FIRST DAY
MONDAY—MORNING SESSION

REPORT ON ALCOHOLIC BEVERAGES

By J. W. SALE (U. S. Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Referee*

Recently, the writer's attention was called to the omission from the sixth edition of "Methods of Analysis, A.O.A.C." of the official method for alcohol in beer, based on the use of the immersion refractometer, which appeared in the fifth edition of the book, Chapter XIV, sec. **5(c)**, pages 150–1. This is an error which is being called to the attention of the Editorial Board. The reference to the tables should be changed to apply to the sixth edition, as sec. **14.5(c)** the present reference table being **44.24** and **44.25.**

Attention of the Associate Referee on Color in Beer is called to the fact that we now have two methods for the determination of color in beer, namely, **14.2**—Color Official, and the tentative procedure adopted last year and published in *This Journal*, **30**, p. 68. Consideration should be given to making this latter method official and to deleting the former one.

In a letter of February 12, 1947, Mr. Allan D. Dickson, Associate Referee on Methods for Testing Soluble Starches used in Diastatic Power Determination of Malt, advised that available methods were not in shape for collaborative study. Moreover, the procedures do not lend themselves very well to collaborative work. Under the circumstances it appears desirable to discontinue this associate refereeship until some fundamental work has been conducted on soluble starches. The subject can then be reopened and collaborative studies resumed.

RECOMMENDATIONS*

The recommendations of the Associate Referees which are incorporated in the individual reports meet with the approval of the writer and are repeated here.

Malt Beverages, Brewing Materials, and Allied Products:

It is recommended—

(1) That the official, first action, methods for malt (*This Journal*, **30**, p. 55) be adopted as official, final action.

(2) That the official, first action, methods for beer (*This Journal*, **30**, p. 55) be adopted as official, final action.

(3) That the official, first action, methods for hops (*This Journal*, **30**, p. 56) be adopted as official, final action.

* For report of Subcommittee D and action by the Association, see *This Journal*, 31, 55 (1948).

(4) That the official, first action, methods for brewing sugars and sirups, (*This Journal,* **30,** p. 56), be adopted as official (final action).

(5) That the official, first action, methods for wort (*This Journal,* **30,** p. 56) be adopted as official (final action).

(6) That study of methods for determination of essential oil in hops be continued.

(7) That the study of the tentative method for color (*This Journal,* **30,** 68, 1947), and the photoelectric beer color evaluation, as well as work on beer turbidity methods, be continued.

(8) That the direct (non-ashing) orthophenanthroline method described in Proceedings of the Eleventh Annual Meeting of the American Society of Brewing Chemists, pages 32 and 37, for the determination of iron in beer, be studied collaboratively.

(9) That study of methods for testing soluble starches used in diastatic power determination of malt be discontinued.

(10) That study of methods for determination of total solids and yeast solids be continued in accordance with this year's report of the Associate Referee.

(11) That the Milos test for caramel, **14.35,** be deleted, first action.

(12) That the Mathers test for caramel, described in this year's report of the Associate Referee on Wine, be studied collaboratively with respect to its application to beer.

(13) That the study of carbon dioxide in beer be continued.

(14) That the official modified Denigés' method for methanol, section **16.25,** be studied together with the directions in **39.161–39.162,** to bring about uniformity in these procedures.

Wines:

It is recommended—

(1) That study of the spectrophotometric examination of wines be discontinued.

(2) That chromatographic studies in wines be continued.

(3) That study of methods for methanol in wines and distilled liquors be continued.

(4) That the official Milos test for caramel **(15.38)** be deleted (first action).

(5) That the Mathers test, as described in the report of the Associate Referee this year, be adopted as official, first action.

(6) That the tentative confirmatory test for caramel **(15.39)** be modified as described in the report of the Associate Referee for this year.

Distilled Liquors:

It is recommended—

(1) That the study of the obscuration method for determining the true proof of blended spirits be continued.

(2) That the study of methods of analysis with reference to the aging or maturing of whiskey in laminated (plywood) barrels be continued.

(3) That method **16.46 (b)** be changed as recommended by the Associate Referee in this year's report and that study of cordials and liqueurs be continued.

(4) That the modified Marsh test **16.39,** and the Milos test, official, **16.41** for caramel, be deleted (first action).

(5) That the Fulton test for caramel, described in this year's report of the Associate Referee, be adopted as tentative, for distilled liquors and for cordials and liqueurs.

(6) That the Mathers test for caramel, described in this year's report of the Associate Referee, be adopted as official (first action) for Distilled Liquors.

(7) That the Mathers test for caramel, described in this year's report of the Associate Referee, be adopted as official (first action) for cordials and liqueurs, and that it be included by reference in the chapter on cordials and liqueurs in **16.60,** in place of the modified Marsh test, **16.39,** and the Milos test, **15.38.**

(8) That an Associate Referee be appointed to study a rapid colorimetric method for fusel oil.

(9) That the method of fusel oil, **16.22** (p. 196) be further studied.

No reports were given on malt, on diastatic activity and soluble starches, or on hops.

REPORT ON FERMENTABLE EXTRACT IN BREWING SUGARS AND SYRUPS

By PHILIP P. GRAY (Wallerstein Laboratories, New York, N. Y.), *Associate Referee*

The last report of the Associate Referee[1] recommended that the rapid method for determination of fermentable extract in brewing sugars and syrups be adopted as tentative. Since that time further collaborative studies of the method have been carried out by the subcommittee on sugars and dextrins of the American Society of Brewing Chemists under the chairmanship of the Associate Referee. The results of these collaborative studies were presented at the meeting of the A.S.B.C. in May 1945 and are embodied in the 1945 report of the Associate Referee on Brewing Sugars and Syrups[2] which recommended that both the regular method and the rapid method for determining Fermentable Extract be adopted as

[1] Gray, Philip P., *This Journal,* **28,** 443 (1945).
[2] Laufer, Stephen, *ibid.*, **29,** 285 (1946).

official-first action. The 1946 report of the Associate Referee on Brewing Sugars and Syrups[3] recommended further that both methods be adopted as official, final action.

It is therefore recommended* that further work on Fermentable Extract in Brewing Sugars and Syrups be discontinued.

REPORT ON INORGANIC ELEMENTS IN BEER

By G. H. BENDIX (Continental Can Company, Inc., Chicago, Ill.),
Associate Referee

IRON

Collaborative work on the methods for the determination of iron in beer has not been carried out since 1941 at which time reports were submitted by L. E. Clifcorn (3) describing a procedure involving the wet ashing of 25 grams of beer with nitric and perchloric acids, after which the red thiocyanate complex was developed in an acid methylcellosolve medium. In 1941 Clifcorn recommended this procedure be adopted as tentative.

In 1945 Bendix reported on the results of a survey which indicated a definite trend away from the use of thiocyanate for the determination of traces of iron. A preference for wet ashing techniques in place of dry ashing was also reported, and in addition it was mentioned that the determination of iron in beer had been carried out by Gray and Stone (4) without destruction of organic matter.

The method of Gray and Stone, which used $\alpha\alpha'$ dipyridyl and dispensed with the ashing step, was exceedingly simple and rapid, but it did not immediately receive general acceptance, probably because in 1938 photoelectric colorimetry was in its infancy. Gray and Stone reported that iron in beer exists in the ferrous state, and since dipyridyl responds only to ferrous iron, they were able to use it directly without an ashing treatment.

In 1946 Nissen (5) and Bendix (1) reported to the American Society of Brewing Chemists results obtained by a direct method developed by Nissen using ortho-phenanthroline. Nissen's method also differs from that of Gray and Stone in that hydroxylamine is added to assure iron in the ferrous condition. The method employs photoelectric colorimetry.

Tables 1 and 2 show the results as taken from Nissen's report (5). It may be noted that excellent agreement is obtained both in the case of comparison to the ashing KCNS method and in the case of recoveries of added iron.

The Nissen method, with slight modifications, has been used extensively

[3] *Ibid.*, 30, 214 (1947).
* For report of Subcommittee D and action by the Association, see *This Journal*, 31, 56 (1948).

by the Associate Referee's laboratory and by others,* with complete confidence and satisfaction.

COPPER

In 1945 Bendix (2) reported on collaborative work describing the application of an all-dithizone method for the determination of copper in beer.

TABLE 1.—*Comparison of iron methods*

SAMPLES OF BEER		IRON IN P.P.M.	
NO.	RANGE—(Fe P.P.M.)	DIRECT ORTHOPHENANTHROLINE METHOD	ASHING KCNS METHOD
56 Samples	0.00–0.03	0.02	0.024
4 Samples	0.04–0.06	0.05	0.05
5 Samples	0.07–0.10	0.09	0.07
6 Samples	0.11–0.16	0.12	0.11
3 Samples	0.24–0.26	0.25	0.24
2 Samples	0.32–0.34	0.33	0.27
		0.44	0.35
		0.56	0.51
		0.59	0.53
		0.63	0.63
		0.76	0.74
		0.80	0.76
		0.86	0.88
		1.03	1.05

TABLE 2.—*Recoveries of added iron*

SAMPLE	IRON ADDED, P.P.M.	IRON FOUND P.P.M.	IRON RECOVERED, P.P.M.	IRON RECOVERED, PER CENT
1		0.03		
2	0.05	0.08	0.05	100
3	0.10	0.12	0.09	90
4	0.20	0.24	0.21	105
5	0.40	0.44	0.41	102
6	0.50	0.53	0.50	100
Ave.				99.5

While this method gave results which were not unsatisfactory, several improvements in all-dithizone methods have been devised and will be studied collaboratively by the Referee on metals in foods. The general procedure developed in this work should also be applicable to beer.

* Private Communication from the American Can Company.

TIN

In the Referee's opinion no methods are available for the determination of tin in beer, which offer satisfactory promise for use as an A.O.A.C. referee method. Investigative work involving the use of the polarograph for this determination has been carried out in the Referee's laboratory, but at the present time progress has not been sufficient to warrant collaborative work on a polarographic method.

RECOMMENDATIONS*

It is recommended—

(1) That the direct (non-ashing) orthophenanthroline method be submitted for collaborative work.

(2) That collaborative work on the determination of copper be postponed pending the outcome of proposed work by the Referee on metals in foods.

(3) That no collaborative work be done on the determination of tin in beer until the development of a more promising method.

LITERATURE CITED

(1) BENDIX, G. H., and STRODTZ, N. H., Proceedings of the Eleventh Annual Meeting of the American Society of Brewing Chemists, p. 37.
(2) BENDIX, G. H., *This Journal*, **25**, 456 (1945).
(3) CLIFCORN, L. E., *Ibid.*, **25**, 277 (1942).
(4) GRAY, PHILIP, and STONE, IRWIN, *Ind. Eng. Chem., Anal. Ed.*, **10**, 415 (1938).
(5) NISSEN, B. H., Proceedings of the Eleventh Annual Meeting of the American Society of Brewing Chemists, p. 32.

No report was made on brewing sugars, sirups, wort, spent grains, and yeast.

REPORT ON SOLIDS IN YEAST

By ROBERT I. TENNEY (Wahl-Henius Institute, Chicago, Ill.),
Associate Referee

Inaccuracies in reporting upon the solids content of yeast samples, especially those representing yeast removed from breweries, present several serious problems. Naturally, the financial consideration between the brewer and the processor is affected; but, more important, these figures are entered upon the brewers' records which are inspected from time to time by representatives of the Alcohol Tax Unit of the Treasury Department. A.T.U. Regulations (1) require that yeast removed from breweries shall contain not less than 15 per cent of solids. Furthermore, yeast slurries

* For report of Subcommittee D, and action by the Association, see *This Journal*, 31, 56 (1948).

containing more than 12 per cent of solids are frequently too viscous to transfer through pipe lines and hose properly. The obvious practical solution is to dilute with water, and this is done under conditions which seriously complicate the taking of truly representative samples. Any method proposed by this Society should recognize these difficulties and strive to yield results which can be reasonably duplicated by two or more laboratories that might be participating in any controversy arising about a yeast shipment. The Associate Referee was charged with continuing the investigation of methods for both total solids in yeast and for yeast solids.

The method currently given tentative status as **14.115**, for total solids, was adopted at the 1944 fall meeting and is one which has been in use by the American Society of Brewing Chemists since 1942. It appeared in that Society's published methods (2) after sufficient collaborative data had been obtained to justify its inclusion. Since that time collaborative study has been continued by members of the A.O.A.C. and the A.S.B.C. A portion of this work has been directed toward other methods and a portion toward the variations noted between laboratories.

The Associate Referee is greatly indebted to Fred A. Wilcox, of Wahl-Henius Institute, who as chairman of the Yeast-Analytical Committee within the American Society of Brewing Chemists, made much data available. He is also indebted to the following collaborators and conferees, whose cooperation is gratefully acknowledged.

1. Carroll A. Dayharsh, Jacob Ruppert Brewery, New York, N. Y.
2. Clarence Estes, Anheuser-Busch, Inc., St. Louis, Mo.
3. Stephen Laufer, Schwarz Laboratories, New York, N. Y.
4. George W. Kirby, Fleischman Laboratories, New York, N. Y.
5. Eugene V. Nay, St. Louis Brewers Yeast Corp., St. Louis, Mo.
6. Joern Olshausen, J. E. Siebel Sons Company, Chicago, Ill.
7. F. O. Rickers, F. & M. Schaefer Brewing Co., Brooklyn, N. Y.

Some of the variations noted in previous collaborative work have been thought to be due to autolysis of the samples. To avoid this possibility and yet permit comparison of the method between widely separated laboratories, a large sample was divided and the separate portions autoclaved at 15 pounds for 30 minutes. Results obtained by five laboratories using method **14.115** are shown in Table 1.

From this table, the method appears capable of giving close agreement between laboratories. The maximum deviation between different laboratories in this case is equal to the greatest difference in duplicates for one collaborator.

Earlier collaborative attempts between widely separated laboratories did not give as consistent results. While a part of this disagreement may be blamed upon lack of familiarity with the new procedure and failure to follow it precisely, it is now thought that sampling had as great an influence as any other factor.

Yeast slurries must be recognized as difficult materials to sample. They settle out fairly rapidly and chemical changes due to continued enzyme action are bound to occur in transit unless special precautions are taken. The significance of autolysis as it affects both total solids and yeast solids is currently being studied by a committee of the American Society of Brewing Chemists.

Carroll Dayharsh has proposed a toluene distillation type of procedure for determining total solids in yeast and has submitted a good many figures to the Associate Referee in support of his views. The speed with which such an analysis may be conducted is certainly a point in its favor, though results differing from those of the tentative A.O.A.C. method may be anticipated. In general, it may be said to agree more closely to those

TABLE 1.—*Total solids in autoclaved yeast slurry*

COLLABORATOR	RESULTS		DIFFERENCE	AVERAGE
	Per cent	*Per cent*	*Per cent*	*Per cent*
I	22.2	22.1	0.1	22.15
II	23.1	22.9	0.2	23.0
III	22.9	22.4	0.5	22.65
IV	22.3		—	22.3
V	22.5		—	22.5

Lowest result, 22.1%, high 23.1%
Overall average, 22.5%
Maximum deviation, 0.5%

methods in which some of the solubles are washed from the yeast with water before drying. The method has not yet been subjected to collaborative study, but it will be included in future A.S.B.C. work.

The situation with respect to yeast solids is entirely different. Here we approach a question of definition not unlike that involved in crude fiber determinations. The Associate Referee holds that total solids should, by definition, include all nonvolatile substances in the slurry except obvious extraneous matter known to be not representative of the whole. Any method which washes the yeast before drying is an attempt to remove a portion of these total solids, and hence is an approach to yeast solids.

To date, no method of washing out a portion of the slurry selectively has proven capable of interlaboratory agreement. The report to the American Society of Brewing Chemists last May (3) pointed out that the sediment from a brewery fermenter commonly termed yeast consisted of a mixture of unbroken yeast cells, broken yeast cells with contents partially or totally spilled, beer, hop resins, separated proteins, and gums. The question arises, "How much of this mixture is yeast?" The effects of several solvents was compared but no suggested method was developed.

