TABLE I

Yields of Yeast on Nitrogen-Supplemented Peanut Water

	Carbon/Nitrogen Ratio	Grams of yeast per 100 g. of sugars*
Unsupplemented	63/1	41.3
$(NH_4)_2$ SO_4 added	16/1	48.5
$(NH_4)_2$ SO_4 added	8/1	47.8

*Average values from several experiments.

Continuous Propagation

In a system of three bottles similar to that used for sweet potato experiments (8), two of which were provided with air (as in Figure 1), it was found entirely practicable to feed continuously peanut water of about 0.8% sugar supplemented with nitrogen to an actively growing culture of *T. utilis* and withdraw yeast liquor continuously. Feeding at a rate of 50% of the volume in the propagator per hour, the sugar was completely utilized and a yield of 40.6 grams of yeast per 100 grams of sugar was produced. At increased rates the yield dropped considerably. Continuous feed appears to be the most efficient method of using equipment and time for handling large quantities of waste water in the propagation of yeast.

Quality of Yeast Produced

Although maximum yields occur in a nutrient medium having a carbon/nitrogen ratio of 16/1, a higher-protein yeast is formed in one having a carbon/nitrogen ratio of 8/1. Estimating protein as 6.25 times the total nitrogen of dried yeast, 54.3% protein is formed at a C/N ratio of 8/1 as compared to 46.8% at 16/1 (calculated to moisture free basis), which correspond to values found by other workers for *T. utilis* (7, 9) and commercial yeast (13).

The vitamin content of dried yeast grown in peanut water is apparently similar to dried yeast from other sources (2, 11); thiamine averaged 72 gamma per gram, and riboflavin 40.9 gamma per gram in samples containing about 5% moisture.

Summary

Peanut protein waste liquor supplemented only with an ammonium salt was found to be an excellent medium for the propagation of the food yeast, *Torulopsis utilis*, in batch and continuous processes.

When nitrogen was provided to give a carbon/nitrogen ratio of 8/1, 100 grams of sugar yielded 48 grams of a high-protein yeast that was comparable in food value and vitamin content to food yeasts from other sources.

Acknowledgment

The protein analyses of dried yeast were made by V. Orr. The thiamine and riboflavin determinations were made by L. H. Charbonnet and M. Murray, using photofluorometric methods.

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The Fatty Acids of Menhaden Oil*

II. The Separation of the Methyl Esters of Menhaden Oil Into Saturated, Monoethylenic, and Polyethylenic Fractions by Low Temperature Crystallization. The Composition of the Saturated and Monoethylenic Fractions.

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THE fatty acids of fish oils constitute some of the most complex mixtures of fatty acids encountered in the field of fat chemistry. The saturated acids alone embrace carbon series from C_{12} to C_{24}. The unsaturated acids occur in an equally wide range of carbon series, and in addition include unsaturation from one to six double bonds. Thus, with menhaden oil for an example, we have shown (1) that the C_{18} series of unsaturated acids includes acids of from one to four double bonds. Previous work from a number of laboratories has indicated that the C_{20} and C_{22} series of this oil and of other fish oils are even more complex in nature, the unsaturation culminating in the presence of docosapentenoic and docosahexenoic acids in the C_{22} series.

The acids of two and more double bonds in a fish oil may be isolated by the lithium soap acetone procedures, originally proposed by Tsujimoto in 1920 (2). Debromination of the ether-insoluble bromides of the esters of the oil, as carried out by Brown and Beal (3) in their study of menhaden oil fatty acids, results in a mixture of esters of three and more double bonds. In the previous report of this series (1) we

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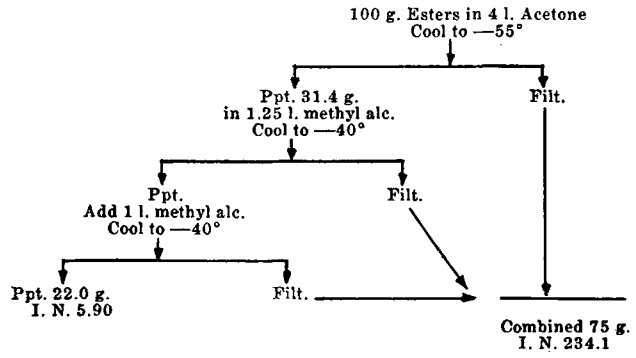
CHART I.

