THE EFFECT OF REFINING ON THE DIGESTIBILITY OF EDIBLE OILS AND FATS.

By F. G. T. Menezes and B. N. Banerjee.

The manner in which oils and fats are digested and absorbed is a subject of acute controversy. Fats and oils are suggested to be hydrolysed (Pfluger, *Pflugers Arch.*, 1900, **80**, 111; **82**, 303) by lipase of the pancreas and the intestines and then absorbed in the form of soap. While in another way, they are absorbed (Munk, *Lehrbuch d Physiol*, 1897, 199) in the form of fine emulsion in which the bile salts play an important part. The subject has been reviewed by Bloor (*Chem. Rev.*, 1925, **2**, 243), Leathes and Raper (The fats, 1925), Verzar (*Nutrition Abs. & Rev.*, 1932–33, **2**, 441, *J. Physiol.*, 1935, **84**, 41P), Verzar *et al.*, (*Pflugers Arch.*, 1936, **237**, 14), and Anderson *et al.*, (*Physiol. Rev.*, 1937, **17**, 335) where the facts and gaps in our knowledge regarding the digestion and absorption of fats have been dealt with. Studies on different animal species including human being with a variety of food fats have presented ample proof that food fat is readily digestible (Langworthy, *Ind. Eng. Chem.*, 1923, **15**, 276; Holmes, *U.S. Dept. Agric. Bull.*, 630, 687, 613, 781; Levine and Smith, *J. Biol. Chem.*, 1927, **72**, 223; Krakower, *Amer. J. Physiol.*, 1934, **107**, 49). The action of lipase and bile salts has been studied by a number of workers (Verzar, Bloor, *loc. cit.*). Recent studies indicate that fat absorption depends on active processes in the intestinal mucosa, wherein lipoids and hormones also take part. It is influenced by the composition of the diet, or by the balance of the constituents that affect the processes of digestion and absorption. Lyman (*J. Biol, Chem.* 1917, **32**, 7) fed dogs with glyceryl stearate and palmitate and observed great difference in absorption. He doubts whether absorption is dependent on the melting point of the fat as suggested

by Langworthy. McCay and Paul (*J. Nutrition*, 1938, **15**, 377) fed guinea-pigs with fats at a 6% level and found that fats and oils of low melting points, were utilised better than those of high melting points, and suggest that this might be due to inability to absorb to any great extent the higher melting fatty acids. Barbour (*J. Biol. Chem.*, 1934, **106**, 281) fed rats with peanut oil at a level of 20% in a diet otherwise normal, and did not find accumulation of fat in the liver, but the arachidic acid of the oil was almost quantitatively excreted. Irwin, Steenbock, et al., (*J. Nutrition*, 1936, **12**, 85 & 103) have determined the rate of absorption of different fats in rats. The technique adopted by them for measuring absorption, involved the feeding of a definite quantity of fat, and subsequent analysis of the intestinal residues. In a four hour test, there was appreciable difference in the rate of absorption of olive oil and oleo stock. Holt et al., (*J. Pediatrics*, 1935, **6**, 427) have reported on the factors that influence the absorption of fats in normal infants. The size of the particle of fat had no influence on fat absorption. Volatile fatty acids and glycerides were well absorbed. Short chain fatty acid glycerides were more completely absorbed than longer chain fatty acid glycerides. They suggest that chemical composition rather than melting point is an important factor in absorption. Tidwell *et al.*, (*J. Pediatrics* 1935, **6**, 481) found that premature infants and twins had marked difficulty in fat absorption. In such infants olive oil and soya bean oil were better absorbed than butter fat.

It will be seen from the above researches that in normal healthy subjects edible oils and fats or proprietary mixtures show relatively small difference in digestion and absorption. There are circumstances, however, where such study is very helpful. Again, the physiological effect of a retarded fat digestion and absorption is not known. In the refining or the hydrogenation processes of oils and fats, it is necessary to follow up the effect of the various treatments on digestion and absorption. Sudborough *et al.*, (*J. Indian Inst. Sci.*, 1918 & 1922, **2**, 213; **5**, 119) observed that certain crude oils after refining treatment with alkali did not undergo appreciable hydrolysis when acted upon by castor seed lipase, in the absence of an activator. Addition of volatile

fatty acids (which had been removed during neutralisation and washing) as activator was found to restore the digestibility of these oils. It was therefore assumed that the volatile free acids present in the crude oil act as a natural activator on castor seed lipase. During the processes of refining and particularly deodorisation, the oil is subjected to several changes of temperature for varying periods of time. In the hydrogenation process, the addition of hydrogen molecule takes place and the fat is exposed to high temperatures. In the present paper, we have studied the effect of refining, deodorisation and hydrogenation processes on some common Indian edible oils.

