was extended to 30 minutes, the bottom interface of the foots layer became indistinct (or torn), probably because meal particles were dragged from the layer by the centrifugal force. Otherwise they are held within the foots by the forces of interfacial tension. These observations suggest therefore that the method could be improved by removing the meal by centrifuging or filtering after the oil has been heated to 65°C. to disperse or dissolve any petroleum naphtha-soluble gums which may have been separated with the meal; i.e., redisperse the material which it is the intent of the method to determine.

If there is meal present which has to be removed before applying the test, it is apparent that the usefulness of centrifugal foots results on such oil would depend upon the result being used in connection with the measured meal content, provided the main object is to obtain an estimate of probable refining loss (i.e., by combining those results with f.f.a. content). This requirement undoubtedly detracts from the potential value of the test as applied to cottonseed oil for this purpose, although the authors have found it especially useful when so applied to soybean oil, all of which in our experience has been substantially meal-free.

However, aside from this complicating effect of meal content in cottonseed oil, it seems likely that wider variations in the chemical nature of the acetone insoluble material in crude cottonseed oil and in the pigments, and especially variations in the gossypol content, may be such that it is beyond reasonable expectation to find anything approaching the same degree of correlation of refining loss and centrifugal foots results (combined with f.f.a.), as we have found in the instance of crude soybean oil (3). That conclusion indeed seems indicated by the poor agreement between "calculated loss" and the official cup loss results on the present series of samples even when the factors used in the equation for "calculated loss" have been chosen to give agreement on the average on most oils.

Still, where there may be a need for an empirical measure of the phosphatide content of crude cottonseed oil, the centrifugal foots value determined on the sample from which meal had been separated should have value as a simple rapid test. Or, as a rapid measure of "foots," as the term is used in reference to raw linseed oil, embracing both phosphatides and meal, this test may find application in programs involving the evaluation of crude cottonseed oil with respect to these components.

### Acknowledgment

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# Photometric Determination of Esterase in Animal or Plant Tissues and in Microorganisms

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THE method described here is similar in principle to the colorimetric method for phosphatase (1) and β-glucuronidase (2). Phenolphthalein dibutyrate is hydrolyzed to phenolphthalein by animal or plant esterase without any compensating activation, the esterase inactivated by trichloroacetic acid, proteins removed, and the phenolphthalein estimated at pH 10 by measuring the extinction with a Pulfrich photometer with filter S 53, cell depth 49.96 mm., and comparing with this value a calibration curve established for phenolphthalein solutions at pH 10.

### Preparation of the Substrate

Phenolphthalein dibutyrate is synthesized by adding butyryl chloride (13 gm.) drop by drop to phenolphthalein (10 gm.) dissolved in water-free benzene (80 ml.) in the presence of pyridine as a catalyst at higher temperatures (under reflux). The pyridine (6 ml.) is added to the reacting mixture also drop by drop after the main portion of the butyryl chloride has already reacted. The major part of the solvent is evaporated *in vacuo* at room temperature. The residue is diluted with not too much water (100 ml.). Then 3 to 4 times as much ethyl ether (as water) is added. To remove the pyridine the solution is shaken 8 to 10 times with about 200 ml. of water. The water is removed and the solution evaporated *in vacuo* at room temperature. The residue is taken up with a little methanol and the phenolphthalein dibutyrate crystallized from it at −8 to −10°C. By recrystallizing the initial product (8 gm.), to which traces of unchanged phenolphthalein still adhere, from the solution in methanol, 6.5 gm. of chemically pure phenolphthalein dibutyrate are obtained in colorless long thin flakes (see Figure 1) with m.p. 91.5°C. (uncorrected), constituting a 65% yield. The crystalline substance is not soluble in water, somewhat in methanol and ethanol at room temperatures, easily soluble at higher temperatures and also easily soluble in ethyl ether, benzene, and chloroform.

*Analysis of the crystallized phenolphthalein dibutyrate.* The carbon and hydrogen content of the refined crystals correspond closely to their theoretical values.

$C_{28}H_{26}O_6$ C calculated 73.77%, found 73.70%.
H calculated 5.72%, found 5.76%.

### Esterase Estimation Procedure

*Solutions required.*

1. *N trichloroacetic acid.* Check titer with N sodium hydroxide, using phenolphthalein as indicator.