Crystallization of Menhaden Oil Esters.
Preparation of Saturated and Monoethylenic Fractions.



have described low temperature crystallization procedures for the isolation of a number of the saturated and monoethylenic acids of menhaden oil. Pure hexadecenoic and octadecenoic acids were prepared and described.

Processes for the preparation of mixtures of unsaturated acids from fish oils by direct crystallization of the acids or their methyl esters have been described by Brown (4). Thus by crystallization of a solution of 100 g. of menhaden oil fatty acids in 2 liters of petroleum ether and the taking off of crystal fractions at various low temperatures down to —65° there resulted a crystal fraction consisting mainly of monoethylenic acids. Most of the saturated acids had been removed at —40° and above.

In studying the composition of menhaden oil fatty acids, we thought that valuable information could be obtained by separating the acids (or their esters) directly into substantially pure saturated (STD), monoethylenic (ME), and polyethylenic (PE) fractions by crystallization at low temperatures. The only practical method of doing this previously was by a combination of the lead soap ether (or alcohol) and the lithium soap acetone procedures whereby, theoretically at least, the STD lead soaps would come down in cold ether (or alcohol) and the ME acids would be precipitated as lithium soaps in acetone. Both methods, however, have their serious drawbacks, especially when applied to the complex mixtures of acids found in a fish oil.

In the present report we have described procedures for the separation of menhaden oil methyl esters into the three fractions noted above. Two of these, the STD and ME ester fractions, were subsequently distilled and their composition studied. The range and relative amounts of the several carbon series are described. The crystallization procedure is shown to be adapted and practical for this method of study of the composition of a fatty acid mixture of this kind.

Experimental

Six kilograms of the same specimen of menhaden oil used previously (1) was esterified in two batches. A 50 g. sample of the crude esters was distilled and gave the following constants: I.N. 177.0; m.m.w. 288.9. The recovery of crude esters was 5870 g.

Separation of the Methyl Esters of Menhaden Oil Into Saturated, Monoethylenic, and Polyethylenic Fractions.

Several procedures were tried for isolating the STD esters. Whatever method was employed, the yield of esters of iodine number 4-6 was 22-23 g. from 100 g. of the original ester mixture. The best procedure consisted of relatively complete removal of the STD material by cooling to —55° in acetone and recrystallizing the resulting crystal fraction twice from methanol at —40°. The procedure, described in Chart I, was applied to a total of 900 g. of the original esters in nine batches of 100 g. each. The final precipitates from five of these runs were combined and

CHART II.

Crystallization of Menhaden Oil Esters.
Preparation of the Monoethylenic Fraction.

