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OXIDATIVE RANCIDIFICATION OF MARINE OILS

by

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INTRODUCTION

Of the total fatty acid content of marine fats, 16—20%, as a rule, consist of saturated acids, mainly palmitic, 45—60% are monoenoic, and 20—35% polyenoic acids. The presence of monoenoic C_{20} and polyenoic C_{20} and C_{22} fatty acids is a characteristic feature of marine oils. When exposed to air, fish oils are particularly liable to oxidative rancidification, due to their fairly great content of highly unsaturated fatty acids, such as pentaenoic and hexaenoic acids.

When atmospheric oxygen is absorbed by the unsaturated fatty acids, it is preferentially added in the form of hydroperoxide groups to the carbon atom adjacent to (in α position to) the carbon atoms linked together by double bonds. These hydroperoxides are the primary oxidation products in the rancidification process. They exert a destructive action on easily oxidizable compounds, such as e.g. Vitamin E and Vitamin A, and in higher concentrations may be directly poisonous.

Hydroperoxides of fatty acids are unstable compounds which slowly decompose, via radicals, even at lower temperatures.

Hydroperoxides are tasteless. On the other hand, their decomposition products, which mainly consists of carbonylic compounds, will give to the oil a disagreeable, rancid taste, even when present in minute amounts. These split-off "carbonyls" are of different chain length and of different degree of unsaturation, according to the position in the fatty acid molecule where the hydroperoxide groups were introduced.

Investigations carried out, amongst others by O. Lundberg et al. at the Hormel Institute (1) seem to indicate that the unsaturated aldehydes are chiefly responsible for the rancid taste.

The hydroperoxides may be determined titrimetrically for instance according to Wheeler's method. It is however extremely difficult to find a simple routine method which will give even an approximately quantitative measure of the complex mixture of carbonylic compounds present in rancid fat. One has to be content with methods measuring either one single aldehyde or a limited number of aldehydes in the mixture, in which the relative amount of the various aldehydes, and therefore presumably also the intensity of the rancid taste, will vary greatly, depending upon the fatty acid composition of the fat.

This implies that by means of such simple methods we may surely get a useful picture of the relative degree of rancidity within definite groups of fat with very little variation in the fatty acid composition. But if it is a question of comparing the rancidity of widely different types of fat, e.g. marine and vegetable fats, the methods thus far employed have been most unsatisfactory.

There is, then, an urgent need for a simple routine method for the determination of rancidity, which will give a satisfactory index for the total carbonyl content of the fat, and which in addition should differentiate, quantitatively, between saturated and unsaturated carbonyls, since the presence of this latter group seems to be the main cause of the rancid taste.

Since the 2,4-dinitrophenylhydrazine method suggested by Henick et al. (2) appears to offer the possibility of a more comprehensive chemico-physical characterization of the degrees of rancidity of fats, it was thought expedient to investigate the usefulness of this method for the determination of carbonyls in medicinal cod liver oils.

There has been some doubt, however, as to the value of Henick's method for the determination of carbonyls in rancid fat, especially concerning the question whether — and if so to what extent — the peroxides are broken down to carbonyls during the analysis itself.

In this report is described a modification of Henick's method. It further deals with the breaking-down of the peroxides to carbonyls during the analytical operation and during the storage of the fat, and with the tendency of the peroxides (in medicinal liver oils) to decompose at different temperatures and at different peroxide levels, and finally with the transformation of carbonyls at higher temperatures (60—280° C) in the absence of oxygen.

DETERMINATION OF RANCIDITY BY THE 2,4-DINITROPHENYLHYDRAZINE METHOD

The method is based on the condensation of the carbonyl group, ($>C=0$) with 2,4-dinitrophenylhydrazine in acid medium (trichloracetic acid) with the formation of hydrazones. The alkali salts of these hydrazones are red coloured, and their molar extinctions are practically identical both for saturated and for α, β-unsaturated straight-chained monoaldehydes at a wave length of 430$m\mu$ (see Table 1, Fig. 1). But these salts have different, well-defined maxima for the molar extinction, at 435 and 460$m\mu$ for saturated and unsaturated carbonyls respectively. In unknown mixtures of these types of aldehydes, the wave length at maximum absorbtion ($\lambda_{E\max}$) should serve as a useful index for the molar fraction

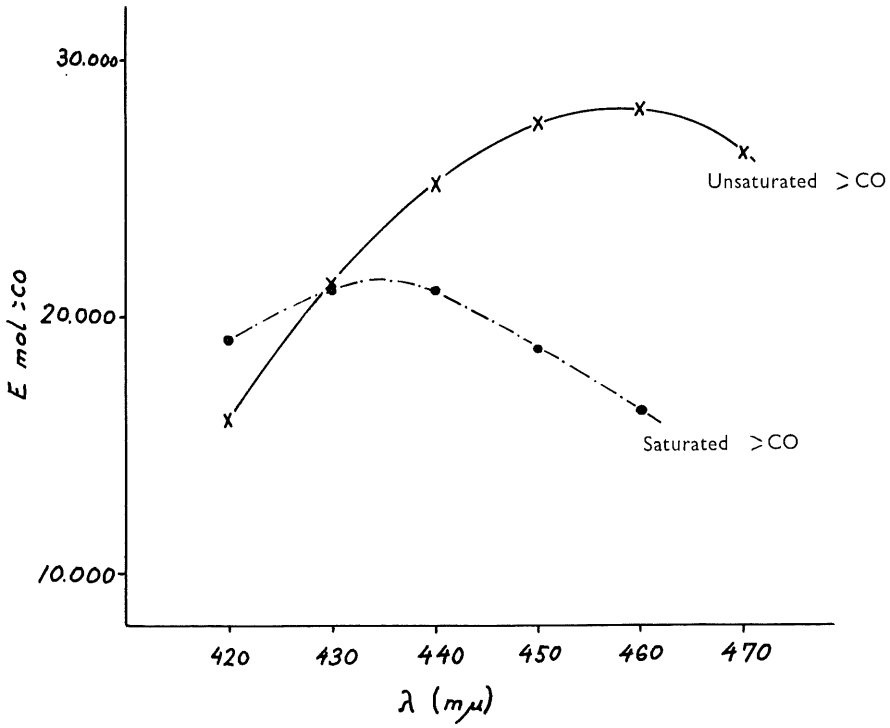

Fig. 1. Molar Extinction of monoaldehydes at varying wave lengths.

of saturated and α, β-unsaturated monocarbonyls in the rancid fat. In mixtures containing only the mentioned type of aldehydes, the ratio between the *E*-values at 460$m\mu$ and 430$m\mu$ will also indicate the molar fraction of saturated and unsaturated carbonyls. Small amounts of irrelevant absorbtion at 430 and/or 460$m\mu$, which in rancid oils might be attributed to other types of aldehydes with diverging $\lambda_{E\max}$, could invalidate the calculation of the molar fraction of carbonyls from the ratio $E_{460m\mu}/E_{430m\mu}$.

Henick's method gave good and reproducible *E* values for pure straight-chained saturated and α, β-unsaturated monocarbonyls. When used for the determination of carbonyls in liver oils the method did not give so clearly reproducible *E* values as was desired. This circumstance, which might be due to a decomposition of peroxides during the analytical operation itself, therefore had to be further investigated.

HENICK'S METHOD

Henick's method (2) is carried out in the following way: In a 50 ml volumetric flask is poured 3.0 ml trichloracetic acid solution (4.3 g/l), 5.0 ml 2,4-dinitrophenylhydrazine solution (0.5 g/l) and 5.0 ml of a

solution of the fat to be examined — both fat and reagents to be dissolved in carbonyl-free benzene. The flask is stoppered with a ground-in glass stopper and placed for 30 minutes in a thermostat at 60° C. It is left to cool to room temperature and then filled to the mark with carbonyl-free alcoholic potassium hydroxide (1 g/100 ml). After mixing, the flask is left standing for exactly 10 minutes at room temperature. The extinction of the red colour of the hydrazones is read at 430 and 460$m\mu$, together with the wave length for the maximum extinction ($\lambda_{E\max}$), in a spectrophotometer against a blank, prepared in exactly the same way as the sample, but with 5.0 ml carbonyl-free benzene instead of the fat solution.

MODIFICATION OF HENICK'S METHOD

A. *PURIFICATION OF THE SOLVENTS:*

Henick and his collaborators prepare the carbonyl-free ethanol by refluxing with Al-granulate and potassium hydroxide for one hour with subsequent distillation. We have found that refluxing for 2—3 hours with 1 g $NaBH_4$ in 2 l ethanol gives lower and more uniform blank values. The benzene is dried over $CaCl_2$ and purified by refluxing for 2—3 hours with 5 g 2,4-dinitrophenylhydrazine and 1 g trichloracetic acid per liter before distillation, as prescribed by Henick's method.

B. *CONCENTRATION OF CARBONYL VERSUS COLOUR INTENSITY:*

When the concentration of carbonyl in the analysis exceeds a certain limit, Beer's law is not any longer valid. The colour intensity and the amount of carbonyls are then not proportional. We have demonstrated this in analyses carried out with pure aldehydes, as shown in Fig. 2. Since the blank values are relatively high ($E_{430n\mu}=0.2$ to 0.3) the most reliable E readings are obtained from the upper part of the linear range of the curve, between $E=0.8$ and $E=1.1$.

C. *REMOVAL OF OXYGEN FROM THE REACTION FLASK:*

In Henick's method the oxygen (air) is not removed from the reaction flask during the analysis. This is of no consequence for the analysis of pure aldehydes. However, when fats containing peroxides are being analysed, experience shows that reproducible results will be obtained only if the oxygen is completely removed from the reaction flask. This would indicate that even traces of dissolved oxygen have an influence on the mechanism of the radical reactions during the break-down of the peroxides.

We therefore remove the oxygen completely from the reaction flask by means of the contrivance shown in Fig. 3. A glass stopcock, 4 mm

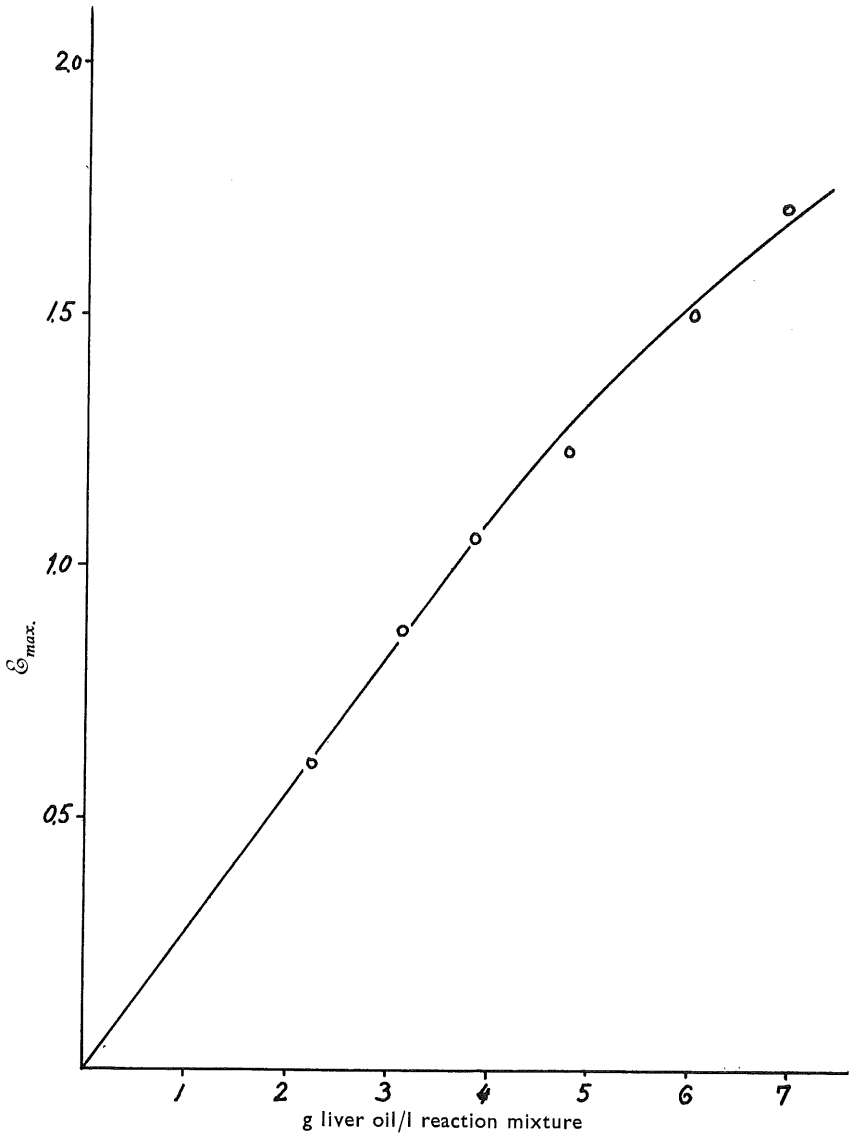

Fig. 2. Colour versus Carbonyl Concentration.

