minution methods studied included grinding at moisture contents from 12 to 31%, rolling and re-rolling at various moisture levels from 4 to 13%, and rolling at temperatures from 90 to 150°F.

Grinding of cottonseed flakes, at 18 to 31% moisture content and at pH 8.2, through a peanut butter mill reduces the free-gossypol content to between 0.047 and 0.090%, depending upon the moisture content and the number of times the material is passed through the mill. Concurrently the nitrogen solubilities of these materials were reduced from approximately 96% to approximately 80%.

The moisture content of cottonseed meats during rolling significantly affects the free-gossypol content of the meals. The free-gossypol contents of the flaked meats are inversely related to the moisture content during rolling. The free-gossypol contents of these flaked meats are in the same relative order during all stages of processing, indicating that moisture content during rolling is critical and its influence cannot be overcome during subsequent processing steps.

The effects of multiple re-rolling to reduce free-gossypol content can apparently be overcome by subsequent processing. The values for materials which had been rolled only once fell on the same smooth curve as those for materials which had been rolled three times, after all of these materials had been mixed for two hours at 31% moisture and pH 8.2 and 90°F.

The nitrogen solubility of cottonseed meals is slightly reduced by rolling at moisture contents in excess of 10%.

There is an indication that the nitrogen solubility of flaked cottonseed meats decreased during the initial phase of mixing at pH 8.2 and 31% moisture and then increases to approximately its original value.

Mixing in the presence of alkali at moisture contents of 24 to 31% and at a temperature of 90°F. gave a significant reduction in the free gossypol content of cottonseed meals without a corresponding decrease in nitrogen solubility.

Increased temperatures of flaking, up to 150° F., are accompanied by a reduction in the free-gossypol content of the flakes produced. At the higher temperatures there is some reduction in nitrogen solubility (to approximately 85%).

Prolonged mixing (for 120 min.) in the presence of 31% moisture and at pH 8.2 and 120 or 150°F. significantly reduced the nitrogen solubilities of cottonseed meals when compared to mixing at 90°F.

Cooking at temperatures as low as 150°F. at 31% moisture content and pH 8.2 significantly reduced the nitrogen solubilities of cottonseed meals (to approximately 70%). Most of these meals had free-gossypol contents of less than 0.04%.

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REFERENCES

1. American Oil Chemists' Society, "Official and Tentative Methods of Analysis," 2nd ed., rev. to 1955, Chicago 1946–55.
2. Heywang, B. W., Southwest Poultry Experiment Station, Glendale, Ariz., unpublished data.
3. King, W. H., Knoepfler, N. B., Hoffpauir, C. L., and McCourtney, E. J., J. Am. Oil Chemists' Soc., in press.
4. King, W. H., Wolford, L. T., Thurber, F. H., Altschul, A. M., Watts, A. B., Pope, C. W., and Conly, Jean, J. Am. Oil Chemists' Soc., *33*, 71–74 (1956).
5. Lyman, C. M., Chang, W. Y., and Couch, J. R., J. Nutrition, *49*, 679-690 (1953).
6. Pons, W. A. Jr., Hoffpauir, C. L., and O'Connor, R. T., J. Am. Oil Chemists' Soc., *27*, 390–393 (1950).
7. Reuther, C. G. Jr., LeBlanc, M. F. H. Jr., Batson, D. M., and Knoepfler, N. B., J. Am. Oil Chemists' Soc., *30*, 28–32 (1953).

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Some Properties of the Lipase Present In Germinating Rapeseed[1]

L. R. WETTER, Prairie Regional Laboratory, National Research Council of Canada, Saskatoon, Saskatchewan, Canada

A PRELIMINARY SURVEY has indicated that the various oilseeds grown in Western Canada have extremely low contents of lipase. According to the literature, the lipase activity of some oilseeds increase during germination. Ramakrishnan (13), for example, reported a qualitative increase in the lipase content of the groundnut when it was germinated. Gershtein (3) has reported that sprouting tung seed increases five-fold in lipase activity after 40 days and then there is a gradual decline; Johnston and Sell (7) found that the activity increased eight and a half times after 32 days. On the other hand, oats produce a lipase that reaches maximum activity after 8 hrs. of germination and then drops off rapidly (6). Germinating cottonseed produced a marked increase in lipase along with a decrease in total lipids (12).

One of the oilseeds grown in Western Canada, rapeseed, possesses very little lipase activity in the dormant seed. In common with the behavior of other oilseeds already cited however it exhibits a marked increase in lipase activity on germination. This paper deals with quantitative aspects of this increase and also discusses some of the properties of the enzyme.

