c) Ideally, if acids are to be analyzed, the standard should be elaidic acid; if esters, elaidates; and if triglycerides, trielaidin.

d) The procedure whereby each and every sample is converted to some other form, in order to complete the analysis on the same basis as the standardization, *i.e.*, converting triglycerides to methyl esters to agree with a methyl elaidate standard, is not to be recommended.

e) Considerably more collaborative work is required before a specific procedure can be selected for recommendation to the Society as a tentative A.O.C.S. method.

It was agreed at the New Orleans meeting that the immediate activities should consist of a) composition of a specific procedure by the chairman, b) examination of this procedure by each committee member and revision in accordance with collected comments and criticisms, and c) collaborative test of the agreed specific procedure, probably with simple mixtures of acids, esters, or triglycerides, using the corresponding standard elaidic acid, elaidate, or trielaidin for instrument calibration. Action on these proposals is to be initiated immediately, and a second round of collaborative testing is planned later during the coming year.

Acknowledgment

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Thermal-Diffusion Fractionation: Oils, Fats, and Some Derivatives[1]

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RECENT REPORTS of the effectiveness of thermal diffusion in concentrating certain components of petroleum oils (5, 7) or crude natural oils (3, 4)[3] have suggested the possible usefulness of this principle in separating biochemical substances, especially the lipides and their derivatives. In this laboratory studies have included the fractionation of mixtures containing acids, esters, alcohols (12), and natural isoprenoid compounds such as carotenoids, squalene, phytol, or terpene oils (13). This paper reports experiments with fat and oil components and derivatives: mono-, di-, and triglycerides, fatty acids, methyl esters of fatty acids from soybean oil, polymeric triglycerides, and certain trace components, particularly pigments, in crude vegetable or marine oils.

Experimental

Apparatus. A thermal-diffusion column used in this work is described elsewhere (12). It was constructed with parallel, stainless-steel plates, heated with condensing steam (atmospheric pressure) and cooled with tap water. Oils placed in the column were allowed to diffuse for 72 hrs.; then the contents were removed in 10 fractions of 2 ml. each.

Analytical Methods. Refractive index values were determined with an Abbé refractometer thermostated at 40 ± 0.2°C. Acid values and saponification values were determined by standard methods. Monoglyceride and glycerol values were obtained by the method of Pohle and Mehlenbacher (9).

Spectrophotometric measurements were obtained with a Beckman instrument, Model D. U. Absorbance values were determined in the usual manner for the oils dissolved in commercial hexane (Skellysolve B), then the absorptivity values of the oil were calculated after the manner of O'Connor *et al.* (8). This permitted the direct comparison of the color of fractions when absorbance was of necessity determined at different solute (oil) concentrations.

Materials. Glyceryl trioleate (Emery 2230) was obtained from Emery Industries Inc., Cincinnati, O., and glyceryl (mono) oleate (S-1097) was obtained from Glyco Products Company, Brooklyn, N. Y.

Crude methyl esters were prepared from soybean oil by methanolysis at room temperature for 24 hrs. with 1.6 equivalents of methanol and 0.5% potassium hydroxide as catalyst. The glycerol layer that separated was drawn off, then the crude methyl ester preparation was washed three times with warm water and dried over anhydrous sodium sulfate.

Mixed oleic and linoleic acids (Neofat 3R)—Armour Chemical Division, Chicago, Ill.

Dehydrated castor oil (Castung 103GH) and thermally polymerized, dehydrated castor oil (Castung 403UV)—The Baker Castor Oil Company, New York, N. Y.

Cod-liver oil refined, and vitamins A and D (Nopco XX)—Nopco Chemical Company, Harrison, N. J.

Crude vegetable and marine oils as follows:

Wheat germ oil—Viobin Corporation, Monticello, Ill.

Sesame and safflower oils—Pacific Vegetable Oil Corporation, San Francisco, Calif.

Tung, palm, and refined rapeseed oils—Balfour, Guthrie and Company, New York, N. Y.