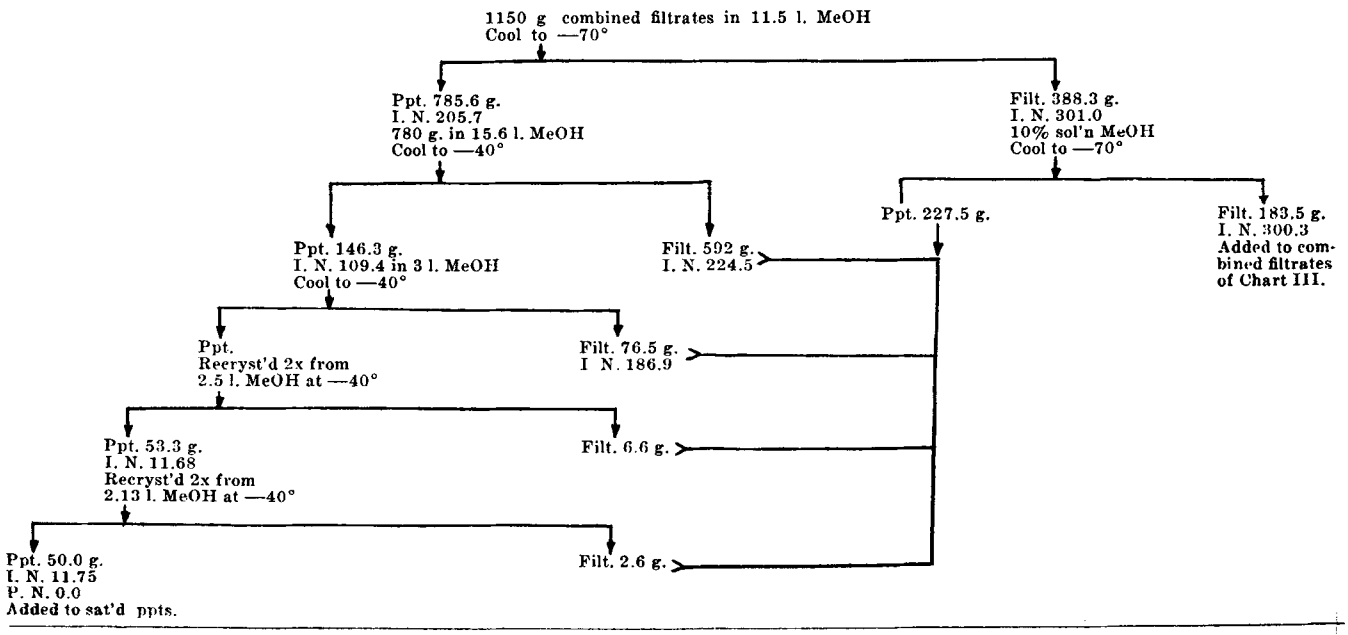
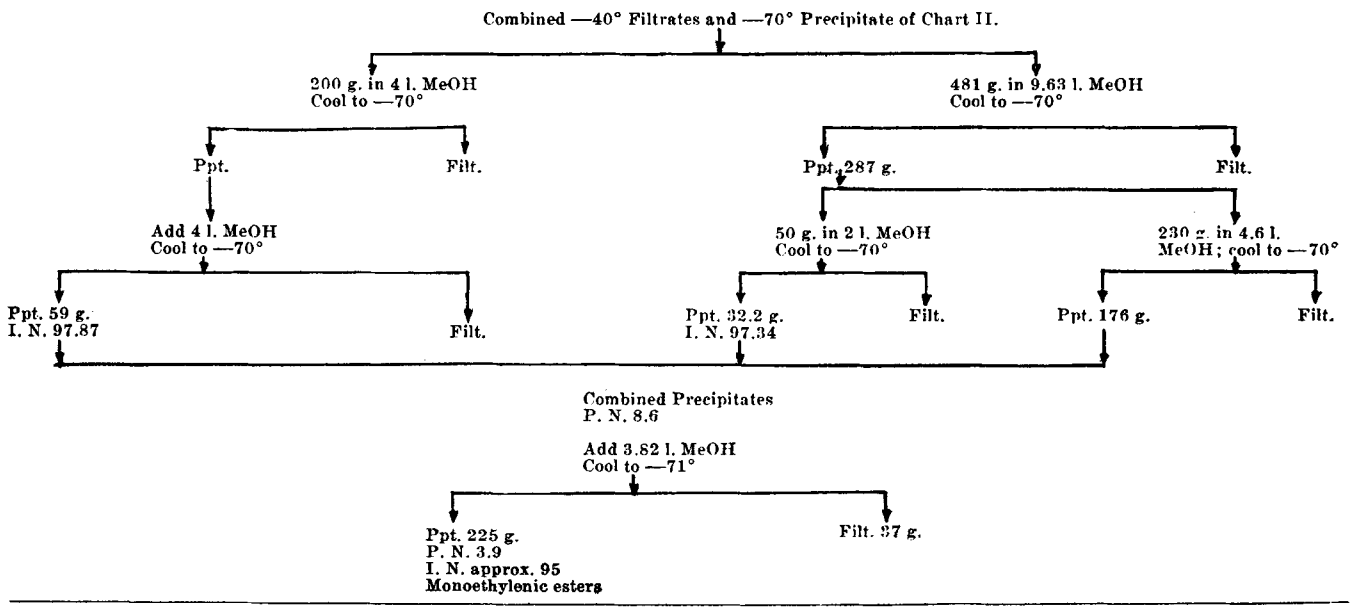


CHART III.

Crystallization of Menhaden Oil Esters.
Further Separation of Monoethylenic Fraction.



crystallized once more from methanol, yielding 201.2 g. of STD esters of I.N. 7.3 and 13.8 g. of filtrate esters of I.N. 139.0.

The final crystal fractions from this operation were combined with similar products from other experiments. From a total of 1,700 g. of original crude esters there were obtained 436 g. of STD and 1,225 g. of filtrate esters. In the course of working up the latter (see Charts II and III), an additional 53 g. of STD esters was obtained which was added to the above, making a total of 489 g., the constants of which, after distillation, are described in Chart IV. These esters contained less than 5% of unsaturated esters and no PE esters, as is shown by the zero polybromide number.