Coconut, gingili, groundnut, and cotton-seed oils have been used in these studies. Two samples of groundnut oil, hydrogenated to one of ghee consistency and another of harder quality melting at about 43°C., have also been examined.

Among the different methods followed for the study of digestion of edible fats, digestion with pancreatic lipase suited best for these trials. Pancreatic lipase has been extensively studied, and its mode of action greatly resembles body conditions of temperature, pH etc. In this laboratory, Giri and Dastur (*Ind. J. Med. Res.*, 1937, **25**, 427) have studied some of the edible oils and fats and offer very good data for relative study and comparison.

Preparation of the pancreatic lipase. — Fresh swine pancreas was separated from adhering fatty material and then minced well in a mincing machine. It was subsequently treated with successive quantities of acetone, acetone- ether (1 : 1) mixture and ether, according to the procedure of Willstätter and Waldschmidt- Leitz (*Zeitschr f. Physiol Chem.*, 1923, **125**, 132). The defatted material was ground to a fine powder and sieved through a 60 mesh sieve. The powder was shaken well for 4 hours with 10 parts of 87% glycerol, the mixture centrifuged, and the supernatant liquid preserved in the cold. This extract was diluted ten times, as and when required for use.

Method of hydrolysis. — According to Willstätter, Waldschmidt-Leitz, and Memmen (*Zeitschr f. Physiol. Chem.*, 1923, **125**, 111) 2.5

gms. of oil were weighed into a conical ground-glass stoppered flask of 30 c.c. capacity and 2 c.c. of n NH_3-NH_4Cl buffer (0.66 c.c. n NH_3+1.34 c.c. n NH_4Cl) of pH 8.9 was added. When the hydrolysis was carried out in presence of external activator, 0.5 c.c. of 2% $CaCl_2$ solution and 0.5 c. c. of 3% albumin solution were added. When the hydrolysis was done without any external activator, the last two were substituted by 1 c. c. of water. The contents of the flasks were shaken to mix intimately and maintained at 30°C. in a thermostatic bath for about 20 minutes. 10 c.c. of freshly diluted enzyme solution (also maintained at 30°C.) were then added, the flask shaken well for 30 seconds and a 2 c.c. aliquot transferred into a 50 c.c. Erlenmeyer flask containing 20 c.c. of alcohol-ether (5 : 1) mixture. The contents of the flask were titrated against standard 0.1 n alcoholic potash, using phenolphthalein as indicator. The reaction flask was maintained at 30°C. in the thermostat, for 2 to 8 hours, and 2 c.c. aliquots removed at regular intervals and titrated. The titre of the first aliquot gives the blank and was subtracted from the subsequent readings.

Effect of refining and deodorizing (heating) on the digestibility of coconut oil. — Coconut oil was pressed in the cold and filtered free from any suspended matter. This " crude oil " was thereupon refined as follows. It was first freed from albuminoid impurities by washing repeatedly with 5% brine at 60°C. The oil was washed free from salt with hot water (at 60°C.), and dried by shaking with anhydrous Na_2SO_4. The acid value of the oil was then determined, and on the basis of this value, the free fatty acids were neutralized according to the technique described by Morrow (Biochemical Laboratory Methods, 1935). The oil was washed and dried as before, and clarified by shaking with a 1 : 1 mixture of kiesselguhr and norit. This "refined oil" was then heated in vacuo at 170°C. for 4 hours with a view to imitate, as far as possible, the heating effect of deodorization. In practice however, superheated steam is used to drive out the odour. The resulting sample had a peculiar burnt odour, much like that of deodorized cocoanut oil in the market. This sample will be referred to as " heated oil " in what follows.