bore, is fitted with fused-on inlet and outlet tubes. The latter one with ground edge is joined to the reaction flask. Through a thin glass tube (outside diameter less than 4 mm) purified nitrogen is introduced into the flask, at first *above* the reaction mixture, later also *into* the mixture, when the flask has been placed in the thermostat. As soon as benzene is being condensed in the stopcock itself, the tube is taken out and the

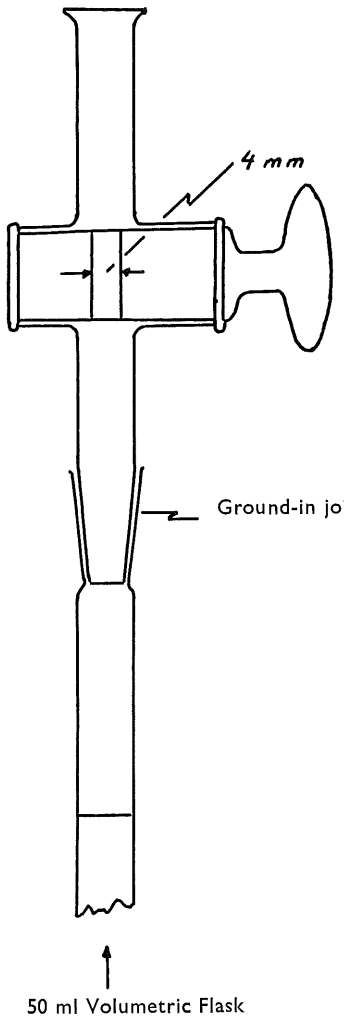

Fig. 3. Contrivance for ensuring Oxygen free Operation in the Henick Test.

cock is closed. By this complete removal of the oxygen from the reaction flask, reliable and strictly reproducible *E* values have been obtained, even in liver oils with a high peroxide content.

D. *REACTION TIME AND REACTION TEMPERATURE:*

Henick's method prescribes a temperature of 60° C and a reaction time of 30 minutes for the formation of the hydrazones, followed by a slow cooling of the reaction mixture to room temperature. If it is desired to save time by cooling the reaction mixture rapidly, for instance under the water tap, at the same time securing a complete breakdown of the peroxides during the analysis, our tests have shown that the time of reaction (at 60° C) should be increased to 40 minutes. The same values for *E* are also obtained by heating to 65° C for 30 minutes, with subsequent rapid cooling. These latter conditions have been adopted in our procedure.

E. *SUMMARY. MODIFICATIONS OF HENICK'S METHOD:*

1. Ethanol with a minimum of carbonyl is obtained by distillation after refluxing with 0.5 per cent sodium borohydride (1 g $NaBH_4$ per 2 l alcohol) for 2—3 hours.

2. The extinctions used for the determinations should lie in the range 0.8—1.1, on account of the relatively high blank values.

3. Reliable and reproducible extinction values are only obtained when the solutions are completely freed from oxygen when the peroxides decompose during the analysis (see Section C and Fig. 3).

4. To insure complete decomposition of the peroxides within 30 minutes it is necessary to conduct the reaction at a temperature of 65° C.

Potassium carbonate is but slightly soluble in ethanol. When the potassium hydroxide contains appreciable amounts of carbonate, and when carbonic acid is absorbed from the air, the reaction mixture may become supersaturated with regard to carbonate and will then be turbid.

As the amount of carbonyl reacting in the analysis is very small (order of magnitude 0.05 mg CO/50 ml) very careful rinsing of pipets and flasks is required. After thorough cleaning the glass utensils should be flushed with carbonyl-free ethanol and benzene.

The modified Henick method outlined above gave E values accurate to $\pm$ 3 per cent.

READING THE RED HYDRAZONE COLOUR IN LOVINBOND TINTOMETER

In the lack of a spectrophotometer, the red colour of the alkaline hydrazones may be read in a Lovibond Tintometer. The reading is done in a 1 cm cell and the result is calculated to a concentration oi 10 g liver oil per liter of reaction mixture. Disregarding the blank, (which is low and fairly constant) one has found that the value read in the Tintometer increases at a greater rate than the concentration. This value must therefore be corrected acording to the formula:

$$R_{corrected} = \frac{R}{1+0.026R}$$

where R is the reading of the red value in the 1 cm cell. This gives the formula:

$$RV_{1\ cm}^{10\ g/l} = \frac{R}{1+0.026R} \cdot \frac{10\,g/l}{x\,g/l}$$

where x signifies the concentration of liver oil in g/l in the solution being measured, and 10 g/l is the standard concentration. A series of measurements have shown that

$$RV_{1\ cm}^{10\ g/l} = 6 \cdot E_{430m\mu}$$

MOLAR FRACTION OF SATURATED AND α, β-UNSATURATED CARBONYL

Henick's corrected values (2) for the molar extinction of saturated and α, β-unsaturated n-aliphatic monoaldehydes, on which the following investigations are based, are given in Table 1.

Table 1. *Henick's corrected values for molar extinction of straight-chained monoaldehydes.*

	E molar		$\lambda_{E\max}$
	$\lambda = 430m\mu$	$\lambda = 460m\mu$	
Saturated aldehydes	21.000	16.300	435
α, β-unsaturated aldehydes (crotonal)	21.350	28.100	460

It follows from the data of the table that the molar extinction for saturated and α, β-unsaturated n-aliphatic monoaldehydes is practically identical at $\lambda = 430_{m\mu}$. The extinction at this wave length is therefore a measure of the total carbonyl content of the sample.

Since $\lambda_{E\max}$ increases linearly with the molar fraction of unsaturated carbonyl, from $\lambda = 435m\mu$ to $\lambda = 460m\mu$, one gets the following relation between $\lambda_{E\max}$ and molar fraction of unsaturated carbonyl:

$$\text{a) Mol. fraction unsat. carbonyl} = \frac{\lambda_{E\max} - 435}{25}$$

where $\lambda_{E\max}$ is read on the extinction curve.

Theoretically the molar fraction of α, β-unsaturated carbonyl could also be calculated from the ratio between the E values at 430 and $460m\mu$ according to the formula:

$$\text{b) Mol. fraction unsat. carbonyl} = 1.85 \cdot \frac{E_{460}}{E_{430}} - 1.44$$

Combining equations a) and b) one gets

$$\text{c) } \lambda_{E\max} = 46.24 \cdot \frac{E_{460}}{E_{430}} + 399 .$$

Relations b) and c) will only give satisfactory results on the condition that saturated and α, β-unsaturated aldehydes predominate in the aldehyde mixture, because a small displacement in the ratio E_{460}/E_{430}, caused by the presence of other types of carbonyls will have a great influence on the calculated values for $\lambda_{E\max}$ and on the molar fractions. Direct reading of $\lambda_{E\max}$ from the extinction curves is therefore regarded as a safer way of arriving at the molar fraction of saturated and unsaturated carbonyls in rancid fat.

DETERMINATION OF CARBONYL IN LIVER OIL IN THE PRESENCE OF PEROXIDES

Decomposition of peroxide—formation of carbonyl: According to published data there is considerable doubt as to whether, or if so to what extent, the peroxides are broken down and converted to carbonyls during the Henick analysis. A clarification of this question is obviously of the greatest importance in judging the usefulness of the method.

Linow (9) and also Mizuno & Chipault (10) have eliminated the peroxides by reduction before proceeding with the carbonyl determination. We have found it more expedient to remove the aldehydes beforehand, and afterwards to investigate the behaviour of the pure peroxides during the Henick analysis. The aldehydes in the liver oil were washed out in the form of sodium aldoxim salts in the following way:

15 g liver oil are dissolved in 40 ml carbonyl-free benzene + 40 ml carbonyl-free ethanol, and the free fatty acids in the oil are neutralized (to pH = ab. 9.0). In 60 ml distilled water, cooled to 0° C by the addition of lumps of pure ice, are dissolved 3 g hydroxylamine hydrochloride (ab. 0.043 mol.) and 1.75 g sodium hydroxide (also ab. 0.043 mol.). The cooled hydroxylamine solution is added to the neutralized fat solution, which has also been cooled, and the mixture is shaken under pure nitrogen at 0° C overnight. The aqueous phase should then have a pH of ab. 8.5 to 9.0. The mixture is transferred to a clean, cooled separating funnel, and the aqueous phase is drawn off. The fat solution is then washed 3 times, under pure nitrogen, with a solution consisting of 1 g sodium acetate in 80 ml ice-cooled distilled water + 20 ml cooled carbonyl-free ethanol. Finally the solution is washed with cold, distilled water. The benzene-fat solution is then dried over anhydrous sodium sulphate, transferred to a clean 50 ml volumetric flask, which is then filled to the mark with carbonyl-free benzene. 15 ml of this fat-solution are evaporated under vacuum, at room temperature, nearly to dryness, and the peroxide value is determined according to Wheeler's method. Another aliquot of the fat-solution is evaporated to determine its fat content. The remainder of the solution may be employed for the Henick analysis.

The aldehydes in the liver oil should now be completely eliminated. Identical peroxide values were found before and after the elimination procedure.

The result of determinations carried out on liver oils with greatly varying peroxide values, after elimination of the aldehydes, are given in Table 2. It shows that the decomposition of peroxides during the Henick analysis proceeds according to the following relation:

$$\frac{P(m.\text{mol/kg liver oil})}{4.72 \pm 0.2} = E^{10\ g/l}_{\substack{1\ cm \\ 430\ m\mu}}$$

4.72 m.mol. hydroperoxides thus give $E_{430m\mu} = 1$, corresponding to 132 mg or 4.72 m.mol CO/kg liver oil. By the decomposition of 1 m.mol of hydroperoxide is obtained 1 m.mol of CO during the analysis.

It now became important to investigate, if the decomposition of hydroperoxide under oxygen-free storage of the liver oil itself would proceed in the same manner as during the analytical practice.

Table 2. *Conversion of liver oil peroxides to aldehydes during the Henick analysis.*

Sample No.	$E_{430m\mu}$ (Henick)		Peroxide value of oil after aldehyde removal	$\frac{P}{E_{430m\mu}}$
	Before removal of aldehydes	After removal of aldehydes		
		5,4	25,5	4,72
1	8,9	5,6	25,3	4,52
		5,45	26,6	4,88
		5,4	26,5	4,90
2	11,45	10,0	45,5	4,55
3	8,65	6,65	32,0	4,81
4	2,40	2,17	9,75	4,50
5	8,2	8,2	39,8	4,85
6	4,2	3,82	18,2	4,77
7	13,8	13.0	61,5	4,72
				Mean value 4,72 ± 0,2

For this purpose the liver oil already analysed was blown with air at room temperature, until 4 different peroxide levels had been attained — from about 10 to about 60 m.mol peroxide/kg. Samples of oil from each of these stages were transferred to glass ampullae, flushed with pure nitrogen, evacuated and sealed by fusion. The tips of the ampullae were lacquered to prevent suction of air through accidental cracks. They were then stored at 20° C and 37° C for 170 and 80 days respectively. The peroxide values and the $E_{430m\mu}$ as determined before and after storage are given in Table 3.

The results show that we find the same values for $E_{430m\mu}$ before and after about 2/3 of the peroxides have been broken down. Thus under these conditions the decomposition of the liver oil during storage and during the analytical operation proceeds according to the same patterns of reaction.