The combined filtrates above represent the ME and PE esters present in the original mixture, plus a small amount of STD esters, not yet removed. They were separated into ME and PE fractions by the procedures described in Charts II and III. The final crystal fractions from these experiments gave an I.N. of about 95 and a polybromide number of 3.9; the actual yield was 225 g., which represents the ME fraction of the specimen. The P.N. of 3.9 indicates the presence of 3-4% of PE esters in this product, which, in turn, would account for about 10 of the total iodine number. Thus, the average iodine number of the ME fraction exclusive of contaminating PE esters is close to 85, which is about that of methyl oleate.

The filtrates resulting from the crystallizations in Chart III and the —70° filtrate of Chart II were combined and amounted to 801 g. Further attempts to crystallize this material were unsuccessful since only a heavy, thick syrup resulted on cooling to the temperature of dry ice.

A resumé of the final products from 1,700 g. of original esters is shown in Chart IV.

CHART IV.

Separation of Menhaden Oil Esters Into Saturated Monoethylenic, and Polyethylenic Fractions.
Menhaden Oil Esters, 1,700 g.

Saturated	Monoethylenic	Polyethylenic
489 g. I. N. 4.8 P. N. 0.0 30.9%	239.0 g.* I. N. about 95 P. N. 3.9 15.1%	853 g.* I. N. 285.2 P. N. 80.5 54.0%

*These values have been corrected to account for the fact that only 1,150 g. of the 1,225 g. of filtrate esters, mentioned previously, were crystallized. Thus the recovery from 1,225 g. would have been 53 g. STD, 239 g. ME, and 853 g. PE esters, or a total of 1,145 g. The loss here is 80 g., part of which is mechanical and part as a result of sampling, etc. Percentages above were *approximate* values, based on the actual recovery of 1,581 g. from 1,700 g. of original esters.

TABLE I.

Fractional Distillation, Saturated Esters of Menhaden Oil

Fraction	Weight g.	Iodine No.	M. Mol. Wt. Esters	Composition g. C_{14}	C_{16}	C_{18}	C_{20}	C_{22}	C_{24}
1	6.1	1.3	245.6	6.1	.....	.....	.....	.....	.....
2	53.5	0.1	242.5	53.5	.....	.....	.....	.....	.....
3	21.1	0.7	250.3	14.7	6.4	.....	.....	.....	.....
4	291.9	0.4	269.6	7.5	284.4	.....	.....	.....	.....
5	12.2	7.0	281.9	.....	6.9	5.3	.....	.....	.....
6	43.8	5.0	294.7	.....	5.4	38.4	.....	.....	.....
7	6.8	37.6	327.9	.....	.....	.....	6.5	0.3	.....
Hold-up	10.7	69.4	346.4	.....	.....	.....	3.0	7.7	.....
Residue	9.6	58.8	381.3	.....	.....	.....	.....	0.4	9.2
Totals	455.7			81.8	303.1	43.7	9.5	8.4	9.2
Wt. % of STD esters				18.0	66.5	9.6	2.1	1.9	2.0
Wt. % of original esters (1581 g.)				5.1	19.2	2.8	0.6	0.5	0.6
Wt. % from (1)				6.8	15.5	3.1	.....	.....	.....

Investigation of the Saturated and Monoethylenic Fractions.

The STD and ME esters of Chart IV were fractionally distilled, using the efficient column described previously (1). The analytical constants and the composition of the fractions are listed in Tables I and II.

From the data in Table I it is concluded that the saturated acids of menhaden oil include series from C_{14} to C_{24}. The recovery of myristic acid, 5.1%, however, is incomplete, since in our previous report we found 6.8% of this acid. However, an additional 1.8% was recovered in the ME fraction (Table II). Methyl palmitate comprised 66.5% of the STD fraction. We are unable to explain this high recovery, 19.2% of the total esters, since in our previous work we found only 15.5% of this ester. The findings for methyl stearate, 2.8 and 3.1%, respectively, are in good agreement. We believe the data in Table I to verify fully the presence of arachidic, behenic, and tetracosanoic acids in this oil; however, they are present only in very small amounts, since the iodine numbers of fraction 7, hold-up, and residue (37.6, 69.4 and 58.8, respectively) show them to be composed of up to one-half unsaturated esters. Thus, it is likely that not over 0.3% of any of these acids can occur in the oil.

