

MALABAR SARDINE OIL.

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I. INTRODUCTION.

The fish oil manufactured on the Malabar coast is obtained from one species only, the West Coast sardine (*Clupea longiceps*). The mature oil-bearing sardines are found along the West Coast from Cape Comorin to Goa. The fishing season begins in September and ends by the middle of February or earlier. From data collected at the Madras Experimental Station at Tanur, it appears that the shoals tend to increase annually for about six consecutive years and then diminish until after nine or ten years the oily sardine disappears for a season.

A mature oil-bearing sardine, when fresh from the sea, weighs about 45 grams, and is about 10 cm. long exclusive of the tail. The fresh fish contains about 60 per cent. of water, and in some cases as much as 25 per cent. of oil. The oil content, however, varies; in some cases it may be as low as 8 per cent., but the average may be taken as 16 per cent.

The ordinary 'dug outs' used in sardine and mackerel fishing are about 24 feet \times 4.5 feet and a catch weighs about one ton. Each boat carries six men and may go as far as five miles from shore. The net is made of strong cotton yarn with a small mesh. The whole operation takes about seven hours, and two hours may elapse between the actual catch and landing the fish on shore and usually a further three or four hours before the rendering operation is started.

The old method of rendering the oil was very crude and consisted in collecting the fish in heaps and allowing them to undergo putrefaction. The oil cells decayed and the oil oozed out. The oil produced by this process had a dark colour, an offensive nauseating odour and usually a high acid value, in some cases as high as seventy. At the present time most of those engaged in the manufacture of the oil adopt a method first introduced by the Government Experimental Station at Tanur. This process consists of heating the fish for thirty to forty-five minutes with a little water in iron pans (150 to 250 gallons) provided with an open steam coil or more commonly heated over a free fire. The

heated mass is cooled by adding water and is then put into coir bags and subjected to a pressure of about 90 lbs. to the square inch in a screw press. The oil which exudes contains much mucilagenous matter and is carried along gutters to settling tanks and allowed to settle for at least a week, when a clear, dark coloured oil is obtained above the separated water and mucilage. An appreciable amount of 'stearin' also separates and the clear oil constitutes the fish oil of commerce.

During the process of heating with water in the iron pans a small quantity of oil separates on the surface and if this is removed at once a pale yellow coloured oil with a faint odour is obtained. Its acidity is usually under 1 per cent. calculated as oleic acid and it constitutes the first-grade oil of commerce. It is used medicinally to a certain extent as a substitute for cod-liver oil.

The grading of the oil is largely based on the colour, and as a rule the deeper the colour the more disagreeable the odour and the higher the acid value. Good oils with pale colour, not too strong in odour and low acid value are obtained if the oil is rendered within four hours of the catch, so that putrefaction is avoided and also if, during the cooking, the fish is kept for not more than half an hour in contact with the water.

The press cake left after the removal of the oil and water is fish guano. It is usually sun-dried and then sold as a fertiliser. In some cases it is found contaminated with much sand, but if free from sand its average nitrogen and phosphorus content is :—

Nitrogen 6.5 to 9.0 per cent.
Phosphorus pentoxide	... 7.8 to 10.0 " "

At the present time there are about 250 petty installations producing oil on the Coast and the output of oil is stated to be about 6,000 tons per annum.

In Malabar the oil is known as 'Vilakkennai' or burning oil, as it is used by the fisher-folk as an illuminant in place of kerosene. Before the war large quantities¹ of the oil were exported to Germany and Belgium ostensibly for admixture with linseed oil in the paint industry. At the present time the oil is largely used in India itself both in jute mills and tanneries. The first-grade oil is used in the local canning factories and is also sold as a substitute for cod-liver oil. In addition the lowest grade oils, 'foots' and 'stearin' are converted into a soap which is used for insecticidal purposes on tea and coffee estates.

¹ In 1913-14 377,744 gallons, valued at Rs. 2,30,000, were exported.

The guano is largely exported ¹ mainly to the Straits Settlement, Ceylon and Japan.

At the present time the West Coast fish oil is cheap when compared with most of the vegetable fixed oils of both North and South India.

The following table gives the prices of some of the commoner Indian oils :—

TABLE I.

	Rs. per ton.
Rape-seed oil	... 650 (naked).
Ground-nut oil	... 650 "
Cocanut oil	... 720 "
Castor oil	... 730 (in drums).
Mohua oil	... 700 ...
Gingelly oil	... 850 (naked).
Linseed oil	... 1,000 (in drums).

Second-grade fish oil can be obtained at about Rs. 200-250 per ton or from the Government factory at Rs. 300 per ton. The 'stearin' from the oil can be purchased for Rs. 100-150 per ton.

It is obvious that if fish oil could be refined by simple processes so that it could take the place of some of these oils and if the supplies could be appreciably increased, then the fish oil industry would be capable of great development.

The following is a list of some of the possible ways in which fish oil could be utilised :—

1. Refining the oil by removing colour and odour, splitting the refined oil by means of castor seed lipase or by Twitchell's reagent and utilising the glycerine and fatty acids.