Each of the three samples of coconut oil prepared above viz., crude oil, refined oil, and heated oil, was hydrolysed with pancreatic lipase both with and without activator according to the method described above. The results are shown in Table I.

TABLE I.

Coconut Oil.

Time	Crude		Refined		Heated	
	Unactivated	Activated	Unactivated	Activated	Unactivated	Activated
10 min.	0.600	0.695	0.505	0.690	0.165	0.660
20 „	0.975	1.145	0.950	1.125	0.365	1.065
30 „	1.275	1.545	1.250	1.505	0.480	1.475
45 „	1.635	2.000	1.615	1.950	0.660	1.825
60 „	1.910	2.350	1.875	2.325	0.885	2.175
120 „	2.630	3.290	2.515	3.125	1.340	3.085

It will be noticed from the figures that refining does not cause any appreciable alteration in the digestibility of coconut oil either with or without external activator. Represented graphically, the curves for both crude and refined oils, in either case are nearly identical. (Fig. 1). But the heated oil when hydrolysed in the absence of activator, undergoes very poor digestion; when however, to this heated oil, activator is added, then the digestibility is restored to the original rate, the corresponding curve resembling that of crude or refined oil under similar conditions. This clearly demonstrates that on heating, some substance, which acts as an activator to the pancreatic lipase, is rendered inactive.

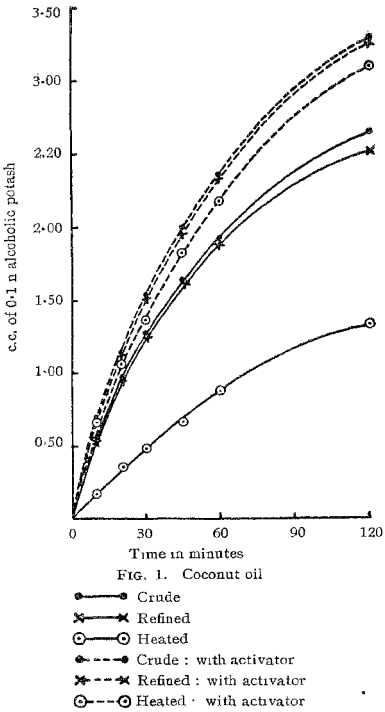


FIG. 1. Coconut oil

Rate of fall of digestibility of coconut oil on heating.—Having found that on heating, coconut oil suffers a remarkable loss in its rate of digestion (in absence of activator), it was thought desirable to study the course of change, i.e., of inactivation of the natural activator.

Samples of "crude oil" were heated in vacuo at temperatures of 140° and 160°C. Aliquots were taken at intervals of every one hour. The heating was carried on for 6 hours. Test samples were hydrolysed in the the absence of activator. The results are shown in Tables II and III, and graphically in Fig. 2, 3 and 3a.

TABLE II.

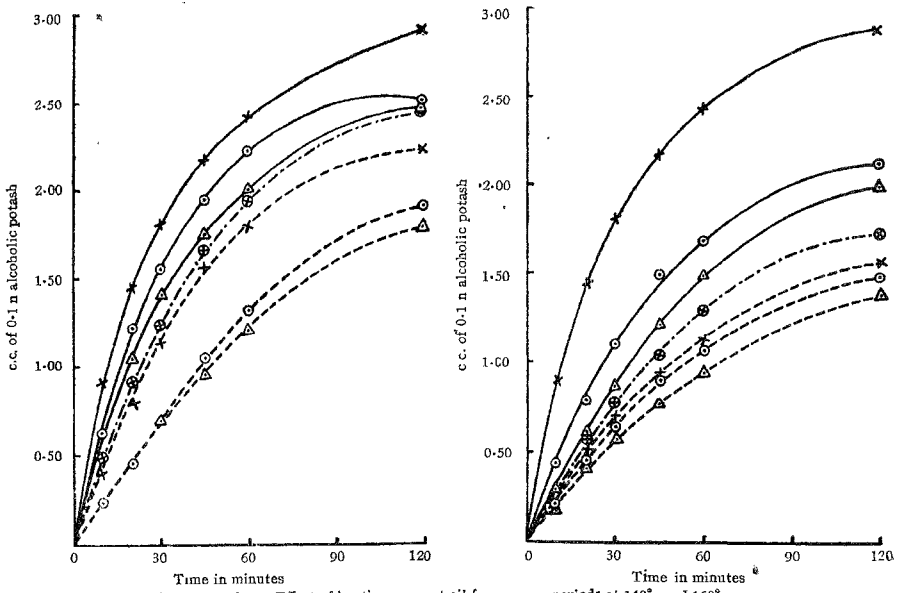
Coconut oil heated at 140° C. *for* 1, 2, 3...6 *hours.*