Table 3. *Destruction of liver oil peroxides at low temperature, (below 40°C) in inert atmosphere (repeated evacuation and flushing with nitrogen).*

Initial values		After decomposition (storage)	
Peroxide value m.mol/kg oil	Henick $E_{430m\mu}$	Peroxide value m.mol/kg oil	Henick $E_{430m\mu}$
10,2	2,4	4,1	2,5
18,8	4,2	7,5	4,35
39,0	8,2	12,0	7,9
60,8	12,8	15,1	12,6
Mean values 32,2	6,90	9,7	6,85

ACTUAL AND POTENTIAL ALDEHYDE RANCIDITY

As the decomposition of hydroperoxides during the Henick aldehyde analysis proceeds in the same way as in oil stored without access to oxygen, the following relation may be postulated:

$$E^{total}_{430m\mu} = E^{aldehyde}_{430m\mu} + \frac{P(m.\,\mathrm{mol/kg})}{4.72}$$

where $E^{aldehyde}_{430m\mu}$ and $\frac{P}{4.72}$ is an index of actual and potential aldehyde rancidity. Now since $E_{430m\mu} = 1$ corresponds to 132 mg CO per kg of liver oil, we have, generally

$$\text{mg CO/kg liver oil} = \left(E^{total}_{430m\mu} - \frac{P}{4.72}\right) \cdot 132$$

The content of unsaturated carbonyl in the liver oil will then be expressed by:

$$\text{mg unsaturat. CO/kg liver oil} = \left(E^{total}_{430m\mu} - \frac{P}{4.72}\right) \cdot \left(\frac{\lambda_{E\max} - 435}{25}\right) \cdot 132$$

where $\frac{\lambda_{E\max} - 435}{25}$ represents the molar fraction of unsaturated carbonyl.

THE RANCID TASTE OF COD LIVER OIL ALDEHYDES

In oxidized fat, the carbonyls are responsible for the organoleptic rancidity. In one test, the peroxides in an inferior grade of medicinal liver oil (with peroxide value = 6 m.mol per kg, Kreis value = ab. 15 and taste = ab. 4) were decomposed at low temperature in acid medium, and the total carbonyl content was washed out as aldoxim salts according to the procedure just described (to Kreis value = 0). After this treatment the typical rancid taste of liver oil was eliminated.

In a second test, four different medicinal liver oils were filled in small bottles and store in the dark at room temperature. About 3 per cent of the content of each flask was poured out every day to simulate daily use. Rancidity tests and taste scoring was carried out at the start and then regularly once a week. The results of these tests are shown in Table 4. From this table will be noticed that $\lambda_{E\max}$ and thus also the mol. percentage of the unsaturated carbonyl increases rapidly with the iodine value.

By submitting the $E_{430m\mu}$ values and the taste score to a statistical treatment, including also the ideal case: S (taste) $= 1$ and $E_{430m\mu} = 0$, one

Table 4. *Taste "score" and mol. % unsaturated carbonyl in cod liver oils. "Score" range of Norwegian Cod Liver Oil Stat. 1—6. Low peroxide value (P about 2) for all oil samples.*

Sample No.	Analysis after number of weeks	Taste score	$E_{430m\mu}$ (Henick)	λ_{Emax} Reading	Mol. % unsatur. carbonyl	Iodine value (Wijs)
Ideal oil		1	0	—	—	—
1	0	2,5	1,85	444,5—445	40	170,7
	1	3,0	2,15			
	2	3,5	2,40			
	3	3,5	2,90			
2	0	3,0	1,65	447,5—448	51	176,5
	1	3,5	1,80			
	2	3,75	2,15			
	3	4,0	2,40			
3	0	3,5	1,85	448 —449	54	177,2
	1	4,5	1,95			
	2	4,5	2,55			
	3	5,0	2,95			
4	0	5,0	2,45	449— 450	58	178,3
	1	5,5	2,75			
	2	5,5	2,95			
	3	5,75	3,15			

arrives at a relation between S and $E_{430m\mu}$ for the different liver oils. This relation, and also the percentage unsaturated carbonyl for each separate oil is given in Table 5.

According to the relations of Table 5, the coefficients in front of $E_{430m\mu}$ must constitute a relative measure of the intensity of taste of the aldehyde mixtures. From Fig. 4 will also be seen that this coefficient increases practically linearly with the molar percentage of unsaturated carbonyl. It would therefore seem most probable that the unsaturated carbonyls are the chief contributors to organoleptic rancidity.

Table 5. *Taste "score" versus unsaturated carbonyls.*

	mol % unsaturated carbonyl
Liver oil no. 1: S (taste) $= 0.91 \times E_{430m\mu} + 1$	40
" " " 2: S $= 1.27 \times E_{430m\mu} + 1$	51
" " " 3: S $= 1.39 \times E_{430m\mu} + 1$	54
" " " 4: S $= 1.56 \times E_{430m\mu} + 1$	58

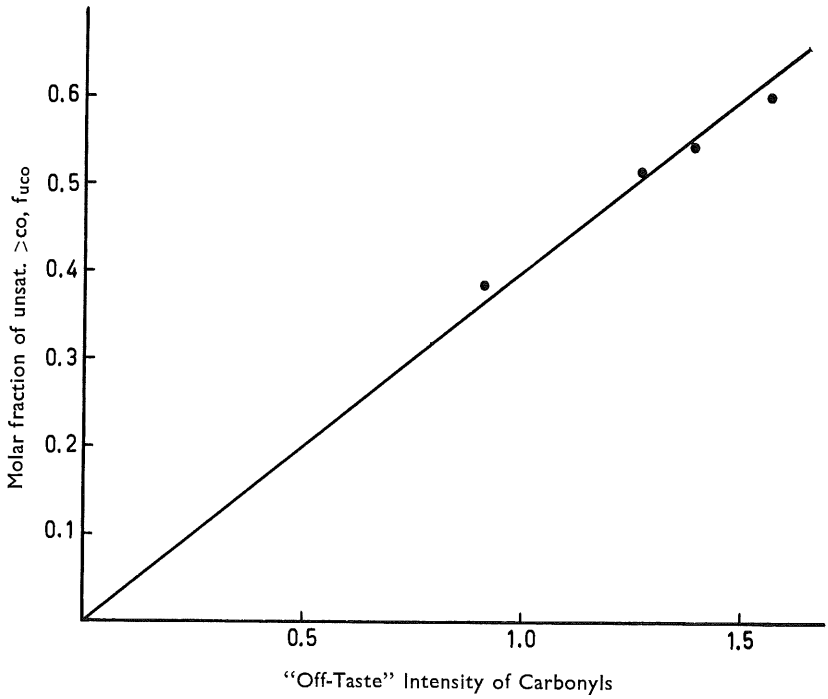

Fig. 4. "Off Taste" versus Molar Fraction Unsaturated Carbonyls.

Assuming that the unsaturated aldehyde mixture in these liver oils possess the same intensity of taste (F) we get the following relation:

$$S\ (\text{taste}) = FE_{430m\mu}^{unsat.} + 1 = Ff_{uco}E_{430m\mu} + 1$$

where f_{uco} is the molar fraction of unsaturated carbonyl in the liver oil.

From the four expressions for the relation between taste and $E_{430m\mu}$ (Table 5) one gets a mean value, F = ab. 2.5, and substituting this value for F, one finds that

$$S = 2.5f_{uco} \cdot E_{430m\mu} + 1$$

The taste is here indicated by a "score" ranging from 1—6. The constant 2.5 depends mainly upon the established scale for the taste, but possibly also to a certain degree upon variation in taste intensity of the unsaturated aldehydes in different types of fats.

This formula for predicting the organoleptic rancidity in medicinal cod liver oil has been derived from a limited test material, and hence needs confirmation through a greater number of oil samples, the iodine value of which should vary from say 155 to 175.

DECOMPOSITION OF HYDROPEROXIDES IN COD LIVER OIL WITH PEROXIDE VALUE 15.5 M. MOL PEROXIDE/KG AT DIFFERENT TEMPERATURES

The break-down of peroxides in a given sample of liver oil depends upon both temperature, peroxide concentration and the ease with which the different types of peroxides tend to decompose.

To find the time required for complete break-down of the hydroperoxides ($P \rightarrow 0$) in a liver oil with peroxide value 15.5 m.mol/kg, a great number of oil samples were filled in glass ampullae, which were then flushed with pure nitrogen, evacuated and sealed by fusion and stored at different temperatures, from 60 to 145° C. Ampullae were then taken out for peroxide determinations after certain intervals of time. The total number of days required for complete break-down of the peroxides at different temperatures is given in Table 6. In Fig. 5 $\log D$ (day $= 24h$) has been shown in ordinates against storage temperature as abscissa. It will be seen that $\log D$ decreases linearly with the temperature, according to the relation:

$$\log D = 3.3 - 0.032t \ (°C)$$

DECOMPOSITION RATE OF COD LIVER OIL HYDROPEROXIDES AT DIFFERENT TEMPERATURES AND PEROXIDE LEVELS

Cold-cleared cod liver oil was blown with a weak air current, just sufficient to mix the oil, at 20° C, to different peroxide levels. The amount of peroxide in the oil increased by about 2.3 m.mol/kg per day. After blowing, the liver oil was stored without access to air (in pure nitrogen-vacuum) in sealed glass ampullae for different periods of time and at different temperatures. The content of hydroperoxides before and after storage was determined according to Wheeler's method.

Table 6. *Time (log. days) required for complete break-down of peroxides at different temperatures.*

Temperature °C	Storage time for $P \rightarrow 0$ days (D)	log D
60	24	1,38
70	11	1,04
80	4,5	0,65
100	1,0	0,00
120	0,25	−0,60
145	0,04	−1,40

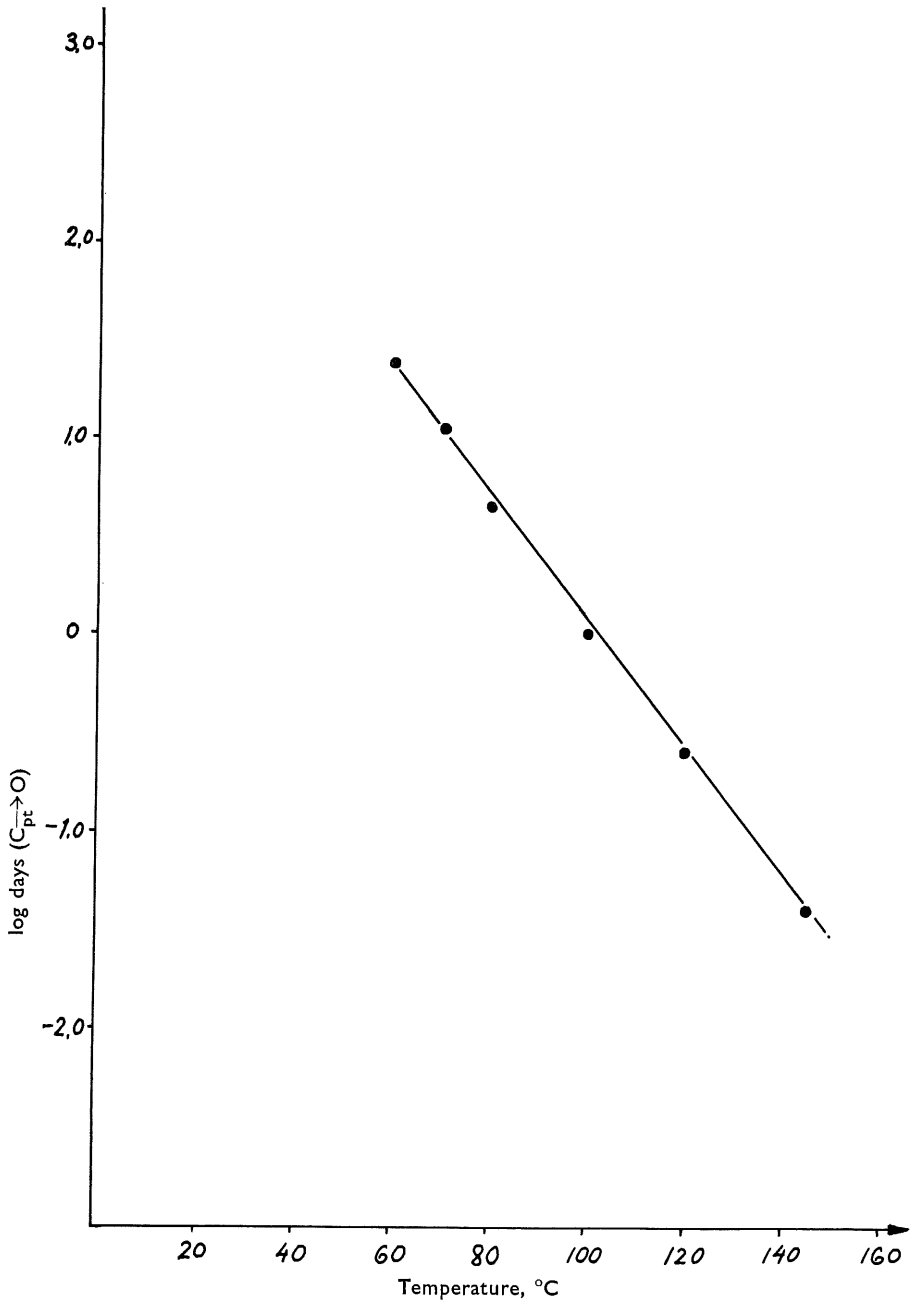

Fig. 5. Time (log days) required for complete Break-down of Peroxides at different Temperatures