2. Hardening the fatty acids from (1) and using the products for soap and candle manufacture.

3. If the fatty acids themselves cannot be readily hardened, then the refined oil may be hardened and used as such for soap and candle manufacture or split by the Twitchell process and the glycerine recovered and the hardened fatty acids utilised.

4. Sulphonating the oil and using the sulphonated product in the place of Turkey-red oil in tanneries and dye-houses.

¹ In 1913-14 4,726 tons, valued at Rs. 4,00,000, were exported.

5. Using the oil either in the crude or refined state in the paint industry as a partial or complete substitute for linseed oil.

6. Using the partially hardened oil for edible purposes.

The experiments described in this paper can be classified under the first four headings in this scheme.

II. MALABAR SARDINE OIL AND ITS REFINING.

A. K. Menon¹ has given the following analyses of three samples of Malabar sardine oil :—

TABLE II.

OIL	Sp. gr. 24°	Acid value	Saponifica- tion value	Iodine value	Refractive index at 29.5
A-1 quality skimmed ...	0.9251	1.69	194.7	172.6	1.476
No. 1 ,, ,, ...	0.9241	5.63	195.4	173.2	1.475
,, 2 ,, pressed ...	0.9242	36.10	195.9	165.5	1.474

In addition the physical and chemical characteristics of the fatty acids obtained from the oils are also given by Menon.

Table III gives the results of the analyses of several samples of Malabar sardine oil made in these laboratories.

Refining the oil.—The object of refining is to remove suspended matter, to produce an oil of pale colour, little or no odour and practically free from acidity. The oils used were second grade oils only. It was considered inadvisable to attempt to refine lower grade oils, as with oils of acid value 30–70 the amount of soap formed on alkali treatment is so large that the quantity of refined oil is correspondingly small, especially as appreciable amounts of oil remain adhering to the soap.

The following method of refining has been adopted and has given satisfactory results :—

¹ Indian Science Congress, 1920.

TABLE III.

West Coast sardine oils—Analytical data.

Quality of oil	Colour	Sp. gr. 15.5/15.5	Refractive index $n_D^{40^\circ}$	Acid value	Saponifi- cation value	Iodine value	Insoluble bromide	Unsapo- nifiable matter	Fatty acids		
									Titre	Neutrali- sation number	Mean molecular weight
First grade	... Pale yellow	2.7	194.0	172	33.0	...	33.4	195	287
Second grade	... Deep brown	8.0	195.0	169	28.6	...	33.2	194	289
Do.	... do.	11.0	194.5	155	27.2	...	33.2	194	289
Do.	... do.	26.0	194.0	154	33.3	195	287
Oil with stearin	... Light brown ...	0.9336	1.4726	0.53	192.2	160	39.3	...	32.8
Clear oil	... Deep yellow ...	0.9330	1.4734	1.1	193.0	168	44.3	1.8	30.6
Oil with stearin	... Yellow ...	0.9328	1.4736	0.56	193.0	170	44.6	...	30.6

The majority of these oils were supplied by the Assistant Director of Fisheries, Calicut.

The acid value of the oil is determined and sufficient 10 per cent. caustic soda solution to neutralise the free acids is gradually added to the oil, which is maintained at a temperature of 60° and stirred. After the addition of the alkali is complete the stirring is continued for half an hour. At the end of this time the heating is discontinued and the mixture kept at rest. After two to three hours three distinct layers are obtained—

- (a) An upper layer of soap,
- (b) A middle layer of oil, and
- (c) A lower aqueous layer and some mucilage.

The upper layer of soap is skimmed off, the lower mucilageous layer syphoned off and the middle oily layer washed several times with hot water in order to remove traces of soap. The washing is continued until the wash-water gives no alkaline reaction to phenol-phthalein. The oil is then steamed for six to eight hours to deodorise it as much as possible and finally heated to about 105° to drive off last traces of moisture. On the small scale the steaming was carried out in a glass flask and on the large scale in a steam jacketted pan provided with an open steam coil. The oil may be finally treated with Fuller's earth; this does not improve the colour appreciably but serves to remove last traces of soap.

Table IV gives the results of a number of experiments made in this way.

The oil obtained has a pale or golden yellow colour, only a faint odour and an acid value of 0.1 to 0.4.¹ The other constants of the oil are very slightly affected by this treatment.

The soap produced during the refining process has a certain amount of oil mixed with it. The loss entailed by an acid value of eleven is practically 5.5, whereas the actual loss found experimentally was 8.5 to 9 per cent., so that in many cases the soap may retain as much as 70 per cent. of its own weight of oil. The soap formed during the process of refining together with the adhering oil may be used for the manufacture of insecticidal soaps (cf. p. 20) or attempts may be made to remove the oil from the soap. One method which has been suggested is the addition of water glass, sodium silicate. We have tried this process on a small scale and the results are fairly satisfactory. From 200 grams of soap containing 25 per cent. of oil, twenty grams of oil were recovered by using 50 per cent. sodium silicate solution at a temperature of 80° .

¹ When the refined oil is kept for six months there is only a slight increase in the acid value

TABLE IV.

Experiments on refining sardine oil.