Time	Crude (unheated)	Heated for					
		1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	6 hrs.
10 min.	0.900	0.620	0.490	0.390	0.380	0.235	0.240
20 ,,	1.445	1.210	1.035	0.905	0.780	0.450	0.455
30 ,,	1.800	1.550	1.415	1.235	1.130	0.685	0.695
45 ,,	2.165	1.935	1.705	1.650	1.565	1.050	0.970
60 ,,	2.430	2.210	1.995	1.925	1.790	1.305	1.200
120 ,,	2.895	2.485	2.450	2.425	2.200	1.885	1.775

TABLE III.

Coconut oil heated at 160°C. *for* 1, 2, 3...6 *hours.*

Time	Crude (unheated)	Heated for					
		1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	6 hrs.
10 min.	0.900	0.445	0.285	0.285	0.250	0.220	0.190
20 ,,	1.445	0.790	0.615	0.580	0.510	0.465	0.420
30 ,,	1.800	1.110	0.860	0.775	0.700	0.650	0.595
45 ,,	2.165	1.500	1.210	1.030	0.925	0.890	0.775
60 ,,	2.430	1.690	1.485	1.235	1.125	1.060	0.955
120 ,,	2.895	2.120	1.980	1.720	1.550	1.480	1.375



FIGS 2 and 3: Effect of heating coconut oil for varying periods at 140°c and 160°c

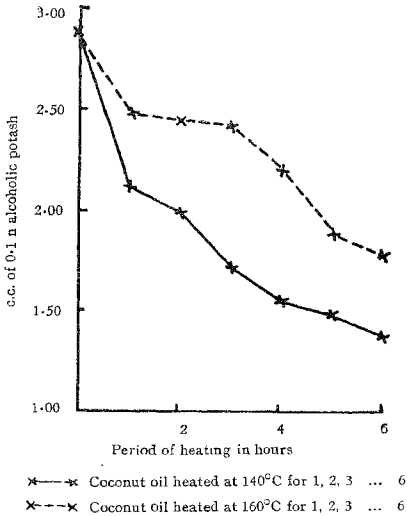


FIG. 3a. Relation between digestibility and time of heating (Coconut oil)
(The values are of 2 hours digestion of samples heated at 140°C and 160°C for varying periods of time)

The figures indicate a gradual fall in the digestibility with rise in temperature and also with the duration of heating.

Similarly, the rates of hydrolysis of cotton-seed oil, gingili oil, and groundnut oil, when crude, refined and deodorized were determined. For deodorization, these oils were steamed for four hours at 100°C. instead of being heated in vacuo. Two samples of hydrogenated groundnut oil of ghee consistency and of hard consistency melting at 43°C. were also examined. The digestions with the hydrogenated samples, were done at 37°C., as good emulsification could be secured only at the higher temperature. However, the harder sample could not be hydrolysed, unactivated, even at the higher temperature, as the oil solidified quickly. The results are given in Tables IV, V, VI and VII and graphically represented in Figs. 4 to 7.

TABLE IV.

Gingili Oil.

Time	Crude		Refined		Deodorized	
	Unacti-vated	Acti-vated	Unacti-vated	Acti-vated	Unacti-vated	Acti-vated
30 min.	0.090	0.400	0.200	0.795	0.305	1.375
1 hr.	0.175	0.625	0.320	1.015	0.360	1.580
2 hrs.	0.315	0.875	0.505	1.240	0.465	1.800
4 ,,	0.535	1.150	0.710	1.425	0.590	2.065
6 ,,	0.710	1.320	0.845	1.490	0.680	2.140
8 ,,	0.770	1.385	0.880	1.495	0.770	2.170

TABLE V.

Groundnut Oil.