Table 7. *Liver oil, air-blown at 18—20°C to different peroxide levels and stored in oxygen-free atmosphere (under N_2-vacuum) at different temperatures (in glass ampullae).*

Storage		Peroxide value, m. mol/kg			% Decrease, $-\frac{\Delta C}{C_0} \cdot 100$
Temp. °C	Time days (D)	Initial C	After storage C	Decrease $-\Delta C$	
	175	10,0	9,75	0,25	2,5
	323	10,0	9,5	0,5	5,0
	175	18,6	17,9	0,7	3,75
−10	322	18,6	17,0	1,6	8,6
	175	39,0	36,6	2,4	6,15
	337	39,0	33,2	5,8	14,85
	175	60,8	56,3	4,5	7,4
	334	60,8	49,1	11,7	19,25
	101	10,0	9,4	0,6	6,0
	202	10,0	8,6	1,4	14,0
	105	18,6	17,0	1,6	8,6
	201	18,6	15,7	2,9	15,6
	347	18,6	14,2	4,4	23,6
0	91	39,0	33,0	6,0	15,4
	203	39,0	29,5	9,5	24,4
	337	39,0	28,2	10,8	27,7
	88	60,8	49,5	11,3	18,6
	200	60,8	43,7	17,1	28,1
	334	60,8	39,0	21,8	35,9
	24	10,0	8,1	1,9	19,0
	50	10,0	6,3	3,7	37,0
	152	10,0	5,2	4,8	48,0
	171	10,0	5,0	5,0	50,0
	17	18,6	15,15	3,45	18,5
	59	18,6	11,9	6,7	36,0
	126	18,6	9,9	8,7	46,7
	170	18,6	9,6	9,0	48,5
20	9	39,0	34,0	5,0	12,8
	20	39,0	30,7	8,3	21,3
	57	39,0	22,4	16,6	42,6
	176	39,0	15,7	23,3	59,8
	6	60,8	55,0	5,8	9,5
	21	60,8	45,1	15,7	25,8
	54	60,8	33,7	27,1	44,5
	173	60,8	20,5	40,3	66,5

Table 7. (*Continued.*)

Storage		Peroxide value, m. mol/kg			% Decrease $-\frac{\Delta C}{C_0} \cdot 100$
Temp. °C	Time days D	Initial C	After storage C	Decrease $-\Delta C$	
	6	10,0	7,9	2,1	21,0
	16	10,0	6,2	3,8	38,0
	31	10,0	4,8	5,2	52,0
	43	10,0	4,1	5,9	59,0
	60	10,0	3,4	6,6	66,0
	88	10,0	2,9	7,1	71,0
	6	18,6	14,7	3,9	21,0
	16	18,6	11,6	7,0	37,6
	31	18,6	9,0	9,6	51,6
37	87	18,6	5,4	13,2	71,0
	6	39,0	29,6	9,4	24,1
	10	39,0	24,9	14,1	36,2
	17	39,0	20,7	18,3	46,9
	39	39,0	14,5	24,5	62,8
	88	39,0	8,3	30,7	78,7
	4	60,8	51,0	9,8	16,1
	10	60,8	38,5	22,3	36,7
	17	60,8	32,1	28,7	47,2
	43	60,8	18,8	42,0	69,0
	88	60,8	9,7	51,1	84,0
	2	10,0	5,6	4,4	44,0
	4	10,0	4,1	5,9	59,0
	7	10,0	2,9	7,1	71,0
	11	10,0	2,1	7,9	79,0
	12	10,0	1,9	8,1	81,0
	16	10,0	1,5	8,5	85,0
	2	18,6	10,4	8,2	44,1
	4	18,6	7,5	11,1	59,7
	7	18,6	5,5	13,1	70,5
	11	18,6	3,9	14,7	79,1
	16	18,6	3,2	15,4	83,0
60	2	39,0	19,4	19,6	50,2
	4	39,0	13,5	25,5	65,4
	6	39,0	10,9	28,1	72,0
	8	39,0	8,3	30,7	78,8
	13	39,0	4,8	34,2	87,7
	17	39,0	3,6	35,4	90,8
	1	60,8	37,1	23,7	39,0
	2	60,8	27,3	33,5	55,1
	3	60,8	20,6	40,2	66,2
	4	60,8	18,6	42,2	69,5
	8	60,8	8,8	52,0	85,5
	12	60,8	5,1	55,7	91,7
	17	60,8	2,7	58,1	95,5

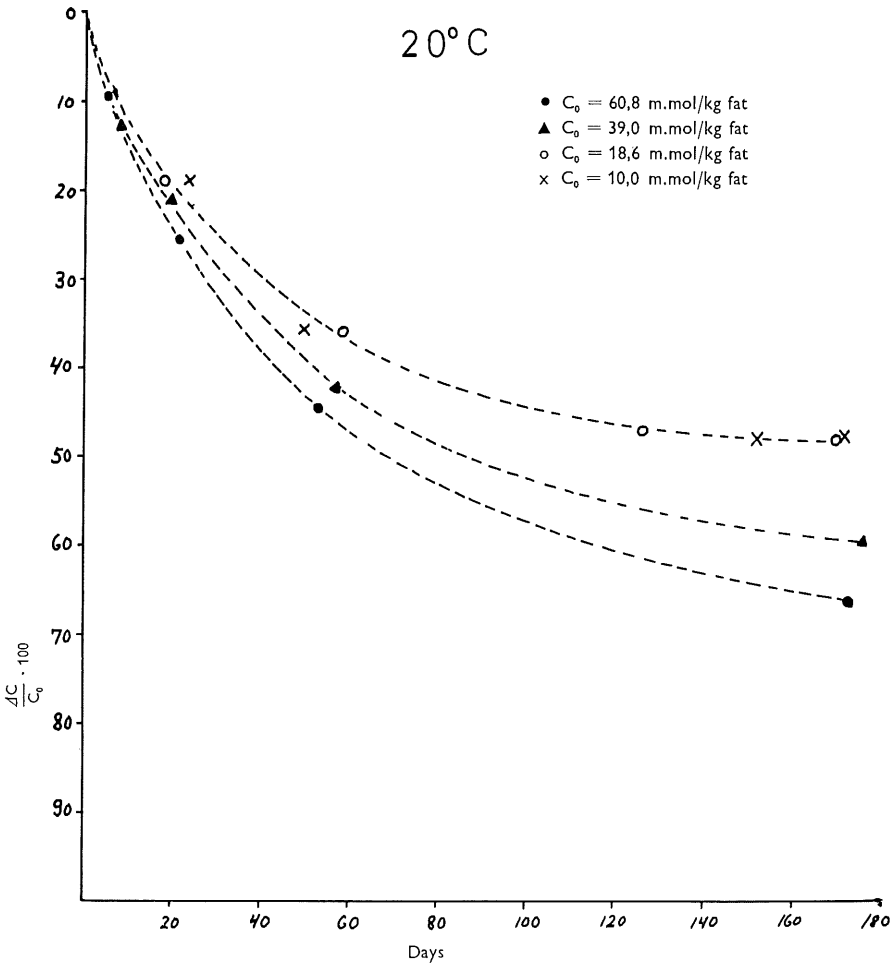

Fig. 6. Break-down of Peroxides in Liver Oil with different initial Peroxide Content, at 20° C

Peroxide values for the oil before storage varied from 10.0 to 60.8 m.mol/kg. The storage temperature during the investigation varied from −10 to +60° C.

The results of these tests are given in Table 7. The percentage decrease in peroxide content — depending upon temperature and storage time — for the different peroxide levels is indicated in column 6 and is also shown graphically in Figs. 6, 7 and 8.

From these diagrams will be seen that the percentage decrease of peroxide is greatest for the initial peroxide value of $C_0 = 60.8$. It is somewhat less for $C_0 = 39.0$ and lowest for $C_0 = 18.6$ and $C_0 = 10.0$, the decrease being practically equal for these last two levels. This seems to indicate

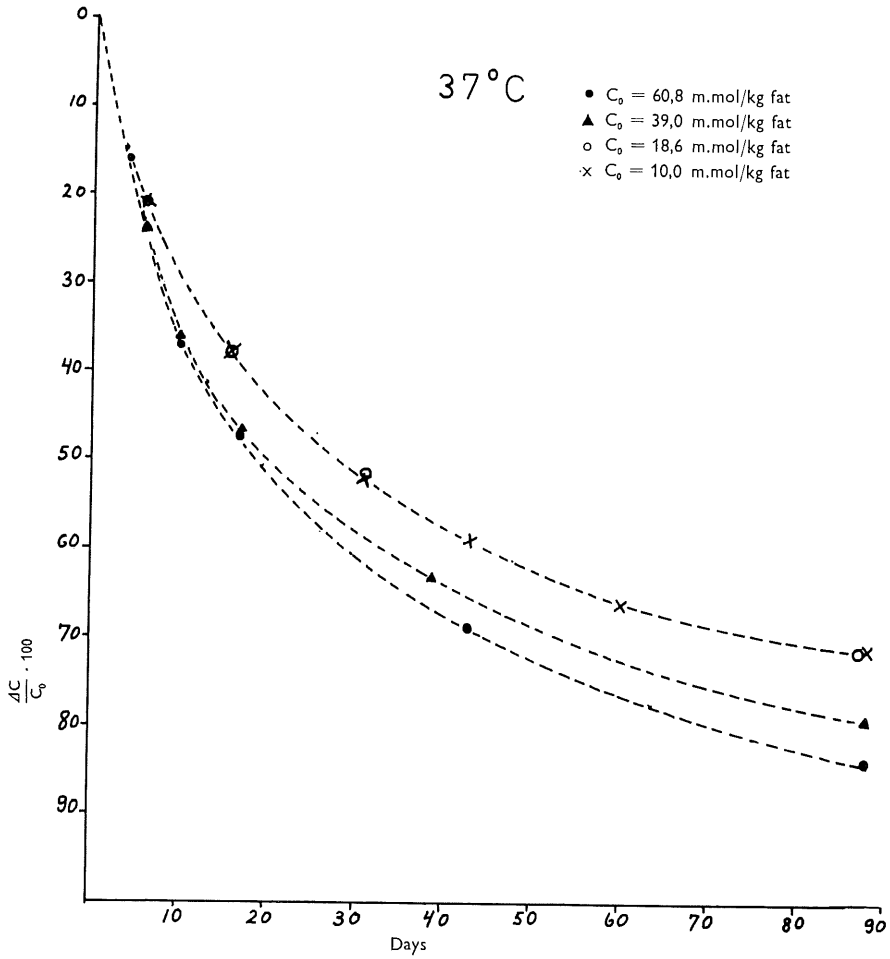

Fig. 7. Break-down of Peroxides in Liver Oil with different initial Peroxide Content, at 37° C.

that the composition of the various types of peroxides in the liver oil is identical for $C_0 = 10.0$ and $C_0 = 18.6$, while they have a different percentage composition for higher peroxide levels. To get a clearer picture of these findings, the peroxide values of the liver oils have been given in Table 8, calculated for 10, 20, 30 ... % decomposition of each initial peroxide level and for the corresponding number of storage days. The values have been interpolated from the continuous curves, Figs. 6, 7 and 8.