No. of Experiment	Weight of oil	Acid value of oil	Concentration of alkali	Temperature in °C.	Method of stirring	Weight of residue	Number of washings	Weight of refined oil	Acid value of refined oil	Percentage loss	Remarks
M 1	500 grams	8.0	10 per cent.	59	Hand	...	3	395 grams	0.3	39.0	37 grams of salt were added before the soap settled, and mixture left for 1.5 hours.
M 2	500 "	8.0	10 "	54	do.	230 grams	3	310 "	0.31	38.0	38 grams of salt were used and mixture left over night.
M 3	500 "	8.0	10 "	62	do.	120 "	5	375 "	0.35	25.0	Colour of refined oil rather dark.
M 4	500 "	8.0	10 "	58	do.	110 "	5	385 "	0.36	23.0	Colour of refined oil rather dark.
M 5	500 "	8.0	10 "	70	do.	200 "	7	325 "	...	35.0	Colour of refined oil quite dark.
M 6	500 "	8.0	15 "	50	do.	88 "	3	434 "	0.32	13.0	...
M 7	500 "	8.0	10 "	50	Mechanical	96 "	3	410 "	0.25	18.0	Colour of refined oil very good.
M 8	500 "	8.0	15 "	68	do.	88 "	3	440 "	0.25	12.0	Colour of refined oil very good.
M 9	500 "	8.0	15 "	65	do.	88 "	3	442 "	0.25	11.6	Colour not so good as MS.
M 10	4000 "	8.0	15 "	56	do.	...	3	3510 "	0.30	22.2	Colour good.
M 11	500 "	8.0	16 "	61	do.	60 "	3	450 "	1.1	10.0	Colour rather darker than M10.
M 12	500 "	8.0	20 "	65	do.	50 "	3	460 "	0.9	8.0	Colour rather darker than M10.
K 1	300 "	8.4	10 "	60-65	Hand	42 "	4	270 "	0.1	10.0	In Experiments K1 to K7 the residue is the soap only.
K 2	5000 "	11.0	10 "	do.	do.	685 "	4	4560 "	0.2	9.0	
K 3	8000 "	11.0	10 "	do.	do.	940 "	4	7300 "	0.2	8.8	
K 4	2000 "	11.0	10 "	do.	do.	240 "	4	1830 "	0.2	8.5	
K 5	210 lbs.	11.0	10 "	do.	Mechanical	30 lbs.	5	191 lbs.	0.2	9.1	
K 6	2194 grams	10.5	10 "	do.	Hand	349 grams	4	1965 grams	0.3	9.0	
K 7	126.5 lbs.	10.5	10 "	77	Mechanical	21.5 lbs.	5	114 lbs.	0.4	9.9	

As the fish oil is highly unsaturated and tends to oxidise on exposure to the air, the operations entailed in refining should be carried out in the minimum time possible. The process of steaming, if conducted in a more or less closed vessel, entails only slight oxidation as the oil is protected from the oxidising action of the air by the steam itself.

Experiments have shown that Fuller's earth (5 per cent.) and good quality animal charcoal (2 per cent.) have so slight an effect upon the colour of the alkali refined oil that their use does not appear to be a practical proposition.

Steam at 100° or superheated steam under reduced pressure (60 mm.) have a pronounced deodorising effect, but it has been found impossible to remove all traces of odour. Hydrogen at 100° has much the same effect. If the temperature at which the deodorising is carried out is too high then the oil is apt to become a deeper yellow in colour.

It has been found that a second treatment with a small amount of caustic soda, e.g. 20 cc. of 10 per cent. solution for 400 grams of oil, produces a distinct improvement in the colour.

III. HYDROLYSIS OF SARDINE OIL BY CASTOR-SEED LIPASE.

Sardine oil, like other oils consisting of glycerides, can be hydrolysed to fatty acids and glycerine by most of the usual methods, but so far few experiments have been made on hydrolysing it by means of castor seed lipase, a process which gives good results with most vegetable oils.¹

The results of a series of experiments made with both crude oil, grade 2, and the same oil, after treatment with caustic soda and washing but without steaming are given in Table V. In each experiment four grams of crushed castor-seed and 100 grams of oil were used together with 40 cc. of water containing 0.066 gram of acetic acid as activator and the mixture was stirred during the whole course of the reaction.

¹ Cf. this Journal, 1919, 2, 241.

TABLE V.

Hydrolysis of sardine oil with crushed castor-seeds.

No. of Experiment	Acid value of oil	Saponification value of oil	Temperature in degrees Centigrade	Per cent. hydrolysis after hours				
				1	4	20	27	48
1	5.03	34.0	36-37	25.4	38.5	65.5	65.6	...
2	5.03	34.0	23-25	19.1	40.0	76.0	Emulsion broke	
3	0.02	34.0	36-37	25.2	43.0	54.2	61.7	61.7
4	0.02	34.0	23-25	18.3	37.0	60.0	64.0	73.2
5 Ground-nut oil	0.01	33.3	36-37	43.0	80.2	96.8

Experiments 1 and 2 were made with the crude oil and experiments 3 and 4 with the alkali-treated oil.