2. *0.20 M glycine buffer solution* (Sørensen) for pH 8.0 (with esterase in pancreas, saliva, milk, and blood plasma, respectively) and for pH 8.3 (with es-

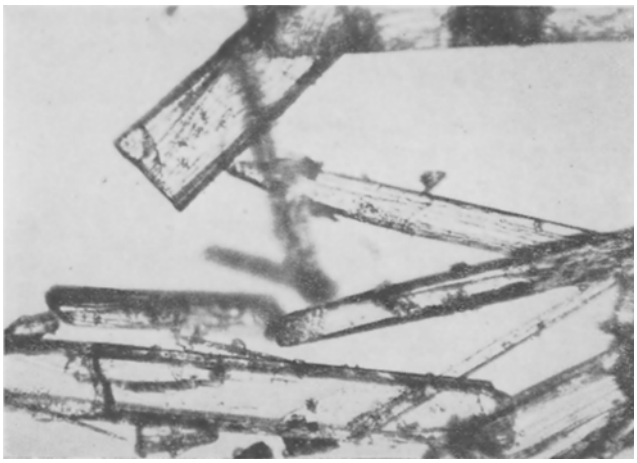

FIG. 1. Crystals of phenolphthalein dibutyrate.

terase in oats and other plants). The standard glycine solutions as well as the buffer solution keep only a short time and have to be prepared immediately before using.

3. *Substrate solution.* 36.668 mg. of phenolphthalein dibutyrate dissolved in 10 ml. of purest dehydrated methanol (Merck). The solution keeps in a refrigerator for 8 to 10 days.

*Sample.* Take 10 to 200 mg. of finely ground animal or plant tissue, according to esterase content, or an equivalent amount of body fluid (saliva, milk, blood plasma) and suspend in distilled water in a 20-ml. volumetric flask. Add 5 ml. of 0.2 M glycine buffer solution for pH 8.0 (with animal esterase) or for pH 8.3 (with plant esterase). Dilute to 15 ml. with distilled water and place flask in a thermostat at 37°C. After equalization of temperature add that amount of phenolphthalein dibutyrate in methanol (0.5 ml. of substrate solution) which corresponds to that contained in 20 ml. (total volume of sample) of an 0.0002 M solution of phenolphthalein dibutyrate. Shake well and let react at 37°C. for 1 to 2 hours or even longer, shaking the mixture at frequent intervals. At the end of the desired reaction time inhibit esterase activity by adding 2 ml. of N trichloroacetic acid and fill flask to 20 ml. with distilled water. Remove protein precipitate by centrifuging for 10 minutes. Transfer 15 ml. of the clear solution to a 20-ml. volumetric flask already filled with 2 ml. of N sodium hydroxide solution, constantly agitating the flask. By adding the sodium hydroxide solution, the desired pH value of 10 is obtained. Check this value at the beginning of each test series after filling the test vessel to the mark. Remove any turbidity appearing in the sample by centrifuging. Measure extinction value of red coloring caused by the liberated phenolphthalein in a Pulfrich photometer, filter S 53, cell depth 49.96 mm. Compare calibration curve (Figure 2) with this value to find the amount of liberated phenolphthalein, computing therefrom the activity percentage.

A practical hint: The phenolphthalein dibutyrate emulsion adheres tenaciously to the walls of the test vessels. To obviate any errors all used vessels have to be cleaned with a mixture of methanol and sodium or potassium hydroxide solution until no red coloring reappears.

The size of the sample containing esterase must be chosen so that the amount of phenolphthalein liberated by hydrolysis lies between 0.010 and 0.040 mg. (see Figure 5). When the amount of liberated phenolphthalein exceeds 0.040 mg., the test must be repeated with a smaller sample.

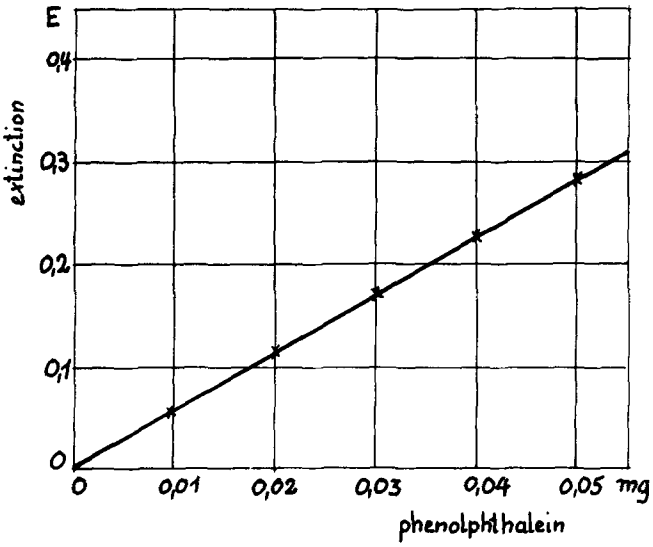


FIG. 2. Calibration curve for phenolphthalein solutions at pH 10; Pulfrich photometer, filter S 53, cell depth 49.96 mm.

