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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