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

rapidly with the increased content of these compounds.

These observations led to the conclusion that the flavor reversion was due to these high-molecular-weight, carbonyl compounds which are present either in the raw oil or are formed during refining, breaking down into low-molecular-weight, carbonyl compounds with an intense flavor (12).

Since these high-molecular-weight oxidation products are secondary products formed from primary oxidation products either in the raw material or during refining, it is important to be able to determine the extent of oxidation of the raw material and of the fat during the refining process as well as of the finished product. The extent of oxidation at different stages of the refining process has been studied earlier by spectrophotometric methods (5, 9, 13), using the results of basic research on the oxidation products of linoleates and linolenates.

Since it is often difficult to interpret the spectrophotometric result and there is a relatively small range of readings for the variations which may occur when refining reasonably good fats and oils, an attempt has been made to find other methods. When checking the extent of oxidation of a fat, both the primary and the secondary oxidation products should be determined. Rather reliable methods have been available for a long time for determination of the peroxides, the primary oxidation products.

Analysis of the secondary oxidation products is rather more complicated since the course of the breakdown of the peroxides in a fat is not yet entirely clear, but they can be estimated by determination of the carbonyl compounds in the fat. Of the better known analytical methods the most reliable and the most used are those based on the colorimetric determination of the 2,4-dinitrophenylhydrazones in alkaline solution. The strong acids used in the preparation of the hydrazones and the raised temperature however cause decomposition of part of the peroxides present, and these methods are therefore less suitable for the analysis of oils containing peroxides.

The analytical method described below is based on the reaction of carbonyl compounds with benzidine acetate (11) and is carried out directly on a solution of the fat without raising the temperature or adding strong acid. It does not attempt to give a numerically correct value for the content of carbonyl compounds or to give a direct measure of the taste of the fat. (Not all carbonyl compounds give a taste, and those that do vary considerably in type and intensity of taste.)

Benzidine reacts mainly with aldehydes under the experimental conditions described here, and the reactive material is therefore referred to below as aldehyde.

Reaction Between Benzidine Acetate and Saturated and Unsaturated Aldehydes

Reactions with benzidine acetate have been investigated, using solutions of the following pure aldehydes in aldehyde-free absolute alcohol: heptanal 0.2–1.0 millimole/litre, 2-nonenal 0.02–0.1 millimole/litre, citral/2:4-hexadienal, and cinnamaldehyde 0.01–0.05 millimole/litre. Benzidine solution (0.5 ml. 1% solution) in acetic acid/ethyl alcohol (1:1) was added to these solutions (5 ml.). The extinction was measured after 6 min. at 350 mμ (λ max.) against a blank with the reagent. The molar absorptions obtained with the respective aldehydes are given in Table I.

It is apparent that the color intensity is lowest for the saturated aldehydes, considerably higher for aldehydes with one double bond, and highest for aldehydes with two double bonds. The absorption of all the reaction products followed Beers law within the above concentration limits.

TABLE I

Molar Absorption of the Reaction Products Between Benzidine and Aldehydes

Aldehyde	Molar absorption 350 mμ
Heptanal	1,370
2-Nonenal	8,000
Citral	16,000
2-4 Hexadienal	15,500
Cinnamaldehyde	18,000

Application of the Benzidine Method to Oils

The values given here are calculated, using cinnamaldehyde as standard. The numerical value for the aldehyde content of a sample is therefore low.

Method

Solvent: aldehyde-free absolute alcohol/2,2,4-trimethylpentane 1:1.

Reagent: 1% solution of benzidine in glacial acetic acid/ethyl alcohol, 1:1.

Apparatus: Beckman DU 1-cm. Pyrex cell.

The oil (1.5 g. less for high values) is weighed into a 25-ml. measuring flask and diluted to the mark with solvent. Part of this solution (5 ml.) is pipetted into a test-tube, the reagent solution (0.5 ml.) is added, and the extinction at 350 mμ is measured after 6 min. against a blank containing the reagent. The absorption of the fat itself at 350 mμ must be corrected for, and this is measured on a part of the oil solution.