It is superfluous to run a blank since phenolphthalein is not present in any animal or plant tissues, and special checks have shown that phenolphthalein dibutyrate is not saponified in the pH range of 7.5 to 9.5 even after many hours' reaction at 37°C. in the absence of any enzymes.

## The Kinetics of the Reaction

*The influence of the pH value of the reaction velocity.* The influence of the pH value of the hydrolysis of phenolphthalein dibutyrate by animal and plant esterase is rather varied. Maximum efficiency lies at pH = 8.0 with esterases of animal origin tested so far, and at pH = 8.3 with plant esterases (Figure 3).

*Phenolphthalein dibutyrate hydrolysis as a function of time.* Kinetic tests performed show (Figure 4) that it is impossible to establish an enzyme unit by

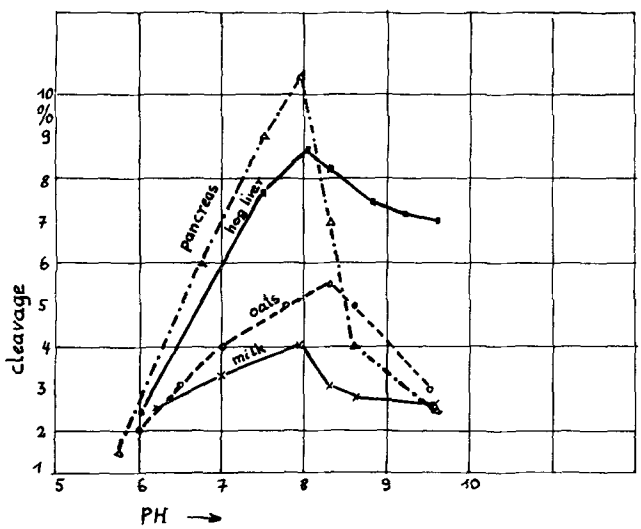


FIG. 3. Relation between phenolphthalein dibutyrate cleavage and pH value.

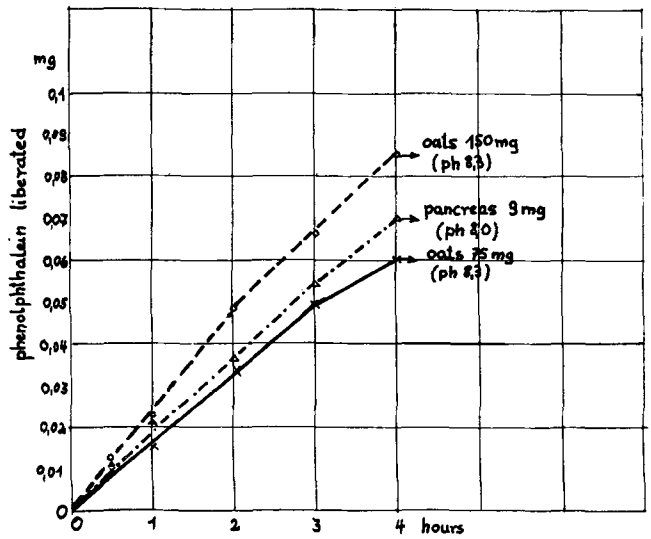


FIG. 4. Hydrolysis of phenolphthalein dibutyrate by different amounts of esterases of various origins.

means of a simple kinetic law of reaction. The phenolphthalein dibutyrate unit must therefore be determined empirically.

### Determination of Esterase Concentration

The estimation of esterase is based on the empirical relation plotted in Figure 5 between the reaction time and the amount of phenolphthalein liberated from phenolphthalein dibutyrate by 1 phenolphthalein dibutyrate unit (ph. dib. u.) at optimal reaction conditions.

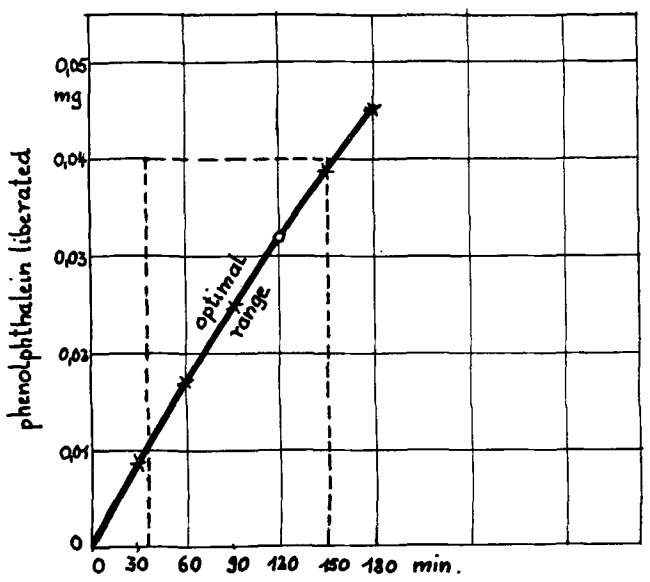


FIG. 5. Cleavage of phenolphthalein dibutyrate by 1 ph. dib. u.