The results are interesting as they show that on the whole the crude oil hydrolyses somewhat more readily than the alkali-treated oil, but that in neither case are the results as good as those obtained with a vegetable oil such as groundnut oil (Experiment 5). The experiments made at 36-37° were conducted in an electrically heated incubator provided with stirring gear and the remainder at room temperature. The experiments made at 36-37° prove that an equilibrium is reached after 20 to 27 hours, as after that time the percentage hydrolysis remains fairly constant. This may be due to the destruction of the lipase by the proteolytic enzymes present in the crushed seeds.

A second series of experiments was made using Nicloux' ferment,¹ The ferment was prepared by grinding 60 grams of decorticated castor seeds with 120 grams of cotton seed oil and pressing through mull cloth in order to remove most of the aleurone.

In experiments 6-13 the amount of ferment used for 100 grams of oil corresponded with 4 grams of crushed seed, but in experiment 14 the quantity of ferment was doubled. Where acetic acid was used as activator the quantity was 0.068 gram in 40 cc. of water for 100 grams of oil and when manganese sulphate was employed the quantity was 0.08 gram of the crystallised salt per 100 grams of oil.

¹ This journal, 1919, 2, 251.

The acid and saponification values represent the number of cc. of 0.1 N. alkali required for 1 gram of the oil and the method of calculating the hydrolysis is the same as that already described.¹ In all cases the result given is the mean of two experiments.

Experiments 6 to 9 and experiment 14 were made with crude oil and experiments 10 to 13 with alkali-treated oil.

TABLE VI.

Hydrolysis of sardine oil with Nicloux' ferment.

No. of Experiment	Acid value of oil	Temperature in degrees Centigrade	Activator	Percentage hydrolysis after hours					
				1	4	20	27	48	72
6	5.03	36-37	Acetic acid	19.5	34.5	53.2	55.0	59.0	66.0
7	5.03	36-37	Mang. sulphate	...	38.9	63.2	63.9
8	5.03	23-26	Acetic acid	19.6	36.4	61.0	75.6	82.3	...
9	5.03	23-26	Mang. sulphate	17.7	40.4	72.3	77.4	79.3	82.0
10	0.02	36-37	Acetic acid	14.6	26.5	44.1	48.0	58.3	59.0
11	0.02	36-37	Mang. sulphate	...	33.3	46.5	46.8
12	0.02	23-26	Acetic acid	17.2	27.2	46.0	52.0	62.3	68.4
13	0.02	23-26	Mang. sulphate	...	37.4	53.3	56.3	60.8	63.0
14	5.03	23-26	Do.	...	55.9	80.0	84.5	100	...

The results show that with Nicloux' ferment the sardine oil is not hydrolysed as rapidly as most vegetable oils.² With ferment equivalent to four grams of seed the hydrolysis is slow and after a time tends to reach an equilibrium, when either acetic acid or manganese sulphate is used as activator. This equilibrium in the case of the crude oil appears to be about 65 per cent. hydrolysis at 36-37° and about 82 per cent. at 23-26°. With the alkali-refined oil, the equilibria at the two temperatures are lower. As was to be expected an increase in the concentration of the ferment as compared with the oil has a marked effect and complete hydrolysis can be effected in forty-eight hours at 23-26° when ferment corresponding with eight grams of seed is used for each 100 grams of oil (Experiment 14).

A third set of experiments has been made with Nicloux' ferment prepared by grinding the castor-seeds with refined fish oil instead of cotton-seed oil. The results obtained are given in Table VII. The results are similar to those given in Table VI, and with ferment equivalent to four grams of seed the crude oil is hydrolysed a little more readily than the refined oil.

¹ This Journal, 1919. p. 243.

² *Ibid.*, p. 252-255.

TABLE VII.

Hydrolysis of sardine oil with Nicloux' ferment prepared by using sardine oil.

Temperature, 21-25°. Saponification value of oil = 34.

No. of Experiment	Acid value of oil	Ferment corresponding with grams of seed per 100 grams of oil	Activator	Percentage hydrolysis after hours						
				1	4	8	20	27	48	72
17	0.5	4	Acetic acid	...	39.4	...	60.0	66.5	78.0	81.6
18	0.5	4	Mang. sulphate	...	40.0	...	69.4	73.5	82.7	84.7
1a	2.5	4	Acetic acid	28.4	39.8	49.0	60.0	62.6	79.0	...
1b	2.5	4	Do.	27.4	38.7	48.9	55.9	62.5	73.5	...
1c	2.5	4	Do.	27.0	37.4	51.0	57.6	62.7	66.7	...
15	0.5	8	Do.	...	48.7	...	72.0	76.1	94.0	95.0
16	0.5	8	Mang. sulphate	...	53.6	...	76.9	83.2	94.7	...
6a	2.5	8	Acetic acid	40.6	58.0	69.4	77.1	86.9	97.5	...
6b	2.5	8	Do.	41.9	56.8	69.8	75.7	84.0	95.6	...
2a	0.01	4	Do.	15.6	31.6	40.7	52.3	55.2	61.3	...
2b	0.01	4	Do.	16.1	32.3	41.0	54.7	59.3	69.0	..
2c	0.01	4	Do.	16.5	33.7	44.7	57.8	59.4	67.5	...
19	0.02	8	Mang. sulphate	...	44.8	...	66.1	74.3	79.1	80.4
4a	0.01	8	Acetic acid.	35.6	59.7	70.5	80.5	85.2	95.0	...
4b	0.01	8	Do.	40.7	56.9	67.1	76.6	83.1	87.0	...
4c	0.01	8	Do.	36.0	59.6	70.2	81.2	85.9	94.2	...
3a	0.01	4	Do.	23.6	42.3	52.2	60.2	63.1	73.2	...
3b	0.01	4	Do.	18.5	38.3	50.1	55.9	59.3	61.3	...
3c	0.01	4	Do.	19.8	39.4	53.7	59.8	62.1	68.9	...
5a	0.01	8	Do.	40.3	60.1	74.4	86.8	89.4	97.2	...
5b	0.01	8	Do.	36.6	56.1	65.6	78.8	84.1	86.0	...
5c	0.01	8	Do.	37.8	58.0	70.9	85.6	87.1	94.8	...

