The increase was much less in seeds germinated in the absence of light. A zero-order reaction rate for the enzyme was observed when tributyrin was employed as the substrate. Optimum activity occurred at pH 8.5. The Michaelis-Menton was calculated to be 4.06 × 10^{-4} M, which is close to values reported for other lipases. While most substances tested had no effect on fat-splitting activity, NaCN, NaN_3, and glutathione behaved as activators.

Acknowledgment

The author wishes to acknowledge his indebtedness to A. S. Sieben for technical assistance.

REFERENCES

1. Balls, A. K., Matlack, M. B., and Tucker, I. W., J. Biol. Chem., *122*, 125 (1937-38).
2. Bamann, E., and Ullmann, E., Biochem. Z., *312*, 9 (1942).
3. Gershtein, L. A., Doklady Akad. Nauk. S.S.S.R., *72*, 1091 (1950).
4. Gertler, W., Fermentforschung, *15*, 171 (1936).
5. Goldman, M. L., Burton, T. H., and Rayman, M. M., Food Res., *19*, 503 (1954).
6. Janecke, H., Starke, *3*, 29 (1951).
7. Johnston, F. A. Jr., and Sell, H. M., Plant Physiol., *19*, 694 (1944).
8. Kistiakowsky, G. B., Mangelsdorf, P. C. Jr., Rosenburg, A. J., and Shaw, W. H. R., J. Am. Chem. Soc., *74*, 5015 (1952).
9. Lineweaver, H., and Burk, D., J. Am. Chem. Soc., *56*, 658 (1934).
10. Martin, H. F., and Peers, F. G., Biochem. J., *55*, 523 (1953).
11. McConnell, W. B., Spencer, E. Y., and Trew, J. A., Can. J. Chem., *31*, 697 ((1953).
12. Olcott, H. S., and Fontaine, T. D., J. Am. Chem. Soc., *63*, 825 (1941).
13. Ramakrishnan, C. V., Science and Culture (India), *19*, 566 (1954).
14. Rose, W. G., J. Am. Oil Chem. Soc., *28*, 47 (1951).
15. Schoenheyder, F., and Volqvartz, K., Enzymologia, *11*, 178 (1944).
16. Singer, T. P., J. Biol. Chem., *174*, 11 (1948).
17. Sobotka, H., and Glick, D., J. Biol. Chem., *105*, 199 (1934).
18. Weinstein, S. S., and Wynne, A. M., J. Biol. Chem., *112*, 649 (1935-36).

[Received September 4, 1956]

Error in the Determination of Active Ingredient in Detergent Products

L. U. ROSS and E. W. BLANK, Research and Development Department,
Colgate-Palmolive Company, Jersey City, New Jersey

THE DETERMINATION of active ingredient in commercial detergent products by gravimetric separation of the alcohol-soluble material is an accepted procedure (1). The alcohol soluble is titrated for NaCl content, and a correction is made. Experience in this laboratory indicates that when Na_2CO_3, $NaHCO_3$, and $Na_2B_4O_7$ are present, similar interferences arise by virtue of their solubility in alcohol. The establishment of a correction when such salts are present in the alcohol soluble is a difficult and impractical operation.

The appreciable solubility of NaCl in alcohol is an accepted fact. The literature reveals little information regarding the solubility of Na_2CO_3, $NaHCO_3$, and $Na_2B_4O_7$ in methyl and ethyl alcohol (2).

The magnitude of the error involved in assuming the alcohol soluble to be equivalent to active ingredient is shown by the results presented in Table I for three typical detergents. In each case the result for active ingredient determined as alcohol soluble is considerably greater than that determined by ultraviolet absorption. By extracting the alcohol-soluble material with 1:1 acetone-ethyl ether, values are obtained which more closely approximate those determined by absorption in the ultraviolet.

TABLE I

Comparison of Results for the Active Ingredient Content of Detergent Products Employing Three Methods of Determination

Determination	Detergent Product		
	No. 1	No. 2	No. 3
Alcohol soluble	22.18	6.27	42.32
1:1 Acetone-ethyl ether soluble	15.11	3.98	41.34
Active ingredient (alkyl aryl sulfonate) by U. V. absorption	14.25	3.72	41.33
Na_2CO_3	3.82	12.99	5.00
$NaHCO_3$	Nil	31.53	Nil
NaCl	0.79	1.06	0.10
Borax	14.18	Nil	Nil

Procedure

Obtain the dry alcohol-soluble material in the usual manner (1). It is not necessary to heat to constant weight. Add 75 ml. of 1:1 acetone-ethyl ether mixture to the alcohol-soluble residue and warm on the steam bath. Agitate with glass stirring rod, and filter warm through a Whatman No. 40 paper or equivalent. Wash the flask and paper with small, additional volumes of warm 1:1 acetone-ethyl ether. Evaporate the combined filtrate and washings on the steam bath, and dry in an oven to constant weight at 80 ± 2°C.

Discussion

The treatment with 1:1 acetone-ethyl ether removes NaCl, Na_2CO_3, $NaHCO_3$, NaOH, and $Na_2B_4O_7$ in addition to traces of other inorganic salts. In developing this purification procedure, the removal of Na_2CO_3 and $NaHCO_3$ was demonstrated by running infrared spectra of the alcohol-soluble material before and after purification. Carbonate ion absorptions at 4.05, 5.65, 7.0, 11.38, and 14.3 microns were eliminated as a result of the purification treatment.

The addition of 1:1 acetone-ethyl ether directly to the product under analysis is not recommended because of the limited solubility of active ingredient in the mixture. By working with the alcohol-soluble material, visual observation will prevent errors resulting from partial solubility of the active ingredient.

Acknowledgment

Thanks are due K. Kellenbach for running infrared spectra.

REFERENCES

1. A.O.C.S. Official Method Db 2-48. This method was designed for soap in admixture with synthetic detergents, but the principle is the same when soap is not present. Soap does not dissolve in 1:1 acetone-ethyl ether mixture, and this solvent can be employed to separate soap from surface-active agents when both are present.
2. Blank and Boggie, Oil & Soap, *20*, 125–128 (1943).

[Received September 22, 1956]