OIL SPLITTING BY CASTOR-SEED LIPASE.

By J. J. Sudborough and H. E. Watson.

PART V. THE SPLITTING OF CRUDE AND REFINED OILS BY LIPASE.

With D. Y. Athawale, Ittyerah Joseph, K. R. Rama Iyer, S. Palaniyandy Pillai and K. R. Narayana Iyer.

In Part I[1] emphasis has been laid on the importance of a co-enzyme or activator, such as acetic acid or manganous sulphate, in order to bring about appreciable hydrolysis of vegetable oils by castor-seed lipase and in Part II[2] examples are given in the case of ground-nut oil showing the differences between experiments in which castor-seed alone is used and those in which, in addition, acetic acid is used as activator (Experiments 12 and 14).

During the last two years various experiments made with crude and refined oils have shown appreciable differences in the rates at which these are hydrolysed by castor-seed lipase in the absence of acetic acid. In practically all cases the crude oils show distinct hydrolysis after twenty-four hours, whereas samples of the same oil, which have been refined by alkali treatment, show little or no hydrolysis under similar conditions.

Table I gives the results of a number of typical experiments.

From these experiments it is clear that many unrefined vegetable oils contain substances which can produce appreciable hydrolysis within twenty-four hours when the oils are stirred with water and crushed castor-seed. In the absence of the crushed seed such oils show no appreciable hydrolysis after twenty-four hours. The substances present in the crude oils are therefore of the nature of co-enzymes or activators and cannot replace the lipase.

[1] This Journal, 1919, **2**, 223.
[2] *Ibid.*, 247.

TABLE I

Hydrolysis of crude and refined oils by crushed castor-seeds in the absence of an activator.

No. of Experiment	Oil used	Crude or refined	Acid value	Saponification value	Accelerator	Percentage hydrolysis after hours					
						1	4	29	45	53	72
A3	Coconut	Crude	1·3	45·9	*Nil*	1·8	17·8	...	43·4	56·4	...
A4	Do.	do.	1·3	45·9	do.	1·8	16·0	16·4	29·7	34·7	34·1
B3	Do.	do.	1·3	45·9	do.	2·2	5·6	20·9	40·1	...	50·4
C1	Do.	Refined	0·1	46·0	do.	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
C2	Do.	do.	0·1	46·0	Acetic acid	25·1	...	73·0	84·1	...	90·7
B2	Do.	Crude	1·3	45·9	do.	27·2	61·9	91·4	93·0	...	94·0
18*a*	Mahua[3]	do.	2·6	33·8	*Nil*	5·4	6·7	7·5	9·0	...	...
18*b*	Do.	do.	2·6	33·8	do.	5·8	7·1	7·4	8·3	...	...
19*a*	Do.	Refined	0·16	34·9	do.	0·5	0·7	1·0	...	...	...
110*a*	Do.	do.	0·16	34·9	do.	0·5	0·7	1·1	...	...	...
110*c*	Do.	do.	0·16	34·9	Acetic acid	17·5	31·9	52·4	68·1	...	...
15*b*	Do.	Crude	2·6	33·8	do.	30·3	54·1	88·5	95·4	...	...
R3	Ground-nut	do.	4·0	34·5	*Nil*	4·8	3·4	3·9	4·2	...	...
R4	Do.	do.	4·0	34·5	do.	1·7	1·0	1·1	5·2	...	...
R2	Do.	do.	4·0	34·5	Acetic acid	17·5	49·2	...	92·2	...	...
R5	Hongay[4]	do.	2·9	34·4	*Nil*	3·2	3·6	...	8·6	...	...
R6	Do.	do.	2·9	34·4	do.	4·0	6·1	...	6·5	...	...
R7	Do.	do.	2·9	34·4	Acetic acid	7·6	34·5	...	91·6	...	...
P15	Illipe[5]	do.	2·0	34·85	*Nil*	8·7	36·2	...	85·3	...	...
P16	Do.	do.	2·0	34·85	do.	8·6	39·8	...	83·1	...	...
P19	Do.	Refined	0·18	34·85	do.	0·6	0·6	0·95	...	...	...
P20	Do.	do.	0·18	34·85	do.	0·4	0·6	0·8	...	...	...
P17	Do.	do.	0·18	34·85	Acetic acid	18·9	44·8	71·7	...	...	...
J74*A*	Maroti[6]	Crude	5·6	36·75	*Nil*	21·9	...	53·4[1]	57·0	...	...
J48*A*	Do.	do.	5·1	36·75	do.	18·0	24·3	33·3[1]	37·6	...	...
J48*B*	Do.	do.	5·1	36·75	do.	18·1	22·3	32·2[1]	36·6	...	...
J46	Do.	do.	5·1	36·75	Acetic acid	40·6	66·7	81·5[1]	85·1	...	...
J74*B*	Do.	Refined	0·03	36·75	*Nil*	0·3	...	0·36[1]	0·42	...	...
J72*c*	Do.	do.	0·03	36·75	Acetic acid	22·0	58·1	78·0[1]	82·7	...	...
A80	Cotton seed	do.	0·06	34·5	*Nil*	1·1	2·0	3·0	3·0	...	...
A81	Do.	do.	0·06	34·5	do.	1·1	1·6	3·0	3·0	...	...
A79	Do.	do.	0·06	34·5	Acetic acid	36·5	57·2	93·0	98·6	...	...
A74	Do.	Crude	3·3	34·5	*Nil*	...	39·2	...	75·0	...	...
A73	Do.	Refined[2]	0·56	34·5	do.	...	34·4	...	70·0	...	...
	Poppy seed oil	Crude	0	...	Acetic acid	29·4	72·8	99·4	101·0	...	...
	Do.	do.	...	...	*Nil*	0·7	1·8	2·6	3·0	...	3·8