The same collaborators shown in Table 1 also determined yeast solids by a water washing technique on the autoclaved yeast. The results varied from a low of 7.839 per cent to a high of 16.9 per cent although interlaboratory variations did not exceed 0.4 per cent. Therefore a satisfactory procedure for determining yeast solids is as lacking as is a definition to which all will agree.

RECOMMENDATIONS*

In view of the described situations it is recommended:

(1) That method **14.115** for total solids in yeast be continued in tentative status pending the outcome of further study.

(2) That the influence of autolysis upon this determination be studied in detail.

(3) That a non-miscible solvent distillation method be studied as a possible alternate.

(4) That study be continued seeking a definition and method for determination of yeast solids.

(5) That the attention of laboratories be called to the necessity of taking truly representative samples from yeast slurries by inclusion in the method of a paragraph substantially as follows:

SAMPLING

Yeast slurries tend to settle rather rapidly and are frequently quite viscous. These facts must be kept in mind when drawing samples from tanks and other large containers. While satisfactory aliquots may be obtained for laboratory purposes from small samples after thorough agitation mere agitation is not usually sufficient to insure a uniform mix in a tank or vat. In taking the sample a device which will permit samples to be drawn from several portions and depths of the tank should be employed. A satisfactory device is a watertight trier such as is used in grain sampling. Or a continuous sampling device may be attached to a hose or pipe line used in transferring the yeast from tank to transport car or truck tank and this composite thoroughly mixed before a smaller sample is withdrawn.

REFERENCES

(1) Article 21, Section 192. 149, Regulation 18, Alcohol Tax Unit, Treasury Department (1940).
(2) *Book of Methods*, Yeast I. American Society of Brewing Chemists (1944).
(3) TENNEY, *et al.*, *Proceedings Annual Meeting*, A.S.B.C., 1947.

No reports were given on beer, or on acidity and *p*H of beer.

No reports were given on color and turbidity in beer, or carbon dioxide in beer.

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* For report of Subcommittee D and action by the Association, see *This Journal*, 31, 56 (1948).

REPORT ON CARAMEL IN WINES AND DISTILLED LIQUORS

By PETER VALAER (Bureau of Internal Revenue, Washington 25, D. C.), *Associate Referee*

Chemical work was initiated during the year 1945 for the purpose of improving the accuracy and making more concise the Chemical Methods for the Qualitative Detection of Caramel in wines and in spirituous liquors. The new procedures which will be set forth in detail below are applicable for the qualitative indication of caramel in any fluid substance, aqueous or alcoholic.

The Milos test designed for the qualitative determination of caramel is at present an A.O.A.C. official method, made so after the report of the collaborators and recommended by the Associate Referee and presented at the annual A.O.A.C. meeting in October, 1944[1] and at the same time a supporting test was adopted which became "Confirmatory test-tentative." Reference was also made in Chapter 14 on "Malt Beverages" etc., to "Caramel," Milos test—official, p. 158. In the same report it was recommended that cyclohexanol test be also accepted, and it is now included with the Marsh test in Chapter 16 "Distilled Liquors." All these appeared for the first time in the A.O.A.C. Methods of Analysis, 6th Edition, 1945. Although the Milos procedure, as it appears in the above volume (page 188), and its attendant "confirmatory-tentative," has given useful service, the collaborators and the Associate Referee feel, however, that the Milos test could be advantageously replaced by a shorter and still more conclusive method, accompanied by one good confirmatory test for caramel, instead of the present number of small tests.

This new proposed method devised by Alex P. Mathers, Bureau of Internal Revenue, Washington, D. C., and referred to as the "Mather's Test," is recommended as an official substitute for the present Milos test, to be sustained by the present confirmatory-tentative (p. 189), this last being only slightly changed. The additional tests, such as paraldehyde and the others which accompany this confirmatory test, are omitted in favor of the test using 2, 4-dinitrophenylhydrazine reagent.

The details of the methods are described in *This Journal*, **31**, 76, 1948, under "Changes in Methods of Analysis."

These changes have been justified after a great deal of analytical work during the past two years which tended to prove that the new procedures are more conclusive, and saving in time and page coverage; and it is the consensus of opinion of the collaborators that they are, on the whole, better than those caramel methods which now appear in the 6th Edition (1945) of Methods of Analysis.

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[1] *This Journal*, **28**, 467 (1945).

In order to make a beginning for this investigation and to support any recommendations made for the proposed tests with collaborative results by as many chemists as possible, in 1946 and 1947, eight large bottles each containing several liters of wine and whiskey were prepared and analyzed as thoroughly as possible with existing caramel methods, the basic material in each case being caramel free. Into two of these batches one of wine and one of whiskey was incorporated a small amount of caramel coloring, the color of the basic material due to natural ingredients was much darker than the color produced by the caramel used.

Each collaborator or collaborating laboratory was sent a 4 fluid-ounce sample of each of the eight prepared batches consisting of four wines and four whiskies.

The letter of transmittal accompanying the eight samples sent to each collaborator requested that the samples be analyzed for caramel coloring and that report be made as to which method was considered best.

Suggestions and comments were requested as to how the methods may be improved and how these methods compare with other methods used for the detection of the presence of caramel coloring matter in beverage products.

The tests described in this report have been thoroughly tried out with all the difficult coloring substances encountered over several years, including those which interfere with the usual caramel tests and those which might possibly be mistaken for caramel.

Key to Unknown Samples

Sample		
1	W-1	*Sample W-1* consisted of odds and ends of old commercial blackberry wines and some other old fruit wines, to which was added a small amount of commercial caramel coloring.
2	W-2	*Sample W-2* consisted of old darkened miscellaneous fruit wines sweetened with very old and dark grape concentrate, to which was added very dark pokeberry juice and very darkest brown sugar (raw).
3	W-3	*Sample W-3* consisted of very old plum wine which had become almost black with age.
4	W-4	*Sample W-4* consisted of old plum wine, very old Elderberry wine, molasses, and the darkest specimens of raw Cuban sugar.
5	S-1	*Sample S-1* consisted of straight aged whiskey, whose natural color was heavily fortified with toasted white oak chips.
6	S-2	*Sample S-2* consisted of straight-aged whiskey, heavily colored with a strong hydro-alcoholic infusion of white oak chips.
7	S-3	*Sample S-3* consisted of straight aged whiskey, further colored with an infusion of uncharred chips and a small amount of caramel.
8	S-4	*Sample S-4* consisted of straight aged whiskey, further colored with an infusion of charred chips, uncharred chips, molasses, brown (raw) sugar, and an infusion of peat-dried barley malt.

All of the above samples gave positive indications with Marsh and Williams reagents, although only 2 actually contained caramel.

COLLABORATORS

The following chemists collaborated in this investigation:

Romig, Milos, and others, in Alcohol-Tax, New York Laboratory.
Riley and Watson, in Alcohol-Tax, Louisville, Laboratory.
Quillen in Alcohol-Tax, Baltimore Laboratory.
Fonner, Carr, and others in Alcohol-Tax, Chicago Laboratory.
Nealon and Forrest, in Alcohol-Tax, Detroit Laboratory.
Mallory, Love, and others, in Alcohol-Tax, San Francisco Laboratory.
Holman in Alcohol-Tax, Atlanta Laboratory.
Ringstrom in Alcohol-Tax, Seattle Laboratory.
Morawski and others, in Alcohol-Tax, Boston Laboratory.
Mathers, Blaisdell, Burritt, and Valaer, in Alcohol-Tax, Washington Laboratory.
John Wilson, Washington, Food and Drug Laboratory.
Loughrey, Boston, Food and Drug Laboratory.

Since all the collaborators found caramel in W-1 (wine) and in S-3 (spirits) and did not find caramel in any of the six other samples by the two proposed methods, it is pointless to present their findings in any usual tabulated form. Other tests were made by other methods and excesses were reported, which is not surprising when we consider the unusually difficult interfering substances that were incorporated in these unknown samples.

COMMENTS OF COLLABORATORS

Although opinions and comments were requested a number of collaborators merely reported without further statements; however some of them commented to the following extent:

Nealon and Forrest, stated that "both methods were rapid and excellent for the separation of caramel coloring matter from wine and spirits; there seems to be little, if any, foreign matter precipitated with the caramel, which renders the confirmatory tests more positive. However, since the Mathers test is somewhat more rapid and neutralization of the sample is unnecessary, it is to be preferred over the A.O.A.C. Method."

Blakely and Clapp were of the opinion that a stronger indication of caramel was obtained with Mathers test than with the A.O.A.C. tentative-confirmatory; there was some objection expressed to the washing required by the latter.

Fonner and Carr found "both methods rapid and simple, with the A.O.A.C. procedure satisfactory except requiring more washings."

Ringstrom found "both methods worked very well on the samples and both gave clean clear-cut results; however the Mathers method required less washing than the A.O.A.C. method to remove natural coloring from the wine samples, and consequently it is the shortest good method for determining the presence of caramel coloring."

J. H. Loughrey "liked the $Zn(OH)_2$ precipitation; even though it brings down only a small % (about 14%) of the caramel. I particularly prefer the 2,4-di-nitro-phenylhydrazine as the precipitating test; it is much easier to prepare and keep in solution; the precipitate with caramel is quite definite."

Quillen stated, "I am very much impressed with the Mathers method, especially its simplicity, the ease and rapidity with which the test can be performed."

Morawski and Forbes were of the opinion that "the Mathers and A.O.A.C. methods are equally efficient. Many tests were carried out with both methods taking Bottled in Bond whiskey of a tintometer reading of 16, and adding caramel to portions, thus increasing the total color to 17 and 18. This was done in order to satisfy ourselves as to the sensitiveness of the methods." "Both methods are good but we are inclined to use the Mathers test because of the smoother operation and less time involved to complete the tests."

Holman, besides analyzing the samples, made other experiments by adding caramel to various samples and "in each instance the results were very satisfactory."

Riley and Watson, "In the Mathers test, instructions call for the addition of 50 ml or more of alcohol to the 10 ml sample and to the residue dissolved in 10 ml of water. The Babcock bottles in the District laboratory have a total capacity of 50 ml, which would permit the addition of only 40 ml of alcohol. Approximately the same alcoholic content could be obtained by dissolving the residue in the centrifuge bottle in 8 ml of water, instead of 10 ml, and adding about 40 ml of alcohol, to make a total volume of not over 50 ml. (So far we have found the Babcock cream bottle satisfactory; a larger centrifuge bottle is sometimes used.)

Mathers method states: "Repeat this process until the upper alcoholic layer is quite clear and colorless." Does this mean completely or entirely clear and free from all color and opalescence? (The upper solution should be without color—opalescence is of no consequence.)

On some samples the gelatinous residue by the Mathers method was not firmly held in the centrifuge bottle and would break loose when the supernatant liquid was decanted. Better results were obtained by inserting a small glass tube into the centrifuge bottle and removing most of the supernatant liquid by a slight vacuum or siphon action then decanting the small amount of supernatant liquid which remains. The failure to obtain a firmly packed residue in all cases may be due to inadequate speed of the centrifuge employed.

In the A.O.A.C. confirmatory test considerable KOH was required to neutralize sample W-3, which left little room for reagent and alcohol in the centrifuge bottle. Use of a stronger KOH solution on samples containing considerable acidity would reduce the final volume. Some difficulty was experienced in dissolving the residue first obtained in the A.O.A.C. confirmatory method. Very hot water and heating in boiling water plus shaking was required.

The Mathers test required less time than the A.O.A.C. confirmatory test, because less washing was required; there is no difficulty in dissolving the residue and it is not necessary to neutralize the sample before starting the test."

Practically all the collaborators believed that the new reagent (2,4-dinitro-phenyl-hydrazine) was more satisfactory in operation than the older phenyl-hydrazine reagent (p. 189) and find this new reagent much more stable, keeping apparently indefinitely.

The unusual number of chemists that participated in this collaborative investigation were practically all of the opinion that the Mathers method was one of the quickest and best methods yet devised for caramel detection, and when sustained by the present confirmatory test tentative (p. 189), and the final caramel solutions from each method confirmed by the 2,4-dinitro-phenyl-hydrazine reagent, it would produce one of the strongest combinations of tests for caramel so far obtained.

In Chapter 16 on "Distilled Liquors," the Marsh test-tentative for artificial color **(16.38)** has been an A.O.A.C. procedure for a number of years and has been quite useful in that it shows the presence of artificial color, caramel, sometimes coal tar, and other coloring matter.

The official modified Marsh test **16.39** (8) was originally designed to determine whether a positive reaction given by Marsh reagent was caramel or was given by the presence of coloring matter from raw or untoasted white oak chips, or due to long storage in plain uncharred barrels. This can now be replaced by the simpler test, cyclohexanol reagent, and Fulton's reagent, and the Mathers test or the A.O.A.C. tentative-confirmatory, or all of them, to make assurance doubly sure.

A cross reference to these last two tests would be sufficient in the Distilled Spirits chapter in the same manner as the Milos test-official is referred to in the 6th Edition, p. 202.

It is best, in applying the Mathers test to lightly colored spirituous liquors such as imported blended Scotch whiskies, to use 50 ml of the sample and to reduce its volume to about 10 to 12 ml on a steam bath, hastened by aid of a current of air (electric fan) and filter it; and on this approximately 10 ml continue with the Mathers or A.O.A.C. confirmatory tests, as outlined above.

The Cyclo-hexanol test-tentative **(16.40),** has proved its worth and should be retained; but the Fulton test, which up to this time has not sought entrance into the A.O.A.C. Methods of Analysis should be placed next to the cyclohexanol test as a supporting or an extra check.

The Fulton test was devised principally for the same purpose as the cyclohexanol test, to distinguish between caramel and uncharred white oak color in distilled spirits, since uncharred white oak gives a color in the lower layer of the Marsh test and the Williams test.

The Williams test, extensively used by A.T.U. Junior inspectors, was devised by John F. Williams (U.S. Customs service) and designed to show not only the presence of artificial color in commercial spirituous liquors, but also gives a fairly accurate determination of proof. The Williams reagent consists of 70 parts of normal amyl alcohol (Pentasol); 28 parts of toluol; and 2 parts of dilute HCl (conc. HCl dil. 1 to 10). In testing for color in spirits it is used in the same manner as the Marsh reagent.

It is true that at present not a great deal of spirituous liquor is colored or "quick-aged" with uncharred chips. However, blended Scotch whiskey, Scotch grain spirits, and Scotch malt whiskey is usually long aged in plain barrels, if the barrels are previously unused or not much used, more or less color will be extracted by the whiskey over the traditionally long years of storage, some of which will show up in the lower layer of Marsh and Williams reagent but not in the lower layers of Cyclohexanol-tentative test **(16.40,** p. 202), or in the Fulton reagent, which is herewith proposed as an additional test for caramel and other coloring matter.

Caramel is added to Scotch whiskey only when it is necessary to produce a certain standard depth of color, for the sake of uniformity of this product.

The formula for the Fulton's Acetone Reagent is as follows:

FULTON TEST

REAGENTS

(a) *Acetone reagent.*—Acetone, 500 ml; amyl alcohol, 200 ml; ethyl acetate, 200 ml; syrupy H_3PO_4, 50 ml; water, 50 ml.
(b) Sodium di-hydrogen phosphate.—NaH_2PO_4, 25 g; water, 100 ml.

DETERMINATION

To ca 5 ml of spirits add 10 ml of the acetone reagent and 3 ml of the NaH_2PO_4 soln, shake and let stand. Color in the lower layer indicates caramel.

This reagent does not register a positive test with vegetable matter, uncharred chip color, charred or toasted chip color; but with some coal tar dyes occasionally used for coloring whiskey there may be a weak positive indication.