Calculation of the amount of aldehyde present:

$$E = 1.1\ E_a - E_b$$

where E_a is the extinction of the test sample and E_b is the extinction of the fat solution. The value for E gives the corresponding amount of cinnamaldehyde from a standard curve.

TABLE II

Analysis of Vegetable Oils with Varying Sample Size

Sample	Weight of sample in g.	1.1 E_a[a]	E_b[a]	Ald.V.
Rapeseed oil	0.08	0.154	0.060	3.44
Rapeseed oil	0.16	0.297	0.100	3.58
Rapeseed oil	0.24	0.440	0.145	3.58
Rapeseed oil	0.32	0.578	0.180	3.62
Rapeseed oil	0.40	0.726	0.230	3.60
Cottonseed oil	0.04	0.338	0.040	21.60
Cottonseed oil	0.08	0.660	0.060	21.80
Cottonseed oil	0.12	0.980	0.083	21.70
Cottonseed oil	0.16	1.285	0.107	21.40
Cottonseed oil	0.20	1.615	0.130	21.60

[a] E_a = extinction of the test sample at 350 mμ.
E_b = extinction of the fat solution at 350 mμ.
$E = 1.1\ E_a - E_b$.

Table II lists analyses of cottonseed oil and rapeseed oil with varying sample sizes. The content of carbonyl compounds is expressed in millimoles of aldehyde in 10 kg. of fat (Ald.V.). It is apparent from the table that the absorption of the reaction product of benzidine with the aldehydes present in the fat followed Beers law. Known amounts of 2-nonenal

added to the fat could be determined with satisfactory accuracy (Table III). The reproducibility of the method was also good (Table IV).

TABLE III

Analyses of Samples with Added 2-Nonenal

Sample	2-Nonenal added	Aldehyde found	2-Nonenal found	Difference
Rapeseed oil	—	1.20	—	
Rapeseed oil	0.75	1.90	0.70	−0.05
Rapeseed oil	0.33	1.55	0.35	+0.02
Rapeseed oil	0.16	1.33	0.13	−0.03

TABLE IV

Replicate Analyses of a Rapeseed Oil

Number	1.1 E_a	E_b	Ald.V.
1	0.545	0.185	3.50
2	0.545	0.180	3.54
3	0.556	0.195	3.50
4	0.550	0.185	3.54
5	0.556	0.195	3.50
6	0.545	0.185	3.50
7	0.550	0.185	3.54

Effect of Preparation and Refining on the Extent of Oxidation of the Oils

Some examples will be given below of determinations of aldehyde and peroxide in samples of oils taken during preparation and refining. In the tables the aldehyde content (Ald.V.) has been given in millimoles/10 kg. of oil and the peroxide content (P.V.) in millimoles/kg. of oil. The peroxide content has been determined on 2-g. samples of fat, mainly by Lea's cold method but without evacuation.

Pressing and Extraction Process

Determination of the aldehyde content and the peroxide number of freshly prepared raw rapeseed oil showed that even the fresh oil contained oxidation products (Ald.V. about 1 and P.V. about 0.7). These oxidation products may have been present in the seed or formed during the pressing or extraction process. To examine the effect of pressing and extraction on the content of oxidation products in the oil, samples of whole seed, crushed and heated seed, presscake, and flakes were extracted under mild conditions. The oils obtained from these samples were analyzed, and the values were compared with the analytical values for oils prepared in the factory from the same batch of raw material.

The analyses showed that the oils prepared in the laboratory also contained small amounts of peroxides and aldehydes (Ald.V. about 0.5 and P.V. about 0.4), which indicates that the oil is oxidized in the seed. Pressing did not increase the content of oxidation products in the oil, but the extraction increased the Ald.V. from about 0.4 to about 1.7.

Refining Process

The analytical results from some samples of rapeseed oil taken from each step in the refining are given in Table V. All the raw oils shown in the table contained approximately equal amounts of peroxides and aldehydes. However during alkali refining the aldehyde value increased in all batches while the peroxide number increased in batches 3 and 5 only. From the results of a large number of similar analyses made here it seems that the changes in the aldehyde content and peroxide number during alkali refining are the net result of both formation and decomposition of peroxides in the material. Excessive oxidation during alkali refining usually shows as an increase of the peroxide number but may also give a marked increase in the aldehyde value. If the alkali refining is properly done, there should be no appreciable increase in peroxide number or aldehyde value.