The ME esters of Table II, likewise, include series from C_{14} to C_{24}. However, the 13.2% reported as C_{14} is mainly methyl myristate as is shown by the low iodine number of fractions 1 and 2. The C_{14} component of fraction 3 is also assumed to be myristate. Together these make up 1.8% of the original esters. The 0.1% of tetradecenoate, found in the previous work (1), has thus been lost, and may well have gone into the PE fraction. Likewise, the recoveries of methyl hexadecenoate and oleate, 2.3 and 6.4%, respectively, are considerably lower than found previously, 14.1 and 15.9%. It is concluded that these esters have been carried over into the PE fraction. Thus, about 80% of the hexadecenoate and 60% of the oleate have been lost as a result of their solubility in the PE filtrates.

The iodine numbers of fractions 5-8, hold-up and residue, show the presence of appreciable amounts of esters with more than one double bond. However, we believe that the evidence for the presence of ME acids of these higher series is good. Fraction 6 is mainly methyl eicosenoate, mixed with some oleate. The dihydroxy derivative of the acids of this fraction was prepared and was found to melt at 129.2-129.5°; mixed melting point with dihydroxystearic acid was 116.3-118.3. The neutralization equivalent was 346.8; theory for dihydroxyeicosanoic acid 344.3. Baldwin and Parks (5) reported the preparation of this acid from menhaden oil and stated the melting point to be 115.5-116°. Their acid was shown to be 11,12-dihydroxyeicosanoic acid.

We also prepared the dihydroxy acid from fraction 8 but found it to behave as the corresponding derivative of the C_{20} acid. Thus we were unable to prove by actual identification the presence of C_{22} and C_{24} monoethylenic acids. Since these fractions, especially the hold-up and residue, contain more highly unsaturated esters and, also, small amounts of esters altered during the course of the distillation, melting point evidence on this hydroxy acid is not conclusive.

From the data above it is clear that the ME fraction, as prepared by the crystallization procedure, is contaminated by the presence of methyl myristate on the one hand and of small amounts of PE esters on the other; further, it does not contain all of the C_{14}, C_{16} and C_{18} ME esters present in the original ester mixture. No information is available on the recovery of the three higher series of ME esters; these ME esters should decrease rapidly in solubility with increase in molecular weight; hence their recovery should be more efficient with increase in molecular weight. In spite of the limitations thus shown we feel that no other known method would approach the crystallization method in effecting the separation described above. It should be further pointed out that the PE fraction of P.N. 80.5 and containing 75-80% PE acids, should possess unique drying properties.

Summary

The methyl esters of menhaden oil have been separated into three fractions by low temperature crystallization; saturated esters, monoethylenic esters, and polyethylenic esters, the contents of the fractions being roughly 30.9, 15.1 and 54.0%, respectively. The saturated fraction was contaminated with about 5% of the ME esters. The ME fraction, in turn, contained small amounts of methyl myristate and PE esters; further, the recovery of ME esters by the procedure was incomplete.

The saturated and ME fractions were separately distilled, and observations were made on the nature

TABLE II.

Fractional Distillation, Monoethylenic Esters of Menhaden Oil

Fraction	Weight g.	Iodine No.	M. Mol. Wt. Esters	Composition g.					
				C_{14}	C_{16}	C_{18}	C_{20}	C_{22}	C_{24}
Original	.....	ca. 95	304.7	.....	.....	.....	.....	.....	.....
1	9.2	0.7	241.8	9.2	.....	.....	.....	.....	.....
2	18.4	1.1	242.7	18.4	.....	.....	.....	.....	.....
3	13.3	57.0	267.7	1.2	12.1	.....	.....	.....	.....
4	119.2	85.0	290.8	.....	24.2	95.0	.....	.....	.....
5	3.2	117.9**	305.5	.....	.....	2.1	1.1	.....	.....
6	27.4	103.5**	320.5	.....	.....	3.7	23.7	.....	.....
7	11.9	134.8**	345.6	.....	.....	.....	2.9	9.0	.....
8	8.0	108.0**	358.1	.....	.....	.....	.....	6.4	1.6
Hold-up	0.5	108.3***	.....	.....	.....	.....	.....	.....	0.5
Residue	6.3	115.9***	.....	.....	.....	.....	.....	.....	6.3
Totals	217.4	.....	.....	28.8	36.3	100.8	27.7	15.4	8.4
% of monoethylenic esters				13.2*	16.7	46.4	12.7	7.1	3.9
% of original esters (1581 g.)				1.8*	2.3	6.4	1.7	1.0	0.5
% from (1)				0.1	14.1	15.9	.....	.....	.....

