

OIL SPLITTING BY CASTOR-SEED LIPASE.

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

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In Part I¹ 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 II² 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	20	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
18a	Mahua ³	do.	2.6	33.8	Nil	5.4	6.7	7.5	9.0
18b	Do.	do.	2.6	33.8	do.	5.8	7.1	7.4	8.3
19a	Do.	Refined	0.16	34.9	do.	0.5	0.7	1.0
110a	Do.	do.	0.16	34.9	do.	0.5	0.7	1.1
110c	Do.	do.	0.16	34.9	Acetic acid	17.5	31.9	52.4	68.1
15b	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
J74A	Maroti ⁶	Crude	5.6	36.75	Nil	21.9	...	53.4 ¹	57.0
J48A	Do.	do.	5.1	36.75	do.	18.0	24.3	33.3 ¹	37.6
J48B	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
J74B	Do.	Refined	0.03	36.75	Nil	0.3	...	0.36 ¹	0.42
J72c	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 Mahua = *Bassia latifolia*, Roxb.

⁵ Illipe = *Bassia longifolia*, Linn.

⁴ Hongay = *Pongamia glabra*, Vent.

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.