Materials and Methods

Materials. Polish rapeseed *(Brassica campestris* L.) was used throughout in this investigation unless otherwise designated. The seeds were germinated either in the dark or in the light at approximately 25°C. on white Ottawa sand in large Pyrex dishes (10 by 15 in.). A glass plate cover was employed to maintain the humidity near saturation. During germination no nutrients were added, and the moisture content was maintained in the dish with distilled water.

After the desired period of growth, distilled water was used to wash the seedlings free of sand. Then

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TABLE I
The Lipase Activity of Polish Rapeseed at Various Stages of Germination

Days of germination	Lipase activity[a] of material grown in the	
	Dark	Light
0	0.157	0.157
1	0.231	0.164
2	0.533	2.79
3	1.60	15.18
5	4.75	15.35[b]
7	2.95	8.63[b]

[a] Expressed as mM. butyric acid released per hour per gram dry weight.
[b] For Argentine rapeseed the values were 2.79 and 1.60 for 5 and 7 days, respectively.

they were blended in acetone at −10°C. in a Waring blendor. The temperature of the suspension was kept below 0°C. while the seedlings were homogenized (the time was usually 10 min.). The homogenate was filtered in the cold and washed free of oil and pigments with cold acetone. The finely ground plant tissue was dried and stored in the cold until ready for assay. Ungerminated rapeseed powders were prepared by the same method.

The substrate was prepared from a commercial sample of tributyrin with no further purification. For assay purposes the emulsion was prepared by homogenizing 10 g. of tributyrin and 1 g. of vegetable lecithin in 90 ml. of water in a "Vir Tis" homogenizer[2] for 4 min. at 45,000 r.p.m. The emulsion was made up to 100 ml. and stored in the cold until ready for use. The substrate emulsion was stable for periods of at least a month.

Methods. The assay for lipase activity was essentially the same as described by Goldman and coworkers (5). The enzyme suspension was prepared by weighing out the required amount of acetone-powder and homogenizing it in 0.9% NaCl for 2 min. in the "Vir Tis" homogenizer. The suspension and sufficient distilled water to give a final volume of 50 ml. after the substrate has been added were placed in a 100-ml., double-walled beaker. Water was circulated around the beaker from a thermostated bath maintaining the contents of the beaker at 35°C.

The enzyme and water were first titrated with base (approximately 0.1 N) to a pH of 8.5 and allowed to equilibrate at 35°C., then 10 ml. of substrate were added and the time was recorded as zero. As the reaction progressed, the pH was kept at 8.5 by the addition of 0.1 N NaOH from a Beckman model K automatic titrator. The automatic titrator eliminated the need for buffers and, in addition, made provision for a closer observation of the release of fatty acids by the enzyme. Early in the study it was found that the blank was negligible during the 1-hr. period required for the assay, therefore no regular blanks were determined in agreement with the practice of Balls *et al.* (1). Lipase activity was expressed as mM. of butyric acid released after 1 hr. of incubation at 35°C., pH 8.5, and a substrate concentration as specified above (10).

Results

The effect of germination on lipase content was studied by growing 25-g. lots of Polish rapeseed on white sand as described. These were grown either in the dark or in the greenhouse under optimum light conditions with the temperature approximately the same in both cases. Samples were harvested at specified time-intervals and prepared as acetone powders.

The seeds germinated very rapidly; in all cases the emergence of the root tip could be observed after 12 hrs. The seedlings grown in the light did not grow any faster but, as shown in Table I, the production of lipase was more rapid and reached a higher value than those grown in the dark. The triglyceride-splitting enzyme increased in activity 30 times in 5 days in the etiolated seedlings, and those grown in the light showed a hundred-fold increase in the same time. By the seventh day the activity had started to decline.

The species differences are pronounced. Two samples of Argentine rapeseed *(Brassica napus* L.*)*, which were germinated in the light for 5 and 7 days, respectively, possessed a much lower lipase activity (Table I) whereas the initial lipase content in the ungerminated seed was only slightly lower (0.116-mM. butyric acid released per hour per gram dry weight).

Protein nitrogen values indicate no significant change during germination. Both germinated and non-germinated seeds contained about 55 mg. of nitrogen per gram of acetone-extracted meal. The properties of oil extracted in acetone from etiolated seedlings however exhibited a profound change, suggesting that the lipase which was produced was hydrolyzing the oil. Both the iodine and saponification values decreased with germination time.

One of the most active preparations (3-day germination in the light) was used for the studies of the general properties of the lipase. In all cases the enzyme was homogenized in 0.9% (w./v.) NaCl immediately before carrying out the assay. The substrate was prepared beforehand, kept at 3°C. and used as required.