TABLE V

Aldehyde and Peroxide Content of Rapeseed Oils During Refining

Nr	Crude oil		Alkali-refined oil		Bleached oil		Deodorized oil	
	Ald.V.	P.V.	Ald.V.	P.V.	Ald.V.	P.V.	Ald.V.	P.V.
1	0.8	0.5	1.7	0.4	2.3	0.0	1.1	0.0
2	0.9	0.4	2.6	0.4	2.0	0.1	1.3	0.0
3	0.8	0.4	1.5	0.6	2.4	0.3	1.9	0.0
4	0.7	0.7	1.3	0.7	3.3	0.5	2.6	0.1
5	0.5	0.3	1.5	0.7	2.3	0.7	2.5	0.1
6	0.8	0.4	1.9	0.4	4.6	0.7	3.5	0.2

TABLE VI

Refining Results on Crude Oils with High Content of Oxidation Products

Sample	Crude oil		Alkali-refined oil		Bleached oil		Deodorized oil	
	Ald.V.	P.V.	Ald.V.	P.V.	Ald.V.	P.V.	Ald.V.	P.V.
Rapeseed oil	1.1	2.0	2.5	2.5	6.3	0.4	4.1	0.1
Peanut oil	1.4	2.0	2.7	1.7	5.8	0.6	4.0	0.1
Cottonseed oil	0.8[a]	10.0	1.5	9.5	17.0	0.4	9.3	0.1
Soybean oil	4.8	1.2	5.0	1.4	6.6	0.6	4.7	0.1

[a] Semi-refined.

The acid-activated clays used for bleaching rapeseed oil decompose the peroxides and at the same time adsorb part of the oxidation products. It is therefore difficult to judge the effect of bleaching. However a comparison of the total content of oxidation products, the degree of oxidation before and after bleaching gives a measure of the effect. On thermal decomposition of the peroxides in rapeseed oil, 1 millimol of peroxide gives 1.5 millimols of aldehyde. If the total content of oxidation products is expressed as millimols of aldehyde in 10 kg., then the degree of oxidation will be equal to the aldehyde value + 15 × the peroxide number. According to this formula the table shows that bleaching removed 70% of the oxidation products from batches 1 and 2 but less from the other batches. The effect was even negative in batches 5 and 6. If the bleaching is properly done, the bleaching effect should be at least 70%, and the peroxide value of the oil should be 0.

Freshly deodorized oils normally show no peroxide number. If a peroxide number is found in deodorized oils, it is usually formed during or after cooling of the oil. The aldehyde values given in the table for the deodorized oils vary considerably and seem to be wholly dependent on the aldehyde value and the peroxide number before deodorization.

Deodorizing bleached oils with P.V.O. removes 50% of the aldehydes. Any peroxide present in bleached oils will give rise to nonvolatile aldehydes, and the aldehyde value of the deodorized oil is then usually equal to 0.5 × the aldehyde value + 2 × the peroxide number before deodorization. If a higher aldehyde content is obtained after deodorization than that given by the formula, then deodorization is incomplete or there has been a leakage of the air.

The analyses show that the usual methods of refining cannot remove more than about 85% of the total content of oxidation products in crude rapeseed oils of normal quality. In addition to the analyses on rapeseed oil of normal grade, some analyses have also been made on oils of inferior quality and on imported

oils with high contents of oxidation products. Some of these results are given in Table VI. The rules given above for refining rapeseed oils of normal quality also appear to be applicable to the refining of other oils with variable degrees of oxidation. It is clear from Tables V and VI that the aldehyde content of the deodorized oil is dependent on the content of oxidation products in the raw oil and on the way in which the refining is done.