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[3] The concentration of carotenoids in palm oil, of tocopherol in acidulated deodorizer sludge, and of steroids in soya acid oil have also been accomplished at the Chemical and Physical Research Division Laboratory of the Standard Oil Company, Ohio (14).

Corn oil—Corn Products Refining Company, Argo, Ill.
Cottonseed oil—Southern Cotton Oil Company, New York, N. Y.
Linseed, soybean, menhaden, and sperm oils—Archer-Daniels-Midland Company, Minneapolis, Minn.
Peanut oil—Wesson Oil and Snowdrift Sales Company, Chicago, Ill.
Olive oil—Magnus, Mabee, and Reynard Inc., New York, N. Y.
Castor and coconut oils—Spencer Kellogg and Sons Inc., Buffalo, N. Y.
Cod-liver oil—Peder Devold Oil Company, Brooklyn, N. Y.

Results

Commercial Monoglycerides. A commercial, oleic monoglyceride preparation, S-1097, on analysis was found to contain some diglyceride, triglyceride, free oleic acid, and free glycerol. This material was then fractionated stepwise by thermal diffusion to study the separability of its components. In the first step two fractions were obtained: A, the top 50%; and B, the bottom 50% from the column. Each fraction was then allowed to diffuse again. The results (Table I) showed that free glycerol concentrated toward the bottom of the column and that monoglyceride tended to move in the same direction. Saponification values (not shown) were comparatively high in the top fractions, and it was therefore evident that the triglycerides had concentrated there. Some additional free acid which formed showed little tendency to separate from the major (ester) components.

TABLE I

Crude Glyceryl (Mono) Oleate: Composition of Fractions

Fraction	Monoglyceride	Glycerol	Acids (as oleic)
	%	%	%
1. Glyceryl (mono) oleate	32	5	5
2. Fraction A from 1 (top 50%)	30	4	9
Top 10% of A	18	1	11
¼ point fraction of A	28	4	11
¾ point fraction of A	30	5	9
Bottom 10% of A	34	8	8
3. Fraction B from 1 (bottom 50%)	34	6	8
Top 10% of B	19	2	10
¼ point fraction of B	31	7	12
¾ point fraction of B	33	9	10
Bottom 10% of B	37	9	8

Mixed Esters. The glyceryl trioleate preparation showed small changes in saponification values during the 72-hour period of thermal diffusion (Table II). From an equal-volume mixture with the monoglyceride, S-1097, a pale yellow top fraction, S. V. 191, was obtained (theoretical S. V. for triolein = 190). In all cases the top fractions showed less color than the starting materials.

TABLE II

Ester Mixture: Saponification Values of Fractions

Feed material	Saponification values		
	Top	Feed	Btm.
1. Glyceryl trioleate	195	190	189
2. Glyceryl (mono) oleate	169	145	138
3. 1 + 2 (50:50)	191	167	133
4. Methanolysis product of soybean oil	199	—	142

The crude methanolysis product from soybean oil underwent some fractionation as shown by saponification values (Table II) and refractive index range (top, 1.450; bottom, 1.461). However the nature of this shift was not ascertained. Brown impurities concentrated in the bottom 30% of the column, leaving the almost colorless esters in the upper 70%.

Brominated Acids and Esters. Since previous work had shown thermal diffusion to be ineffective for separation of the C_{18} unsaturated acids, oleic and linoleic (12), the behavior of bromine derivatives of unsaturated fatty acids and esters was studied. A 60-g. portion of free fatty acids from saponified corn oil was brominated in ether solution at 5°C., and the excess bromine was removed with thiosulfate. After transferring the product to hexane solution and chilling, the crystalline tetrabromide fraction was removed. The remaining mixture ($n_D^{40} = 1.4950$), free of solvent, was diluted with an equal volume of oleic acid (tech. grade "Emersol 233," $n_D^{40} = 1.4530$), and the mixture was subjected to thermal diffusion at a maximum, hot-wall temperature of 93°C. for 72 hrs. The 10 fractions thus obtained showed, from top to bottom of the column, sharply increasing refractive indexes (Curve A of Figure 1) and sharply decreasing iodine values (Table III). After debromination these fractions showed increasing iodine values, top to bottom. By contrast the original fatty acids showed no tendency to fractionate when al-