It has been found that Mathers test is better for some kinds of samples and the tentative-confirmatory test gives better results for other kinds of material, so it is recommended that both be applied to any sample in question, followed by the 2, 4-di-nitro-phenyl-hydrazine reagent confirmatory on any final brown solution obtained in both tests.

Substances like very old fruit wines which are unusually difficult to remove can be conclusively diagnosed by taking a much larger sample and condensing it to 10 ml or less. A large quantity of the sample does not increase the indication; except that when caramel is present the strength of the test is correspondingly increased with the amount of the sample taken.

RECOMMENDATIONS*

It is recommended—

Wines:

(1) That the official Milos test for caramel **(15.38)** be deleted, first action.

(2) That the Mathers test for caramel as described (*This Journal*, **31**, 76, 1948), be adopted as official, first action.

(3) That the tentative confirmatory test for caramel **(15.39)** be modified as described on page 77, *loc. cit.*

Distilled liquors:

(4) That method **16.46(b)** be changed as recommended in the Associate Referee's report.

(5) That the official modified Marsh test and the official Milos test, **16.39** and **16.41**, for caramel be deleted, first action.

* For report of Subcommittee D and action by the Association, see *This Journal*, **31**, 57 (1948).

(6) That the Fulton test for caramel, as described by the Associate Referee, be adopted as tentative for distilled liquors and for cordials and liqueurs.

(7) That the Mathers test for caramel, described in this year's report of the Associate Referee, be adopted as official, first action, for distilled liquors.

(8) That the Mathers test for caramel, as described by the Associate Referee, be adopted as official, first action, for cordials and liqueurs, and that it be included by reference in the chapter on cordials and liqueurs in sec. **16.60,** in place of the modified Marsh test, **16.39,** and the Milos test, **15.38.**

REPORT ON FUSEL OIL IN DISTILLED SPIRITS

By G. F. BEYER (Bureau of Internal Revenue, Washington 25, D. C.), *Associate Referee*

In view of the lengthy and time-consuming procedure of the fusel oil determination, experiments were made with a view toward shortening the method without interfering with its accuracy.

The procedure suggested is as follows:

To 50 ml of the sample add 10–12 ml of 0.5 *N* NaOH, add 60–70 ml dist. water and distil slowly until ca 75 ml have been received. Add ca 100 ml H_2O and saturate with NaCl. Ext. this salt soln 4 times with CCl_4, using 40, 30, 20, and 10 ml, respectively, and wash the CCl_4 3 times with sat. NaCl soln and twice with satd. Na_2SO_4 soln. Transfer the CCl_4 to Erlenmeyer flask containing 50 ml of the oxidizing soln and oxidize for 4 hrs. under a reflux condenser. Ground glass connections in all cases should be used and no grease.

Add 15–20 grains (granules) of #20 carborundum and 50 ml distd. water thru the condenser and distil until only ca 50 ml remains. Add 50 ml of H_2O and distil again until 35–50 ml are left.

Experiments have also shown that certain losses occur; that is, there is a 3–5 per cent loss in the CCl_4 up to the time of oxidation, and then it seems that a small amount of the fusel oil is washed out with the salt solution.

This procedure was carried out in parallel with that described in the *Methods of Analysis, A.O.A.C.,* and the results agreed with each other within the limits of experimental error.

In view of the results obtained, therefore, it is recommended* that a further study be made with a view toward shortening the method, and also of determining how to prevent the loss during the washing of the CCl_4 with saturated salt solution.

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* For report of Subcommittee D and action by the Association, see *This Journal,* 31, 57 (1948).

No report was given on spectrophotometric examination of wines and distilled spirits or on chromatographic absorption of wines.

No report was given on methanol in wines and distilled liquors.

REPORT ON CORDIALS AND LIQUEURS

By JOHN B. WILSON (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

The composition of various cordials and liqueurs varies widely. As defined by the Federal Alcohol Administration such products are "obtained by mixing or redistilling neutral spirits, brandy, gin, or other distilled spirits with or over fruits, flowers, plants, or pure juices therefrom, or other natural flavoring materials, or with extracts derived from infusions, percolations, or maceration of such materials, and to which sugar or dextrose or both have been added in an amount not less than 2½ per cent by weight of the finished product. Synthetic or imitation flavoring materials shall not be included." (Regulations 5, Art. II, Sec. 21, Class 6, page 8).

Alcohol in such products may vary from about 25 to 70 per cent and the sugar content from the lower limit of 2.5 per cent imposed under the law, to 50 per cent or even more, as found in "Surfine" French liqueurs.

It cannot be assumed that the coefficient of expansion of such mixtures is the same as that of a water solution containing the same relative volume of alcohol, and therefore it becomes obligatory to measure the sample at 15.56°C. and to make up the distillate to volume at 15.56°C., in order to obtain the correct percentage of alcohol by volume in cordials, unless the solids content approximates the minimum of 2.5 per cent.

RECOMMENDATIONS*

It is recommended:

(1) That **16.46(b)** (p. 203, *Methods of Analysis*, 1945) be changed to read as follows:

16.46(b) By Volume.—Proceed as directed under **16.6** except calibrate pycnometer used at 15.56°C. and measure sample at that temperature.

(2) That the study of cordials and liqueurs be continued.

REPORT ON FRUITS AND FRUIT PRODUCTS

By R. A. OSBORN (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Referee*

RECOMMENDATIONS†

It is recommended:

(1) That work on methods for determining fruit and sugar content in frozen dessert fruits be continued.

* For report of Subcommittee D and action by the Association, see *This Journal*, 31, 57 (1948).
† For report of Subcommittee D, and action by the Association, see *This Journal*, 31, 61 (1948).

(2) That further work be done on methods for the electrometric titration of acidity.

(3) That further collaborative study be made of methods of separating and determining fruit acids.

(4) That work on methods for the determination of water-insoluble solids be continued.

No reports were given on titration of acids, fruit acids, fruit and sugar in frozen fruits, or on water-insoluble solids.

REPORT ON CACAO PRODUCTS

By W. O. WINKLER (Food and Drug Administration, Federal Security Agency, Washington, D. C.) *Referee*

METHODS OF ANALYSIS

There are six projects in the analysis of cacao products which are being studied at the present time. These studies are on lecithin, maltose, lactose, cacao ingredients, pectic acid, and fat in refractory materials.

Lecithin.—A formal report was not made this year, but the Associate Referee made some comments. Last year collaborative work was carried on by the Associate Referee on a method proposed by him. Results were low, about 80 per cent in most cases. Supposing that the old lecithin was at fault, he bought a new supply this year. After careful mixing with chocolate at 40°C., the best recovery he could obtain was 81 per cent. Consequently, no samples were sent out this year. This type of problem has vexed us ever since the beginning of the work on lecithin. It has been reported that lecithin forms a loose combination with proteins, and this may be the reason for the difficulty in recovery.

Maltose and lactose in presence of other reducing sugars.—The Associate Referees on these materials are not ready to make formal reports but the Referee has knowledge that a considerable amount of spade work has been done on these projects.

Fat in refractory materials.—A report was submitted by the Associate Referee on this subject. He has modified the method suggested by Miss M. L. Offutt, the former Associate Referee, to use a Palkin extractor after the preliminary treatment, and has obtained very promising results by two collaborators. Further collaborative work is recommended.

Cacao Ingredient.—The present tentative method for estimating the cacao ingredient is based on the residue left after extracting fat, milk, and sugar solids with various solvents. The method is applicable to present standard cacao products, but where products contain other ingredients such as starch or cereal solids the method is not applicable. The constituents which are most characteristic of cacao are theobromine, cacao red,

tannins, and modified tannins or phlobaphenes. The Referee has made a study to devise a method for determining the tannins, phlobaphenes, and most of the cacao red together; and a method has been developed which he believes is applicable to all types of products and the quantities of material determined are a considerable portion of the fat-free cacao. The details of the method proposed as a tentative procedure are published in "Tannins and Pigments," *This Journal* No. **31**, page 78 (1948). Preliminary results are given in Table 1.

TABLE 1.—*Per cent of cacao constituents*

Special reagent—40% formaldehyde solution

NO.	KIND OF COCAO BEAN	TANNIN AND PIGMENT PRECIPITATE
Sample		*per cent**
(1)	Java	13.6
(2)	Unknown blend	13.38
(3)	⅔ Accra; ⅓ Bahia	12.25

* Percentage based on dry fat-free cacao.

Pectic Acid.—In the past some difficulty has been experienced in the filtration of the pectic acid precipitate. Even though the precipitate flocculated well and filtered well at first, it tended to become colloidal before washing was complete. This occurred at times regardless of the fact that saponification was made at temperatures below 20°C. The Referee has found that the washing can be done satisfactorily by using a 2 per cent salt solution in place of water and washing until free of acid. The precipitate is dried and weighed and is then ignited at about 600°C. in the muffle furnace, cooled, and reweighed. The salt is not affected at this temperature.

RECOMMENDATIONS*

It is recommended:

(1) That methods for the determination of lecithin in cacao products be further studied.

(2) That work on methods for maltose and lactose in the presence of other reducing sugars be continued.

(3) That the three procedures for fat in refractory beverage bases, described in the Associate Referee's report, be further studied.

(4) That further work be done on the determination of pectic acid in cacao products with particular reference to milk chocolate.

(5) That the method for tannins and pigments, as a means of estimating cacao ingredient, be adopted as tentative.

* For report of Subcommittee D and action by the Association, see *This Journal*, **31**, 57 (1948).

No reports were given on lecithin, malt solids, pectic acid, cacao ingredients, or lactose; see Referee report, p. 186.

REPORT ON FAT IN CACAO PRODUCTS

By CARL B. STONE, (Food and Drug Administration, Federal Security Agency, Cincinnati, Ohio), *Associate Referee*

Committee D recommended at the last meeting that the work on the determination of fat in beverage bases and other refractory materials be continued.

The work this year covered methods reported in the *Journal*[1] by Miss Offutt, and several others. The General Referee suggested that the beverage material be thoroughly ground with sand and then placed in a continuous fat extractor. This was tried with a sample of malted milk flavored with chocolate and extracted with mixed ethers for a period of 16 hours. The duplicate determinations gave results of 1.78 per cent and 1.23 per cent.

Using the same beverage base the two methods suggested by Miss Offutt were then tried with the following results:

No.	*Product*	*Hillig Method*	*Roese-Gottlieb Method*
		Per cent	*Per cent*
INV. 53-792 H	Chocolate Malted Milk	4.80	5.00
		5.25	5.04
			4.89

The Roese-Gottlieb method had a tendency to form an emulsion causing a difficult separation.

In view of this, the author thought it might be well to try using a Palkin extraction apparatus for the final removal of the fat. The Roese-Gottlieb method was used up to the point where the sample was transferred to an extraction set-up in place of the large separator suggested in the other method. The following results were secured on sample 53–792 H, after complete extraction with the mixed ether. The extracted fats were dried and reextracted in order to purify the fat material:

Analyst	*Fat %*
C. B. Stone	4.84
	4.91
H. C. Van Dame	4.88

In view of the limited results that have been secured, it is recommended* that all three procedures be studied further on several refractory beverage bases.

[1] *This Journal*, **28**, 482 (1945).
* For report of Subcommittee D and action by the Association, see *This Journal*, **31**, 58 (1948).

REPORT ON SUGAR AND SUGAR PRODUCTS

By CARL F. SNYDER (National Bureau of Standards, Washington 25, D. C.), *Referee*

The Associate Referees for the following subjects have submitted reports which will be presented at this meeting: Unfermented Reducing Substances in Molasses, Confectionery, Reducing Sugars, and Color and Turbidity in Sugar Products. In regard to the recommendations made in these reports, the Referee concurs. It is regretted that circumstances have prevented the Associate Referees on Honey and on Corn Sugar and Corn Sirup from presenting reports, but it is hoped that work in both of these fields may be carried out during the coming year.

THE INTERNATIONAL SUGAR SCALE

The Associate Referee, Dr. F. W. Zerban, of the New York Sugar Trade Laboratory, has called attention to the existence of uncertainties in the official method for estimating sucrose by direct polarization, **34.18**, and to the desirability of this Association officially adopting the International Sugar Scale. The method in question, employed chiefly in the analysis of raw sugars, was first introduced into the *Methods of Analysis* in the second edition in 1925, and has been included in all subsequent editions. Most of the changes that have been made during this period were of an editorial nature.

The method consists of the detailed directions for the direct polarization of raw sugars adopted by the International Commission for Uniform Methods of Sugar Analysis at its third session in Paris, 1900.[1] Included in these directions was the manner of verifying the scale of the saccharimeter by means of a solution of pure sucrose containing 26 grams in 100 ml soln. and polarized in a 200 mm tube at 20°C. The 100° point on the sugar scale was fixed as the reading of this so-called normal sucrose solution.

Following this meeting of the International Commission, Herzfeld and Schönrock,[2] on the basis of this definition of the sugar scale, standardized a number of quartz control plates on the saccharimeter and then measured their optical rotation in circular degrees on a polarimeter using sodium light. They reported that a quartz plate which read 100° on the saccharimeter with white light filtered through the bichromate cell specified by the Commission read 34.657 circular degrees on a polarimeter illuminated by sodium light. This version of the saccharimeter scale, which for all practical purposes had its 100° point fixed by the reading of a standard quartz plate, has been referred to as the Herzfeld-Schönrock scale. This scale superceded the existing Ventzke scale, whose basis of standardization was so vague that it led to uncertainty in the fixing of the 100° point.

[1] *Z. Ver deut. Zucker-Ind.*, **50** (N. F. 37), 357 (1900); **63** (*N. F.* 50), 25 (1913); *J. Ind. Eng. Chem.* **5**, 167 (1913); *This Journal*, **18**, 162 (1935); National Bureau of Standards Circular C440, 1942, pp. 768, 774.
[2] *Z. Ver deut. Zucker-Ind.*, **50**, 826 (1900); **54**, 521 (1904).

An exhaustive study of the standardization of the saccharimeter scale was undertaken by Bates and Jackson,[3] whose preliminary report to the International Commission in 1912 showed evidence that the Herzfeld-Schönrock values were not quite correct. Their complete work, published in 1916, showed that the normal sucrose solution read 99.895 on instruments graduated on the basis of the Herzfeld-Schönrock values. This value was later verified by Balch and Hill[4] and by Zerban, Gamble, and Hardin,[4] who found values of 99.907 and 99.912, respectively. The average of the three determinations is 99.905. In addition to the saccharimetric values, Bates and Jackson determined the value for the optical rotation in circular degrees of the quartz control plate having a reading of 100 degrees on the saccharimeter.

The next meeting of the International Commission was held in 1932 in Amsterdam.[5] At this session the Commission adopted a standard scale for the saccharimeter, designating it the "International Sugar Scale" and the readings thereon as degrees sugar (°S). The rotation of the normal sucrose solution under the conditions defined by the Commission in 1900 was adopted as the primary basis of calibration of the 100° point on the scale, accepting the value 99.90° as the equivalent value on the Herzfeld-Schönrock scale. In addition, the following values were adopted, the normal quartz plate which reads 100°S on the saccharimeter has a rotation of 40.690 circular degrees for mercury light of wave length $\lambda = 5461$A and a rotation of 34.620 circular degrees for sodium light of wave length $\lambda = 5892.5$A.

This action of the Commission virtually eliminated the uncertainty due to the existence of the several versions of the standardization of the sugar scale with the result that the new scale was accepted internationally. In view of these facts, it is essential that this Association officially adopt the International Sugar Scale, and a recommendation to that effect is presented.

Another question of importance considered at the Amsterdam meeting was the subject of the elimination of errors due to lead clarification in polarizing raw sugars. It was resolved to subject the matter to further study, using raw sugars from the various countries. The reports covering this collaborative work were presented at the ninth session in London in 1936.[6]

After a long discussion, the recommendation was adopted that in polarizing raw sugars on instruments calibrated on the International Sugar Scale clarification must be effected by dry basic lead acetate, the reagent being added after the sugar solution has been made to volume. In conformity with this it is recommended that official method **34.18** for raw sugars be amended accordingly.