[1] Twenty-four hours only.

[2] Refined but kept for several months during which time the acid number had risen from 0·06 to 0·56.

[3] Mohua = *Bassia latifolia*, Roxb.

[4] Hongay = *Pongamia glabra*, Vent.

[5] Illipe = *Bassia longifolia*, Linn.

[6] Maroti = *Hydnocarpus Wightiana*, Blume.

[7] Throughout this paper acid and saponification values are given in terms of c.c. of 0.1 N alkali required for 1 gram of oil (cf. This Journal 1919, **2**, 245).

The acid values of most of the crude oils are much higher than those of the corresponding refined oils, which are not appreciably hydrolysed under similar conditions, and hence it is presumably the free fatty acids present which can act as the co-enzyme. The free insoluble fatty acids were isolated from a specimen of crude Illipe oil, and, after washing, were introduced into a sample of the refined oil, but this mixture was not appreciably hydrolysed in the absence of acetic acid. In a similar manner the acids, formed by hydrolysing the oil to 90 per cent., were isolated, washed with water and used as activator, but their accelerating action on the hydrolysis of refined Illipe oil was negligible. It is thus clear that the water-insoluble fatty acids derived from Illipe oil cannot function as activators.

Experiments were then tried to obtain any volatile fatty acids present in the crude oils and to use these in place of acetic acid.

In the case of coconut oil, illipe oil and mohua oil it was found possible to isolate volatile acids, and when aqueous solutions of such acids were used in place of acetic acid it was found that crushed castor-seeds produced appreciable hydrolysis.

The results of comparative experiments are given in Table II.

TABLE II.

Hydrolysis of refined oils by crushed castor-seeds in presence of fatty acids from crude oils.

