 4, 1964—Accepted August 6, 1964]