Note.—Experiments 17 to 6b were made with second-grade crude oil, experiments 2a to 4c with alkali refined oil thoroughly washed, and experiments 3a to 5c with alkali refined oil which had been treated with Fuller's earth.

Experiments 1*a*, 2*a*, 3*a*, 4*a*, 5*a* were made at the same time with the same ferment preparation and similarly with 1*b* to 5*b* and 1*c* to 5*c*. Also 6*a* and 6*b* were made at the same time.

IV. HYDROGENATION OF SARDINE OIL.

The process of hydrogenation consists in adding hydrogen to the molecules of the glycerides of unsaturated acids such as oleic, linolic, linolenic and clupanodonic. Most fish oils are characterised by high iodine values, i.e. they are highly unsaturated and contain appreciable amounts of the glyceride of the most unsaturated acid, clupanodonic acid, $C_{17}H_{27}\cdot CO_2H$. The iodine value of West Coast sardine oil indicates that the oil belongs to this type and hence should be capable of adding on appreciable amounts of hydrogen.

In Europe and America large quantities of fish oils, e.g. whale oil, are hardened by the process of reduction with hydrogen in the presence of a finely divided nickel catalyst. The degree of hardening depends upon the amount of hydrogen taken up, and products varying from butterlike fats to hard, brittle solids can be thus manufactured. The harder products can be used in place of tallow for soap and candle manufacture, and, if the original oils are sufficiently refined, the softer reduction products can be utilised for margarine manufacture, when mixed with other fats.

We have carried out a number of experiments with Malabar sardine oil in order to ascertain the readiness with which it can be hardened and the nature and value of the products formed.

The method of reduction, and the preparation of hydrogen and of catalysts were exactly as described in an earlier paper.¹

The oil used in all the experiments was of good colour and practically acid free; it had been refined by the process described on p. 24.

In using a catalyst of the type B for a number of experiments it was observed that the removal of the catalyst by filtration at the end of the reduction became more and more difficult after each experiment, probably owing to the nickel becoming more finely divided and thus blocking up the pores of the filter paper.

The results of the experiments with the three types of catalyst confirm the results obtained with vegetable oils². The most efficient of the three catalysts is the one prepared by precipitating nickel carbonate on kieselguhr and final reduction at 300°.

¹ This Journal, 1922, 5, 47.

² *Ibid*, p. 63.

TABLE VIII.

Hydrogenation of sardine oil with nickel catalysts.

No. of Experiment	1	2	3	4	5	6	7	8
Type of Catalyst ¹	A	A	B	B	C	C	C	C
Time in hours	0.25	88
	0.50	60	66
	1	55	40	44
	2	88	92	36	26	26
	4	120	119	66	72	20	18	15
	6	45	46	11	9	11
	8	55	58	30	34	3	5	7
	10	44	46	26	28
	12	36	35

The original iodine value was 155, and in each experiment two grams of nickel were used for each 100 grams of oil.

A series of experiments was carried out in order to ascertain for how many hours a catalyst of type C could retain its activity unimpaired when used for reducing sardine oil and it was found that signs of deterioration could be detected after it has been used for forty-four hours. With a catalyst of this type the product has the consistency of tallow after one hour's reduction at 180° under atmospheric pressure.

Experiments have also been made with catalysts of the type C but using less catalyst, viz. 1 per cent. of nickel on the weight of the oil taken. After four hours' reduction a product with an iodine value of forty was obtained, indicating that a reduction in the amount of catalyst diminishes the rate of hydrogenation. The result of increasing the proportion of Kieselguhr from 75 to 85 per cent. was also to diminish the rate of hydrogenation although the ratio nickel to oil was kept constant.

Iodine values and refractive indices of hardened sardine oils.

The table and graph representing the relation between these two constants have already been given² and are reproduced here in Table IX.³

¹ For description cf. 1922, 5, 61.

² *Ibid.*, p. 58.

³ For curve see Fig. I, in vol. 5, opposite p. 51.

TABLE IX.

WEST COAST SARDINE OIL.

Relation between iodine value and refractive index.