[3] *N. Bur. Standards Sci. Paper* No. 268, p. 67 (1916).
[4] *This Journal*, **12**, 106 (1929).
[5] *Int. Sugar Jour.*, **35**, 19 (1933).
[6] *Ibid.*, **39**, 32S (1937).

In incorporating the above recommendations it is deemed advisable to combine in a separate section of the method the directions for the verification of the saccharimeter scale as well as the values for the normal quartz plate.

The recommended changes in Methods **34.18** and **34.19** are given in detail in *This Journal*, **31**, (1948), together with other corrections, under "Changes in Methods of Analysis," on pages 110 and 111.

RECOMMENDATIONS*

It is recommended—

(1) That the Java method (*This Journal*, **29**, 242) for unfermented reducing substances in molasses be adopted as tentative, and that work be continued.

(2) That the study for the determination of moisture be continued.

(3) That study be continued on tables of density of sucrose solution at various temperatures.

(4) That the Zerban and Martin values for refractive indices of dextrose and invert sugar solutions (*This Journal*, **30**, 77 (1947)), be subjected to collaborative study.

(5) That the study of the applicability of electro-deposition to the direct quantitative determination of dextrin in honey and honeydew honey be discontinued.

(6) That the study of the characteristic properties of dextrins of honey and honeydew honey be discontinued.

(7) That the official method for the determination of free acid in honey, **34.99**, be studied collaboratively with a view to establishing the end point more accurately.

(8) That study be made of methods for the detection of adulterants in honey, particularly commercial syrups.

(9) That collaborative study of the method described in last year's report of the Associate Referee, for the determination of resinous glaze in confectionery be continued, with special reference to large amounts of lac.

(10) That study be continued on the determination of dextrose, maltose, and dextrins, by copper reduction methods in pure sugar mixtures.

(11) That the tentative methods, **34.133–34.155**, inclusive, be subjected to collaborative study.

(12) That the procedures in N.B.S. Circular C440, pp. 342–334, for measurement of transmission of solutions of commercial sugar products, be considered with a view to their future adoption as tentative methods.

(13) That the Somogyi Modification (*J. Biol. Chem.*, V. 160, p. 61, 1945) of the tentative micro method for reducing sugars **(34.63)** be studied.

(14) That changes in accordance with the International Sugar Scale be adopted as official, final (special action) in sections **34.18** and **34.19.**

* For report of Subcommittee D and action of the Association, see *This Journal*, 31, 61 (1948).

REPORT ON UNFERMENTED REDUCING SUBSTANCES IN MOLASSES

By F. W. ZERBAN (New York Sugar Trade Laboratory, Inc., New York, N. Y.), *Associate Referee*

In a series of reports on this subject[1] it was shown that the two methods of the Association generally used for the determination of reducing sugars, namely, those of Munson and Walker, and of Quisumbing and Thomas, do not give reliable results when applied to the determination of unfermentable reducing substances in molasses. It was suspected that the discrepancies might be due at least partly to differences in the Fleischmann's baker's yeast secured locally by the collaborators in various cities. In 1945[2] it was decided to exclude this factor by using dried distillery slop for the determination of the reducing substances, instead of first fermenting molasses with yeast and making the determinations on the residue. In this study the Quisumbing and Thomas method again gave unsatisfactory results, but the copper reduction procedure prescribed in the method of the Java Sugar Experiment Station[3] proved so promising that the entire method, comprising fermentation and copper reduction, was applied in this year's work to molasses.

Six collaborators expressed willingness to take part in this work and one sample each of cane blackstrap and of refiner's sirup was sent to them, with the directions as given in the following method:

UNFERMENTED REDUCING SUBSTANCES IN MOLASSES

*Method of Java Sugar Experiment Station**

REAGENTS

(a) *Baker's yeast, free from starch.*—Fleischmann's, sold in packages of 1 lb. each by Standard Brands, Inc., was found suitable. It keeps fresh for a few days if kept in the refrigerator.

(b) *Neutral lead acetate soln.*—Dissolve 20 g $Pb(C_2H_3O_2)_2 \cdot 3H_2O$ in H_2O to 100 ml.

(c) *Anhydrous potassium oxalate.*

(d) *Soxhlet soln.* See section **34.33(a)** and **(b)**.

(e) *Potassium iodide soln.*—Dissolve 20 g KI in H_2O to 100 ml.

(f) *Dilute sulfuric acid.*—5 volumes of water plus 1 volume of conc. H_2SO_4.

(g) *Standard thiosulfate soln.*—0.1 *N*.—See sections **43.28** and **43.29.**

FERMENTATION

Transfer 12 g of blackstrap molasses (or 8 g High-test molasses) to a 500-ml volumetric flask, using in all 75 ml of H_2O. Add 25 g of coarsely chopped, fresh baker's yeast and mix thoroughly with the molasses soln. Close the flask with a stopper provided with a delivery tube the other end of which dips about 1 cm below the surface of H_2O in a beaker; or use any other type of fermentation trap. Place the flask in a water bath kept at 30°C. and allow to ferment for at least 4 hours, shaking the flask from time to time. When fermentation is complete dilute with

[1] *This Journal*, **23**, 562 (1940); **24**, 656 (1941); **26**, 112 (1943).
[2] *This Journal*, **29**, 242 (1946).
[3] "Methoden van Onderzoek," 6th ed. (1931), p. 365.
* "Methoden van Onderzoek bij de Java-Suikerindustrie," 6th ed. (1931), p. 365.

H_2O, clarify with 15 ml of neutral $Pb(C_2H_3O_2)_2$ soln, make to the mark at 20°C., add a teaspoonful of Filter-Cel, shake well and filter, discarding the first runnings. Delead the entire filtrate with ca 0.5 g of anhydrous $K_2C_2O_4$ and filter again with the aid of Filter-Cel. Test the filtrate for Pb. If necessary add more oxalate and refilter.

DETERMINATION

Transfer 25 ml of the final filtrate to a 250-ml Erlenmeyer flask, mix with 20 ml of combined Soxhlet soln, and wash the wall of the flask down with 5 ml of H_2O, making 50 ml in all. Add a few pieces of ignited pumice stone and place the flask on a wire gauze covered with an asbestos plate which has a hole in the center, slightly smaller than the bottom of the flask. As the source of heat use either a Bunsen burner or preferably an electric heater with temperature control. Heat to boiling in 3 min and boil gently for exactly 2 min longer. Close the flask immediately with a stopper provided with a Bunsen valve and cool quickly under the water tap to prevent reoxidation. Add 15 ml of KI soln and then 10 ml of dilute H_2SO_4. Titrate the liberated I at once with 0.1 *N* $Na_2S_2O_3$ soln, adding starch indicator toward the end of the titration.

Run a blank with 75 ml of H_2O instead of molasses soln, adding yeast, etc., as described. Deduct the titer of the sample from the titer of the blank, and find the mg invert sugar corresponding to the difference, from the table. The result, divided by 6 (in the case of High-Test molasses, by 4) gives directly the per cent of unfermentable reducing substances in the molasses, in terms of invert sugar.

Copper reduction, modified method (II).—In two other 25-ml aliquots of the final filtrate carry out the copper reduction exactly as described, but at the end of the 2-min boiling period filter at once thru a Gooch crucible with asbestos mat, or thru a fritted glass crucible, wash with hot water, and determine the copper in the precipitate, either by the thiosulfate method (*Methods of Analysis*, pp. 572, 573, sec. **34.41** and **34.42**), or by the permanganate method (*ibid.*, sec. **34.43** and **34.44**). Report the results of each experiment as milligrams metallic copper.

Milligrams of Invert Sugar Corresponding to Millimeters of 0.1 N *Thiosulfate*

0.1 *N* THIOSULFATE	INVERT SUGAR	0.1 *N* THIOSULFATE	INVERT SUGAR
ml	*mg*	*ml*	*mg*
1	3.2	14	47.3
2	6.4	15	50.8
3	9.7	16	54.3
4	13.0	17	58.0
5	16.4	18	61.8
6	19.8	19	65.5
7	23.2	20	69.4
8	26.5	21	73.3
9	29.9	22	77.2
10	33.4	23	81.2
11	36.8	24	85.2
12	40.3	25	89.2
13	43.8	—	—

Reports have been received from five of the six collaborators, and the results, expressed as per cent of equivalent invert sugar in the original product, are shown in Table 1.

TABLE 1.—*Per cent unfermented reducing substances, expressed as invert sugar*

In blackstrap and refiner's sirup

ANALYST	METHOD I		METHOD II	
	BLACKSTRAP	REFINER'S SIRUP	BLACKSTRAP	REFINER'S SIRUP
1. Sam Byall	4.73	4.28	4.84	4.49
New Orleans, La.	4.84	4.39	5.01	4.30
2. J. K. Dale,	4.54	3.92	4.33	3.92
Terre Haute, Ind.	4.47	3.92	4.31	4.07
3. Carl Erb,	4.54	3.99	4.82	4.00
New York, N. Y.	4.48	3.93	4.86	4.08
4. W. J. Hughes,	4.43	3.99	4.73	4.08
New York, N. Y.	4.49	3.99	4.86	4.00
		3.99	4.73	
5. D. J. Smith,	4.23	3.54	4.79	3.60
Boston, Mass.	4.28	3.57	4.80	3.62
	4.25	3.57		
Averages:				
1	4.79	4.34	4.93	4.40
2	4.51	3.92	4.32	4.00
3	4.51	3.96	4.84	4.04
4	4.46	3.99	4.77	4.04
5	4.25	3.57	4.80	3.61
Grand Averages	4.50	3.96	4.73	4.02

The deviations of the individual averages from the grand averages are shown in Table 2.

As in previous years, each individual collaborator checked his own results very closely. The agreement between the results of the different analysts by both variants of the Java method is of the same order as found previously with the same method. Considering the many manipulations required in carrying out the method, the agreement may be deemed satisfactory.

In both years Analyst 1 obtained in every case the highest results, and Analyst 5 in six cases out of eight the lowest results of all. These tendencies could in the previous series of analyses not be ascribed to differences in the yeast, since the analyses were made upon distillery slop. It is therefore improbable that the yeast used was responsible for the same tendencies observed this year. However, it appears advisable to settle this point definitely in future work, by arranging for the exchange of yeast samples among the collaborators.

TABLE 2.—*Deviations of individual averages from grand averages*

Per cent of invert sugar

ANALYST	METHOD I		METHOD II	
	BLACKSTRAP	REFINER'S SIRUP	BLACKSTRAP	REFINER'S SIRUP
1	+0.29	+0.38	+0.20	+0.38
2	+0.01	−0.04	−0.41	−0.02
3	+0.01	0.00	+0.11	+0.02
4	−0.05	+0.03	+0.04	+0.02
5	−0.25	−0.39	+0.07	−0.41
Average deviations	+0.06	+0.08	+0.08	+0.08
	−0.06	−0.09	−0.08	−0.09
	±0.12	±0.17	±0.16	±0.17
Maximum deviations	0.29	0.39	0.41	0.41

Method II gave on the average slightly higher results than Method I, and both averages and maximum deviations were slightly higher. Since in addition the modified method requires much more time, it offers no particular advantage over the original method.

RECOMMENDATIONS*

On the basis of the results obtained in 1945, and again this year, it is recommended:

(1) That the method of the Java Sugar Experiment Station for the determination of unfermented reducing substances in molasses be adopted as a tentative method.

(2) That the method be subjected to further collaborative study, with arrangements for the exchange of yeast samples among the collaborators.

No reports were made on drying methods, densimetric and refractometric methods, or honey.

REPORT ON CONFECTIONERY

By CHARLES A. WOOD (Food and Drug Administration, Federal Security Agency, New York, N. Y.), *Associate Referee*

In considering the method for lac[1] in candies, it occurred to the writer that removal of sugars, etc., from the impure lac residue thus obtained could be more easily accomplished than by the indicated washing and redrying.

* For report of Subcommittee D, and action by the Association, see *This Journal*, **31**, 109 (1948).

[1] *This Journal*, **30**, 289 (1946).

It was found that the impure lac residue could be dissolved in a mixture of isoamyl alcohol and benzol and this could be washed free of water soluble matter in a separatory funnel.

Recovery of known amounts of shellac seemed satisfactory, as shown in Table 1.

TABLE 1.—*Recovery of known amounts of lac*

TYPE OF MATERIAL	AMOUNT OF LAC ADDED	AMOUNT OF LAC RECOVERED
	gm	*gm*
200 g sucrose*	.208	0.216
50 g sugar candy†	.533	0.526

* Dissolved in amyl alcohol-benzol mixture with lac.
† Carried entirely through determination.

It appeared this modification was suitable, therefore the details were included in the method for collaborative testing, as follows:

METHOD FOR LAC IN CANDIES

Place 50 g of candy in a 400 ml beaker. Add 50 ml of a mixture of benzol and absolute alcohol (50% by volume) and cover with a watch-glass. Place on the steam bath, heat to boiling, and simmer for a few minutes, stirring occasionally. Decant the liquid into a tared round glass dish of about 100 ml capacity, having a flat bottom about 2¾ in diam. Repeat, using a similar mixture; and finally rinse twice with two 25 ml portions of absolute alcohol, simmering and stirring each rinsing liquid. With moist sugar candy, avoid overheating to prevent pieces from sticking together.

Add each liquid to the glass dish previously placed over the steam to evaporate the alcohol-benzol mixture. Allow to remain on bath until alcohol is just removed, rotating the dish as it goes to dryness in order to spread the extract uniformly over the bottom surface. Avoid baking the shellac on the dish. If fat appears to be present wash with 3–15 ml portions of petroleum benzene, stirring, and warming. Decant thru a rapid filter.

Add 50 ml of a mixture of 25 ml isoamyl alcohol and 25 ml benzol, rinsing any solid matter off and filter back into dish. Heat on steam bath with stirring, cool somewhat, and transfer the soln with suspended matter to a suitable (125 ml) separatory funnel. Rinse the dish with 25 ml water, adding it to the funnel; shake well, and reject the wash water. Repeat washing *two times* (or until washings are colorless) with water, rinsing the dish well around sides with the first portions of the liquid. Finally, filter the soln of the shellac into the tared dish, rinsing the separator and filter with a little absolute alcohol. Evaporate to dryness on the steam bath, rotating the dish on going to dryness to give a uniform film.

If much fat was extracted in original benzol extraction, wash the final shellac residue with 25 ml petroleum ether, warming and stirring. Decant, dry on steam bath and 100° oven, and weigh.

Three samples for collaborative testing were prepared.

Sample A—sugar candy with 0.912% lac added
Sample B—sugar candy with 0.456% lac added plus 8 ml almond oil to simulate conditions in marzipen-type candies
Sample C—commercial glazed chocolate-covered peanuts.

Results are shown in Table 2.

TABLE 2.—*Collaborators' results on lac in candy*

COLLABORATOR	LAC FOUND—PER CENT		
	A	B	C
J. L. Hogan, F&D Adm., N.Y.	0.92	0.47 0.47 0.47	.08 .09
C. A. Wood, F&D Adm., N.Y.	0.94 0.91	0.47 0.47	0.20 0.12
K. Breen, N.Y. State Lab., Albany	0.73 0.51	0.48 0.50	0.18 0.15

Results appear fairly good on samples B and C. In the higher range, at times, incomplete recovery is obtained. Possibly, the solubility limit in the isoamyl alcohol-benzol mixture is approached, and this should be further investigated.

It is recommended* that the method be further studied especially with reference to large amounts of lac.

REPORT ON REDUCING SUGARS

By EMMA J. MCDONALD (National Bureau of Standards, Washington 25, D. C.), *Associate Referee*

I. DENSITY AND REFRACTIVE INDICES OF LACTOSE SOLUTIONS

During the past year the Associate Referee has spent considerable time on densimetric and refractive index studies of lactose solutions. This disaccharide, commonly known as milk sugar, for many years has been of commercial importance in the pharmaceutical and in the baby food industries. During the past few years the demand for lactose has greatly increased because of its use in penicillium manufacture. It is expected that the density and refractive index tables which will soon be published will be of use to the investigator working with pure solutions, as well as to the analyst dealing with solutions in which the total solids may be expressed as lactose.

