THE ENZYME SPLITTING OF FATS

By Albert P. Sachs

The splitting of fats into glycerin and fatty acids is known in practical work as "saponification" which should more strictly be applied to the conversion of fats or fatty acids into soaps, i.e. the metallic salts of the fatty acids. The better term for the splitting of fats is "hydrolysis" and this is consistently used in scientific work. The chemical change which the fats (triglycerides) undergo on conversion into their hydrolysis products may be represented as follows:

$$\begin{matrix} \mathrm{OR} \\ \mathrm{C_3H_5\ OR'} \\ \mathrm{OR''} \end{matrix} + 3\mathrm{MOH} = \mathrm{C_3H_5(OH)_3} + \begin{cases} \mathrm{ROM} \\ \mathrm{R'OM} \\ \mathrm{R''OM} \end{cases}$$

C_3H_5 (OR) (OR′) (OR″) represents a triglyceride in which the acidyl groups R, R′, R″ may be the same or different; MOH represents either water (HOH) or any metal hydroxid (NaOH or CaOH, where Ca = ½Ca). The products on the right hand are the glycerin formed and the free fatty acid or soap (or mixtures of free fatty acids or of soaps) formed.

The above equation is not necessarily that which actually occurs, but represents merely the end result; in the case of hydrolysis by steam under pressure it may and probably does represent the end result of several successive reactions, namely:

C_3H_5 (OR) (OR′) (OR″)+HOH=C_3H_5 (OR) (OR′) (OH)+R″OH

C_3H_5 (OR) (OR′) (OH)+HOH=C_3H_5 (OR) $(OH)_2$+R′OH

C_3H_5 (OR) $(OH)_2$+HOH=C_3H_5 $(OH)_3$+ROH

In discussing the enzyme hydrolysis of fats we shall touch on this reaction again.

Hydrolysis is usually carried out by one of the following methods, which are by no means of equal technological value and importance. Thus for instance, hydrolysis by ammonia under pressure, is theoretically very intriguing because the ammonia soap which is formed can be decomposed into free fatty acid and ammonia by blowing steam through the soap; all the ammonia is recoverable. On paper we have thus an effective means of hydrolyzing fats into free fatty acids and glycerin without using up any chemicals at all, but the process is apparently not commercially feasible.

The methods are:

1. By steam alone at elevated temperatures; fifteen atmospheres pressure and 200° C. are necessary, and the product is darkened.

2. By means of steam and lime in open kettles (i.e. not under pressure nor at elevated temperatures). About 9.7 per cent actual lime, CaO, is theoretically necessary, but in practise 12-14 per cent of lime must be used to obtain complete saponification.

3. By means of steam at moderately elevated temperatures and a

catalyst or accelerator. Examples are steam at eight atmospheres with 3 per cent of lime or at twelve atmospheres with 1 per cent of lime. Saponification, i.e. the formation of calcium soaps is complete to the extent of the lime present, but free fatty acids are also present due to the insufficient lime present to combine with all the fatty acids. Magnesia or zinc oxide with or without zinc dust are also used, as is lime with zinc oxide or zinc dust, which seems to diminish discoloration.

4. By means of excess alkali in open heated vessels. A variant of this is the use in the laboratory of alcoholic caustic potash or caustic soda for saponifying small samples of fat completely.

5. By ammonia under pressure with subsequent removal of ammonia by blowing out with steam.

6. By acids. The use of muriatic acid (HCl) is not commercial, but that of 4-6 per cent of 66-67°Bé sulfuric acid (H_2SO_4) is commercial.

7. By Twitchell's reagent and similar hydrocarbon-oleic sulfonic acid reagents, etc.

8. By enzymes (also called "ferments").

The water hydrolysis, open kettle lime process and the ammonia processes are not used commercially; all the others find some use in commercial plants, though by no means to the same extent. The enzyme splitting of fats is one of the less important commercial methods but is nevertheless extremely interesting and is subject to possible improvements which may greatly increase its use.

Much work had previously been done on a scientific basis in studying the lipolytic (fat-splitting) action of certain ferments or enzymes, but without any attempt or indeed possibility of applying the results to technical purposes when Connstein, Hoyer and Wartenberg (Ber. d. deut. chem. Ges. 1902, vol. 35, pp. 3988-4006) reported their work. They stated that by using the enzyme present in castor beans (merely by grinding up the beans) fats could be hydrolyzed so effectively that the fatty acids yielded a pure white stearin directly by chilling and pressing while the olein was lighter than the commercial olein available; the glycerin was obtained directly in 40-50 per cent concentration with a small quantity of salts and some albuminous substances and peptones formed from the albuminoid substances by the peptonizing ferment present. The organic impurities could be removed by bone-black. The actual commercial results have proved by experience to be neither so favorable nor so simple as the first laboratory results seemed to indicate.