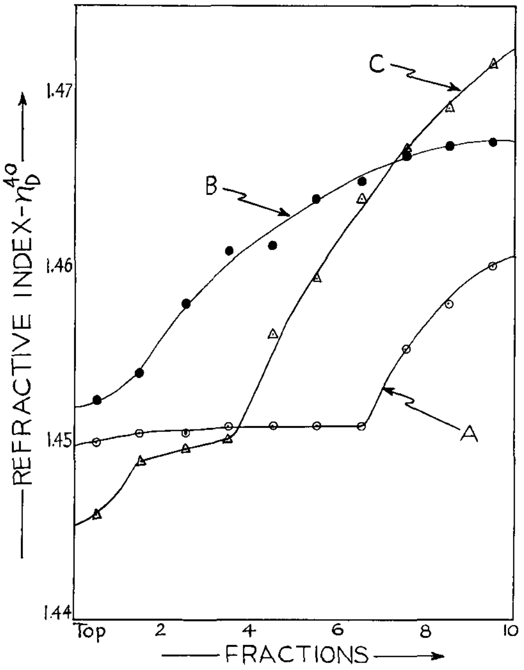

FIG. 1. Brominated fatty acids and methyl esters from corn oil; separation as shown by refractive index: A. brominated acids from corn oil dissolved in oleic acid, B. brominated methyl esters of corn oil acids, and C. debrominated fractions of B.

TABLE III

Brominated Fatty-Acids and Methyl Esters from Corn Oil: Iodine Values of Fractions

Fraction	Fatty acids	Brominated fatty-acids	Fatty acids after debromination	Methyl esters after debromination
1 Top	130	73	73	51
2	—	70	74	101
3	130	66	75	114
4	—	49	79	120
5/6	—	36	83	126
7	—	29	91	118
8	130	22	99	131
9	130	16	108	135
10	132	12	115	140
Feed	130	37[a]	87[a]	110[b]

[a] I.V. of mixture after dilution with an equal volume of oleic acid (tech.).
[b] I.V. of methyl esters before bromination = 123.

lowed to diffuse for 72 hrs. It was concluded that the brominated acids diffused downward while untreated acids (mainly oleic) diffused upward in the thermal-diffusion column.

To observe whether a mixture of bromides might also be separable, a sample of methyl esters derived from whole corn oil was brominated and subjected to diffusion. Substantial separation was evidenced by the refractive-index values of the fractions, before and after debromination (Curves B and C of Figure 1) and by iodine values after debromination (Table III). A high degree of color developing in the bottom fractions indicated that the bromides were not completely stable at 93°C.

Polymers. Dehydrated castor oil (Castung 103GH, $n_D^{40} = 1.4751$), thermally polymerized, dehydrated castor oil (Castung 403UV, $n_D^{40} = 1.4782$) and an equal-volume mixture of these two materials ($n_D^{40} = 1.4767$) all showed significant refractive-index changes ($\Delta n_D^{40} \times 10^4$): 20, 12, and 28, respectively. Since the changes were distributed linearly as a function of column length, the separation of major components was indicated. Material balances based on refractive-index values were within the range of experimental error. The bottom fractions were darker in color and more viscous than the feed or top fractions. Thus partial separations of monomeric from polymeric triglycerides were apparent, despite the high viscosities of these materials, with the polymers concentrating toward the bottom of the column.