The lactose used in the investigation was prepared by recrystallization of the commercial product from aqueous solution. This was carried out at temperatures where only the lactose hydrate is formed. Lactose hydrate contains one molecule of water of crystallization, or 5 per cent. The water content of the samples used in the investigation was determined from time to time by drying a sample in a vacuum at 120°C. It was found that some decomposition took place, as indicated by a slight discoloring of the

* For report of Subcommittee D and action of the Association, see *This Journal*, 31, 61 (1948).

sugar. Comparative rates of drying were run at 130, 120, and 85°C. 120°C. was chosen as the optimum temperature, since there appeared to be less decomposition during the loss of water at this temperature. The importance of drying to a constant weight rather than for a given time should be emphasized, since the rates of drying of samples placed in the oven at the same time was not identical.

The solubility of lactose determines the range where accurate measurements of solution properties can be obtained. At 20°C. a saturated solution contains 16 per cent of lactose hydrate. We, therefore, carried out density and refractive index measurements with high precision in the range of unsaturation. The values that will be reported for supersaturated solutions, although not obtained with the same precision, are suitable for many purposes.

The density measurements were carried out in a 126 ml flask, whose volume had been accurately determined. All weighings were reduced to vacuum. The air was removed from the solution before making completely to volume. This was accomplished by placing the flask in an environment of reduced pressure and carefully rotating it.

Refractive index measurements were made with a Zeiss immersion refractometer under carefully controlled conditions of temperature, when working with unsaturated solutions at 20° and 25°C. An Abbé instrument was used for more concentrated solutions at 25°C. and for the entire range of concentration at 15°C. These latter measurements were made in order to determine the temperature coefficient. Attempts to determine the refractive indices of supersaturated solutions at higher temperatures failed because of the ease with which the lactose crystalizes. The data on density and refractive indices for each temperature has been subjected to the method of least squares, and equations thus prepared have been used for calculating these physical constants at various percentage concentrations. In the range of unsaturation the disagreement between the observed densities and those calculated by the formulas thus derived has a maximum value of 3 in the 5th decimal place. At higher concentrations, the data of Schmoeger has been analysed by the same method. Since this work was reported only to the 4th place and since the difficulties of preparing the solution are considerable, it is remarkable that the deviation of the observed data from that of the curve represented by the formula is on an average less than .0002 with a maximum of .0007 for a concentration of 50 per cent. The formula expressing the refractive index of an unsaturated solution as a function of the percentage concentration gives values differing from those observed by a maximum of 2 in the 5th decimal place. This is well within the precision of the measurements. For the higher concentrations at 25°C., and over the entire range of concentration at 15°C. where the values are reported only to the 4th place, the maximum deviation is 2 in this decimal place.

II. THE DETERMINATION OF LACTOSE AND LACTOSE-SUCROSE MIXTURES BY THE MUNSON-WALKER GENERAL METHOD

In continuation of his work on dextrose, levulose, invert sugar, and invert sugar-sucrose mixtures which are at present included in the Book of Methods, Dr. Hammond, of the Bureau of Standards, has now about completed a study of the copper equivalents of lactose and lactose-sucrose mixtures by the Munson-Walker method. The copper has been determined electrolytically, and hence this table will be of value to analysts who determine copper directly rather than as the oxide.

III. MICRO METHOD FOR REDUCING SUGARS

The micro method for reducing sugars proposed by Somogyi is now included in the Book of Methods[1] as a tentative method. A copper-carbonate-bicarbonate reagent is employed in this method. In 1945 the author[2] proposed a reagent containing disodium phosphate and sodium hydroxide which had the advantage of stability over a long period of time. Since this would be of great value to the analyst who is required to make an occasional determination it is recommended* that this modification of the method be studied.

No report was made on corn sirup and corn sugar.

REPORT ON COLOR AND TURBIDITY IN SUGAR PRODUCTS

By JOSEPH F. BREWSTER (National Bureau of Standards, Washington 25, D. C.), *Associate Referee*

Certain changes in nomenclature and symbols used in colorimetry and spectrophotometry have been suggested with the view of simplifying the expression of results. The proposed amendment applies to the section of the author's 1946 report[1] headed "Definitions and Symbols." The changes are to be as follows:

Fourth item: For "transmittance" read "transmittancy."
Fifth item: Eliminate and substitute "$A = -\log_{10} T = \log_{10} 1/T$ = absorbancy."
Sixth item: Eliminate and substitute therefor "$a = A/bc = \log_{10} 1/T/bc$ = absorbancy index." It will be noted that a replaces $-\log t$ formerly used.

It is recommended* that these proposed changes be given consideration when the method for the measurement of color in solutions of sugar products is recommended for adoption either as a tentative or official method.

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[1] *Methods of Analysis*, sixth ed., 34.63–34.65 (p. 578–9).
[2] *J. Biol. Chem.*, 160, 61 (1945).
* For report of Subcommittee D and action by the Association, see *This Journal*, 31, 62 (1948).
[1] *This Journal*, 30, 290 (1947).

REPORT ON FLAVORS AND NON-ALCOHOLIC BEVERAGES

By JOHN B. WILSON (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Referee*

Only a little time was available this year for laboratory work on the problems attending the analysis of flavors and non-alcoholic beverages which are recommended for study by the Association, but it is hoped that several of the investigations can be undertaken during the coming year.

The Referee made a short study of the application of the modified oil separatory trap to the determination of essential oil in emulsions. The following procedure was used:

DETERMINATION OF ESSENTIAL OIL IN EMULSIONS

APPARATUS

Boiling Flask: Use a 500 ml round bottom flask with ground glass joint 24/40.

Oil Trap: Use the modified oil separatory trap described in *This Journal*, **27**: 201 (1944).

Condenser: Use a Liebig condenser.

DETERMINATION

Place about 200 ml of H_2O in the boiling flask; measure 5–10 ml of the emulsion (believed to contain not over 2 ml of essential oil) in a glass-stoppered graduated cylinder; add to the flask and wash out the cylinder into the flask by adding several 5 ml portions of H_2O to the cylinder and shaking to remove the emulsion from the sides.

Fill the oil trap with H_2O until it overflows, connect with the boiling flask and condenser and boil for one hour. When the boiling time is over, remove the heat source and let stand for several minutes. Now draw off enough water to bring the oily layer within the graduated portion of the trap, let stand for 5 min to complete drainage, and measure the quantity of oil from bottommost to topmost points of the menisci.

To try out the procedure several emulsions were prepared as follows:

PRIMARY EMULSION

Mix until homogeneous 55 ml of cold pressed lemon oil, 40 g of gum arabic, and 170 ml of water in a beaker, using an electric soda fountain mixer. Make up to 250 ml in a graduated cylinder, adding more water if necessary. Now pass the mixture four times thru a homogenizer (a hand homogenizer called Aluminum Cream Maker was used).

Three secondary emulsions were prepared from the primary emulsion (A) by diluting various volumes of the latter to known volumes with water. In the case of B, 50 ml was diluted to 100 ml and passed through the homogenizer, giving a concentration of 11 ml oil per 100 ml. With C and D, 25 ml and 20 ml, respectively, were diluted to 100 ml and mixed by shaking. These therefore contain 5.5 and 4.4 ml of oil per 100 ml. The results obtained by the application of the procedure given above are given in Table 1.

The results in Table 1 indicate that the modified oil separatory trap can be applied to the determination of essential oils in emulsions. It is possible

TABLE 1.—*Determination of lemon oil in emulsions*

SAMPLE DESIGNATION	VOLUME	VOLUME OF OIL			RECOVERY	OIL BY VOLUME	
		PRESENT	FOUND	CORRECTED[1]		PRESENT	FOUND CORRECTED
	ml	*ml*	*ml*	*ml*	*per cent*	*per cent*	*per cent*
A	6	1.32	1.10	1.22	92.4	22.0	20.3
	8	1.76	1.53	1.70	96.6		21.3
	8	1.76	1.55	1.72	97.7		21.5
	7.1	1.54	1.35	1.50	97.4		21.1
	9.1	2.00	1.75	1.94	97.0		21.3
B	5.4	0.60	0.53	0.59	98.3	11.0	10.9
	9.4	1.03	0.92	1.02	99.0		10.7
C	6.2	0.34	0.28	0.31	91.2	5.5	5.0
	9.6	0.53	0.47	0.52	98.1		5.3
D	6.0	0.26	0.25	0.28	107.7	4.4	4.7
	9.6	0.42	0.40	0.42	100.0		4.3
				Ave.	97.8		

[1] The factor 0.9 was used, as is used for lemon oil, in the Official Steam Distillation Method for oils of lemon, orange, or lime in oil base flavors, *Methods of Analysis*, 1945, sec. 25.33.

that a factor other than that used for lemon oil in oil base flavors will be required, since there is a tendency toward low results. One-half hour of boiling was found insufficient in the case of emulsions, although 30 to 45 minutes sufficed when no gum was present (*loc cit*).

In the sixth edition of *Methods of Analysis* extensive changes were made in the method for alcohol by volume in the chapter on Distilled Liquors. The changes in technique have made it necessary for the Referee to review the procedures for determining alcohol in flavoring extracts and to bring the directions in Chapter 25 into harmony with the new procedure for spirits upon which they are based.

Vanilla extracts may contain as much as 200 grams of sugar, dextrose, or glycerin per liter, as provided in the advisory standard issued under the Federal Food and Drugs Act of 1906.

Ginger extract contains solid matter derived from ginger root and the remaining extracts treated in this chapter contain essential oils. None of these various mixtures can be expected to have the same coefficient of expansion as water solution containing the corresponding proportion of alcohol. Since the alcohol content is legally expressed at 15.56°C., the only way in which the correct alcohol content of such samples may be ascertained is to measure the sample at 15.56°C. With this purpose in view, recommendations are now being made to bring the alcohol methods in Chapter 25 in line with those in Chapter 15.

In cases where essential oils must be removed by salting out and washing with petroleum ether, the method must remain tentative for the pres-

ent. Other methods are being made official since they refer directly to an official method.

RECOMMENDATIONS*

It is recommended:

(1) That the collaborative study of the reflux method for determination of peel oil in citrus fruit juices and the use of the modified oil separation trap be continued.

(2) That collaborative work be continued on the method for determination of beta-ionone where small amounts are present.

(3) That collaborative studies of the Ripper method for determination of aldehydes in spirits as applied to lemon oils and extracts be continued.

(4) That collaborative studies of the methods proposed by the Referee for determination of esters in lemon extract be continued.

(5) That collaborative studies on the Seeker-Kirby Method for determination of esters in lemon and orange oils (Dept. Agr. Bull. 241) be continued.

(6) That collaborative studies of extract methods containing both isopropyl alcohol and acetone be continued.

(7) That collaborative study of the photometric method for determination of vanillin and coumarin be continued.

(8) That work be continued on the determination of glycerol, vanillin, and coumarin in vanilla and imitation vanilla extracts with special reference to the automatic extraction of vanillin and coumarin.

(9) That the study of emulsion flavors be continued.

(10) That studies on maple concentrates and imitations be continued.

(11) That study of the method for determination of diacetyl, published in *This Journal*, **25**, 255, be continued.

(12) That section **25.2** (p. 365) be changed to read as follows:

"**25.2** Alcohol-Official—Proceed as directed in **16.6** or **16.7** but measure the sample used at 15.56°C. in a pycnometer (see pages 192–193) calibrated at that temperature."

(13) That section **25.23** be changed to read as follows and studied collaboratively:

Measure 50 ml of extract at 15.56°C. in a pycnometer (see page 193) calibrated at that temperature, transfer to 200 ml volumetric flask, washing out the pycnometer several times with H_2O, dilute with H_2O to ca 200 ml; allow mixture to stand until oil separates in a clear layer at top, or centrifuge and add H_2O to bring lower meniscus of oil to mark. Note temperature. Pour mixture into dry Erlenmeyer flask containing 5 g of light $MgCO_3$, stopper, shake well, and filter quickly thru large, dry, folded filter. Pipet 100 ml of filtrate, measured at same temp. into a 300–500 ml distillation flask; add 25 ml of H_2O; and distil almost 100 ml into 100 ml pycnometer (see page 192). Adjust to a convenient temperature, complete volume, mix and determine sp. gr. From the table **44.23** obtain per cent alcohol by volume and compute as under **16.6(b)**. beginning "Compute as follows, etc."

* For report of Subcommittee D and action by the Association, see *This Journal*, **31**, 60 (1948).

(14) That the statement after the colon in line 3 of **25.54** be deleted and the following substituted:

"Fill 50 ml pycnometer (page 193) with sample at 15.56°C., empty into a separatory funnel containing about 10 g of NaCl. Wash out the pycnometer several times with saturated NaCl, using about 100 ml altogether. Extract twice with 50 ml portions of petroleum benzine (b.p. 40–60°). Collect petroleum benzine extract in second separatory funnel and wash with two 25 ml portions of NaCl soln. Combine original NaCl soln with the washings, add a little powdered pumice, and distil into 100 ml pycnometer (page 192). When almost 100 ml has been distilled make up to mark with H_2O at a convenient temperature and determine alcohol from the sp. gr. as directed under **16.6**, using **44.23**."

(15) That **25.64** be changed to read as follows:

"**25.64** Alcohol-Official—Proceed as directed in **16.6(b)**."

No reports were made on beta-ionone; lemon oils and extracts; organic solvents in flavors; glycerol, vanillin, and coumarin in vanilla and imitation vanilla; emulsion flavors; maple flavor concentrate and imitations; or diacetyl. See Referee report for essential oil in emulsions.

REPORT ON WATERS, BRINE, AND SALT

By A. E. Mix (Food and Drug Administration, Federal Security Agency, Washington, 25, D. C.), *Referee*

Some time ago a short reference appeared in one of the chemical journals suggesting the use of dye index No. 1073 for the detection of small amounts of boron in salt.

The Referee used dye No. 1073 and found that the results were of no value. After several trials it was found that index No. 1073 is used for the sulfonated as well as for the unsulfonated dye.

The unsulfonated dye Violet No. 2, when mixed with concentrated sulphuric acid, produces a dark green color.

It was found that when .01 gram of the dye was dissolved in about 25 ml of concentrated sulfuric acid, and portions from 1 ml to 5 or more ml of this mixture each made up to a final volume of 100 ml with sulfuric acid, the resulting solution had a definite green color.

The first trial was made with a spoonful of sodium chloride A.C.S., placed on a watch-glass with a drop of normal sodium hydroxide in the center of the salt; this was dried in a hot air oven for one hour, cooled, and then 10 drops of the dye solution was dropped on top of the salt, while at the same time another lot of salt was treated with a few fine granules of sodium borate, sodium hydroxide, and the dye.

The salt containing the sodium borate showed a definite blue color, while the other salt sample showed a green color.

Some water solution containing .02 milligram of sodium borate treated

with a drop of 1 *N* sodium hydroxide, dried and treated with the dye, showed a definite blue color.

If nitrates interfere, they are removed with concentrated sulfuric acid and hydrazine sulfate. Fluorides retard the action of the dye for about 5 minutes. More work must be done in order to make this a quantitative method.

RECOMMENDATIONS*

It is recommended:

(1) That the method for the determination of boron in water and salt be further studied.

(2) That the determination of fluorine in salt work be completed next year.

No report was given on boron in water or fluorine in salt.

REPORT ON FERTILIZERS

By F. W. QUACKENBUSH (Purdue University Agricultural Experiment Station, Lafayette, Ind.), *Referee*

During the year a new Associate Referee on Sampling was appointed and work was initiated to study sampling methods and containers. Reports were submitted by referees on sampling, phosphoric acid, moisture, nitrogen, and potash. Some others have carried on work, but they felt that further studies should be made before making a report. It is hoped that others will make good progress on their respective assignments and give this group the benefit of their findings next year.