*Definition of phenolphthalein dibutyrate unit.* One ph. dib. u. is defined as the amount of enzyme which, under the stated conditions, splits phenolphthalein dibutyrate at an initial speed liberating 1 mole $\times 10^{-7}$ phenolphthalein (0.0318 mg. corresponding to a 3.31% cleavage of the substrate) from 20 ml. of 0.0002 M phenolphthalein dibutyrate containing 5 ml. of 0.2 M glycine buffer (Sørensen) pH 8.0 (animal esterase) or 8.3 (plant esterase) after 120 minutes at 37°C.

Esterase concentration is stated in terms of ph. dib. units per 1 mg. tissue or per 100 ml. body or organism fluid.

The effect of animal or plant esterase samples on phenolphthalein dibutyrate can be expressed in the following way in terms of ph. dib. u. (3). Supposing the enzyme to be analyzed liberates $x$ mg. of phenol phthalein in $t_1$ minutes, then 1 ph. dib. u. would, according to Figure 5, have liberated the same amount in t minutes. Therefore the amount of enzyme is shown to be $t/t_1$ ph. dib. u.

*Example.* In a test 0.025 mg. of phenolphthalein was liberated after 60 minutes. From the curve of Figure 5 it can be seen that the same amount of phenolphthalein is liberated by 1 ph. dib. u. after 90 minutes. The esterase concentration therefore corresponds to $90/60 = 1.5$ ph. dib. u.

This new method has been tested for its reliability by applying it to a wide range of materials containing esterase, including microorganisms. This test is proved to be suitable for comparative conclusions about the amounts of esterase present in the several animal and plant tissues and body fluids.

### Summary

1. The present paper describes a photometric method for the quantitative analysis of esterase localized to the cell structure of animal or plant organisms or dissolved in their cytoplasm. Phenolphthalein dibutyrate is found to be a suitable substrate. It emulsifies readily by adding a solution in methanol to an excess of water; it is hydrolyzed easily by esterase; it is split to phenolphthalein, which can be measured photometrically and is not present in organs and tissues of any origin; it also has a relatively high resistance against hydroxyl ions at the optimal pH range of the esterases.

The estimation of esterase contained in the sample is based on the empirical relation between the amount of liberated phenolphthalein and the time of reaction under optimal reaction conditions.

The amount of esterase contained in the sample is expressed in terms of phenolphthalein dibutyrate units (ph. dib. u.). One ph. dib. u. is defined as that enzyme amount which, under the conditions mentioned, hydrolyzes phenolphthalein dibutyrate with an initial velocity corresponding to the liberating of 1 mole $\times 10^{-7}$ of phenolphthalein (= 0.0318 mg., corresponding to a 3.31% cleavage of the substrate) from 20 ml. of 0.0002 M phenolphthalein dibutyrate containing 5 ml. of 0.20 M glycine buffer pH 8.0 (animal esterase) or 8.3 (plant esterase) after 120 minutes at 37°C.

The amount of the sample containing esterase must be chosen so that between 0.010 and 0.040 mg. of phenolphthalein are liberated by hydrolysis. The esterase concentration is expressed in terms of phenolphthalein dibutyrate units (ph. dib. u.) per 1 gm. of tissue or per 100 ml. of body or organism fluid.

2. This method does not need any compensating activation because, when using phenolphthalein dibutyrate as substrate, the usual activating substances for pancreas lipase, such as albumin, sodium oleate, and calcium chloride, have an inhibiting effect. With our substrate liver esterase would also be inhibited by them. The action of plant esterase is not interfered with by said activators.

3. For this method the optimal pH value lies at 8.0

for all animal esterases examined so far and at 8.3 for all plant esterases.

4. A procedure is described for preparing the substrate, phenolphthalein dibutyrate, in pure form in the shape of long, thin colorless flakes of m.p. 91.5°C. (uncorrected).