*Calculated as STD esters.
**Fractions 5-8, hold-up and residue contain appreciable amounts of esters with more than one double bond.
***Arbitrarily assigned to C_{24}.

and amounts of the acids in the resulting fractions. The low temperature crystallization technic was shown to be useful in the separation of the complex mixtures of esters found in fish oils.

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Direct Oxidation Tests on Soap

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WHEN it became necessary to test certain cosmetic products for oxidative rancidity, the question arose whether the peroxide test of Lea could be used in preparations where the fatty substance, fatty acid, or soap is emulsified or hydrated in water. This test has been used for years in determining the rancidity of oils while the method of separating oil and testing it by means of the Kreis test is slow and susceptible to change during the separation of the fatty substance. Attempts to make the Kreis test a quantitative test have not until now proved satisfactory for cosmetic products.

The Peroxide Test for Soap Gels

It was to be expected in employing the peroxide test of Lea that the acetic acid used in the solvent mixture would suffice for separating the free fatty acid from the soap-gel sample, thus yielding a solution of free fatty acid in the solvent. It was also to be expected that in the presence of water the hydriodic acid which is liberated from the potassium iodide might split off some free iodine, thus unfortunately increasing the peroxide value. When oxidized fats were emulsified with water in the proportions 1:1 and 1:2 and tested in comparison with the original oxidized oil, it was found that an increase in peroxide value of 0.3 and 0.5 units developed. These increases, while important in cases of very low peroxide values, do not affect practical conclusions in the case of higher values.

Method. Five-tenths to 1.0 gram of soap or 1.0 to 3.0 grams of cosmetic emulsion was weighed into a 40 ml. test tube. The actual amount of sample selected will depend upon the expected peroxide value and upon the water content of the cosmetic emulsion. To the mixture was added 1.0 gram of potassium iodide followed by the mixture of glacial acetic acid: chloroform (2:1). The mixture was boiled until the soap was dissolved (requiring about 10 seconds), and then for exactly 30 seconds longer, timed with a stop-watch. It was then cooled under running water, diluted with 30 ml. freshly boiled water, and titrated with 0.002 N thiosulphate with starch solution as indicator.

A sample of old soapflakes was tested by this method with the following results:

PEROXIDE VALUE

	Sample from package surface	Average sample of mixed flakes
1	5.7	2.0
2	5.3	2.2
3	4.0	2.4
4	5.0	1.8
5	4.0	2.4
Average	4.8	2.16

Blank on reagents: zero.

It will be noted that the greater differences occurred when samples were taken from the surface because of the unevenness of aeration of the various samples. Smaller differences occurred in the case of mixed samples, and it is reasonable to assume that only these are to be taken into account in judging the accuracy of the method.

The procedure described is identical with that of the Lea peroxide test with the exception that instead of oil one gram of soap is substituted. The result is calculated directly per gram of soap. If only the state or extent of oxidation of a soap sample is to be determined, the exposure of the sample need not be considered. If the tendency of the sample to become rancid, however, is to be estimated, it is necessary carefully to control the exposure of the product to the action of oxygen or air.

The following tables provide general preliminary figures concerning the development of peroxide rancidity under various conditions, using the test proposed in this paper:

TABLE 1

Marseilles Type (Peanut-Coconut, 25% Water, Shavings)

Under quartz lamp									
Hours	0	1	2	3	then 24 in open air without lamp				
P. V.	4.4	8.0	10.0	14.8	44.0				
200-watt lamp									
Hours	0	24	48	72	96	144	192	336	
	0.0	19	50	60	61	84	120	80	
Dark drying oven (at 105°C.)									
Hours	0	8	18	36					
P. V.	0	6.0	3.0	3.5					

TABLE 2

Soap Flakes (Olive, Coconut Oil, 5% Water)

Under quartz lamp			
Hours	0	8 with lamp 20 without	11 with 60 without
P. V.	0.0	86	144
Remarks	0.04% NaOH		2% FFA
Hours	16 with 100 without	23 with 100 without	23 with 2 wks. without
P. V.	175	230	130
Remarks		no discoloration	yellow 7.7% FFA

Under 200-watt daylight lamp (at 70°C.)							
Hours	0	24	72	120	264	336	580
P. V.	0.0	13.5	22	42	120	62	118

TABLE 3

Milled Soap (Tallow-Coconut Shavings)

Under 200-watt daylight lamp					
Hours	0	21	26	46	and 6 under quartz lamp
P. V.	0.0	8.0	10.0	12.0	114