No. of Experiment	Refined oil used	Acid value	Saponification value	Activator used	Amount of activator in grams	Percentage hydrolysis after hours			
						1	5	28	48
P19	Illipe	0·18	34·8	*Nil.*	...	0·6	0·6	0·95	...
P23	Do.	0·18	34·8	Fatty acids obtained by refining	6	0·7	1·7	3·1	...
P29	Do.	0·27	34·7	*Nil.*	...	0·3	0·2	0·5	...
P31	Do.	0·27	34·7	Steam distillate from crude oil	0·061 calculated as acetic acid	16·8	35·9	67·0	74·5
P32	Do.	0·27	34·7	do.	do.	20·4	37·6	64·0	77·7
P27	Do.	0·27	34·7	do.	0·03 calculated as acetic acid	9·7	25·4	46·5	52·9
P28	Do.	0·27	34·7	do.	do.	11·4	30·8	47·9	55·2
P6	Do.	0·11	34·8	Acetic Acid	0·058	20·6	51·3	75·4	79·0
R6	Hongay	2·9	34·4	*Nil.*	...	4·0	6·1	6·6[1]	6·5
R9	Do.	2·9	34·4	Steam distillate from crude oil	0·16 calculated as acetic acid	6·5	14·8	55·1[1]	60·1

[1] After twenty-four hours.

The oil (ground-nut) used in Experiments R3 and R4 was examined for free volatile acids. One litre of the oil was steam distilled and the steam condensed. The first 100 cc. of distillate neutralised 0·42 cc. of 0·1 N. sodium hydroxide. The acid content of the distillate is therefore 0·0025 gram calculated as acetic acid, a quantity of acid too small to act as activator for four grams of seed.

The crude Illipe oil (*Bassia longifolia*, Linn.) used in Experiments P15 and P16 was also examined as regards volatile acid content. 700 cc. of the oil were steam distilled and 100 cc. of distillate collected. 10 cc. of this distillate neutralised 1·4 cc. of 0·1 N. alkali, i.e. its acid concentration calculated as acetic acid was about half that of the acetic acid solution usually used as activator. When 40 cc. of the distillate were used with 100 grams of refined oil and 4 grams of crushed seed the percentage hydrolysis after forty-eight hours was 52·9–55·2 (Experiments P27 and P28) as compared with 0·5 per cent. for water alone (Experiment P29). A further quantity of two litres of crude oil was steam distilled and the first 100 cc. collected. This distillate was practically the same concentration as the acetic acid solution used as activator, and when 36 cc. of this distillate was used instead of acetic acid the percentage hydrolysis was 74 to 77 in forty-eight hours (Experiments P31 and P32), a result equal to those obtained by using 36 cc. of water containing 0·058 gram of acetic acid (Experiment P6).

Similarly with crude hongay oil (oil from seeds of *Pongamia glabra*, Vent.). One litre of the crude oil was distilled and the first 200 cc. of distillate collected in four fractions of 50 cc. each. These four fractions required respectively 2·78, 1·87, 1·51 and 1·27 cc. of 0·1 N. alkali. Another litre of the oil was steam distilled and 60 cc. of distillate collected, 10 cc. was used for titration and 50 cc. was used as activator in conjunction with 20 grams of refined oil and 0·8 gram of crushed seed. The acid content of the 50 cc. calculated as acetic acid was 0·16 gram and after forty-eight hours the percentage hydrolysis was 60·1.

SUMMARY.

1. Certain samples of crude oils have been found to hydrolyse distinctly when stirred with crushed castor-seeds and water without the addition of any activator. No such action takes place if the oil is previously refined with alkali or if the castor-seeds are not added.

2. The hydrolysis has been shown to be due to the volatile free fatty acids in the crude oil which form an activator. The free acids insoluble in water have no effect.

3. The volatile acids removed from illipe and hongay oils by steam distillation and used as activators give results comparable with those obtained by the use of equivalent quantities of acetic acid.

PART VI. EXPERIMENTS ON OIL-SPLITTING BY CASTOR-SEED LIPASE WITH MANGANOUS SULPHATE AS ACTIVATOR.