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# A Method for the Quantitative Determination of Relative Wear of Soap Bars

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As a result of current interest on the part of manufacturers of fine toilet soaps in those properties of their product which are of importance, consumer-wise, in a highly competitive business, the perfection of techniques by means of which soap lathering characteristics may be evaluated has been the object of extensive research. But although a variety of methods for the estimation of foam producing power of soaps, measurement of foam stability, and study of foaming properties of soap solutions have been reported, the question of lathering power of soap bars has received little attention. Accordingly, since the lathering behavior of soap bars is influenced not only by the chemical characteristics of constituent materials but also by physical fixations proceeding from various manufacturing circumstances, tests involving measurement of foam production of soap solutions are insufficient in bar lathering studies, and need of a test applicable to whole bars is indicated. The significance of a means of determining relative wear of soap bars as an analytical device in bar lathering studies becomes apparent when one observes that to obtain lather from a bar of soap, a yield of soap solution must, by some wearing action, be effected.

Examination of the scientific literature pertaining to soaps, detergents, and allied subjects, failed to reveal the existence of a method for the quantitative determination of relative wear of soap bars. Yet, the effects of variations in soap processing on lathering properties of the bars must be disclosed by information derived from whole bars. This paper therefore is presented for the purpose of describing what is believed to be an accurate, quantitative method for the determination of relative wear of soap bars of all sizes and shapes.

## Selection of Samples

While the several bars comprising each of the nine groups of samples (A to I, inclusive) were prepared with the intention that they be alike in all respects. sample members of particular groups were intentionally caused to exhibit properties different from those of samples of other groups by a variety of alterations in the manner of treatment of the soap during the course of its being made into finished bars. Small variations in the dimensions of the sample bars belonging to a given group could not be avoided but were duly taken into account in the final calculations. Data compiled during the testing of the samples are given in Table I.

TABLE I

Wearing Characteristics of Soap Bars

| Group | Sample | Original thickness, inches | Final thickness, inches | Strokes | Wear Number |
|---|---|---|---|---|---|
| A | 1 | 1.053 | 0.903 | 40 | 3.8 |
| | 2 | 1.061 | 0.912 | 40 | 3.8 |
| | 3 | 1.056 | 0.903 | 40 | 3.9 |
| B | 1 | 1.114 | 0.939 | 40 | 4.3 |
| | 2 | 1.110 | 0.936 | 40 | 4.3 |
| C | 1 | 1.088 | 0.894 | 40 | 4.9 |
| | 2 | 1.095 | 0.900 | 40 | 4.9 |
| D | 1 | 1.078 | 0.942 | 30 | 4.5 |
| | 2 | 1.048 | 0.876 | 40 | 4.5 |
| | 3 | 1.078 | 0.901 | 40 | 4.5 |
| E | 1 | 1.101 | 0.928 | 39 | 4.4 |
| | 2 | 1.107 | 0.929 | 40 | 4.4 |
| F | 1 | 0.964 | 0.803 | 40 | 4.6 |
| | 2 | 0.967 | 0.806 | 40 | 4.5 |
| G | 1 | 1.101 | 0.923 | 40 | 4.4 |
| | 2 | 1.109 | 0.932 | 40 | 4.4 |
| | 3 | 1.109 | 0.928 | 40 | 4.5 |
| | 4 | 1.103 | 0.922 | 40 | 4.5 |
| | 5 | 1.110 | 0.932 | 40 | 4.4 |
| H | 1 | 1.112 | 0.915 | 40 | 4.9 |
| | 2 | 1.097 | 0.995 | 20 | 4.9 |
| I | 1 | 1.107 | 0.921 | 40 | 4.6 |
| | 2 | 1.112 | 0.925 | 40 | 4.6 |

## Apparatus

In Figure 1 the main parts of the wear-testing apparatus are shown. The frame, in which the sample is secured, and the sponge paddle were constructed of ⅛" pressed wood. The paddle handle, frame side blocks, and sample holding pieces were cut from white pine stock. The parts were assembled, and the finished apparatus was painted. Heavy wall neoprene tubing was then slipped over the sample holding pieces, finishing them as shown in the drawing. The paddle was slightly thinned along the edges with sand paper so that it moved easily in the frame grooves. In operation, a little silicone lubricant applied to the upper surface of the paddle proved to be additionally helpful in this connection.

Care in the selection of a sponge material for the wearing surface was found to be necessary, and those sponges which exhibited a tendency to produce uneven wearing of sample bars were discarded. A section, uniformly $1\frac{1}{16}$" thick, cut from a soft, fine textured cellulose sponge of rectangular cross section, was finally chosen and was fastened to the paddle by means of rubber cement.

The entire assembly, ready to use, is shown in Figure 2. A 7½-gallon container for the wash water was