The first property to be investigated was the stability of the enzyme in solution. This was done by carrying out progressive assays on a sample which was homogenized in NaCl and stored at 4°C. Table II

TABLE II
The Stability of Germinated Rapeseed Lipase Solutions Stored at 4°C.

Time of storage (in days)	% Activity
0	100
1	98
2	94
5	87
9	78

shows that 1-day storage has no effect on the activity; however after 9 days approximately 78% of the enzyme remains. Therefore enzyme homogenates used subsequently were freshly prepared to ensure maximum activity in all experiments.

The pH optimum for lipase activity was determined very conveniently by using the automatic titrator, which also eliminated possible buffer effects. Buffer salts have been found to influence the progress of enzyme reactions as is the case for urease (8) and fungal proteases (11), and the elimination of buffer salts from an enzyme reaction is therefore often desirable. Figure 1 shows the effect of pH on the hydrolysis of tributyrin by samples grown in the light and the dark. The pH optimum (8.5) is the same regardless of whether the seed had been germinated in the light or in the dark, thus suggesting that the enzyme is similar for both. No activity was evident at pH 5.0, and it falls off very rapidly at alkaline pH. The rate of inactivation is apparently greater than at acid pH. This optimum is somewhat higher than that found for pancreatic lipase (5). Bamann and Ullmann

[2] Obtained from E. Machlett and Son, New York, N. Y.

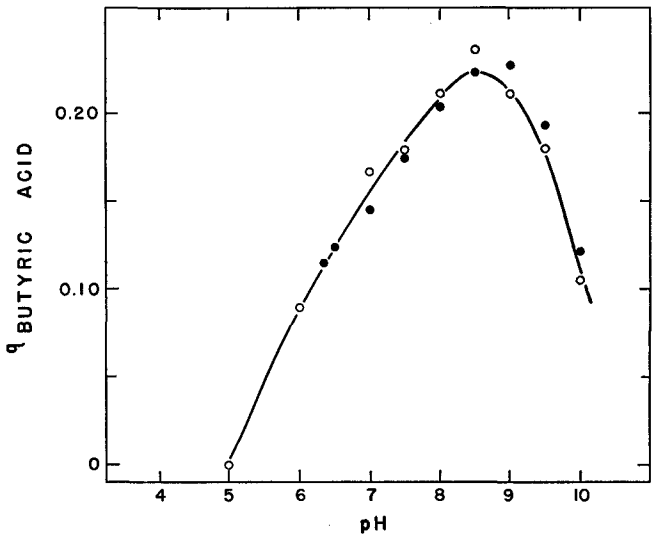

FIG. 1. pH optimum for 3-day germinated rapeseed lipase. The two sets of points are for two different samples, one grown in the dark (●) the other in the light (○).
[q] Butyric acid = mM. of butyric acid released per sample in 30 minutes.

(2) found that in 100 varieties of ripe non-germinated seeds examined, the pH optimum varied from 8.5 to 10.5. Rapeseed lipase falls in the same range and therefore does not differ in this respect from other plant lipases.

The reaction rate during the initial 60 min. of hydrolysis of the substrate is zero-order (Figure 2). The amount of butyric acid released is linear with time for all concentrations of enzyme up to and including 90 mg. of germinated rapeseed per test, but above this the relationship no longer holds and the order of the reaction changes. Some rapeseed lipase preparations exhibited a zero-order rate for a period of 2 hrs.

Another method for determining the order of the reaction is by following the release of fatty acids for varying substrate concentrations. Table III shows

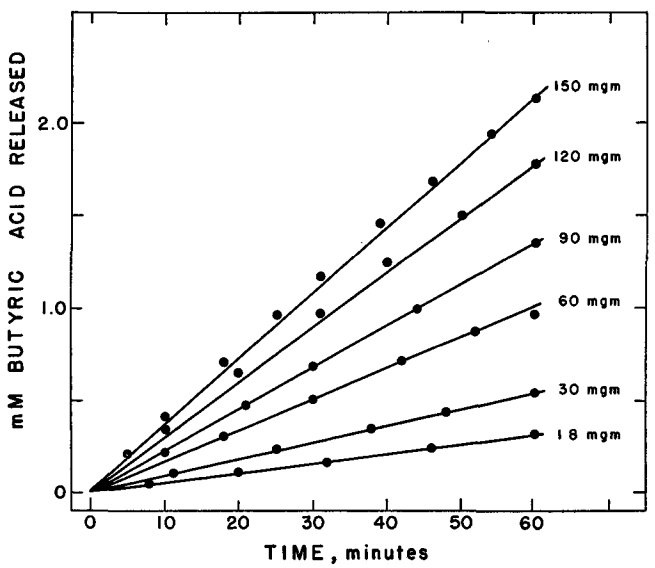

FIG. 2. Influence of enzyme concentration on the rate of hydrolysis. Conditions of experiment: 10 ml. of 10% tributyrin with varying amounts of enzyme. The final volume before titration was 50 ml. The pH was maintained at 8.5 and the reaction temperature was 35° C.