No. of Sample	Iodine value Winkler	$n_D^{60^\circ}$ observed	$n_D^{60^\circ}$ calculated	Difference	Time of hydrogenation in hours
Original oil	159.6	1.4654	1.4628	- 26	...
M 2	152.2	1.4645	1.4620	+ 25	0.3
K 17	149.7	1.4642	1.4618	+ 24	...
K 14	149.6	1.4640	1.4618	+ 22	...
M 3	148.0	1.4638	1.4616	+ 22	0.5
K 1	142.3	1.4631	1.4610	+ 21	...
A 9	138.9	1.4629	1.4607	+ 22	...
M 4	138.8	1.4626	1.4607	+ 19	0.8
K 15	136.0	1.4619	1.4604	+ 15	...
M 5	131.7	1.4617	1.4600	+ 17	1.0
K 16	120.5	1.4600	1.4589	+ 11	...
M 6	114.9	1.4594	1.4583	+ 11	1.3
K 3	113.2	1.4589	1.4581	+ 8	...
K 2	109.7	1.4585	1.4578	+ 7	...
M 7	104.3	1.4580	1.4572	+ 8	1.5
K 8	104.1	1.4578	1.4572	+ 6	...
M 8	91.5	1.4564	1.4560	+ 4	1.8
K 5	90.1	1.4560	1.4558	+ 2	...
M 9	81.3	1.4553	1.4549	+ 4	2.0
K 4	80.1	1.4550	1.4548	+ 2	...
A 5	78.2	1.4548	1.4546	+ 2	1.0
K 9	76.7	1.4547	1.4545	+ 2	...
M 10	74.2	1.4544	1.4542	+ 2	2.5
M 11	64.2	1.4533	1.4532	+ 1	3.5
M 12	57.7	1.4525	1.4526	- 1	4.0
K 6	56.7	1.4523	1.4525	- 2	...
A 8	51.8	1.4523	1.4520	+ 3	1.0
M 13	48.4	1.4515	1.4516	- 1	2.3
A 4	42.0	1.4513	1.4510	+ 3	...
M 14	40.7	1.4507	1.4509	- 2	2.5
K 11	36.9	1.4505	1.4505	0	...
A 6	36.0	1.4506	1.4504	+ 2	...
M 15	35.9	1.4503	1.4504	- 1	2.8
K 12	18.1	1.4486	1.4486	0	...
M 16	18.0	1.4487	1.4486	+ 1	3.0
K 10	17.1	1.4486	1.4485	+ 1	...
A 2	16.7	1.4488	1.4485	+ 3	...
A 3	13.5	1.4485	1.4482	+ 3	...
K 13	12.1	1.4480	1.4480	0	...
A 7	10.5	1.4482	1.4478	+ 4	4.0
	0	...	1.4468

Properties of hardened sardine oils.

The hardened oils form pale yellow pasty masses of ghee-like consistency or pale yellow hard solids or even white brittle solids.

TABLE X.

Analytical constants of hardened sardine oil.

Sample number	Sp. gr. $\frac{40^{\circ}}{40^{\circ}} \text{C}$	n_D^{60}	Acid value	Saponification value	Hegner number	Iodine value	Ether insoluble bromide	Linolic tetrabromide	Fatty acids from the hardened oils			
									Titre	Iodine value	Neutralisation number	Mean molecular weight
K 5	0.9163	1.4560	0.4	192.5	93.40	91.0	0.94 per cent.	16.71 per cent.	34.7 °C	94.1	190.6	294.3
K 13	0.9184	1.4480	0.8	194.7	94.04	12.0	61.0 °C	10.7	190.8	293.9
K 19	0.9179	1.4510	0.4	193.8	93.7	42.0	43.4 °C	43.8	190.7	293.6

Table X gives the results of the analysis of three typical reduction products.

Samples of candles and hard soaps have been made from some of the hydrogenated samples. They are of good quality and, contrary to the statements sometimes made, the soaps do not appear to develop an odour of fish oil when kept for twelve months.

V. HYDROGENATION OF THE FATTY ACIDS FROM SARDINE OIL.

The fatty acids were obtained from second grade raw oil by saponification with alcoholic sodium hydroxide, evaporation to remove alcohol, liberation of the free acids by means of hydrochloric acid, washing until free from mineral acid and drying in an atmosphere of carbon dioxide.

The first catalysts used were of the same type as those employed for reducing the oil (p. 31), i.e. finely divided nickel obtained by precipitating the carbonate on kieselguhr and subsequent reduction in hydrogen at 300°.

As it was found that this type of catalyst rapidly diminished in activity when used for reducing the free acids, later experiments were made with catalysts prepared by precipitating nickel borate on kieselguhr and reducing in hydrogen at 300°.¹

Table XI gives the results of the analyses of samples of hardened or partially hardened fatty acids, obtained by using 2 per cent. of nickel per 100 grams of oil.

TABLE XI.

Hardened fatty acids from sardine oil.

No. of Sample	Type of Catalyst	Time of hydrogenation in hours	Iodine value (Winkler)	Titre degrees Centigrade
8	<i>Nil</i>	<i>Nil</i>	147.7	33.4
3	Carbonate 2	4	69.0	35.0
7	Borate 1	4	59.0	36.0
2	Carbonate 1	4	46.5	38.0
6	Borate 1	4	46.4	38.0
4	Do.	4	33.2	41.8
5	Do.	4	33.1	42.0
1	Carbonate 1	4	26.1	46.8

¹ This volume, p. 62.