RECOMMENDATIONS†

It is recommended—

(1) That the study of sampling equipment and sampling methods be continued. That line 6 of section 2.1 of *Methods of Analysis A.O.A.C.* (1945) under "Directions for Sampling" be changed to read as follows: "If less than 100 bags, sample not less than 20 bags." Other recommendations of the Associate Referee for adding directions on sampling bulk and small package fertilizers are approved (official first action).

(2) That work on methods for phosphoric acid be continued with emphasis on **(a)** the ammonium citrate method and its applicability to basic slag; **(b)** evaluation of sintered, fused, and calcined phosphates as fertilizers; **(c)** aging of molybdate solutions.

(3) That the recommendations of the Associate Referee on P_2O_5 be adopted, official first action.

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* For report of Subcommittee D and action by the Association, see *This Journal*, 31, 62 (1948).
† For report of Subcommittee A and action by the Association, see *This Journal*, 31, 41 (1948).

(4) That attempts to work out improved methods for moisture be continued.

(5) That work on methods for nitrogen be continued with special emphasis on fertilizers which contain ammonium nitrate.

(6) That work on methods for manganese and magnesium be continued.

(7) That work on problems of acid- and base-forming quality of fertilizers be continued.

(8) That work on potash be continued.

(9) That 95 per cent ethyl alcohol be adopted instead of 80 per cent alcohol in the determination of potash:

(a) That section **2.40(d)** p. 31, *Methods of Analysis* (1945) be changed to read: **(d)** *Acid-Alcohol.*—Mix 200 ml. of 95% ethyl alcohol with 20 ml. of concentrated HCl and cool to room temperature (official, first action).

(b) That, in section **2.42(a)**, p. 32, *Methods of Analysis* (1945), all references to 80% alcohol be changed to 95% ethyl alcohol (official, first action).

(10) That work on analysis for sulfur be continued.

(11) That the study of methods for copper and zinc be continued.

(12) That work on methods of analysis for boron be continued.

(13) That editorial correction in section **2.10** (b) of "323.81" to "324.03" line 1, and of "32.38" to "32.40," line 2, be made.

REPORT ON SAMPLING FERTILIZERS*

By H. R. ALLEN (Kentucky Agricultural Experiment Station, Lexington, Ky.) *Associate Referee*

Ross, Hardesty, and Rader[1] reported results of a questionnaire on sampling fertilizers. The most widely used triers were the slotted single-tube trier and the slotted double-tube trier, usually known as the Indiana sampler. These investigators cited published papers on this subject to November 1940. These papers represented work done between the years 1916 and 1927.

Use of the Indiana trier theoretically should provide the most representative sample, since nothing can enter the trier while it is being inserted or removed from the bag. This sampler is cumbersome to use, however, and is harder to clean than the single-tube triers. It is generally conceded that all the triers should have one end closed and pointed. It is probable that a bag should not be sampled in the vertical position with a single-tube trier, since a large portion of the sample might be obtained from the upper portion of the bag.

This report gives analyses of samples taken from the same bags with these two types of triers. The report also compares the moisture content

* This investigation, made in connection with a project of the Kentucky Agricultural Experiment Station, is published by permission of the Director.

[1] Ross, W. H., Hardesty, J. O., Rader, L. F., Jr., *This Journal*, **24**, 499–506 (1941).

of samples sealed in glass when taken and of samples placed in paper envelopes at time of sampling.

Haigh[2] compared analyses of samples taken with the single-tube and double-tube triers, but the fertilizers were lower in grade than those sampled here and the single-tube trier took a smaller core.

EXPERIMENTAL WORK

Comparison of Triers by Analysis of Samples.—Both triers had one end closed and pointed and the other end open. The single-tube trier had a single slot 24 inches long and $\frac{1}{2}$ inch wide and the tube had an inside diameter of $\frac{3}{4}$ inch. The effective opening of the Indiana or double-tube trier was 24 inches, separated by ribs into 3 slots each 8 inches long. The width of the slot was $\frac{1}{2}$ inch and the inside diameter of the tube was $\frac{5}{8}$ inch.

Samples were taken from 3 shipments of fertilizer with the 2 triers. Each shipment was in paper bags and the bags were stacked in the horizontal position. Cores were taken from 6 bags in the first shipment and from 10 bags in the other shipments. For the composite samples, one core was taken from each bag with the single-tube trier and immediately a core was taken from the same bag, and in the same place, with the double-tube trier. Some of the cores were taken parallel to sides of the bags and some were taken diagonally through the bags, but the same procedure was followed with each trier. Cores taken with the single-tube trier constituted one sample from each shipment and those taken with the double-tube trier constituted another sample. The composite samples were taken with the bags in the horizontal position. The single-tube trier was inserted in the bag with the slot down, then the trier was turned over, opened to admit sample, and closed before withdrawal. The samples were sealed in glass jars.

Next, for each shipment, with the bag in a horizontal position, a core was taken with the single-tube trier from 1 bag, then a core with the double-tube trier in the same place from this bag. This was repeated until 3 cores were taken from the same bag with each trier, these cores constituting 2 samples. Next, this procedure was repeated with the bag in the vertical position.

The entire amount of each sample was ground to pass a sieve with 1 mm openings and the samples were analyzed for moisture, nitrogen, total phosphoric acid, and potash. The results, as reported in Table 1, are averages of 2 closely agreeing duplicate determinations.

Comparison of Moisture in Samples Placed in Glass and Paper Containers when Sampled.—The moisture study was thought advisable, since some control agencies send samples to the laboratory in paper containers. Two samples were taken from each of 5 stocks of fertilizer, 10 bags being

[2] Haigh, L. D., *ibid.*, 4, 597–599 (1921).

TABLE 1.—*Analyses of samples taken with single-tube and double-tube triers*

Results in per cent*

SAMPLE NUMBER†	MOISTURE	TOTAL NITROGEN	TOTAL PHOSPHORIC ACID	POTASH
		No. 1 Samples, 6-8-6		
1 A	7.01	6.07	8.55	6.90
1 B	6.97	6.02	8.55	6.81
1 C	6.77	6.20	8.57	6.91
1 D	6.57	6.28	8.65	6.87
1 E	6.67	6.31	8.35	6.96
1 F	6.50	6.27	8.55	7.00
		No. 2 Samples, 4-12-4		
2 A	5.61	4.08	12.35	4.01
2 B	5.58	4.10	12.35	4.03
2 C	5.03	4.10	12.20	3.84
2 D	5.07	4.11	12.25	3.84
2 E	5.03	4.08	12.25	3.92
2 F	4.97	4.08	12.20	3.94
		No. 3 Samples, 6-8-6		
3 A	8.10	5.60	10.70	5.68
3 B	8.12	5.62	10.67	5.74
3 C	7.77	5.74	10.50	5.81
3 D	7.76	5.77	10.50	5.80
3 E	7.50	5.77	10.65	5.77
3 F	7.03	5.75	10.65	5.69

* Results are averages of 2 analyses, except some of the moisture figures.
† A—Composite sample, with single-tube trier, horizontal position
B—Same sample, with double-tube trier, horizontal position
C—1 bag horizontal position, single tube trier
D—Same bag horizontal position, double-tube trier
E—Same bag vertical position, single-tube trier
F—Same bag vertical position, double-tube trier.

Composite samples were from 6 bags for No. 1 samples and from 10 bags each for No. 2 and No. 3 samples.

sampled in each case. Two cores were taken from each bag sampled, from approximately the same place in the bag. One core from each bag constituted a sample which was sealed in glass when taken. The other core from each bag was placed in an envelope[2] made of heavy kraft paper, open at

[2] Spear Safety Envelope, manufactured by Heinrick Envelope Co., Minneapolis, Minn.

one end. The envelope is closed by folding the flap as creased in 3 folds, and the flap is inserted in pocket on side of envelope. The samples were stored in the inspector's car until delivered to the laboratory, at which time the samples in paper were transferred to glass jars which were sealed. The samples were in paper containers from 4 to 6 days.

Each sample was well mixed and about 150 grams were ground in a mortar to pass a sieve with 1 mm openings. The ground part of the sample was kept in a covered container until the grinding was finished. Tops of laboratory bottles holding the samples were sealed with adhesive tape. Duplicate moisture determinations were made, the samples being dried at 98°–100°C. for 3 hours. The results reported in Table 2 are averages of duplicate determinations. The duplicates with one exception agreed within 0.1 per cent moisture.

TABLE 2.—*Per cent moisture of samples placed in glass jars and in paper envelopes when sampled**

SAMPLE NUMBER	SAMPLES IN GLASS	SAMPLES IN PAPER ENVELOPES
4 A and 4 B†	6.53	5.22
5 A and 5 B	6.92	5.48
6 A and 6 B	4.40	3.37
7 A and 7 B	7.82	6.64
8 A and 8 B	9.17	7.58

* Composite samples from 10 bags in each case. Results are averages of 2 analyses.
† A samples placed in glass jars and B samples placed in paper envelopes when taken. B samples transferred to glass jars after 4 to 6 days.

DISCUSSION OF RESULTS

Comparison of Triers.—Comparison analyses made with 2 types of triers were in close agreement, both for the composite samples and for samples representing individual bags. This might indicate very homogeneous mixtures but each stock of fertilizer sampled was not entirely homogeneous. This is shown by the nitrogen results from the individual bags representing shipments 1 and 3 and by the potash results from the individual bags representing shipment 2.

Probably this work should be supplemented with a comparison of different triers on mixtures that have a tendency to segregate and on samples taken before and after segregation.

Comparison of Moisture.—Marked differences were found in moisture in samples sealed in glass and in those placed in paper containers at time of sampling. The latter group contained from 1 to 1.5 per cent less moisture. It is probable that a considerable part of this loss was due to absorption of moisture by the paper containers. These results indicate that samples should be placed in glass or other non-absorptive containers when taken. Decrease of 1.5 per cent moisture for a guarantee of 6 in any

nutrient theoretically means an increase of .09 in percentage results and for a guarantee of 20, the increase is 0.30 per cent.

The writer found even greater differences and in the same direction in comparative samples taken in October and November, 1940. Moisture was compared on samples placed in glass, cellophane and paper. The moisture decrease was less in cellophane than in paper.

SUMMARY

Analyses of samples taken with single-core and double-core triers were in good agreement. Samples from individual bags sampled in horizontal and vertical positions were uniform in analysis.

Samples kept in paper containers for 4 to 6 days after sampling contained from 1 to 1.5 per cent less moisture than samples from same bags which were placed in glass jars and sealed when sampled.

ACKNOWLEDGMENT

The writer is indebted to Miss Lelah Gault for making the phosphoric acid analyses, and to Inspectors Robert Mathews, N. J. Howard, and Neville Hulette for taking the samples.

RECOMMENDATIONS*

It is recommended that—

(1) That study of sampling fertilizers be continued.

(2) That an investigation be made of sampling fertilizers in bulk.

(3) That sec. **2.1**, line 6, be changed to read as follows: "If less than 200 bags, sample not less than 20 bags."

(4) That the following be added to sec. **2.1**, line 7, before the final sentence "In sampling a bulk lot of fertilizer, draw not less than 20 cores from different regions of the lot. In sampling fertilizer packaged in small containers (10 pounds or less) a single package may constitute a sample for the lot."

REPORT ON PHOSPHORIC ACID

A. EFFECT OF CONTINUOUS AGITATION DURING CITRATE DIGESTION ON DETERMINATION OF CITRATE-INSOLUBLE P_2O_5

B. EFFECT OF SULFATE ON DETERMINATION OF P_2O_5 BY THE VOLUMETRIC METHOD

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A. EFFECT OF CONTINUOUS AGITATION DURING CITRATE DIGESTION ON DETERMINATION OF CITRATE-INSOLUBLE P_2O_5

At the 1943 meeting of this Association, MacIntire, Marshall, and

* For report of Subcommittee A and action by the Association, see *This Journal*, 31, 41 (1948).

TABLE 1.—*Summary of published data relating to effect of continuous agitation during citrate digestion on results for citrate-insoluble P_2O_5*

SAMPLE	MATERIAL	TOTAL P_2O_5	CITRATE-INSOLUBLE P_2O_5[a]		
			PERIODIC AGITATION[b]	CONTINUOUS AGITATION[c]	DIFFERENCE
		per cent	*per cent*	*per cent*	*per cent*
	Results given by Jacob, Rader, and Tremearne (9)				
1374	Calcined phosphate[d]	35.17	3.91	3.79	0.12
1351	Calcined phosphate[d]	36.58	11.80	11.79	0.01
1395	Rhenania phosphate	30.42	3.70	0.43	3.27
1396	Rhenania phosphate	25.66	2.18	1.84	0.34
1110	Non-acid phosphate	26.50	11.42	11.00	0.42
1164	Basic slag	18.45	3.74	3.47	0.27
1107	Basic slag	23.36	4.55	3.38	1.17
	Results given by MacIntire, Marshall, and Meyer (11)				
A-1	Superphosphate	20.44	0.03	0.01	0.02
A-2	Superphosphate	20.13	0.02	0.02	0.00
A-4	Superphosphate	18.75	0.06	0.03	0.03
3	Triple superphosphate	47.50	2.99	2.95	0.04
A-10	Ammoniated superphosphate	20.63	1.97	1.72	0.25
A-15	Ammoniated superphosphate	19.37	2.75	2.60	0.15
A-14	Ammoniated superphosphate	19.28	2.88	2.80	0.08
A-11	Ammoniated superphosphate	20.00	2.45	2.25	0.20
A-9	Ammoniated superphosphate	18.79	6.35	6.15	0.20
A-13	Ammoniated superphosphate	19.25	6.93	6.85	0.08
3	Ammoniated superphosphate	41.38	2.40	2.40	0.00
4	Ammoniated superphosphate	41.19	4.58	4.42	0.16
A-5	Limed superphosphate	17.12	4.18	3.68	0.50
1	Monocalcium phosphate	56.32	0.00	0.00	0.00
2	Dicalcium phosphate, crude	51.75	2.60	1.98	0.62
4	Calcined phosphate[d]	37.06	7.54	4.98	2.56
10	Calcined phosphate[d]	36.88	3.65	3.48	0.17
11	Calcined phosphate,[d] reverted	36.63	19.70	19.63	0.07
12	Basic slag, imported	17.13	3.01	2.80	0.21
B-11	Basic slag, fluospar	10.75	9.25	9.25	0.00
13	Calcium metaphosphate	61.88	0.08	0.06	0.02
14	Fused phosphate[e]	26.79	3.75	2.95	0.80
B-12	Bone ash	40.75	35.13	35.00	0.13
—	Phosphate rock[f]	35.31	32.19	31.88	0.31
5	Mixed fertilizer, 6-8-4	21.96	13.35	13.28	0.07
6	Mixed fertilizer, 6-9-4	9.88	0.79	0.75	0.04
4	Mixed fertilizer, 3-10-5	14.88	4.68	4.62	0.06
2	Mixed fertilizer, 5-10-5	15.88	4.88	4.73	0.15
1	Mixed fertilizer, 6-12-6	18.07	3.45	3.40	0.05

[a] In all the determinations, 1-gram samples were digested with 100 ml. of neutral ammonium citrate solution for 1 hour at 65°C. in the presence of filter paper.
[b] Official method; manual shaking at 5-minute intervals.
[c] End-over-end rotation at 12 r.p.m. by Jacob *et al.* and 20 r.p.m. by MacIntire *et al.*
[d] Calcined alpha phosphate.
[e] Fused alpha phosphate.
[f] Florida pebble, National Bureau of Standards sample No. 120.