Time	Crude		Refined		Deodorized	
	Unacti-vated	Acti-vated	Unacti-vated	Acti-vated	Unacti-vated	Acti-vated
30 min	0.195	0.750	0.135	0.965	0.060	0.180
1 hr.	0.285	0.990	0.170	1.280	0.125	0.920
2 hrs.	0.400	1.300	0.220	1.635	0.175	1.190
4 ,,	0.515	1.675	0.265	1.990	0.235	1.535
6 ,,	0.580	1.910	0.285	2.160	0.265	1.720
8 ,,	0.615	2.075	0.285	2.250	0.265	1.815

TABLE VI.

Groundnut Oil—Hydrogenated.

Hydrolysis was carried out at 37°C.

Time	m.p. 33°C (Unrefined)		m.p. 33°C. (Refined)		m.p. 42°C.	
	Unactivated	Activated	Unactivated	Activated	Unactivated	Activated
30 min.	0.090	0.160	0.045	0.245	...	0.365
1 hr.	0.125	0.195	0.080	0.325	...	0.380
2 hrs.	0.150	0.250	0.130	0.435	...	0.455
4 ,,	0.175	0.305	0.165	0.555	...	0.525
6 ,,	0.175	0.345	0.165	0.615	...	0.545
8 ,,	0.175	0.345	0.170	0.625	...	0.545

TABLE VII.

Cotton-seed Oil.

Time	Crude		Refined		Deodorized	
	Unactivated	Activated	Unactivated	Activated	Unactivated	Activated
30 min.	0.080	0.745	0.060	1.070	0.200	1.010
1 hr.	0.150	0.955	0.080	1.370	0.250	1.280
2 hrs.	0.260	1.185	0.120	1.595	0.325	1.550
4 ,,	0.375	1.440	0.185	1.845	0.415	1.795
6 ,,	0.425	1.545	0.200	1.945	0.470	1.900
8 ,,	0.435	1.550	0.210	1.975	0.470	1.930

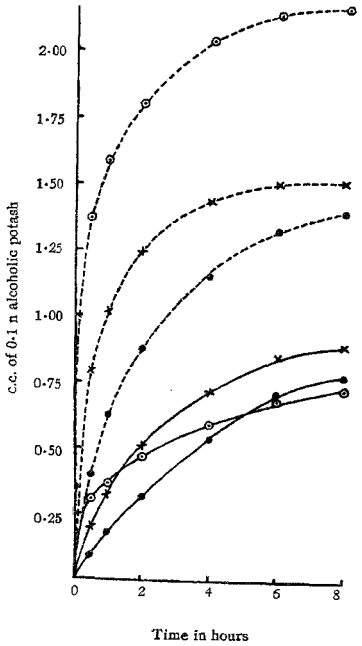


FIG. 4. Gingili Oil

Crude
Refined
Deodorized.

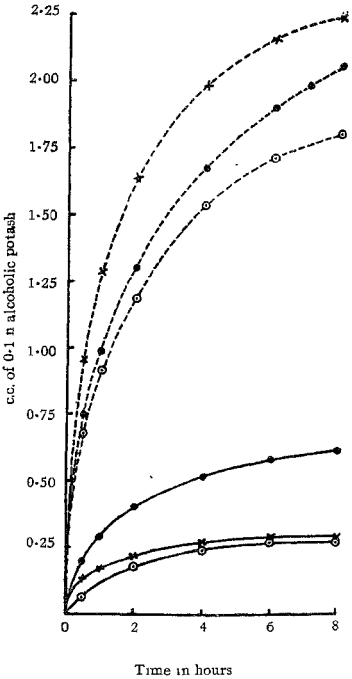


FIG. 5. Groundnut oil

Crude : with activator
Refined : with activator
Deodor. : with activator

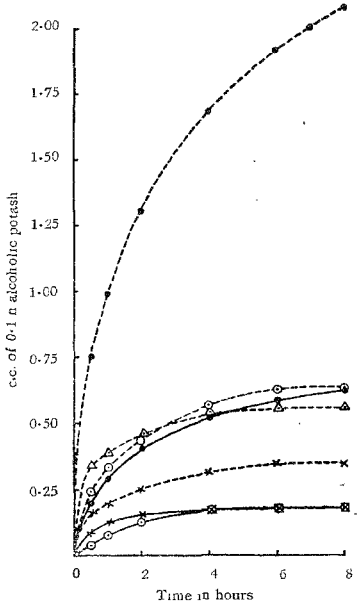


FIG. 6. Hydrogenated groundnut oil

Crude groundnut oil
Hydrogenated (m.p. 33°) unrefined
Hydrogenated (m.p 33°) refined
Crude groundnut oil : with activator
Hydrogenated (m.p. 33°) unrefined : with activator
Hydrogenated (m.p. 33°) refined : with activator
Hydrogenated (m p. 43°) with activator