All the samples of hydrogenated fatty acids were boiled with dilute hydrochloric acid when green solutions were obtained showing the presence of nickel soaps in the hardened fatty acids.

With a catalyst prepared from the carbonate on kieselguhr the activity diminishes considerably even after four hours as shown by a comparison of samples Nos. 1 and 2 which were prepared consecutively by using the same sample of catalyst. The borate catalyst, on the other hand, is more stable and shows deterioration only after twelve to sixteen hours. Samples 4, 5, 6 and 7 were prepared by using the same sample of catalyst and were reduced in the order given.

VI. SARDINE OIL STEARIN.

Samples of the solid which separates when the sardine oil is kept for some time have been collected and examined. These samples were obtained by decanting off the oil and were not pressed so that they contained appreciable amounts of oil.

Before analysis the melted stearin was filtered through paper with the aid of a Buchner funnel and suction.

Table XII gives the analytical data for a sample of filtered stearin.

TABLE XII.

Analytical data for stearin.

D_{40}^{40}	= 0.9227	<i>Fatty acids from stearin.</i>	
n_D^{60}	= 1.4633	Titre	= 34.2°
Acid value	= 6.4	Neutralisation No.	= 183
Saponification value	= 196.5	Mean molecular weight	= 300
Iodine value (Winkler)	= 125		

A sample of first quality stearin gave an acid value of 2.0 and an iodine value of 94. Samples of second quality stearin gave acid values varying from 26 to 78.

Refining the stearin.

1,950 grams of stearin with an acid value of 6.4 was treated with ten grams of sodium hydroxide in the form of a 10 per cent. solution at 80° and gave 1,725 grams of refined stearin with an acid value of 0.03, involving a loss of 11.3 per cent.

Hydrogenation of stearin.

Table XIII gives the results of a series of experiments made by reducing the refined stearin at 180° by means of hydrogen in the presence of 2 per cent. of nickel in the form of a nickel kieselguhr catalyst.

TABLE XIII.

Hydrogenation of sardine oil stearin.

No. of Sample	Time of hydrogenation in hours	Iodine value (Winkler)	Refractive index at 60°
0	<i>Nil</i>	125.0	1.4635
1	0.33	81.6	1.4556
2	1.0	69.0	1.4538
3	2.0	53.3	1.4517
4	3.0	45.3	1.4505
5	4.0	15.6	1.4481
6	4.0	10.0	1.4477

VII. SULPHONATION OF SARDINE OIL.

Castor oil is sulphonated on a commercial scale and the sulphonated product, known as Turkey-red oil, is used both in alizarine dyeing and also in leather dressing. Within recent years other oils have been substituted for castor oil, particularly cheaper vegetable oils and also fish oils.¹

We have carried out a number of experiments with Malabar sardine oil with the object of studying the most suitable conditions for sulphonation and the nature of the products formed as compared with Turkey-red oil.

In each experiment 200 grams of oil were taken in a glass beaker of about 800 cc. capacity placed in a bath through which cold water could be passed or in which ice could be placed in order to keep the reaction temperature fairly constant. Commercial sulphuric acid (sp. gr. 1.82 to 1.83) was allowed to fall drop by drop from a tap funnel into the oil, which was stirred mechanically during the addition of the acid.

¹ Radcliffe, *J. Soc. Dyers Color.*, 1918, **34**, 22; Das and Das, *J. Soc., Leather Trades Chem.*, 1922, **6**, 328. Our work was completed before the latter paper appeared.

The temperature was maintained at 18-26° and the quantity of sulphuric acid was altered in the different experiments, the amount varying from 20 to 70 per cent. on the weight of the oil used.

The addition of the first few drops of acid produced a considerable darkening in colour and as the addition was continued the mixture became viscous and difficult to stir. As a rule stirring was continued for three hours after the addition of all acid. The product was allowed to stand over night (14 to 16 hours) and was then washed with a cold saturated solution of sodium sulphate until the wash liquid was neutral or only faintly acid to litmus paper.

Experiments were made with both second quality crude oil, acid value 11.0, and also with alkali refined oil.

A few experiments were made with castor oil using 35 grams of sulphuric acid per 100 grams of oil for comparison.

The results of the experiments are given in Table XIV. In one or two experiments the mixture was washed with the sodium sulphate solution immediately the whole of the sulphuric acid had been run in. In others the mixture was stirred for a further period of three hours, but was not allowed to stand over night before being washed.

The following conclusions can be drawn from the experiments :—

(1) Almost all the samples for the preparation of which 35 per cent. or more of sulphuric acid was used dissolve in hot water and give a clear solution on the addition of ammonia or caustic soda solution, but all deposit a fine flocculent precipitate when kept.

(2) The sulphonated products are semi-solid, have a brown or reddish brown colour and are entirely free from the characteristic fish-oil odour.

(3) The free fatty acids obtained by saponification with alcoholic soda and decomposing the sodium salts with hydrochloric acid do not contain sulphur and do not give either insoluble hexa- or octa-bromides indicating that the more unsaturated acids are first acted on by the sulphuric acid.