In Table 8, C_1 and C_2 indicate initial and final hydroperoxide concentration for each interval, ΔC the difference between C_1 and C_2, while Δd is the number of days in storage. The table also gives the magnitudes

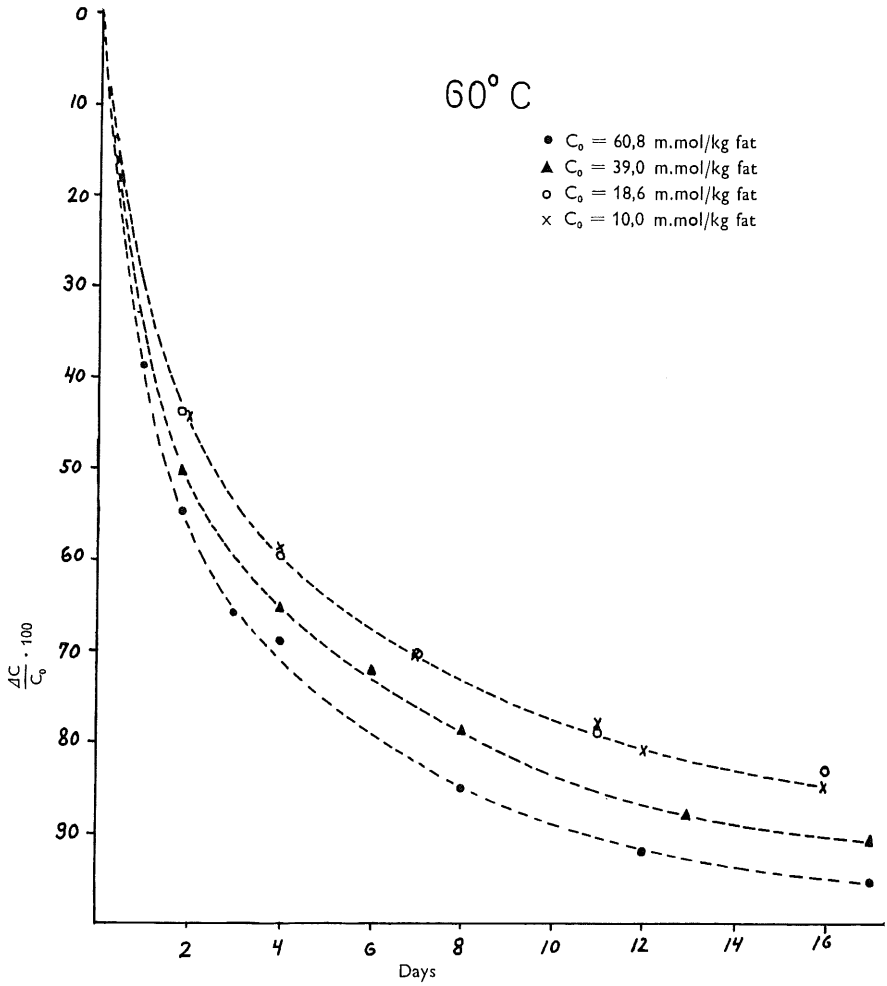

Fig. 8. Break-down of Peroxides in Liver Oil with different initial Peroxide Content, at 60° C.

$\log(\Delta c/\Delta d)$ and $\log c = \log\frac{C_1 + C_2}{2}$. A normal decomposition follows the equation

$$\div\frac{dC}{dD} \cong \frac{\div\Delta C}{\Delta D} = K \cdot \bar{C}^a \quad \text{D:} \quad \log\frac{\div\Delta C}{\Delta D} = \log K + a \cdot \log\bar{C}$$

In Fig. 9 (for 20° C), Fig. 10 (37° C) and Fig. 11 (60° C) the values from Table 8 are shown graphically with $\log\frac{\div\Delta C}{\Delta D}$ as ordinate and $\log\bar{C}$ as abscissa.

In Fig. 10 (37° C) the decomposition has been shown up to 80 per cent, for the two other temperatures up to 30 per cent only. If the dif-

Table 8. % *Decrease of peroxide value with time.* (*Different initial peroxide levels and different storage temperatures.*)

Initial peroxide value C_0	% decrease	C_1	C_1	$\log \overline{C} = \log \frac{C_1 + C_2}{2}$	$-\Delta C = C_1 - C_2$	37° C		60° C		20° C	
						ΔD	$\log \frac{-\Delta C}{\Delta D}$	ΔD	$\log \frac{-\Delta C}{\Delta D}$	ΔD	$\log \frac{-\Delta C}{\Delta D}$
	10	60,8	54,7	1,76	6,08	2,25	+0,43	0,20	1,48	7	−0,06
	20	54,7	48,7	1,71	"	2,50	+0,39	0,25	1,39	9	−0,17
	30	48,7	42,6	1,66	"	2,75	+0,35	0,30	1,31	12	−0,30
60,8	40	42,6	36,5	1,60	"	4,00	+0,18				
	50	36,5	30,4	1,53	"	6,50	−0,03				
	60	30,4	24,3	1,45	"	10,00	−0,23				
	70	24,3	18,2	1,33	"	16,0	−0,42				
	80	18,2	12,2	1,18	"	28,5	−0,67				
	10	39,0	35,1	1,57	3,90	2,25	+0,24	0,25	1,19	8	−0,31
	20	35,1	31,2	1,52	"	2,50	+0,19	0,30	1,11	11	−0,45
	30	31,2	27,3	1,47	"	3,00	+0,11	0,35	1,05	15	−0,59
39,0	40	27,3	23,4	1,40	"	4,00	−0,01				
	50	23,4	19,5	1,33	"	8,00	−0,31				
	60	19,5	15,6	1,24	"	12,50	−0,49				
	70	15,6	11,7	1,13	"	20,0	−0,71				
	80	11,7	7,8	0,99	"	38,5	−0,99				
	10	18,6	16,7	1,25	1,86	2,25	−0,08	0,25	0,872	9	−0,68
	20	16,7	14,9	1,20	"	3,25	−0,24	0,35	0,723	14	−0,88
	30	14,9	13,0	1,14	'	4,50	−0,38	0,50	0,571	18	−0,99
18,6	40	13,0	11,2	1,08	'	7,50	−0,61				
	50	11,2	9,3	1,01	"	10,0	−0,73				
	60	9,3	7,5	0,93	"	17,0	−0,96				
	70	7,5	5,6	0,81	"	32,0	−1,24				
	10	10	9	0,98	1,0	2,25	−0,35	0,25	0,603	9	−0,95
	20	9	8	0,93	"	3,25	−0,51	0,35	0,457	14	−1,15
	30	8	7	0,88	"	4,50	−0,65	0,50	0,301	18	−1,26
10,0	40	7	6	0,81	"	7,50	−0,88				
	50	6	5	0,74	"	10,0	−1,00				
	60	5	4	0,65	"	17,0	−1,23				
	70	4	3	0,55	"	32,0	−1,51				

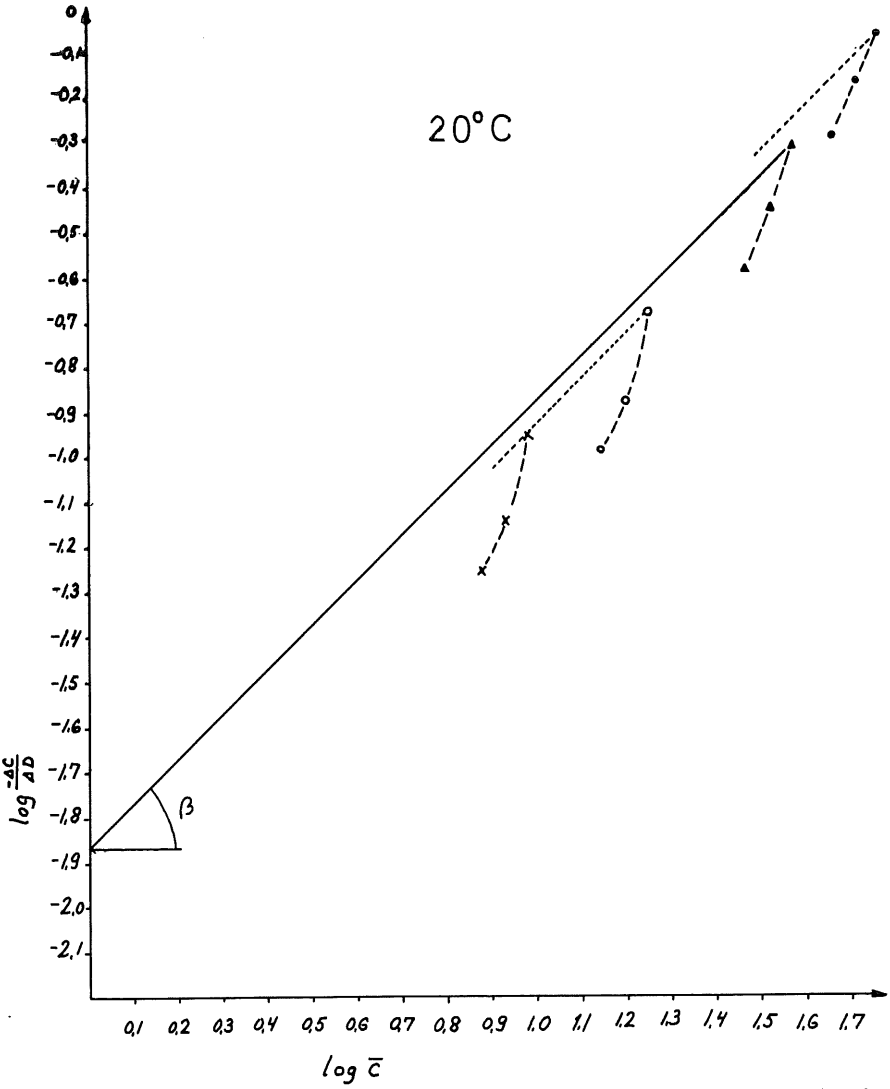

Fig. 9. Decrease of Peroxide Values. Deductions from Table 8. Temperature 20° C.

ferent types of peroxides had shown the same rate of decomposition, all the points in the log $(-\Delta C/\Delta D)/\log \bar{C}$ diagram would have coincided with a straight line (K = const.). This however, is only found to be the case for a certain proportion of the peroxides that breaks down easily (about 10 per cent at $C_0 = 10.0$ and $C_0 = 18.6$, up to 30 per cent at $C_0 = 39.0$ and $C_0 = 60.8$).

In the liver oil we thus find a complex mixture of types of peroxides which decompose more or less rapidly. The relative amounts of these

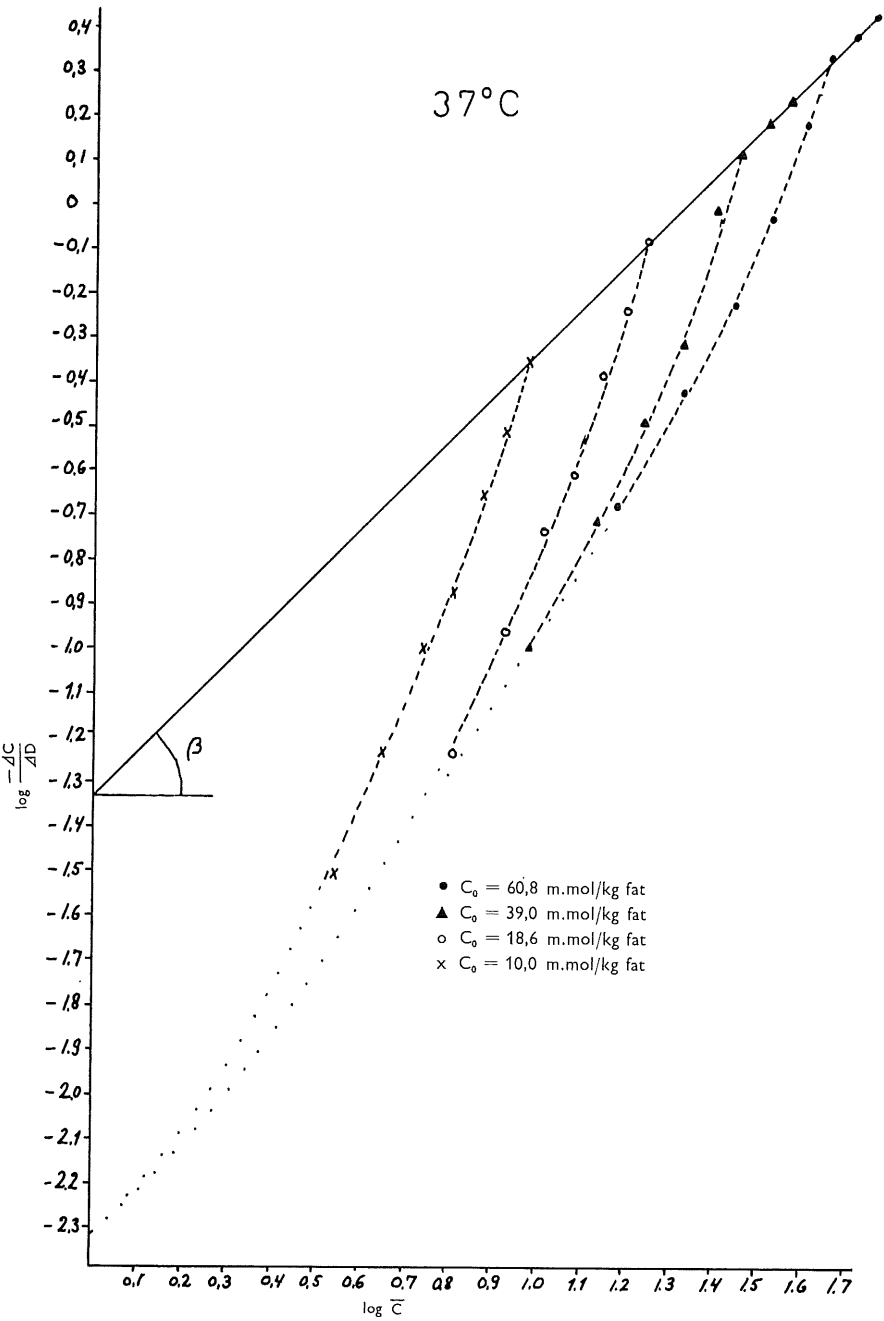

Fig. 10. Decrease of Peroxide Values. Deductions from Table 8. Temperature 37° C.