Let us for a moment consider the problem of the nature of an enzyme. Enzymes are catalysts produced by living matter (i.e. the lipase or fat-splitting enzyme of the castor bean). No enzyme has been prepared in a state of purity. Practically all enzymes are colloids or are intimately associated with substances having colloidal properties. Enzymes are generally

associated with protein matter either as an essential part of the protein molecule or accompanying it. (See K. G. Falk "The Chemistry of Enzyme Actions," Chapter VII.) We know enzymes by their effects and not by their constitution.

Castor beans contain a lipase. At first ground castor beans (including husks) were used but technical difficulties arose, especially the low grade glycerin produced. Decorticating the seeds helped somewhat as the husks do not contain any enzyme but the trouble was not removed.

Nicloux (French patent 335902 of Nov. 14, 1903) introduced an improvement by triturating decorticated castor beans with castor oil or cotton seed oil; the product was filtered and the turbid oily filtrate centrifuged, yielding three layers. There is the excess oil on top, the practically inactive aleurone (starch) grains and membranes at the bottom, while the center active layer contains the cytoplasm (active cellular material) emulsified in oil. Nicloux used magnesium and calcium salts as activators of his ferment preparation (Nicloux's monograph, "Contribution á l'etude de la saponification des corps gras," Paris 1906, contains all the work done up to that time) but since then manganous sulfate has been found to be a more effective activator.

Nicloux's ferment-oil has since then been replaced by a "ferment-milk" due to Hoyer and first described by him in 1907 (Uber fermentative Fettspaltung, Hoppe-Seyler's Ztschr, f. phys. Chem. 1907, vol. 50, p. 1429). Decorticated castor beans are pulped with water in a suitable mill. The resultant emulsion is filtered from coarse suspended matter and the white creamy liquor is permitted to ferment spontaneously at about 24°C. Water separates and a thick creamy layer rises to the top. This is the active material and contains 38 per cent castor oil fatty acids, 58 per cent water and 4 per cent albuminoid substances including the actual lipolytic agent.

As the enzyme is practically insoluble in the oil, present practise is to cold press the seeds first to obtain the valuable first cold pressed castor oil and to work up the press cake for ferment-milk.

This ferment-milk is used as follows: In a lead lined iron tank the liquid fat is stirred up with about 35 per cent of its weight of water, 5-8 per cent, of "ferment" (i.e., "ferment-milk") and 0.2 per cent of manganese sulfate. The preferred working temperature is about 25°C. Once established this is generally maintained especially as the process liberates enough heat to raise the temperature spontaneously 2-3°C. Stirring by air is carried out from time to time in the early stages of the process to prevent separation of the emulsion, but towards the end the emulsion remains permanent. When the desired degree of hydrolysis is obtained (about 80 per cent after 1 day, 90 per cent after 2 days) the temperature is raised to 80-85°C. to kill the enzyme, 0.2-0.3 per cent (on weight of fat) of 66° Bé. sulfuric acid is added and the mixture stirred by air. On permitting to settle three layers form:

1. Aqueous glycerin (12-25 per cent glycerin) at bottom. This contains manganous sulfate, sulfuric acid, water soluble fatty acids and albuminoids.
2. Clear fatty acid layer on top.
3. Middle layer consisting of emulsion of albuminoids, glycerin, fatty material and water.

Several notes may be made at this point on the process. The cytoplasma in contact with water begins to be destroyed at 43°C. Hence the process cannot be carried out above 41° or 42°C. The fat must be liquid; therefore, high-melting fats cannot be hydrolyzed by the enzyme, unless they are admixed with sufficient low melting fats to remain liquid at about 35°C. It is best to operate at least 2-3°C. above the melting point of the fat and the best working temperature is about 25°C. Tallow is difficult to work with. Even with low melting tallows it is difficult to obtain above 75 per cent free fatty acids.

The enzyme works best in a very dilute acid solution. The free fatty acids produced regulate the acidity.

The amount of water used may vary from 30-40 per cent of the weight of the fat. Less than 30 per cent can be used only for fats with a high free fatty acid content such as solvent extracted bone-oil and peanut oil. More than 40 per cent of water dilutes the glycerin unnecessarily.

The action of the enzyme is reversible. If fatty acids and 96 per cent glycerin are mixed and the enzyme added, neutral fat will be produced. Welter (Zeitschr. f. angew. Chem. 1911, vol. 24, p. 385) obtained in this manner 35 per cent of neutral fat from palm kernel oil fatty acids and glycerin. It has been found that the reaction in hydrolysis by enzymes is not stepwise but is represented by the first equation of this article.

The glycerin obtained is generally considered superior to that from the Twitchell process or the autoclave process. However, Contardi (Giorn. di Chimica ind. ed appl. 1925, vol. VII, No. 6) who has had experience with the enzyme process at the Sirio plant at Milano-Bovisa, Italy, considers enzyme glycerin as of low quality due to the nitrogenous substances dissolved in the glycerin which cause distillation difficulties.