TABLE VII

Analysis of Oxidation Products in Hardened Fats

Sample	Nonhardened		Hardened		Degree of hardening, °C.
	Ald.V.	P.V.	Ald.V.	P.V.	
Whale oil	17.7	—	1.8	0.8	40/42
Whale oil	14.0	—	2.8	1.0	32/34
Cottonseed oil	0.8	10	3.6	2.0	34/36

Effect of Hardening on the Content of Oxidation Products in the Oil

As is shown in Table VII, the aldehydes are not reduced completely on hardening oils with high aldehyde or peroxide content. Table VIII shows that the high content of aldehydes in a hardened fat gives poor flavor stability in the refined fat and in products made from it. The table shows average values

TABLE VIII

Effect of the Degree of Oxidation of Unrefined Whale Oil 40/42 on the Flavor Stability of the Margarine

Month	Crude hardened whale oil 40/42		Margarine (20% h.w. 40/42)	Score			Remarks
	Ald.V.	P.V.	Ald.V.	1 w.	3 w.	7 w.	
Sept.	5.1	0.7	3.1	3.8	3.2	2.2	Hardening taste
Oct.	1.5	0.4	2.3	3.9	3.6	3.3	Old
Nov.	1.3	0.3	1.8	3.9	3.7	3.6	Old

for three different months of 1955 of aldehyde and peroxide contents in raw, hardened whale oil 40/42° and in margarine in which there was 20% hardened whale 40/42°, also the average values of taste points for this margarine. The average values for the aldehyde and peroxide contents are calculated from 20 analyses for each month. The taste of the margarine has been judged by the Margarinbolaget tasting panel after 1, 3, and 7 weeks of storage at 18°C., and the average values are calculated from 200 judgments. The scale for judging is 0–6. Four points are given for a margarine without comment.

It can be seen from the table that:

1. the aldehyde content in the margarine varied according to the degree of oxidation of the raw, hardened whale oil;
2. the fresh samples of margarine, in spite of the different aldehyde contents, had a rather similar taste;
3. the taste changes after storage for three weeks and for seven weeks were related to the aldehyde content of the margarine; and
4. the principal comment on the margarine with the highest aldehyde content was hardening taste.

It might perhaps be added that complaints were also received from the consumers about the margarine manufactured in September but that no complaints were made about the October and November margarine.

Conclusions

During the course of this investigation the method described here has been used to determine the content of oxidation products in both refined and unrefined fats of different types, a total of about 1,000 samples from four different refineries. The refined fats have also been tasted by the laboratory staff. It has been found that:

1. refined oils contained nonvolatile oxidation products, regarded here as aldehydes;
2. the content of these aldehydes varied according to the degree of oxidation of the raw material and with the method of refining;
3. there was a correlation between the flavor stability of the refined fat and its aldehyde content.

   (It has been noticed here that the relation of type and intensity of the taste to the aldehyde content varies with the type of fat. Thus rapeseed and soya oils with aldehyde contents over 2 had a noticeable oily, green off-taste after a couple of days while cottonseed oil and peanut oil formed a nutty, metallic, or bitter taste only at higher aldehyde contents. On the other hand, a rapeseed oil with an aldehyde content of 0.5 did not revert. This oil was stored at room temperature in well-closed, completely filled metal drums for four years. During storage the peroxide number had risen to 0.6 and the aldehyde content to 2. The taste of this oil after four years of storage was not inferior to that of freshly refined rapeseed oil with the same aldehyde content.)
4. determination of the peroxide number and the aldehyde content of oils and fats by the methods described gives a good picture of the state of oxidation of both crude and refined material and can be used in controlling the refining process.
5. these analyses give some basis for estimating the effect of refining on a given raw material and for predicting the flavor stability of the refined material.

Summary

The amount of secondary oxidation products in refined and unrefined fats has been determined by reaction with benzidine acetate in iso-octane absolute alcohol solution, and measurement of the absorption at 350 mμ of the yellow color has been made.

An "aldehyde value" has been calculated from this absorption intensity, using cinnamaldehyde as a reference substance. Determination of the aldehyde value and peroxide number of oils before and during refining has given information on the effect of the different refining processes on the state of oxidation of the oils. The effect of hardening on the content of oxidation products of an oil has been investigated. The effect of the amount of oxidation products in the unrefined material on the flavor stability of the refined material and of the margarine made from it has also been studied.

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