Crude Oils. Thermal diffusion of the crude oils produced fractions which differed widely in refractive index and color (Table IV). The fractions evidenced a concentration of trace components, but there was no evidence of fractionation of the mixed triglycerides. The components of higher refractive index and the pigments usually concentrated exponentially toward the bottom of the column to give viscous and dark-colored bottom-fractions. Clear, colorless top-fractions were obtained with all of them as shown by absorptivity value (Table IV). This color improvement in the top fractions was general throughout the visual spectrum and seemed to compare favorably with that obtained in current industrial processing of crude soybean, cottonseed, sesame, and peanut oils (8). Although most oils showed only general absorption, the curves for a few oils had definitive maxima and/or inflections, such as O'Connor *et al.* (1, 8) have attributed to the presence of particular pigment-types. The differential rate of concentration of gossypol-type of materials in the bottom fraction of cottonseed oil sharpened absorption contours which apparently were masked in the original oil (Figure 2). Likewise the major pigment types of soybean oil (8) all concentrated toward the bottom of the column, but their rates of concentration varied. Degradation of pheophytin A and sesamin-type of material in sesame oil was indicated as the λ max shifted during diffusion. Further experimentation on the concentration of carotenoids and other isoprenoid compounds in triglyceride oils is reported in a separate paper (13).

TABLE IV

Crude Vegetable and Marine Oils: Effect of Thermal Diffusion on Refractive Index and Color Distribution

Oil	$\Delta n_D^{40} \times 10^4$	Absorptivity ratios (btm.-top)	
	(btm.-top)	270 mμ	400 mμ
Wheat germ	95	2.1	384
Sesame	62	2.4	37
Tung	52	0.2	4
Corn	46	2.0	16
Cottonseed	44	15.0	267
Safflower	28	1.7	26
Palm	23	—	93
Linseed	20	1.0	55
Rapeseed	15	1.2	5
Peanut	14	1.2	18
Soybean	12	1.5	18
Olive	11	1.1	9
Castor	11	1.7	24
Coconut	4	7.7	6
Sperm	72	2.1	30
Cod liver	49	0.8	13
Refined Cod-liver[a]	5	0.1	11
Menhaden	5	0.7	9

[a] Refined cod-liver oil plus added vitamins A and D (Nopco XX).

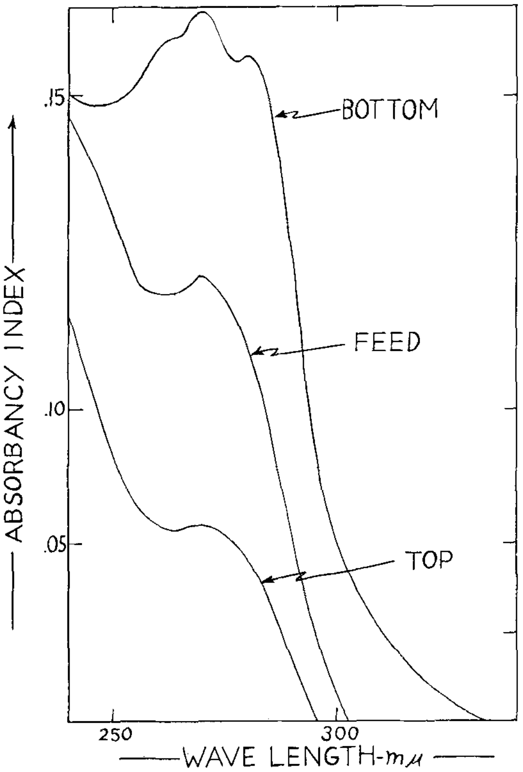

Fig. 2. Cottonseed oil; thermal-diffusion fractions.

In the vegetable oils, except castor and tung, thermal or oxidative degradation as indicated by characteristic trienoic (268–270 mμ) and tetraenoic (316 mμ) absorption maxima was no greater than that observed during conventional bleaching operations (8). Substances absorbing at 270 mμ were not consistent in their movement. Those in most oils moved toward the bottom of the column, thus giving absorptivity ratios above unity (Table IV). But tung oil and the marine oils showed a different pattern in ratios less than unity. The reasons for these differences have not been established.