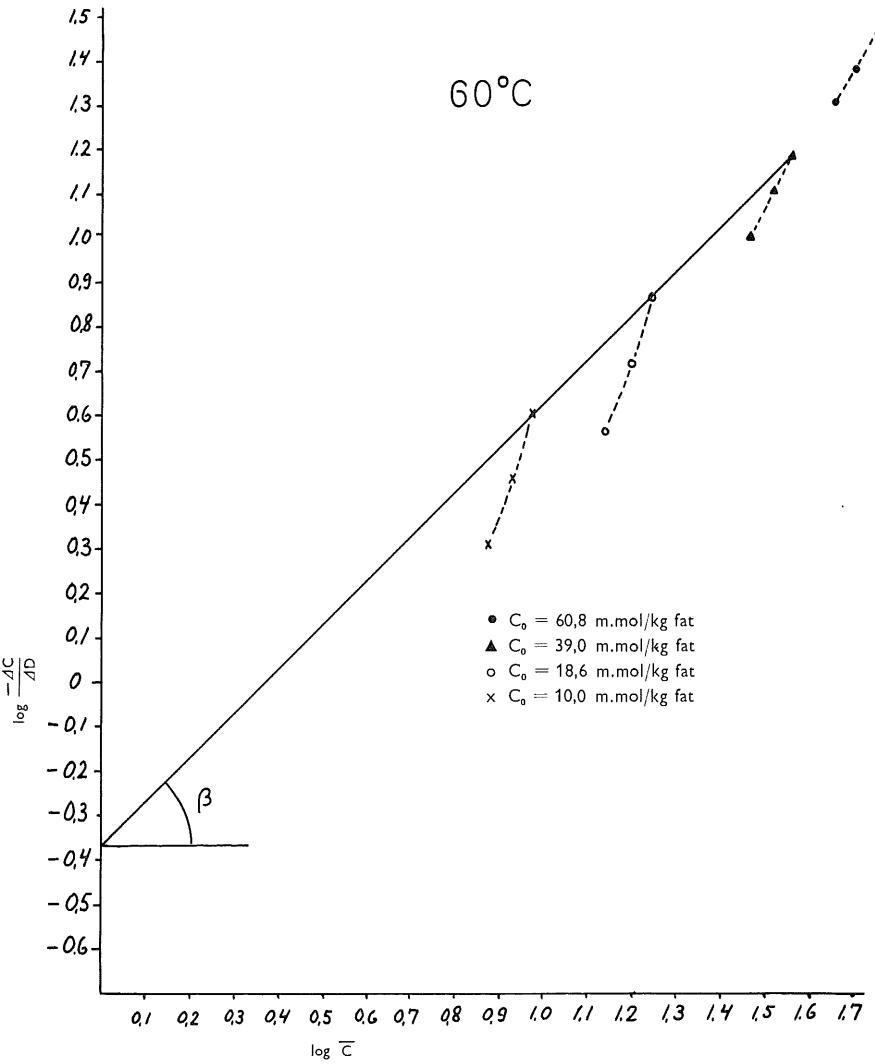

Fig. 11. Decrease of Peroxide Values. Deductions from Table 8. Temperature 60° C.

depend upon the original peroxide level and upon the degree of break-down. If a straight line be drawn (see Fig. 10) between the points that indicate the ratio $\log(-\Delta C/\Delta D)/\log \bar{C}$ at 10 per cent break-down for different initial peroxide levels C_0, the extrapolation of this line intersects the axis of ordinates at $\log K = -1.33$ (for $\log \bar{C} = 0$), which represents the logarithm of the decomposition velocity constant (K) for *all* peroxide levels at 10 per cent peroxide break-down. This line has a gradient of 45° which is the criterion for a reaction of the first order (exponent $a = 1$).

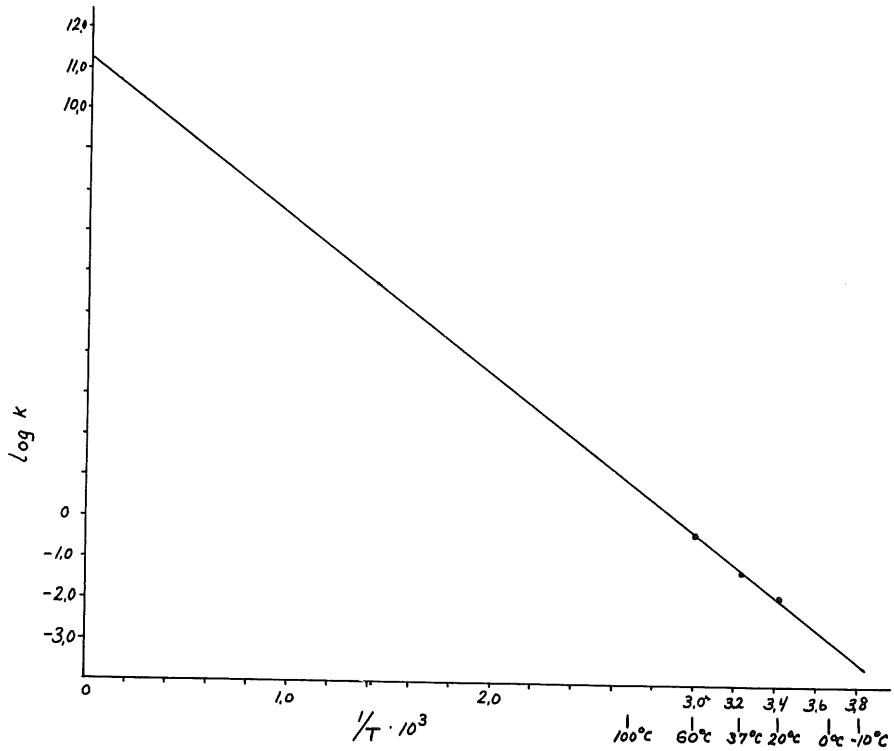

Fig. 12. Graphic Calculation of log K_{max}.

The various points on the curves represent 10, 20, 30 ... per cent decomposition, following the diagram from the top downwards. It will then be seen that for the initial concentrations $C_0 = 39.0$ and $C_0 = 60.8$, the three first points lie on the straight 45° line, i.e. the peroxide mixture that is being broken down is quite similar for these levels of peroxide up to 30 per cent decomposition. For levels $C_0 = 10.0$ and $C_0 = 18.6$, only the first point (10 per cent) lies on the same straight line. This shows that at these lower peroxide levels only about 10 per cent consists of these easily decomposable peroxides.

Drawing lines parallel to the straight 45° line through the points of the log $(-\Delta C/\Delta D)$/log $\bar{C}$ diagram (see Fig. 10), one finds the intersection of the ordinate which represents log K(log $\bar{C} = 0$) for the decomposition degree considered. For the peroxide levels $C_0 = 10.0$ and $C_0 = 18.6$, the points which represent the same percentage decomposition lie on the same parallel, while this is not the case for $C_0 = 39.0$ and $C_0 = 60.8$.

The dotted curves are thus only "resultant curves" for the points lying on the parallel lines mentioned above. If these resultants are prolonged until they intersect the axis of ordinates — an operation which

involves a considerable degree of uncertainty — this section of the ordinate will constitute a measure of the velocity constant of the most stable peroxides. This constant is probably fairly equal for all peroxide levels (C_0), when the decomposition approaches 100 per cent. This supposition is supported by the resultant curves which, all of them, appear to intersect the ordinates axis at the same point.

To find the variation of the velocity constant K with temperature, it was decided to measure the constants for 10 per cent decomposition (the broken-down peroxides being then quite similar for all peroxide levels) at 20°, 37° and 60° C. By a transformation of Arrhenius' equation one gets:

$$\log K = \log K_{max} - \frac{A}{2.303R} \cdot \frac{1}{T}$$

where K are velocity constants, A the activation energy and T the absolute temperature. In Fig. 12 $\log K$ has been chosen as ordinate and $1/T \cdot 10^3$ as absciasa. If the values for $\log K$ are inserted (from Figs. 5, 6 and 7) in this system of coordinates, and if they are extrapolated to $1/T \cdot 10^3 = 0$, one finds $\log K_{max} = 11.2$ for a peroxide decomposition up to 10 per cent, and the tangent of the angle β formed by this line and the abscissa is equal to $A/2.303R = 3.85\ 10^3$. At the start of the break-down period (in the range 0—10 per cent decomposition) the following relation between the velocity constant and the absolute temperature holds:

$$\log K = 11.2 - 3.85\ 10^3 \frac{1}{T}$$

If it is desired to measure K as a function of the temperature at other levels of decomposition, one has to proceed in the same way for each peroxide level. With varying composition of the types of peroxides one finds different values for $\log K_{max}$ and for $\tan \beta$.

In this special study on the velocity of decomposition of peroxides it has been necessary to introduce certain approximations. Nevertheless, the results should give a good idea of the factors which affect the decomposition of peroxides in pure fish oil.

THE TRANSFORMATION OF ÅLDEHYDES UNDER OXYGEN-FREE (N_2-VACUUM) STORAGE AT DIFFERENT TEMPERATURES (60—280° C)

An air-blown medicinal liver oil with peroxide value = 33.9 m.mol/kg was filled in glass ampullae and the oxygen present was flushed out with pure nitrogen. The ampullae were then sealed by fusion under vacuum.

Table 9. *Variation in composition (peroxide and carbonyl content) of liver oils during long-time storage at different temperatures*

Oxygen-free storage °C	Storage time	Peroxide value (Wheeler) m. mol per kg liver oil	$E_{430m\mu}$	$E_{460m\mu}$	E_{max}	λ_{Emax} reading	Calculated values (λ_{Emax} reading)		
							Mol. fraction unsat. CO	$E_{430m\mu}$	
								Satur. carbonyl	Unsatur. carbonyl
	0	33,9	8,85	9,7	9,85	451	0,64	3,20	5,65
	days								
	4	12,2	7,2	8,55	8,55	458			
	10	4,7	6,8	8,0	8,0	460			
60	23	1,5	6,8	7,8	7,85	457,5			
	30	0,7	5,6	6,8	6,85	462,5			
	37	1,1	5,6	6,7	6,7	460			
	45	1,1	5,9	7,0	7,0	460			
Final values		1,0	5,7	6,8	6,8	460	1,00	0	5,70
	hours								
	8	1,8	6,6	8,3	8,35	462			
	12	2,3	6,6	7,8	7,8	460			
100	19	1,2	6,6	7,8	7,8	460			
	30	0,4	6,45	7,65	7,65	460			
	43	0,4	6,55	7,7	7,7	460			
Final values		0,4	6,5	7,7	7,7	460	1,00	0	6,50
	min.								
	20	2,8	7,7	9,1	9,1	460			
	30	1,6	7,4	8,9	8,9	460			
	60	0,5	6,9	8,5	8,55	462			
150	90	0,5	6,8	8,1	8,1	460			
	150	0,3	7,0	8,4	8,4	460			
	210	0,3	7,1	8,5	8,5	460			
	270	0,3	7,0	8,2	8,25	458			
Final values		0,3	7,0	8,3	8,35	460	1,00	0	7,0
	min.								
	15	0,8	7,8	9,65	9,7	462			
	35	0,7	7,95	9,55	9,6	458,5			
	55	0,4	7,6	9,15	9,15	460			
180	100	0,5	7,3	8,5	8,6	457,5			
	150	0,4	7,15	8,2	8,3	455			
	200	0,4	7,1	8,0	8,1	453			
	250	0,3	7,2	8,0	8,15	452			
Final values		0,3	7,15	8,0	8,15	452	0,68	2,30	4,85
	min.								
	10	0,4	7,75	8,6	8,8	452			
	20	0,3	7,5	8,05	8,35	449,5			
230	30	0,4	7,7	7,9	8,2	446			
	45	0,3	7,50	7,7	8,0	445			
	80	0,3	7,65	7,7	8,2	445			
Final values		0,3	7,6	7,7	8,10	445	0,40	4,6	3,0
	min.								
	8	0,5	7,5	7,7	8,0	447,5			
280	25	0,3	7,4	7,4	7,8	444			
	40	0,3	6,9	6,7	7,3	443			
	50	0,2	7,2	6,9	7,5	442,5			
Final values		0,25	7,15	6,8	7,4	442,5	0,30	5,0	2,15

