

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<sup>1</sup> 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.<sup>2</sup>

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.

<sup>1</sup> This Journal, 1919, 2, 254 and 261.

<sup>2</sup> 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 <sup>1</sup>	...
87	Do.	0.02	34.93	do.	1.2	5.6	95.1 <sup>2</sup>	...
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 <sup>2</sup>	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

<sup>1</sup> Twenty-eight hours.

<sup>2</sup> 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  $100^{\circ}$  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.

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

*With Ittyerah Joseph, K. R. Rama Iyer, K. R. Narayana Iyer  
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In Part II of this series<sup>1</sup> 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.

<sup>1</sup> This Journal, 1919, 2, 250.