The free fatty acid content of a few crude oils increased slightly during thermal diffusion. Cod-liver oil, for example, had an initial acid value of 3 while the top and bottom fractions had values of 4 and 13, respectively. The direction of concentration of the free acids varied as a function of acid concentration. In crude oils where the acid concentration never exceeded 5%, the acids concentrated toward the bottom of the column. However, in a mixture of one part of mixed unsaturated acids (Neofat 3R, oleic and linoleic acids) to four parts of crude soybean oil, the acids concentrated toward the top of the column. The acid values of the feed, top and bottom fractions of this mixture were 52, 62, and 35, respectively.

Interpretation and Discussion

While some generalizations can be made concerning the directions in which different components will flow during thermal diffusion, certain qualifications are necessary. Most of the fractionations observed were in agreement with the empirical generalizations (5, 7, 12) that cyclic molecules and molecules containing associative-type of functional groups usually concentrate toward the bottom of the thermal-diffusion column relative to linear and non-associative-type of molecules, respectively, despite unfavorable, molecular-weight relationships. The fractionation of mono-, di-, and triglyceride mixtures, or ester mixtures from methanolysis of soybean oil, and the concentration in crude oils of the pigments other than the linear carotenoids (13) followed these principles. The mixed triglycerides of the crude oils tested showed no separation. This would be predictable since they have essentially equivalent molecular weights and since differences in the degree of unsaturation were observed not to be a basis for thermal-diffusion fractionation (12). Free fatty-acids showed no tendency to separate on the basis of differences in unsaturation until molecular weight differences were effected by bromination. However the mobility of free fatty-acids in glyceride-ester systems was more complex as the direction of flow was a function of the acid concentration. This reversal of the direction of flow has been observed previously in hydrocarbon-alcohol systems (10, 11) and has been interpreted in terms of the effect of concentration on the associative nature of the functional group. Thus the empirical generalizations cited are of some value, but until the fundamental bases for liquid, thermal-diffusion separations are more completely understood (2), direct experimentation is still the only reliable approach to thermal-diffusion separation problems.

Potential practical application of thermal diffusion as a separation method in lipide technology was indicated by the fractionation of ester mixtures and the concentration of polymers and pigments in triglyceride oils. These fractionations were accomplished at low-temperature levels and at atmospheric pressure despite the high molecular weights and high viscosities of some of these materials. Also thermal diffusion had the effect of a one-step, physical bleaching process as most of the pigments were concentrated exponentially toward the bottom of the column.

Summary

A number of fats and oils and derivatives therefrom were subjected to thermal diffusion in a column in which one plate was heated with low-pressure steam and the other cooled with tap water. Triglyceride moved toward the top while glycerol and monoglyceride moved toward the bottom when mixtures containing all components were diffused. Mixed triglycerides showed no tendency to separate; neither did fatty-acid mixtures which differed only in degree of unsaturation. However when a fatty acid mixture was brominated, subsequent separation was observed.

Marked color-improvement was observed in the top fractions from various crude oils; the colored substances were concentrated exponentially in the bottom fractions. Polymeric substances from partly polymerized oils also concentrated toward the bottom. Some tentative generalizations on types of separations to be expected are discussed.

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Determination of the Extent of Oxidation of Fats

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ORGANOLEPTIC METHODS have been very useful in judging the quality of refined fats, but they give no information as to the cause of an inferior taste or as to the reasons for variations in quality after refining different batches of the same raw material. An analytical determination of the substances responsible for flavor reversion would provide a more satisfactory criterion of quality. These substances have not been identified, but investigations have shown that part of them are probably oxidation products that are nonvolatile under the conditions of deodorization and are incompletely reduced on hardening (1, 2, 3, 7, 8, and 10).

Chromatographic methods have been used in the Margarinbolaget laboratory to concentrate and fractionate the oxidation products present in freshly refined rapeseed oil. The eluate was shown to contain high-molecular-weight, unsaturated carbonyl compounds. These carbonyl compounds have no distinctive taste themselves but, on being heated with a catalyst, rapidly give rise to substances with an intense flavor; the flavor stability of the oil decreased