With Ittyerah Joseph.

In the account of the earlier experiments [1] attention has been drawn to the fact that, when manganous sulphate is used as activator instead of acetic acid, the percentage hydrolysis is very low during the first five hours, but rapidly increases and at the end of ten hours is equal to that obtained when acetic acid is used as activator.

Subsequent experiments have shown that a crude oil or a refined oil, which has been kept for some time, when hydrolysed with castor-seed lipase in the presence of manganous sulphate as activator undergoes appreciable hydrolysis during the first few hours in exactly the same manner as when acetic acid is employed. If, however, the oil is—

(*a*) subjected to steam distillation, or

(*b*) heated to 100° for half an hour,

then the percentage hydrolysis during the first few hours is very low. It has been found that this diminished hydrolysis during the first few hours can be prevented by any of the following devices :—

(1) Adding a little acetic acid to the manganous sulphate solution, e.g. 0·006 gram acetic acid per 0·2 gram of manganous sulphate.

(2) Adding some of the steam distillate obtained by subjecting the crude oil to steam distillation.

(3) Allowing the steamed oil to stand for some time until the acid value has increased, e.g. for some thirty days.

(4) Grinding the crushed seed, the manganous sulphate and part of the water and then allowing to stand for twelve to eighteen hours before the oil and remainder of the water are added.[2]

The results of a series of experiments are given in Table III. It is clear that in the absence of a small amount of free acid, the hydrolysis is negligible in the first few hours when manganous sulphate is used as activator.

[1] This Journal, 1919, 2, 254 and 261.
[2] Compare this Journal, 1919, 2, 261.

TABLE III.

Experiments on oil-splitting by castor-seed lipase with manganous sulphate as activator.

All experiments were made at room temperature, 24 to 28°, using 100 grams of crude cotton-seed oil, 4 grams of crushed castor-seeds and 40 cc. of water.

No. of Experiment	Treatment of oil	Acid value	Saponification value	Accelerator used	Percentage hydrolysis after hours			
					1	5	24	48
25	Neither steamed nor heated	0·06	34·67	Manganous sulphate 0·2 gram	36·3	64·3	86·5	93·3
2A	Do.	0·06	34·93	do.	23·6	63·6	87·6	100
2B	Do.	0·06	34·93	do.	24·6	63·0	88·1	98·0
28	Do.	0·06	34·93	Acetic acid 0·0574 gram	29·4	62·6	87·8	94·7
86	Steamed	0·02	34·4	Manganous sulphate 0·2 gram	0·9	1·5	89·8 [1]	...
87	Do.	0·02	34·93	do.	1·2	5·6	95·1 [2]	...
38A	Heated to 100° for 30 minutes	0·05	34·93	do.	2·6	48·3	84·4	90·0
38B	Do.	0·05	34·93	do.	2·5	49·6	87·5	92·7
20A	Steamed and kept for 23 days	0·06	34·93	do.	25·3	51·3	70·3	76·0
20B	Do.	0·06	34·93	do.	27·0	52·6	70·6	76·1
37A	Steamed	0·06	34·93	Acetic acid 0·057 gram	34·5	61·8	87·4	...
33B	Do.	0·06	34·93	Manganous sulphate 0·2 gram and acetic acid 0·006 gram	30·0	58·7	88·0	93·6
32B	Do.	0·06	34·93	Manganous sulphate 0·2 gram and steam distillate equivalent to 0·006 gram acetic acid	30·0	59·2	85·3	95·3
40	Steamed and kept for some time	0·05	34·95	Manganous sulphate 0·2 gram	21·0	56·9	81·4	86·3
36 [2]	Steamed	0·05	34·95	Manganous sulphate 0·2 gram kept over night with crushed seed and part of water	33·1	58·3	82·4	94·4

[1] Twenty-eight hours.
[2] Compare also this journal, 1919, 2, 254, Experiments, 92–99.