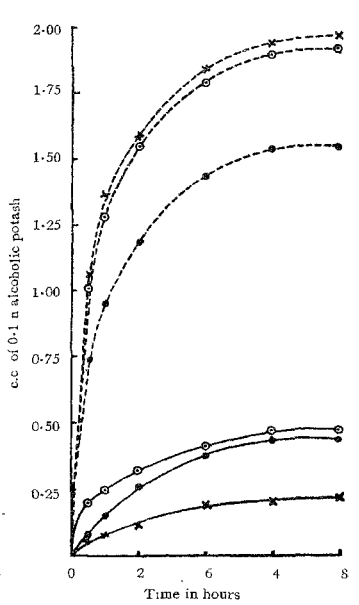


FIG 7. Cotton-seed oil

Crude
Refined
Deodorized.
Crude : with activator
Refined : with activator
Deodor. : with activator

DISCUSSION AND SUMMARY.

The rates of digestion of different oils are different after refining treatments. While clarification, neutralization and decolorisation do not affect the digestibility of coconut oil, both groundnut oil and cotton-seed oil are appreciably affected. The digestibility of gingili oil improves on

refining. The gingili oil in question had a rather high acid value, and the lower values for the crude oil were thought to be due to rancidity as judged by its high acid value. A second sample of oil from fresh healthy seeds also gave similar results thus disproving any effect of high acid value. Deodorization, while appreciably lowering the rate of digestion of coconut oil as also groundnut oil, and to some extent gingili oil, actually improved the digestibility of cotton-seed oil. In the case of gingili oil, the deodorized oil, when activated, gave the best results. Thus the processes of refining, deodorization, and hydrogenation retard the digestibility of oils to a more or less extent. It is generally the natural activator that is lost in addition to other changes. The volatile acid, saturated acid and unsaturated acid components of fat or oil, as affected by these processes require to be examined separately. While this lower digestibility will not matter much in the case of a healthy person whose system is strong and normal, and whose bile and other secretions are regular and sufficient, it will not be the same in the case of a person who is in a weak or delicate state of health. If the natural activators are lost or the process of digestion delayed, then it is a strain on the system, the physiological significance of which is not known.

There is another aspect of the question where the condition of the oil or fat requires study. In a hot country like India, oils and fats are liable to go rancid unless consumed soon after preparation. Again, in the processes of frying, roasting and baking, oils and fats are exposed to high temperatures. They are exposed to air and liable to oxidation. Peroxide formation takes place and this is a serious drawback. Oils and fats are the solvents and carriers of vitamins and carotenes. It is known that rancid fats destroy vitamin A (Lease *et al., J. Nutrition*, 1938, **16**, 571). Vitamin A, carotene or their concentrates preserved in different oils and fats, when fed to animals show different degrees of absorption (Lease *et al., J. Nutrition*, 1939, **17**, 91). This study is of great importance, as margarines and vegetable products are fortified with vitamins to a certain extent. Again, medicinal cod liver oil, halibut liver oil and concentrates

have been found not to keep well their vitamin potency in India (Iyengar and Mukherjee, *Ind. Med. Gaz.*, 1939, **74**, 216). Therefore the behaviour of Indian edible oils and fats, when crude, refined, deodorized and hydrogenated with respect to their digestibility and as also as carrier or solvent of vitamin and carotene requires exhaustive study. Steam distillation does not remove the pro-oxidant factor, nor does the addition of antioxidants help in all cases (Lease *et al., loc. cit.*). Generally, the retardation of digestion and instability or loss of Vitamin A in oils or fats on refining treatment run parallel. However, individual oils or fats behave differently and require thorough elucidation.

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