The enzyme process simple as it seems requires a careful technic. Slight changes in conditions produce great differences in results. Contardi gives a striking example of the limitations of our knowledge of the behavior of fat splitting enzymes. The subterranean vaults of the Rotunda at Milan served as the cemetery of the Milan Municipal Hospital and are estimated to have received 150,000 corpses from 1698 to 1783. These vaults were cleared out in 1907-1909. About one-fourth of the corpses, namely part of those in the east crypt had not undergone ordinary disintegration in their rest of more than a century in this vault. Owing to the absence of oxygen, presence of moisture, traces of ammonia, the uniform and moderate temperature, the steapsins (fat-splitting enzymes

present in animals) of the body had met such conditions that they converted all the fat of the tissues to free fatty acids before ordinary decomposition took place and these fatty acids remained unchanged preserving the form of the body and even the expression of the face. After opening of the vault and removal of the bodies disintegration set in immediately. This gruesome incident serves merely to show the undiscovered possibilities of enzyme action under suitable conditions.

Baur proposed the use of jancreas enzyme for fat-splitting. This was not commercially successful, and as the pancreas is our present source of insulin it is unlikely that pancreatic enzymes will be used for commercial fat splitting.

The glycerin obtained, in spite of certain obvious disadvantages, is free from trimethylene glycol. To purify the glycerin it is treated at the boiling point with milk of lime which precipitates manganous sulfate, sulfuric and fatty acids and some nitrogen compounds. After filtration a slight excess of sulfuric acid is added to remove lime. On concentration this glycerin is saturated with calcium sulfate. If this is undesirable the careful limed glycerin is treated with barium hydroxide to remove sulfates and then with oxalic acid to remove lime. On concentration by evaporation in vacuum a commercial glycerin is obtained with 0.2-0.5 per cent ash, and 0.5-1.2 per cent organic impurities. It can be easily purified by distillation.

The chief disadvantage of the process is the formation of the middle layer. These "middles" are collected, rewarmed and diluted and generally a further separation into three layers occurs. If run in connection with a soap factory this material can be run into the soap kettle and worked up with the usual run of fats.

A diagram of an enzyme installation is given in vol. 5 p. 446 of Ullmann's Enzyklopaedie der technischen Chemie, while a whole plant layout for castor bean handling down to the production of fatty acids and glycerin is given on a 1:125 scale in F. Goldschmidt "Chemie, Analyse, Technologie der Fettsäuren, des Glyzerins, etc.," vol. 2, page 54.

The costs for enzyme splitting are not known exactly but for comparative purposes it may be stated that Ullmann gives 3 marks per 100 kilos ($6.49 per ton) of fat hydrolyzed by enzymes, while he gives 1½ marks ($3.25 per ton) for the Twitchell method.

The advantages of the enzyme method are:

1. Light color of fatty acids.

2. Operation at lower temperatures with resultant less wear on equipment and lower amortization and repair charges.

The disadvantage is the formation of the special middle layer.

The following table of dydrolysis tests made b ythe Vereinigte Chemi-

schen Werke A.-G. of Charlottenburg, Germany, who operated the first enzyme splitting plant is self-explanatory.

Type of Oil Used	Weight in Kgs.	Water, kgs.	% on oil	$MnSO_4$, kgs.	% on oil	Ferment, kgs.	% on oil	% Hydrolysis	Fatty Acids, kgs.	Glycerin Anhydr., kgs.	% on oil	Middle Layer			
												Wt. Kgs.	% on oil	Fatty Acids	% on oil
Linseed, crude.	877	307	35	1.75	0.2	53	6	90	858	79.7	9.1	26	3.0	6	1.0
Linseed bleached	532	212	40	1.00	0.2	26.5	5	91	511	50.3	9.7	17	3.0	7	1.0
Cottonseed, American	900	360	40	1.80	0.2	54	6	86	870.5	80.5	8.94	34	3.9	14	1.6
English	912	364	40	1.80	0.2	55	6	91	888	82.1	9.0	42	4.5	8	0.9
	780	276	35	1.52	0.2	53	7	89	760	70.2	9.0	24	3.0	7	1.0
Tallow	904	326	35	1.90	0.2	90	10	86	888	78.5	8.7	40	4.4	16	1.8
Palm Kernel	845	338	40	1.70	0.2	68	8	84	833	85.1	10.1	19	2.0	6	0.7
Palm Kernel	816	326	40	1.63	0.2	65	8	90	795	84.3	10.3	28	3.0	8	1.0
Totals and avgs.	6566	2509	38.6	13.1	0.2	46.4	7	88.4	6403.5	610.7	9.4	230	3.5	72	1.1

U. S. ATE 5,000,000,000 POUNDS OF FAT IN 1925

In 1925 over five billion pounds of various kinds of edible fats were consumed by the people of the United States, according to a statement issued by F. M. Barnes, head of the Edible Oils and Fats Division of the Procter and Gamble Company. This is an average of 46.1 pounds per capita, which is an increase of one-fifth pounds per capita over the 1924 figures.

Another interesting indication of America's dietary habits is the swing to vegetable fats. Twenty-five years ago, lard was practically the only cooking fat used in the home, whereas now, according to government figures, vegetable fats make up thirty-four per cent of the actual amount used in American kitchens.