During the course of these experiments it was noticed that certain samples of oil, which had been alkali treated and then steamed to remove odour, gave low percentage hydrolysis values with crushed castor-seed and one of the usual accelerators, e.g. 62 to 66 per cent. in forty-eight hours, whereas the same oil before steaming gave 87 to 88 per cent. hydrolysis within twenty-four hours. It was ultimately found that the cause of the diminished yield was due to catalyst poisons derived from the rubber tubing which was used to join the glass tubes through which the steam was passed, and when all such rubber unions were eliminated the percentage hydrolysis of the steamed oil rose to 95 within forty-eight hours.

The results of a series of experiments are given in Table IV.

TABLE IV.

Effect of steaming on hydrolysis of cotton-seed oil.

Refined oil used. Acid value, 0·05. Saponification value, 34·95. In each experiment 100 grams of oil, 4 grams of crushed castor-seeds, and 40 cc. of water were used.

No. of Experiment	Oil used	Accelerator	Percentage hydrolysis after hours			
			1	5	24	48
28	Unsteamed	Acetic acid 0·057 gram	29·4	62·6	87·8	94·7
25	Do.	Manganous sulphate 0·2 gram	36·3	64·3	86·8	93·3
30	Steamed using rubber connections	do.	1·0	...	62·1	67·5
26A	Do.	do.	1·5	39·0	60·0	64·5
26B	Do.	do.	2·3	35·6	60·4	65·6
39	Do.	do.	13·0	35·4	54·8	61·5
31B	Steamed but no rubber connections	do.	2·4	38·6	77·5	86·5
40	Do.	do.	21·0	56·9	81·4	86·3
37A	Do.	Acetic acid 0·057 gram	34·5	61·8	87·4	...
37B	Do.	do.	33·9	61·4	86·5	92·3
32A	Do.	Manganous sulphate 0·2 gram and distillate from crude oil	32·9	58·5	82·5	95·3
32B	Do.	do.	30·0	59·2	85·3	94·7

Nos. 25, 26A and 26B were carried out at the same time.
Nos. 37B and 39 were carried out at the same time.
Nos. 28 and 30 were carried out at the same time.

SUMMARY.

1. When manganous sulphate is used as an accelerator in the hydrolysis of oils by castor-seed lipase the initial action is very slow if the oil has been freed from volatile fatty acids by alkali treatment or steaming or even if it has been heated to 100° for half an hour.

2. The initial rate of hydrolysis may be increased—

(*a*) by adding about 3 per cent. of acetic acid calculated on the weight of manganous sulphate to the solution of the latter;

(*b*) by adding some of the distillate obtained on steam distilling the crude oil;

(*c*) by allowing the steamed oil to stand until its acid value has perceptibly increased;

(*d*) by grinding the crushed seed with the solution of the manganous sulphate in part of the water and allowing to stand for twelve to eighteen hours before adding the oil and the rest of the water.

3. The activity of a lipase preparation may be greatly reduced by passing into the oil steam which has been in contact with rubber tubing.

PART VII. EXPERIMENTS ON LIPASE HYDROLYSIS WITH A MODIFICATION OF TANAKA'S FERMENT.

With Ittyerah Joseph, K. R. Rama Iyer, K. R. Narayana Iyer and S. Palaniyandy Pillai.

In Part II of this series[1] it has been stated that experiments with Tanaka's dry ferment gave low values for percentage hydrolysis.

Experiments have since been made with a new type of lipase preparation and good results as regards hydrolysis have been obtained, although so far it is doubtful if the glycerine obtained is more easily purified than that obtained by using Nicloux' ferment.

The following is the method adopted for the preparation of the ferment:—A given weight of decorticated castor-seeds is ground in a mortar with ten times its weight of dilute acetic acid (0·027 N.). The milky emulsion so obtained is strained through mull cloth in order to remove aleurone particles and the milky liquid which passes through the cloth is kept for about one hour in a beaker, the clear liquid is decanted and the remaining liquid filtered through paper on a Buchner funnel and finally washed with 1·5 parts of water and weighed.