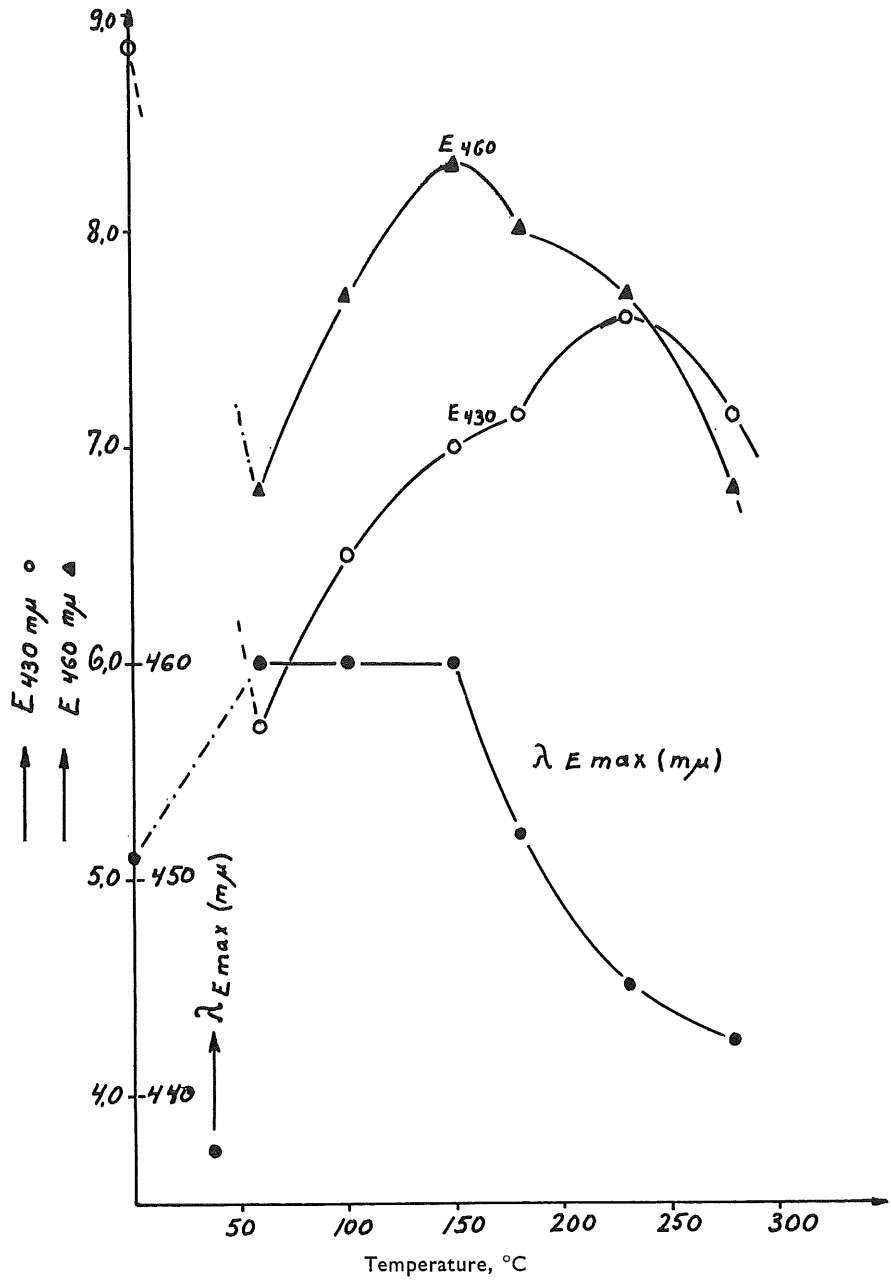

Fig. 13. Variation of Total Extinction ($\lambda = 460m_\mu$ and $430m_\mu$) and λ_{Emax} with Temperature.

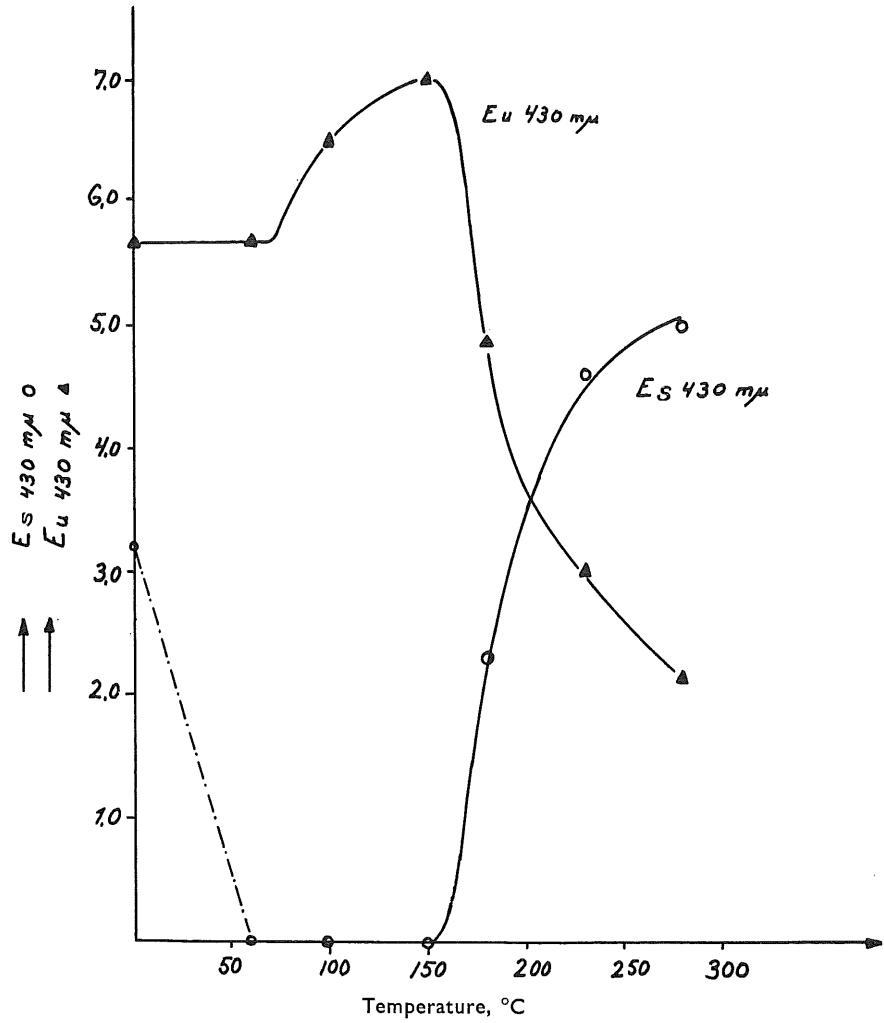

Fig. 14. Variation in Extinction of Saturated and Unsaturated Carbonyls with Temperature.

For additional safety the tip of the ampullae was lacquered to prevent access of air to the oil through accidental crack formation at the fine points. A series of ampullae, filled with this liver oil, were stored at definite test temperatures (60—280° C).

The original sample, and samples from ampullae taken after different storage periods at different temperatures, were analysed for their peroxide value, $E_{430m\mu}$, $E_{460m\mu}$ and λ_{Emax} (Henick). The results of these analyses are given in Table 9 and shown graphically in Figs. 13, 14 and 15. From the table is observed that both extinction and λ_{Emax} tend towards

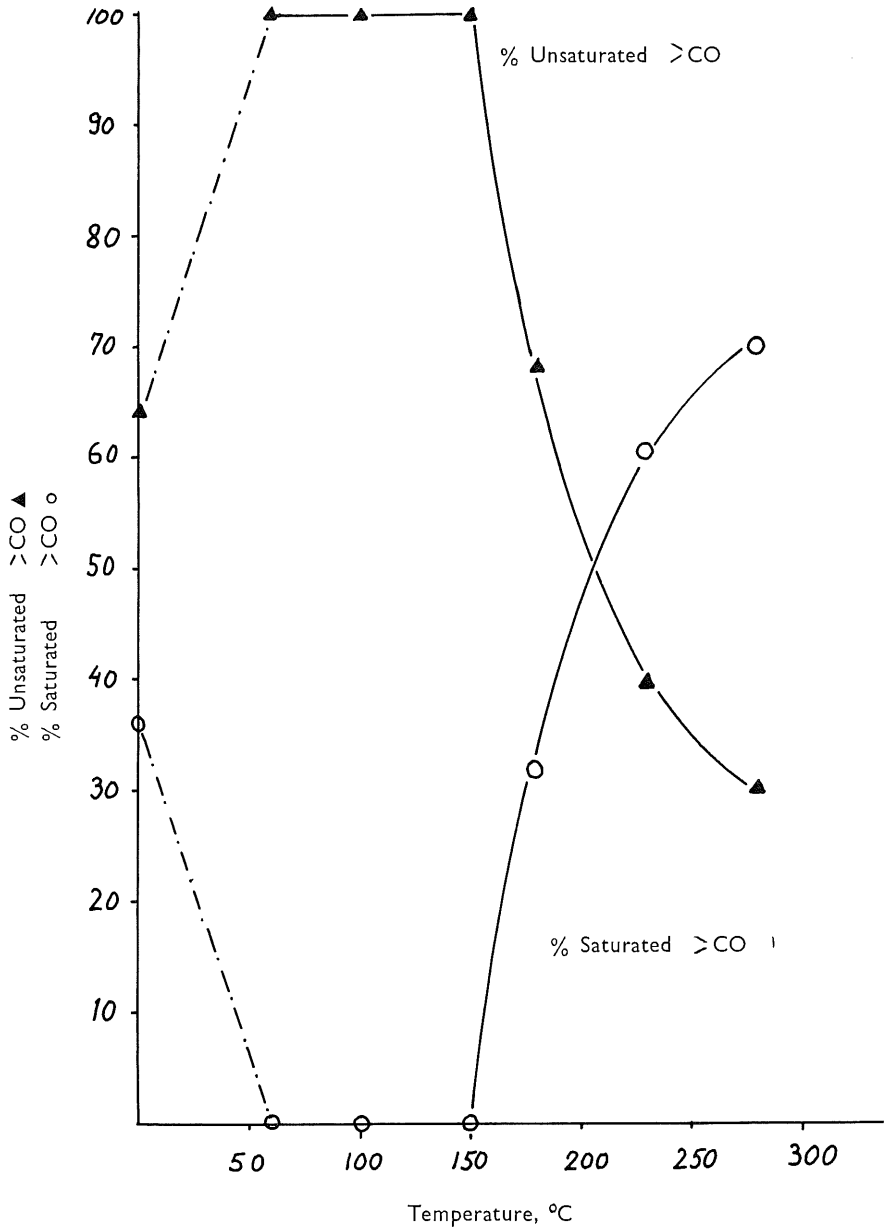

Fig. 15. Variation in Percentage Composition Saturated/Unsaturated Carbonyls with Temperature.

certain stable levels at the different temperatures, provided the liver oil samples have been heated for a sufficiently long time. For each storage temperature from 60° C up to 150° C, $\lambda_{E\max}$ increases from 451$m\mu$ to 460$m\mu$ during storage (see Fig. 13). At temperatures above 150° C on the other hand, $\lambda_{E\max}$ is again displaced towards shorter wave length, to $\lambda_{E\max} = 442.5 m\mu$ at 280° C. This shift of $\lambda_{E\max}$ must be related to transformations of the aldehydes during the heating periods. Making the reasonable assumption that the decomposition products of the peroxides mainly consist of saturated and α, β-unsaturated monocarbonyls, the rading for $\lambda_{E\max}$ should be an acceptable measure for the calculation of the molar fraction saturated/unsaturated carbonyl. This assumption is supported by the practically identical values for the measured and calculated $\lambda_{E\max}$ of the original (unheated) liver oil, namely 451 and 450$m\mu$ respectively (See Table 9). This corresponds to about 0.64 mol. fraction of unsaturated carbonyl, giving $E^{sat.}_{430m\mu}CO = 3.2$ and $E^{unsat.}_{430m\mu}CO = 5.65$.