(4) A comparison of the analytical data for the fatty acids derived from the original oil and from the sulphonated oil shows that the titre, mean molecular weight and acetyl value have all been appreciably raised and the iodine value lowered.

TABLE XIV.

Sulphonation of Malabar sardine oil.

No. of Experiment	Oil used	Sulphuric acid per cent. on weight of oil	Temperature in degrees Centigrade	Total fatty matter percent.	Unsulphonated oil as per cent. of total fatty matter	Ratio of unsulphonated to sulphonated oil	Iodine value of unsulphonated oil	Acids from sulphonated oil					Remarks
								Titre	Iodine value	Acetyl value	Neutralisation No.	Mean molecular weight	
N 1	Crude	35	22-23	83.3	12.0	1:7.3	...	38.9	88.8	...	188	...	Insoluble bromide 1.0 per cent.
K 9	Do.	35	25-26	39.2	89.0	...	159	353	
N 2	Do.	60	18-20	86.3	11.8	1:7.5	...	40.1	76.6	...	137	...	Insoluble bromide <i>nil</i> .
K 10	Do.	70	25-26	40.2	86.0	24.0	153	367	
K 8	Refined	20	25-26	97.6	55.5	1:0.8
N 5	Do.	35	24-25	96.2	10.6	1:8.4
N 8	Do.	35	25	...	12.7
K 1	Do.	35	25-26	95.0	12.2	1:7.2	36.0	39.8	88.0	26.0	159	353	Insoluble bromide <i>nil</i> .
K 2	Do.	35	25-26	90.0	12.2	1:7.2	40.0	40.0	161	349	
K 3	Do.	40	25-26	70.0	12.4	1:7.1	59.0	40.7	156	360	...
K 4	Do.	40	25-26	80.0	12.8	1:6.9	60.3	41.2	154	364	Washed with common salt solution.
N 11	Do.	40	25-27	72.0	23.3	1:3.3	Same as K 7b.
N 12	Do.	40	22-24	71.5	44.8	1:1.2	Same as K 6.
K 6	Do.	40	25-26	72.0	23.3	1:3.3	86.5	37.6	Washed immediately addition of acid was completed.
K 7a	Do.	40	25-26	80.0	19.3	1:4.1	80.0	38.0	Do.
K 7b	Do.	40	25-26	67.0	20.0	1:4.0	...	38.6	Stirring stopped when addition of acid was completed but kept over night before washing.
K 7c	Do.	40	25-26	93.0	12.5	1:7.0	
N 9	Do.	50	30	87.8	11.4	1:7.8
N 10	Do.	50	24-25	91.4	11.2	1:8.0
K 5	Do.	60	25-26	89.0	12.4	1:7.0	36.0	42.6	84.0	28.8	152	369	...
M 1	Do.	70	20	93.4	11.7	1:7.8
M 2	Do.	70	24	94.8	12.2	1:8.0
M 4	Do.	70	30	91.0	15.3	1:5.5	Made with 1,400 grams
Fatty acids from original fish oil by alkali saponification								33.1	148.5	<i>Nil</i>	195	288	Insoluble bromide 28.6 per cent.

This is in harmony with the results obtained with such oils as cotton-seed, sesame, olive, and whale oils.¹ In the case of whale oil on sulphonation the neutralisation value falls from 190 to 168 and the acetyl value increases from 3.5 to 22.4, whereas with sardine oil the neutralisation value falls from 195 to 159 and the acetyl value increases from zero to 26.0.

(5) More than 35 per cent. of sulphuric acid appears to have no effect.

(6) The stirring after the addition of the acid is completed and the keeping over night affect the amount of sulphonation.

(7) The presence of free fatty acids in the oil does not appear to have any marked influence on the sulphonation.

SUMMARY.

1. The oil from West Coast sardines (*Clupea longiceps*) has been analysed and also the stearin obtained from it.

2. Second quality oil with acid values up to 12 can readily be refined by alkali treatment with a loss of 8 to 10 per cent. The oil so obtained has a yellow colour and can be further deodorised by treatment with superheated steam under reduced pressure, although the oil cannot be completely deodorised by this process.

3. The oil can be hydrolysed by castor-seed lipase, in the form of crushed seed or Nicloux' ferment, but not so readily as most vegetable oils. The fatty acids so obtained have a pale colour.

4. If it is not desired to isolate glycerine the oil can be hardened by reduction with hydrogen in the presence of a nickel-kieselguhr catalyst and products suitable for soap and candle manufacture obtained. The hardened products are quite devoid of the original smell of the fish oil.

5. It is also possible to obtain a white inodorous fat of ghee consistency.

6. The fatty acids obtained from the oil are not readily hardened owing to the rapid deterioration of the catalyst; a nickel borate-kieselguhr catalyst lasts about twelve hours. If, therefore, it is desired to obtain both glycerine and hardened fatty acids it is advisable first to harden the oil and then to split the hardened oil by the Twitchell process.

¹ Radcliffe, *loc. cit.*, pp. 26-9.

7. The stearin is readily hardened and then forms a valuable product for use in the manufacture of soap and candles.

8. The oil can be sulphonated and yields a water-soluble product. This, however, is semi-solid and dark coloured.

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