[1] This Journal, 1919, 2, 250.

In the first preparation from 30 grams of crushed seed 25 grams of ferment and 10 grams of coarse residue were obtained. Thus 4 grams of castor-seeds correspond with 3·3 grams of ferment and 6 grams with 4·95 grams.

Two preliminary experiments on hydrolysing cotton-seed oil with this ferment without the addition of an activator were made and the results are given in Table V. In each experiment 100 cc. of the oil and 36 cc. of water were used. A further set of experiments was made in order to ascertain the number of hours stirring necessary to prevent a separation of the emulsion when stirring was stopped. The results are given in Table V.

TABLE V.

Experiments on splitting cotton-seed oil with the new ferment.

100 grams of oil, 36 cc. of water and no activator were used.
Temperature, 24–28°.

No. of Experiment	Ferment corresponding with grams of castor-seed	Percentage hydrolysis after hours						Stirring stopped after
		1	4	8	24	42	48	
N1	4	19·7	...	...	71·8	89·1	95·0	...
N2	6	26·4	...	...	87·8	92·2	99·5	...
P48	6	29·9	49·3	68·4	83·0	...	86·8	1 hour.
P49	6	31·6	53·6	69·5	86·0	...	93·6	48 hours.
P50	6	28·9	53·7	66·5	84·3	...	87·1	8 ,,
P51	6	24·1	50·5	66·2	86·7	...	86·1	28 ,,
P52	6	27·1	53·8	83·6	85·6	...	89·0	4 ,,

EXPERIMENT P76.—A larger scale experiment was tried using 4·5 kilos of oil. To prepare the ferment 225 grams of castor-seeds were ground in an iron edge runner mill and then mixed with 2250 cc. of 0·027 N. acetic acid. After filtering and washing with 660 cc. of water 335 grams of ferment were obtained. This ferment was added to the 4·5 kilos of oil and 1620 grams of water and the whole stirred in a glazed earthenware jar at a temperature of 24-28°. The following values were obtained:—

Hours	...	1	15	23	40	47	88
Percentage hydrolysis....		32·5	76·6	78·3	84·0	87·1	90·6.

It will be noted that the hydrolysis proceeds rapidly within the first fifteen hours, but afterwards the rate decreases.

The fatty acids and glycerine liquor were separated as described under Experiment No. 120 in Part II.[1] The weight of the fatty acid layer corresponded with 91·0 of the weight of the oil taken, and the volume of glycerine liquor was 1300 cc. When evaporated under reduced pressure 244 cc. of crude glycerine were obtained which gave the following numbers on analysis :—

TABLE VI.

Glycerine from Experiment P76.

		Crude	*Refined.*
Free acidity	=	0·055	0·049 per cent.
Ash	=	0·083	0·043 ,,
Residue at 160°.	=	2·25	1·98 ,,
Glycerol by acetin method	=	87·0	91·6 ,,

These values, especially the ash content, compare favourably with the crude glycerine obtained by the Nicloux' ferment in Experiment No. 120 (*loc. cit.*).

When diluted to 500 cc. purified by treatment with aluminium sulphate (7·5 cc. of a 5 per cent. solution) and then with barium hydroxide and finally concentrated under reduced pressure a yellow product was obtained which, on analysis, gave the numbers given in column 2 of Table VI.

The same ferment has been tried with punna seed oil (*Calophyllum Wightianum*, Wall.) and using a quantity of ferment corresponding with 4 grams of castor-seed for each 100 grams of oil the refined oil gave percentage numbers as follows :—

Hours	...	1	5	24	48
Percentage hydrolysis	...	24–35	42–67	77–92	89–97.