Meyer (11) pointed out the disadvantages of the official method for citrate-insoluble P_2O_5 (*Methods of Analysis*, 1, pp. 23–24), with emphasis on the amount of the analyst's time required for the periodic shaking of the flasks and on the disagreeable features of this operation, as well as the difficulty, if not impossibility, of maintaining strict uniformity of temperature in all portions of the bath and throughout the digestion period. In the same paper a description is given of an electrically-heated cabinet provided with means for continuous end-over-end rotation of the flasks at 20 r.p.m. and for the maintenance of their contents at a constant temperature of 65°C. It is stated that better reproducibility of values for citrate-insoluble P_2O_5 are obtained with the use of this apparatus than with the techniques of the official method.

In an earlier paper[1] Jacob, Rader, and Tremearne (9) report values for citrate-insoluble P_2O_5 in several types of water-insoluble phosphates—calcined phosphate (calcined alpha phosphate), Rhenania phosphate, non-acid phosphate, and basic slag—as determined, respectively, by the official method (shaking at 5-minute intervals) and by continuous end-over-end rotation at 12 r.p.m. in a thermostat. These results, together with those obtained by MacIntire, Marshall, and Meyer (11) on a much wider variety of materials, are summarized in Table 1. It will be noted that continuous agitation gave values identical with or lower than those obtained by the official method. In general, the differences are small and do not exceed 0.5 per cent P_2O_5 except in the case of certain samples of Rhenania phosphate, calcined phosphate, and fused phosphate (fused alpha phosphate).

In view of the fact that the MacIntire-Marshall-Meyer apparatus has been placed on the market and is now used by a number of fertilizer laboratories (including several engaged in State fertilizer-control work), although continuous agitation during the citrate digestion has not yet been adopted officially, the Referee on Fertilizers in his report to the 1946 meeting of the Association (6) recommended "that a study be made of the use of mechanical shakers in the method for citrate-insoluble phosphoric acid to ascertain whether modifications should be made in the method for water-soluble and citrate-insoluble phosphoric acid to permit the use of mechanical shakers." Pursuant to this recommendation a collaborative investigation of the subject was carried out and the results are presented herein.

SAMPLES

The samples submitted to the collaborators are listed in Table 2, which also shows the total P_2O_5 content of the materials. Samples 1, 2, 3, 4, 13, and 14 were ground to pass a 40-mesh sieve and the others to pass a 100-mesh sieve.

[1] Presented at the 1935 meeting of this Association.

TABLE 2.—*Samples for collaborative study of effect of continuous agitation during citrate digestion results for insoluble P_2O_5*

SAMPLE	MATERIAL	TOTAL P_2O_5[a]		
		COLLABORATOR 4	COLLABORATOR 7	AVERAGE
		per cent	*per cent*	*per cent*
1	Ammoniated superphosphate, 3.45% *N*	20.34	20.02	20.18
2	Ammoniated superphosphate, 4.87% *N*	19.69	19.52	19.60
3	Mixed fertilizer, 4-12-6	12.45	12.45	12.45
4	Mixed fertilizer, 4-12-6	12.30	12.32	12.31
5	Dicalcium phosphate	48.60	48.32	48.46
6	Fused alpha phosphate[b]	27.69	27.68	27.68
7	Sintered alpha phosphate[c]	20.47	20.54	20.50
8	Calcined alpha phosphate[d]	37.23[e]	37.25	37.24
9	Phosphate rock-magnesium silicate glass[f]	20.27[g]	20.60	20.43
10	Basic slag, low analysis	8.21[h]	8.42	8.31
11	Basic slag, high analysis	16.12	16.15	16.13
12	Phosphate rock, Florida pebble	30.92	30.70	30.81
13	Superphosphate, Florida pebble	21.14	20.78	20.96
14	Superphosphate, Tennessee brown rock	—	20.44	20.44

[a] By acid digestion of the sample.
[b] 0.34% F.
[c] 0.05% F.
[d] 0.09% F.
[e] 37.30% by NaOH fusion plus acid digestion of the sample.
[f] 1.70% F.
[g] 20.47% by NaOH fusion plus acid digestion of the sample.
[h] 8.33% by NaOH fusion plus acid digestion of the sample.

Superphosphates.—The samples (Nos. 13 and 14) are well-cured run-of-pile materials manufactured from Florida pebble and Tennessee brown-rock phosphates, respectively. Sample 14 was submitted to only one collaborator.

Ammoniated Superphosphates.—The samples (Nos. 1 and 2) were prepared in the laboratory by treating Florida pebble superphosphate (Sample 13) with anhydrous ammonia to yield products containing 3.45 and 4.87 per cent nitrogen, respectively.

Mixed Fertilizers.—The phosphate component in Samples 3 and 4 was supplied by ammoniated superphosphates No. 1 and 2, respectively. The other constituents of the mixed fertilizers are ammonium sulfate, potassium chloride, dolomite, Hyperhumus, and sand (make-weight filler).

Dicalcium Phosphate.—This material (Sample 5) is a high-grade product manufactured by the Tennessee Valley Authority from electric-furnace phosphoric acid.

Alpha Phosphates[2].—The fused alpha phosphate (Sample 6) and the

[2] The term *alpha phosphate* (10) is used to denote the products obtained by heating natural phosphates, usually phosphate rock, at temperatures above 1300°C. in the presence of silica and water vapor for the purpose of volatilizing the fluorine and converting the P_2O_5 into plant-available forms (4, 7, 12, 13, 17). It does not include the product (all glass) obtained by fusing a mixture of phosphate rock and magnesium silicate or low-fluorine phosphate of the Rhenania type. The term now embraces three types of materials—fused, sintered, and calcined—all of which are frequently called "defluorinated phosphate rock" or "defluorinated phosphate." While fused alpha phosphate is currently called "fused tricalcium phosphate," it

sintered alpha phosphate (Sample 7) are commercial-scale products made, respectively, from Tennessee brown-rock and Florida land-pebble phosphates by the Tennessee Valley Authority, Godwin (near Columbia), Tennessee, and by the Coronet Phosphate Company, Plant City, Florida, in 1946. The calcined alpha phosphate (Sample 8) was prepared about 1935 from Tennessee brown-rock phosphate on a pilot-plant scale.

Phosphate Rock-Magnesium Silicate Glass.—The results of experiments on production of available phosphate by fusing phosphate rock with magnesium silicate have been described by Walthall and Bridger (20), and the composition, properties, "solubility," and fertilizing value of several experimental and commercial products of this kind are discussed in a recent paper by Hill *et al.* (8). Large-scale manufacture of the material was achieved in 1946 by the Permanente Metals Corporation, Permanente, California, by fusing a proportioned mixture of Idaho phosphate rock and serpentine in electric furnaces at approximately 1500°C. and quenching the tapped melt to a glass in a violent spray of water. The product is sold under the name of Thermo-Phos. The material (Sample 9) used in the present study is from Permanente's production in late 1946.

Basic Slags.—Sample 10 is a low-analysis material produced in the Birmingham, Alabama, district, while Sample 11 is a high-analysis material imported from Europe prior to World War II.

Phosphate Rock.—The sample (No. 12) is commercial material from the Florida land-pebble district.

COLLABORATORS' DIRECTIONS FOR ANALYSIS

1. Determine citrate-insoluble P_2O_5 as directed in *Methods of Analysis, A.O.A.C.*, 1945, pp. 23–24, sec. **2.15** and **2.16(a)**, in the case of the samples (Nos. 1, 2, 3, 4, 13, and 14) that contain water-soluble P_2O_5. In the case of the samples (Nos. 5 to 12) that contain little or no water-soluble P_2O_5 determine citrate-insoluble P_2O_5 as directed in sec. **2.15** and **2.16(b)**.

2. Repeat the determinations of citrate-insoluble P_2O_5 as follows: Proceed as directed in sec. **2.15** and **2.16(a)** or sec. **2.15** and **2.16(b)**, depending on the nature of the sample, through the point where the flask is first shaken vigorously to reduce the filter paper to a pulp. Next, place the flask in a continuous shaking or stirring device enclosed in a water or air bath provided with means for maintaining the contents of the flask at exactly 65°C., and agitate for exactly 1 hour from the time the sample was introduced into the flask. Then proceed with the determination as before.

3. As it is desired to subject the results for citrate-insoluble P_2O_5 to statistical analysis, it is requested that with each sample and with each method of agitating the contents of the flask the determinations be made in triplicate, *i.e.*, one determination on each of three separate 1-gram portions of the sample for each set of conditions. It is necessary that the result of each determination be reported individually.

has also been designated as "fused rock phosphate," "fused phosphate rock," and "fused phosphate." Sintered alpha phosphate is marketed under the name of "Coronet defluorinated phosphate," but it has also been called "calcined phosphate." Calcined alpha phosphate has not been produced on a commercial scale; it has generally been called "calcined phosphate." The composition, properties, and "solubility" of the alpha phosphates are discussed in the report of the Associate Referee on Phosphoric Acid presented at the 1946 meeting of this Association (10).

NOTES

(a) Most of the collaborators will have access to the constant temperature, continuous agitation (end-over-end rotation) device described by MacIntire, Marshall, and Meyer (11), and it is recommended that this apparatus be used. Also, it is very desirable to have results by other continuous agitation devices provided with means for maintaining the contents of the flask at a constant temperature of 65°C. If you have such devices at your disposal or can readily assemble them, please make additional determinations with their aid. In case devices other than the MacIntire-Marshall-Meyer apparatus are used, please indicate the type and the speed of rotation or stirring.

(b) Determination of total P_2O_5 is desirable but is not required. If such determination is made, prepare the solution as directed in *Methods of Analysis, A.O.A.C.*, 1945, pp. 21–22, sec. **2.8(a)** or **2.8(b)** and proceed as directed in sec. **2.10** and **2.12(a)**, pp. 22–23.

(c) It is desirable that you determine citrate-insoluble P_2O_5 in all the samples. If this is not feasible, however, please study at least those samples marked with an asterisk in the accompanying list.

(d) Your comments and observations concerning this investigation are requested, especially regarding the advantages or disadvantages of mechanical continuous agitation as compared with manual shaking at 5-minute intervals.

COLLABORATORS

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9. North Carolina Department of Agriculture, Raleigh, N. C.

PROCEDURES

With one exception, all the collaborators compared the official method (shaking at 5-minute intervals) with continuous end-over-end rotation in the MacIntire-Marshall-Meyer apparatus at 20 r.p.m. Collaborator 2 compared the official method and continuous stirring at approximately 200 r.p.m. In addition to the official and the MacIntire-Marshall-Meyer methods, Collaborators 3 and 7 made determinations with the aid of other continuous agitation devices enclosed in thermostatically controlled, electrically heated cabinets, as follows:

(1) Ross-Kershaw shaker (18) with movement in the horizontal plane at a rate of approximately 345 oscillations per minute, using tightly stoppered flasks.

(2) Continuous stirring at about 350 r.p.m., using loosely stoppered flasks.

In the determinations by continuous agitation, Collaborators 1–5 and 7 preheated the citrate solution in a water bath, while Collaborators 6, 8, and 9 preheated the solution in the cabinet itself.

TABLE 3.—*Effect of continuous end-over-end rotation during citrate digestion on results for citrate-insoluble P_2O_5*

COLLABORATOR	CITRATE-INSOLUBLE P_2O_5 BY—				DIFFERENCE IN AVERAGE RESULTS[c]
	METHOD I[a]		METHOD II[b]		
	RANGE	AVERAGE	RANGE	AVERAGE	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
	Sample 1. Ammoniated Superphosphate				
1	1.60–1.65	1.62	1.55–1.60	1.58	0.04
3	0.81–0.93	0.87	0.59–0.64	0.62	0.25*
4	1.79–1.82	1.81	1.52–1.56	1.54	0.27*
5	1.35–1.45	1.42	1.15–1.25	1.18	0.24*
6	1.55–1.65	1.60	1.70–1.85	1.76	−0.16*
7	1.70–1.79	1.75	1.60–1.73	1.67	0.08
8	1.38–1.50	1.43	1.38–1.48	1.41	0.02
9	2.12–2.25	2.18	1.95–2.27	2.08	0.10
Average	—	1.59	—	1.48	0.11*
	Sample 2. Ammoniated Superphosphate				
1	3.25–3.35	3.30	2.90–3.00	2.97	0.33*
3	1.89–2.10	2.03	1.54–1.63	1.57	0.46*
4	1.92–2.52	2.22	1.74–1.83	1.78	0.44*
5	1.97–2.00	1.98	1.75–1.80	1.77	0.21
6	2.20–2.40	2.30	2.25–2.55	2.43	−0.13
7	2.45–2.78	2.65	2.83–3.19	3.03	−0.38*
9	3.30–3.50	3.42	3.07–3.22	3.13	0.29*
Average	—	2.56	—	2.38	0.18*
	Sample 3. Mixed Fertilizer				
1	0.15–0.20	0.18	0.15–0.20	0.17	0.01
3	0.19–0.20	0.19	0.18–0.20	0.19	0.00
4	0.15–0.29	0.20	0.08–0.08	0.08	0.12*
5	0.15–0.18	0.17	0.12–0.13	0.13	0.04
6	0.20–0.35	0.28	0.25–0.30	0.28	0.00
7	0.12–0.13	0.13	0.21–0.35	0.28	−0.15*
8	0.27–0.30	0.28	0.24–0.29	0.27	0.01
9	0.47–0.50	0.49	0.40–0.47	0.43	0.06
Average	—	0.24	—	0.23	0.01
	Sample 4. Mixed Fertilizer				
1	0.25–0.30	0.28	0.20–0.20	0.20	0.08*
3	0.28–0.31	0.29	0.18–0.21	0.20	0.09*
4	0.15–0.31	0.21	0.09–0.14	0.11	0.10*
5	0.20–0.23	0.21	0.18–0.19	0.18	0.03
6	0.30–0.35	0.33	0.25–0.30	0.27	0.06*
7	0.25–0.33	0.29	0.29–0.37	0.34	−0.05
9	0.60–0.65	0.62	0.47–0.55	0.51	0.11*
Average	—	0.32	—	0.26	0.06*

[a] Official; manual shaking at 5-minute intervals.
[b] Continuous end-over-end rotation in MacIntire-Marshall-Meyer apparatus (11).
[c] The minus sign denotes that the result by method II is higher than that by method I. The asterisk denotes that the difference is statistically significant at the 5% level.