TABLE III
The Effect of Changing Substrate Concentrations on the Zero-Order Rate Constant

mM. Tributyrin per test	k_o[a]	s[b]
1.34	0.0158	.0007
2.00	0.0158	.0013
3.34	0.0157	.0013
4.00	0.0167	.0014
5.00	0.0161	.0009

[a] k_o is expressed as mM. of butyric acid released per minute.
[b] s is the standard deviation.

that a four-fold change in tributyrin concentration does not result in a significant change in the reaction rate constant. The conditions were the same as those described in Figure 2, except that the amount of substrate was varied while the enzyme concentration was kept constant at 60 mg. of germinated rapeseed per test. The rate can therefore be regarded as zero-order since the change in substrate concentration has no effect.

A noticeable change from the zero-order reaction rate was observed for low tributyrin concentrations. Figure 3 shows the results when 60 mg. of germinated rapeseed were tested with 0.334 mM. of tributyrin for a period of more than an hour. For the first 23 min. the curve follows the zero-order reaction rate as at higher substrate concentrations, and after this time the rate is neither zero nor first order. The change occurs at a point which can be accounted for by the release of one butyric acid molecule per molecule of tributyrin. Also at this point the emulsion clears, indicating that a pronounced physical change has taken place in the substrate. This behavior has also been reported for oat lipase (10) except that hydrolysis ceased completely after 1 mM. of fatty acid had been released for every mM. of triglyceride.

The Michaelis-Menton constant, K_m, was calculated according to the method of Lineweaver and Burk (9) where S/V is plotted against S, giving a straight line from which the constant is calculated. The constant was 4.06×10^{-4} M. Martin and Peers (10) reported a value somewhat higher than this for oat lipase, but the current value agrees with the K_m reported for hog pancreatic lipase by Sobotka and Glick (17).

One distinctive feature of the rapeseed lipase is that the amount of hydrolysis of tributyrin varies directly with the concentration of the enzyme. In contrast, other lipases, such as castor bean lipase (14)

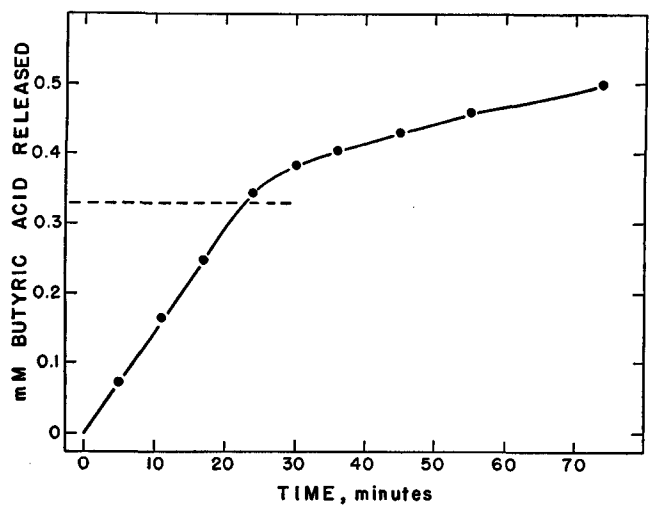

FIG. 3. The hydrolysis curve obtained when low (.334 mM) concentrations of tributyrin are employed.

and pancreatic lipase (5), do not give a linear relationship between enzyme concentration and hydrolysis of substrate.

The action of rapeseed lipase on several substrates was studied. Emulsions of sunflower, olive, and rapeseed oil were prepared in the same way as the tributyrin substrate. The amount of hydrolysis was based on the saponification value of the emulsions. These oils were hydrolyzed less rapidly than was tributyrin (Table IV), and the differences might be accounted

TABLE IV
Action of Germinated Rapeseed Lipase on Various Substrates

Substrate	Percentage of hydrolysis
Triacetin	4.2
Tributyrin	7.8
Triolein	1.6
Olive oil	4.0
Sunflower oil	2.8
Rapeseed	3.1

for by the degree of dispersion of the oils. However visual observation of the emulsions suggested that they were well dispersed. The fact that triacetin is not as readily hydrolyzed as tributyrin suggests that some other factors besides degree of dispersion are involved.