Another series of experiments was made with hongay oil (*Pongamia glabra*, Vent.) with the ferment prepared by the method described on p. 126, and also with the same ferment after it had been kept for twenty-four hours at different temperatures. The results are given in Table VII.

[1] *Ibid.*, 258.

TABLE VII.

Splitting of hongay oil by new ferment.

Oil used = 100 grams. Water = 40 cc. No activator. Acid value of oil = 2·9 cc. Saponification value = 34·4 cc. Ferment used corresponded with 5 grams of castor-seed.

No. of Experiment	Ferment	Temperature in degrees Centigrade	Percentage hydrolysis after hours				
			1	3	20	24	48
R1*a*	Fresh	22–28	14·7	...	68·9	70·6	89·0
R1*b*	Do.	do.	17·2	...	76·9	79·5	94·5
R2*a*	Do.	do.	7·7	11·6	25·1	27·5	39·1
R2*b*	Do.	do.	8·3	13·0	25·2	26·8	36·3
R3*a*	Do.	do.	15·6	24·5	47·4	51·3	61·6
R3*b*	Do.	do.	17·3	25·8	55·3	59·3	71·1
R4	Do.	29–31	18·3	32·5	56·6[1]	61·1	76·5
R5*a*	After 24 hours at 0°	22–28	9·5	13·1	19·3	21·0	26·8
R5*b*	Do.	do.	9·4	12·2	19·4	21·7	27·3
R6*a*	Do.	do.	18·2	24·2	42·5	45·2	53·9
R6*b*	Do.	do.	18·1	25·4	45·1	47·3	53·0
R7*a*	Do.	29–31	18·4	24·4	31·8[1]	35·7	36·3
R7*b*	Do.	do.	19·8	26·3	33·2[1]	35·6	37·7
R8*a*	After 24 hours at 22-28°	22–28	7·3	10·4	15·7	15·8	22·5
R8*b*	Do.	do.	6·9	9·4	16·4	17·9	22·6
R9*a*	Do.	do.	14·6	21·6	39·7	41·0	56·0
R9*b*	Do.	do.	16·7	22·5	41·8	43·2	50·8
R10*a*	After 24 hours at 30°	29–31	10·8	14·8	14·8[1]	15·0	16·8
R10*b*	Do.	do.	7·4	13·3	13·3[1]	13·6	17·8

In Experiments R2*a* and 2*b* the stirring had stopped between the third and twentieth hours and the emulsion had broken.

In Table VIII are given the results obtained with ground-nut oil and in Table IX results obtained with Illipe oil.

[1] After nineteen hours.

TABLE VIII.

Splitting of ground-nut oil by new ferment.

100 grams of oil, 36 cc. of water and no accelerator. Acid value of oil = 3·48 cc. Saponification value = 34·27 cc. Temperature, 24–28°.

No. of Experiment	Ferment equal to gram of seed	Percentage hydrolysis after hours						Remarks
		1	4	9	24	28	48	
P53	5	25·8	47 8	63·7	80·1	84·8	92·8	In Nos. 54-56, 1·5 grams of fresh ferment equal to 3 grams of seed was added after the ninth hour. In Nos. 59-62 and 64 fresh ferment equivalent to 2·5 grams of seed were introduced after the eighth hour.
P54	5 + 3	23·0	49·1	65·2	90·0	94·5	101·0	
P55	2·5 + 3	11·8	29·3	43·0	70·9	76·8	86·5	
P56	2·5 + 3	15·0	32·1	45·2	74·0	79·0	88·0	
P57	5	21·1	43·4	55·6	71·5	73·6	85·3	
P58	5	20·1	43·4	57·5	77·3	78·5	79·6	
P59	5	23·1	45·1	57·6	75·6	80·8	93·7	
P60	5 + 2·5	23·1	49·1	57·2	80·4	81·3	91·4	
P61	2 + 2·5	15·3	29·2	36·4	54·1	58·1	69·0	
P62	2 + 2·5	14·2	26·7	31·1	53·1	59·6	68·2	
P63	5	29·5	51·4	63·0	85·8	89·7	99·0	
P64	5 + 2·5	28·6	52·9	70·7	92·5	97·3	100·0	
P65	2·5	16·2	34·0	45·0	59·2	64·6	74·4	
P66	2·5	19·9	34·7	45·4	60·8	64·1	71·3	

TABLE IX.