After the break-down of the peroxides at 60° C to a stable E level, the $E_{430m\mu}$ was found to be 5.7, which agrees with the values for unsaturated carbonyl in the unheated liver oil. Now since $\lambda_{E\max}$ at the same time has risen to 460$m\mu$, which is the criterion of α, β-unsaturated aldehydes, it seems probable that the saturated carbonyl has disappeared completely through polymerization via the carbonyl groups, while $E_{430m\mu}$ for the unsaturated carbonyl has not altered materially.

$E_{460m\mu}$ for saturated carbonyls in unheated liver oil has been found equal to 2.5. Deducting this value from $E_{460m\mu} = 9.7$ (for the sample of liver oil specified above) one should have expected to find $E_{430m\mu} = 7.2$ for the remaining unsaturated carbonyl, provided the saturated carbonyl had polymerized completely, and no other change had taken place. However a value of $E_{460m\mu} = 6.8$ was actually found, i.e. 5—10 per cent too low, and hence a calculated $\lambda_{E\max} = 454$ mm. The same value for $\lambda_{E\max}$ was found when carrying out the calculation also for the other temperatures, up to 150° C. This discrepancy might possibly be attributed to the formation of limited amounts of other carbonylic compounds, for instance dialdehydes.

However, for the calculation of the molar fractions and the extinctions indicated in columns 8 to 10 in Table 9, only the values for $E_{430m\mu}$ and the reading of $\lambda_{E\max}$ have been utilized. The clearest picture of the modifications of the carbonyls during oxygen-free heating to constant E levels is obtained from Figs. 13, 14 and 15.

In Fig. 13 are shown the levels for $E_{430m\mu}$ and $E_{460m\mu}$ besides the displacement of $\lambda_{E\max}$ as read at the different temperatures. As already mentioned, $\lambda_{E\max}$ increases during storage at 60° C from 451$m\mu$ to 460$m\mu$, and remains constant at this wave length up to 150° C, where one finds

a marked break in the curve with a great (logarithmic) drop in the value λ_{Emax} with increasing temperatures.

In Fig. 14 are shown the calculated values for $E_{430m\mu}$ both for saturated and α, β-unsaturated carbonyls. As already demonstrated and also shown in the diagram, $E^{unsat.}_{430m\mu}$ CO is identical for the original liver oil and for the same oil heated without access to oxygen at 60° C. From about 70—75° C upwards one gets an unexpected increase in $E_{460m\mu}$CO. The most probable explanation for this would be that the liver oil contains a certain amount of more stable peroxides, which are not included in the values from Wheeler's method of analysis (which only determines the hydroperoxides) and that these more stable peroxides only break down at temperatures of 70° C and above.

The saturated carbonyls formed by the decomposition of these peroxides would, as has already been explained, polymerize, while the extinction of the unsaturated carbonyls thus formed would come in addition. Some of the peroxides of this type apparently are very stable, since they are not broken down until about 150° C.

From 150° C upwards the content of unsaturated carbonyls ($E^{unsat.}_{430m\mu}$ CO) decreases and the saturated ones increase correspondingly. This is clearly seen in Fig. 14. The unsaturated carbonyls are thus converted, by polymerization via the double bonds, to more saturated carbonyls. At 200° C, about 50 per cent of the unsaturated compounds will have been converted to more saturated carbonyls (see Fig. 15). That α, β-unsaturated carbonyls polymerize at temperatures above 150° C has previously been shown in studies on the benzidine method (11).

On account of the changes occuring in the saturated carbonyls, determined at temperatures even as low as 60° C, it is very important, in comparative investigations, to work at temperatures as low as is practically possible, say below 40° C.

SUMMARY

MODIFICATIONS OF HENICK'S METHOD

1. a. Carbonyl-free ethanol is obtained by distillation, after refluxing with 0.5 per cent sodium borohydride ($NaBH_4$) for 2—3 hours.

   b. The intensity of the red colour of the alkaline hydrazones obeys Beer's law up to a reading $E = 1.1$ (See Fig. 2). As the blank value is relatively high (about 0.25) the most reliable E-readings are obtained in the upper part of the linear range ($1.1 > E > 0.8$).

c. Reproducible extinction values are obtained only when the reactive solutions are completely oxygen-free. This is obtained by flushing the reaction mixture with pure nitrogen by means of a special contrivance, (see Fig. 3) both before and after having placed the sample in the thermostat.

d. To ensure a total break-down of the peroxides during the analysis within the stipulated 30 minutes, the reaction temperature must be raised to 65° C.

2. The molar extinction for saturated and for α, β-unsaturated straight-chained monocarbonyls are practically equal at the wave length $\lambda = 430 m\mu$ (Fig. 1). The total carbonyl content of the liver oil may therefore be determined from the extinction at this wave length.

3. As $\lambda_{E\max}$ for saturated and α, β-unsaturated carbonyls is $435 m\mu$ and $460 m\mu$ respectively, the molar fraction of the two types of carbonyl is expressed by the formula:

$$\text{Mol. fract. unsat. CO} = \frac{\lambda_{E\max} - 435}{25}$$

4. The total carbonyl content may also be determined from the Lovibond reading of the red colour of the alkaline hydrazones. However, this reading must be corrected according to the formula:

$$RV^{1\,cm}_{\substack{\text{read} \\ \text{corrected}}} = \frac{R}{1 + 0.026R}$$

where R is the reading in a 1 cm cell. The calculated red value in a 1 cm layer for a standard concentration of 10 g liver oil per liter will then be:

$$RV^{1\,cm}_{10\,g/l} = \frac{R}{1 + 0.026R} \cdot \frac{10}{x}$$

where x is the concentration of fat (g/l) in the solution being measured. A series of measurements showed that

$$RV^{1\,cm}_{10\,g/l} = \text{about } 6 \cdot E_{430 m\mu}\,.$$

5. The carbonyls in cod liver oil may be washed out quantitatively as sodium salts of oximes with aqueous, alkaline hydroxylamine solution ($pH = 8.5 - 9.0$) by shaking over-night at low temperature (about 0° C) without any detectable destruction of the peroxides present.

6. a. In liver oils from which the carbonyls have been removed (as shown under 5) the modified Henick analysis gives the following relation between peroxide value P and $E_{430 m\mu}$:

$$\frac{P(m.\,\mathrm{mol/kg})}{4.72} = E_{430m\mu}$$

i.e. *one* peroxide group forms *one* carbonyl group by decomposition.

b. Liver oil containing peroxides, and the same oil, in which the peroxides have been broken down to only a fraction of the original value — by storage at low temperatures, below 40° C — both give the same value of $E_{430m\mu}$ by the modified Henick analysis. This proves that the decomposition of peroxyde during oxygen-free storage follows the same pattern as it does in the analysis.

7. As the peroxides break down with quantitative conversion to carbonyl compounds during the Henick analysis, the true carbonyl content may be arrived at by correcting for the peroxide according to the formula:

$$E_{430m\mu}^{aldehyde} = E_{430m\mu}^{total} - \frac{P(m.\,\mathrm{mol/kg})}{4.72}$$

$$\text{Then: mg CO/kg oil} = \left(E_{430m\mu}^{total} - \frac{P}{4.72}\right)132$$

$$\text{and mg unsat. CO/kg oil} = \left(E_{430m\mu}^{total} - \frac{P}{4.72}\right)132\left(\frac{\lambda_{\mathrm{Emax}} - 435}{25}\right)$$

8. Preliminary investigations seem to indicate that the α, β-unsaturated aldehydes are the chief carriers of organoleptic rancidity in cod liver oil. The following relation between the taste "score" (S) and the amount of unsaturated carbonyls in the cod liver oil was found:

$$S = 2.5 f_{uco} E_{430m\mu} + 1$$

where f_{uco} is the molar fraction of unsaturated carbonyls. The equation holds for the taste score in the range 1—6.

9. Rancid cold liver oil contains a complex mixture of more or less stable peroxides, the relative amounts of which depend upon the fatty acid composition, the original peroxide value and the subsequent degree of decomposition. The time required (days) for complete desctrution of the hydroperoxides in a cod liver oil (peroxide value = 15.5 m.mol/kg) at various temperatures (60—150° C) could be expressed by the relation:

$$\log D_{(days)} = 3.3 - 0.032t(^{0}\mathrm{C})$$

10. a. In a cod liver oil, blown with air at 20°C to peroxide values of 10.0, 18.6, 39.0 and 60.8, the peroxides were decomposed by exposure to different temperatures, up to 60° C, for different periods of time. The percentage decrease in peroxide value per unit time was progressively reduced as the decomposition proceeded, apparently indicating that the peroxides constitute a mixture of varying degree of stability.
    b. The thermo-destruction of the peroxides up to 10–30 % proceeded as a reaction of the 1st order (angle $\beta = 45°$, see Figs. 9, 10 and 11).
    c. The reaction constant (K) for the peroxide decomposition vary with the original peroxide level, and with the degree of decomposition already having taken place, i.e. with the thermal stability of the peroxides. But up to 10 per cent destruction, the rate was found to be fairly equal for various peroxide levels (from $P =$ 10 to $P = 60.8$) and the reaction constant in this range may be calculated according to the formula:

$$\log K = 11.2 - 3.85\ 10^3 \frac{1}{T}$$

    where T is the absolute temperature ($K°$).
11. When liver oils containing peroxides and carbonyls are heated for a sufficient length of time (beyond the time required for complete destruction of the peroxides) the values $E_{430m\mu}$ and $E_{460m\mu}$ (Henick) will tend towards definite levels. At the same time $\lambda_{E\max}$ will rise to about $460m\mu$ when the decomposition temperature is kept below 150° C. At temperatures above 150° C $\lambda_{E\max}$ will again decrease, rapidly at first, later on more slowly, in accordance with the temperature rise. Thus $\lambda_{E\max}$ decreases from $460m\mu$ at temperatures below 150° C to about $442m\mu$ at 280°C (Fig. 13).
12. The relation outlined above may be explained by assuming that the saturated carbonyls are polymerized via the carbonyl groups at lower temperatures, whereas the α, β-unsaturated carbonyls only polymerize via the double bonds at temperatures above 150° C (see figs. 14 and 15).
13. When the unsaturated carbonyls polymerize (at temperatures above 150° C) an equivalent amount of more saturated carbonyl is formed.
14. Long time heating (for about 30 days) of a liver oil in inert atmosphere at 60° C gave the same $E_{430m\mu}$ value for the unsaturated carbonyls as was found in the unheated oil. But if the temperature is increased to 70—75° C or above, the extinction will gradually increase, from an initial value of 5.7 up to 7.0 at 150° C (Fig. 14). This increase may be due to the splitting of more stable peroxides

which remain intact during the Henick analysis (at 65° C). That only unsaturated carbonyls are thus indicated may be due to the polymerization of the saturated compounds, which we have presumed is taking place.

15. To avoid transformations of the saturated aldehyde fraction, all laboratory manipulations must be carried out at as low temperature as is practically feasible, preferably below 40° C.

REFERENCES

(1) O. Lundberg and coll.: Commerc. Fish. Rev. *19*, 7, (1957).
(2) A. S. Henick, M. F. Benca and J. H. Mitchell Jr.: IAOCS *31*, 88, (1954).
(3) A. M. Gaddis, R. Ellis and G. T. Currie: Food Res. *25*, 495, (1960).
(4) U. Holm, K. Ekbom and G. Wode: IAOCS *34*, 606, (1957).
(5) C. H. Lea and P. A. T. Swoboda: J. Sci. Food Agr. *13*, 148, (1962).
(6) E. H. Farmer: Trans. Faraday Soc. *42*, 228, (1946).
(7) M. Keeney: Symposium on Foods: Lipids and their Oxidation, 79 (1962).
(8) D. P. Schwartz, H. S. Haller and M. Keeney: Anal. Chem. *35*, 2191, (1963).
(9) F. Linow, M. Roloff and K. Taüfel: Fette-Seifen-Anstr. m. *66*, 1052, (1964).
(10) G. R. Mizuno and J. R. Chipault: IAOCS *42*, 839, (1965).
(11) L. Aure, K. M. Anthonsen and E. Bjørsvik: 3. Nordiske Harskningssymposium, Sandefjord, August 1961, p. 119.