Activation and inhibition by a number of compounds have been reported for various lipases (4, 16, 18). Some of these compounds have been tested with the rapeseed lipase, and the results (Table V) show

TABLE V
The Effect of Various Compounds on Lipase Activity[a]

Compound	Concentration (M)	Activity
NaCN	0.1	132
NaN_3	0.1	126
Glutathione	0.01	118
n-Amyl alcohol	0.01	111
NaF	0.1	107
Phenol	0.01	103
Cysteine	0.01	103
Glycylglycylglycine	0.1	102
Glycylglycine	0.01	101
None	.....	100
Resorcinol	0.01	92
Glycyl-L-leucine	0.01	92
$CaCl_2 \cdot 2H_2O$	0.01	80
DL-Leucylglycine	0.01	77

[a] Conditions: As in other assays except that 1 ml. of the compounds in the concentration designated were added to 10 ml. of enzyme solution and shaken for 1 hr. prior to assay.

that many of the compounds had no significant effect. Three compounds served as activators (NaCN, NaN_3, and glutathione) while leucylglycine appeared to inhibit the hydrolysis of tributyrin. It was noted that higher concentrations of $CaCl_2$ (0.1 M) had neither an activating nor an inhibiting effect on the lipase activity.

Discussion

The lipase activity of rapeseed is markedly increased by germination. As noted earlier, other oilseeds (3, 7, 12) also show increases in lipase activity but not to the same degree as found in the present study. Along with this increase there is a marked change in the residual oil but no significant change in the amount of nitrogen. It was also observed that the production of lipase is higher in seedlings grown in the light, which probably results from the more active metabolic state of the plant grown in light. There is considerable varietal differences, for example, Argentine *(Brassica napus* L.*)* did not produce as active a lipase as did Polish *(Brassica campestris* L.*)*. This might be related to the fact that Argentine germinates and grows more slowly than does Polish and therefore the increase would not be as marked for the time period used in this investigation.

The lipase found in germinating rapeseed hydrolyzes different triglycerides at different rates. This effect is not entirely a matter of dispersion or of size of the triglyceride molecule because triacetin is no more readily hydrolyzed than is sunflower oil. Schoenheyder and Volqvartz (15) found that pancreatic and milk lipases split tributyrin more rapidly than other triglycerides. They also showed that triacetin is hydrolyzed more slowly, a conclusion which agrees with the present results (Table IV).

The reaction rate is zero-order when tributyrin is employed as the substrate for the experimental conditions described. Schoenheyder and co-workers (15) found that for a number of triglycerides the reaction rate was first-order up to 20% hydrolysis. From their work one might conclude that germinated rapeseed lipase is different from pancreatic, liver, or milk lipase because of the different reaction rate. On the other hand, the variation in reaction rates may result from the methods used for the preparation of the substrate emulsions. Still another fact may be the substrate concentrations employed, which were approximately six times higher in the present investigation. However this appears unlikely because when equivalent concentrations were employed in the present work, a zero-order reaction rate still obtained. In experiments[3] with pancreatic lipase at high substrate concentrations, zero-order rates were also obtained. Goldman *et al.* (5), working with pancreatic lipase and using butterfat as the substrate, found that their data could not be fitted to a zero, first, or second order equation. It would appear therefore that lipases from various sources may have different affinities for the same substrate, possibly depending on the method employed in preparing the emulsions.

Germinated rapeseed (5 days in the light) is not as high in lipase activity as is steapsin (pancreatic lipase). On a weight basis the latter hydrolyzes tributyrin 37 times more rapidly than does germinated rapeseed, but on a nitrogen basis the activity for pancreatic lipase is only about 17 times greater.

Many of the compounds tested as inhibitors or activators had no effect on the activity. Among these were the peptides which reportedly enhance the hydrolysis of triglycerides (4), but since the peptides used in the current work were not the same as reported by other workers, the results may not be strictly comparable. Also of interest is the fact that Ca^{++} did not have any effect on the activity at the concentrations used. NaCN, NaN_3, and glutathione all resulted in an activation which indicates that sulfhydryl groups may be involved, as with wheat germ lipase (16). Weinstein and Wynne (18) showed that KCN activates pancreatic lipase by 50% and that the degree of activation varies with concentration and the length of time the enzyme is in contact with the material. The results described here are of a preliminary nature and demonstrate only that rapeseed lipase possesses some properties in common with lipases from other sources.

Summary

The lipase content of rapeseed germinated in light increases by 100-fold over that of the dormant seed.

[3] Unpublished data by the author.

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

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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).

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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).

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