Splitting of Illipe oil (*Bassia longifolia*, Linn.) *by the new ferment.*

100 grams of oil, 36 cc. of water and no accelerator. Acid value of oil = 2·42 cc. Saponification value = 34·25 cc.

No. of Experiment	Ferment equal to gram of seed	Percentage hydrolysis after hours						Remarks
		1	4	9	24	28	48	
P67	5	24·6	33·4	...	75·4	84·6	96·3	In 69–74 fresh ferment equivalent to 2·5 grams of seed was added at the end of 8·5 hours.
P68	5	25·6	33·7	...	75·3	80·5	92·0	
P69	5 +2·5	22·9	28·0	43·9[1]	87·5	...	99·0	
P70	5 +2·5	21·8	27·1	33·0[1]	83·1	...	97·0	
P71	2·5+2·5	15·8	17·3	22·8[1]	70·0	...	84·5	
P72	2·5+2·5	14·6	16·4	18·3[1]	67·1	...	85·6	
P73	2·5	14·8	16·5	20·9	24·8	...	29·2	
P74	2·5	13·7	16·7	18·9	23·4	...	30·5	

[1] After 8·5 hours.

EXPERIMENT P77.—Similar to Experiment P76 (p. 127) but using four kilos of ground-nut oil of acid value = 3·48 cc. of 0·1 N. alkali.

The percentage hydrolysis values were—

Hours ...	4	20	48
Percentage hydrolysis...	39·7	63·2	85·6

After sixty-eight hours the emulsion was broken, the fatty acids separated and the dilute crude glycerine liquor treated with aluminium sulphate and barium hydroxide, heated to boiling, filtered and concentrated under reduced pressure, again treated with aluminium sulphate and barium hydroxide and after filtering further concentrated under reduced pressure. The glycerine liquor so obtained when diluted and warmed with a little aluminium sulphate solution gave no precipitate.

The weight of the fatty acid layer was 3716 grams = 93 per cent. of the weight of oil taken.

The yield of concentrated glycerine liquor was 285 grams and this gave the following numbers :—

Glycerine content by the acetin method	82·7 per cent.
Total ash	0·06 ,, ,,
Total solids as 160° ...	1·47 ,, ,,
Acidity	0·05

These values are rather better than those given by refined glycerine from the experiments using Nicloux' ferment.

Some further results obtained with this ferment are shortly to be published in a paper on mohua oil.

GENERAL CONCLUSIONS.

1. An active ferment preparation can be obtained by grinding castor-seeds with ten times their weight of dilute acetic acid (1·6 grams per litre), pressing through cloth to remove coarse particles, filtering with the aid of the suction pump and washing with water.

2. This ferment requires no activator.

3. Different preparations vary as regards their activity and it is not always easy to obtain preparations with the same degree of activity.

4. With oils such as cotton-seed and illipe it is not necessary to stir during the whole course of the hydrolysis.

5. As with other preparations the rate of hydrolysis increases with the amount of ferment used, and ferment corresponding with five or six grams of seed per 100 grams of oil usually gives good results.

6. Addition of half the ferment at the beginning of the experiment and the other half after eight or ten hours does not give such good results.

7. Addition of ferment equal to five grams of seed at the beginning and a further addition of fresh ferment equivalent to 2·5 grams of seed after eight or ten hours increases the rate of hydrolysis to a slight extent.

8. The glycerine liquors on the whole appear to be slightly better than those obtained where Nicloux' ferment is used.

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