TABLE 3.—(*continued*)

COLLABORATOR	CITRATE-INSOLUBLE P_2O_5 BY—				DIFFERENCE IN AVERAGE RESULTS[c]
	METHOD I[a]		METHOD II[b]		
	RANGE	AVERAGE	RANGE	AVERAGE	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
	Sample 5. Dicalcium Phosphate				
1	0.60–0.60	0.60	0.60–0.60	0.60	0.00
3	0.62–0.63	0.63	0.61–0.62	0.62	0.01
4	0.54–0.55	0.55	0.55–0.57	0.55	0.00
5	0.41–0.42	0.42	0.40–0.42	0.41	0.01
6	0.65–0.85	0.75	0.70–0.75	0.73	0.02
7	0.56–0.59	0.58	0.65–0.83	0.73	−0.15*
8	0.52–0.67	0.61	0.66–0.69	0.67	−0.06
9	0.72–0.75	0.74	0.70–0.75	0.72	0.02
Average	—	0.61	—	0.63	−0.02
	Sample 6. Fused Alpha Phosphate				
1	5.30–5.35	5.32	5.25–5.35	5.30	0.02
3	5.18–5.20	5.19	4.73–4.82	4.77	0.42*
4	4.79–4.94	4.87	4.38–4.44	4.41	0.46*
5	5.42–5.50	5.45	4.75–4.80	4.77	0.68*
6	5.05–5.30	5.18	5.10–5.15	5.12	0.06
7	5.28–5.57	5.44	4.96–5.18	5.09	0.35*
9	6.15–6.35	6.22	4.75–4.87	4.82	1.40*
Average	—	5.38	—	4.90	0.48*
	Sample 7. Sintered Alpha Phosphate				
1	1.55–1.60	1.57	1.55–1.55	1.55	0.02
3	1.59–1.61	1.60	1.53–1.54	1.53	0.07
4	1.46–1.59	1.50	1.34–1.46	1.40	0.10*
5	1.60–1.65	1.62	1.55–1.60	1.58	0.04
6	1.55–1.70	1.62	1.55–1.65	1.62	0.00
7	1.68.1.72	1.70	1.65–1.67	1.66	0.04
8	1.59–1.61	1.60	1.58–1.73	1.64	−0.04
9	1.62–1.72	1.67	1.47–1.50	1.49	0.18*
Average	—	1.61	—	1.56	0.05*
	Sample 8. Calcined Alpha Phosphate				
1	3.25–3.35	3.30	3.25–3.35	3.30	0.00
3	3.34–3.35	3.34	3.09–3.10	3.10	0.24*
4	2.80–2.86	2.82	2.68–2.76	2.72	0.10*
5	2.62–2.70	2.67	2.62–2.75	2.69	−0.02
6	3.05–3.20	3.12	3.10–3.15	3.15	−0.03
7	3.38–3.54	3.43	3.42–3.56	3.50	−0.07
9	3.67–3.77	3.72	3.20–3.35	3.25	0.47*
Average	—	3.20	—	3.10	0.10*

TABLE 3.—(*continued*)

COLLABORATOR	CITRATE-INSOLUBLE P_2O_5 BY—				DIFFERENCE IN AVERAGE RESULTS[c]
	METHOD I[a]		METHOD II[b]		
	RANGE	AVERAGE	RANGE	AVERAGE	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
	Sample 9. Phosphate Rock-Magnesium Silicate Glass				
1	1.30–1.50	1.43	1.15–1.40	1.27	0.16
3	1.63–2.44	2.00	1.19–1.45	1.29	0.71*
4	2.57–3.34	2.92	2.68–3.07	2.90	0.02
5	1.62–1.65	1.64	1.60–1.65	1.62	0.02
6	1.25–1.45	1.33	1.45–1.55	1.50	−0.17
7	2.67–2.93	2.77	2.54–2.99	2.74	0.03
8	1.02–1.07	1.04	0.97–1.11	1.02	0.02
9	3.32–3.60	3.44	3.32–3.77	3.50	−0.06
Average	—	2.07	—	1.98	0.09
	Sample 10. Basic Slag				
1	3.05–3.05	3.05	3.00–3.05	3.02	0.03
3	3.14–3.16	3.15	3.10–3.12	3.11	0.04
4	3.01–3.01	3.01	2.95–2.95	2.95	0.06*
5	3.10–3.12	3.11	3.00–3.12	3.07	0.04
6	3.15–3.25	3.20	3.20–3.20	3.20	0.00
7	3.08–3.11	3.10	3.16–3.19	3.18	−0.08*
9	3.17–3.25	3.21	3.10–3.15	3.12	0.09*
Average	—	3.12	—	3.09	0.03*
	Sample 11. Basic Slag				
1	1.60–1.65	1.62	1.50–1.50	1.50	0.12*
3	1.55–1.57	1.56	1.72–1.73	1.73	−0.17*
4	1.51–1.55	1.53	1.44–1.56	1.47	0.06
5	1.57–1.62	1.60	1.50–1.52	1.51	0.09*
6	1.60–1.75	1.68	1.60–1.65	1.62	0.06
7	1.60–1.61	1.60	1.55–1.60	1.58	0.02
8	1.48–1.59	1.52	1.50–1.55	1.52	0.00
9	2.07–2.20	2.12	1.50–1.50	1.50	0.62*
Average	—	1.65	—	1.55	0.10*
	Sample 12. Phosphate Rock				
1	27.40–27.70	27.60	27.40–27.60	27.53	0.07
3	28.01–28.01	28.01	27.50–27.57	27.55	0.46*
4	26.91–27.01	26.96	26.61–26.71	26.65	0.31*
5	27.75–27.88	27.84	27.40–27.55	27.50	0.34*
6	27.25–27.70	27.52	27.55–27.70	27.63	−0.11
7	27.65–27.70	27.68	27.65–27.81	27.74	−0.06
9	28.00–28.25	28.15	27.70–27.80	27.75	0.40*
Average	—	27.68	—	27.48	0.20*

TABLE 3.—(*continued*)

COLLABORATOR	CITRATE-INSOLUBLE P_2O_5 BY—				DIFFERENCE IN AVERAGE RESULTS[c]
	METHOD I[a]		METHOD II[b]		
	RANGE	AVERAGE	RANGE	AVERAGE	
	per cent	*per cent*	*per cent*	*per cent*	
			Sample 13. Superphosphate		
1	0.05–0.10	0.07	0.05–0.05	0.05	0.02
3	0.09–0.11	0.10	0.10–0.11	0.11	−0.01
4	0.09–0.09	0.09	0.06–0.10	0.08	0.01
5	0.06–0.07	0.06	0.06–0.08	0.07	−0.01
6	0.10–1.15	0.12	0.10–0.15	0.12	0.00
7	0.05–0.07	0.06	0.04–0.06	0.05	0.01
8	0.05–0.07	0.06	0.06–0.08	0.07	−0.01
9	0.12–0.15	0.13	0.10–0.10	0.10	0.03
Average	—	0.09	—	0.08	0.01
			Sample 14. Superphosphate		
7	1.02–1.08	1.05	1.07–1.15	1.11	−0.06

RESULTS OF ANALYSIS

Table 3 summarizes the results reported by the collaborators for citrate-insoluble P_2O_5 in the samples, as determined by the official method and by continuous agitation in the MacIntire-Marshall-Meyer apparatus. The results obtained by other methods of agitation during the citrate digestion are shown in Table 4.

COMMENTS OF COLLABORATORS

N. S. Chapman.—The citrate-insoluble residues were washed with water at 65°C. Phosphorus was determined volumetrically using solutions standardized against National Bureau of Standards phosphate rock. In a number of cases, measurements were made of the temperature of the liquid in the flasks at the end of the digestion period, in relation to the position of the flask in the bath or chamber. The range in variation from 65°C. was: official method, 0 to +1°; MacIntire-Marshall-Meyer apparatus, 0 to +3°; Ross-Kershaw apparatus, −3 to +1°; continuous stirring, −3 to +3°.

K. L. Elmore.—The following observations were made in carrying out these analyses: The lowering of the temperature of the air bath during introduction of samples in the continuous shaking procedure (MacIntire-Marshall-Meyer apparatus) presents a serious problem. We placed 9 flasks containing 100 ml. of the citrate solution in the bath and brought the temperature of the bath and solutions up to 65°C. as specified. In adding the sample charges to the solutions the temperature dropped to 58°C. due to opening the doors of the bath to introduce the charges. Fifty minutes of the 1-hour shaking period were consumed before the bath again reached 65°C. To obviate this difficulty the same samples were rerun by preheating the solutions to 65°C. in flasks set in a water bath. This procedure resulted in a tem-

TABLE 4.—*Effect of different methods of agitation during citrate digestion on results for citrate-insoluble P_2O_5*

COLLABORATOR	CITRATE-INSOLUBLE P_2O_5 BY METHOD				DIFFERENCE IN RESULTS BY METHOD I AND METHOD—		
	I[a]	II[b]	III[c]	IV[d]	II[e]	III[e]	IV[e]
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
				Sample 1. Ammoniated Superphosphate			
2	1.65	—	—	1.23	—	—	0.42*
3	0.87	0.62	0.73	0.82	0.25*	0.14	0.05
7	1.75	1.67	1.78	1.69	0.08	−0.03	0.06
				Sample 2. Ammoniated Superphosphate			
2	2.44	—	—	1.93	—	—	0.51*
3	2.03	1.57	1.53	1.46	0.46*	0.50*	0.57*
7	2.65	3.03	2.95	2.77	−0.38	−0.30*	−0.12
				Sample 3. Mixed Fertilizer			
2	0.31	—	—	0.20	—	—	0.11*
3	0.19	0.19	0.19	0.21	0.00	0.00	−0.02
7	0.13	0.28	0.31	0.18	−0.15*	−0.18*	−0.05
				Sample 4. Mixed Fertilizer			
2	0.28	—	—	0.28	—	—	0.00
3	0.29	0.20	0.20	0.22	0.09*	0.09*	0.07*
7	0.29	0.34	0.39	0.35	−0.05	−0.10*	−0.06
				Sample 5. Dicalcium Phosphate			
2	0.63	—	—	0.66	—	—	−0.03
3	0.63	0.62	0.61	0.62	0.01	0.02	0.01
7	0.58	0.73	0.65	0.74	−0.15*	−0.07	−0.16*
				Sample 6. Fused Alpha Phosphate			
2	5.09	—	—	4.82	—	—	0.27
3	5.19	4.77	4.84	4.87	0.42*	0.35*	0.32*
7	5.44	5.09	5.25	4.89	0.35*	0.19*	0.55*
				Sample 7. Sintered Alpha Phosphate			
2	1.49	—	—	1.74	—	—	−0.25*
3	1.60	1.53	1.54	1.56	0.07	0.06*	0.04*
7	1.70	1.66	1.71	1.69	0.04	−0.01	0.01
				Sample 8. Calcined Alpha Phosphate			
2	3.24	—	—	3.18	—	—	0.06
3	3.34	3.10	3.15	3.13	0.24*	0.19*	0.21*
7	3.43	3.50	3.50	3.28	−0.07	−0.07	0.15*

[a] Official; manual shaking at 5-minute intervals.
[b] Continuous end-over-end rotation in MacIntire-Marshall-Meyer apparatus (11).
[c] Continuous shaking in horizontal plane in Ross-Kershaw apparatus (18).
[d] Continuous stirring.
[e] The minus sign denotes that the result by this method is higher than that by method I. The asterisk denotes that the difference is statistically significant at the 5% level.

TABLE 4.—(*continued*)

COLLABORATOR	CITRATE-INSOLUBLE P_2O_5 BY METHOD				DIFFERENCE IN RESULTS BY METHOD I AND METHOD—		
	I[a]	II[b]	III[c]	IV[d]	II[e]	III[e]	IV[e]
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
	Sample 9. Phosphate Rock-Magnesium Silicate Glass						
2	1.56	—	—	1.66	—	—	−0.10
3	2.00	1.29	1.72	1.28	0.71*	0.28	0.72*
7	2.77	2.74	2.65	2.96	0.03	0.12	−0.19
	Sample 10. Basic Slag						
2	3.16	—	—	3.37	—	—	−0.21
3	3.15	3.11	3.12	3.12	0.04	0.03*	0.03*
7	3.10	3.18	3.12	3.24	−0.08*	−0.02	−0.14*
	Sample 11. Basic Slag						
2	1.71	—	—	1.70	—	—	0.01
3	1.56	1.73	1.73	1.73	−0.17*	−0.17*	−0.17*
7	1.60	1.58	1.56	1.69	0.02	0.04	−0.09*
	Sample 12. Phosphate Rock						
2	26.56	—	—	26.50	—	—	0.06
3	28.01	27.55	27.91	27.67	0.46*	0.10*	0.34*
7	27.68	27.74	27.92	27.45	−0.06	−0.24*	0.23*
	Sample 13. Superphosphate						
2	0.09	—	—	0.07	—	—	0.02
3	0.10	0.11	0.10	0.09	−0.01	0.00	0.01
7	0.06	0.05	0.01	0.10	0.01	0.05	−0.04
	Sample 14. Superphosphate						
7	1.05	1.11	1.07	1.23	−0.06	−0.02	−0.18*

perature drop of only 2°C., which was regained in the first 5 minutes of the 1-hour shaking period. The following table shows the effects of the two techniques.

SAMPLE	MATERIAL	CITRATE-INSOLUBLE P_2O_5 BY PREHEATING SOLUTION IN—	
		WATER BATH	DIGESTION CHAMBER
		per cent	*per cent*
1	Ammoniated superphosphate	1.54	1.87
2	Ammoniated superphosphate	1.78	2.48
3	Mixed fertilizer	0.08	0.12

We also determined P_2O_5 insoluble in 2 per cent citric acid (Wagner method) on the more basic materials for comparison with the official citrate method, with the following results:

SAMPLE	MATERIAL	P_2O_5 INSOLUBLE IN—	
		CITRATE	CITRIC ACID
		per cent	*per cent*
9	Phosphate rock-magnesium silicate glass	2.92	0.00
10	Basic slag	3.01	1.40
11	Basic slag	1.53	0.77

L. J. Hardin and H. S. Johnson, Jr.—All preliminary leachings of samples, filtrations of ammonium citrate digestates, and of "yellows" were made by suction, using the Shimer filter. All ammonium citrate digestates were washed with 5 per cent ammonium nitrate at 65°C. All phosphorus determinations were made volumetrically using solutions standardized against National Bureau of Standards phosphate rock. The temperature of our cabinet fluctuates ±1° when set for 65°C. and varies ±1.5° when set for 50°. It is considered that the values for some of the samples, namely 3, 4, and 13, are too small for consideration in the evaluation of the two methods.

R. M. Pinckney and L. F. Rader, Jr.—The citrate-insoluble residues were washed with water at 65°C. Phosphorus was determined volumetrically using solutions standardized against National Bureau of Standards phosphate rock. A comparison was made of the results for insoluble P_2O_5 in the samples as determined by the official citrate and citric acid methods, respectively. The data are given in the following table which also shows the *p*H values of the solutions at the end of the citrate digestion.

SAMPLE	MATERIAL	P_2O_5 INSOLUBLE IN—		*p*H OF CITRATE SOLUTION AFTER DIGESTION[c]
		CITRATE[a]	CITRIC ACID[b]	
		per cent	*per cent*	
1	Ammoniated superphosphate	1.75	0.38	7.00
2	Ammoniated superphosphate	2.65	0.47	7.05
3	Mixed fertilizer	0.13	0.29	7.10
4	Mixed fertilizer	0.29	0.24	7.10
5	Dicalcium phosphate	0.58	1.32	7.15
6	Fused alpha phosphate	5.44	4.25	7.65
7	Sintered alpha phosphate	1.70	1.15	7.50
8	Calcined alpha phosphate	3.43	2.56	7.95
9	Phosphate rock-magnesium silicate glass	2.77	0.38	8.20
10	Basic slag	3.10	2.87	8.12
11	Basic slag	1.60	1.34	8.00
12	Phosphate rock	27.68	23.57	7.05
13	Superphosphate	0.06	0.47	6.90
14	Superphosphate	1.05	2.34	6.85

[a] Official method; manual shaking at 5-minute intervals.
[b] With samples 1–4 and 13–14 the determinations were made on the water-insoluble residues.
[c] In a blank determination no change occurred in the *p*H value (6.98) of the ammonium citrate solution during the digestion period.

North Carolina Department of Agriculture.—Experience in this laboratory has been that the constant temperature, continuous agitation (end-over-end rotation)

cabinet now on the market, embodies a number of difficulties which offset its possible advantages. One of the major difficulties is preheating. It has been observed here that when a set of flasks is put into the cabinet for processing, the temperature of the cabinet promptly rises to the desired level and so registers on the cabinet thermometer. It has been further found, however, that the actual solutions in the flasks do not reach the proper temperature until approximately 40 or 50 minutes have elapsed. This trouble indicates the need for a convenient, quick preheating arrangement. Other difficulties are the trouble and liability of handling flasks inverted and with the stopper downward. The spring cups and pistons for holding the flasks in place unfortunately in the present design are also excellent stopper pullers. There is always the high possibility that stoppers will be pulled out of the flasks when removing them from the cabinet.

INTERPRETATION OF RESULTS

Official Method vs. Continuous End-Over-End Rotation.—Among the 99 comparisons (averages of replicate determinations) between the official method (shaking at 5-minute intervals) and continuous end-over-end rotation in the MacIntire-Marshall-Meyer apparatus (Table 3), 69 (70 per cent) show lower results for citrate-insoluble P_2O_5 by continuous agitation, 21 by the official method, and 9 show no difference. The distribution of the differences, without regard for signs, is as follows:

DIFFERENCE	COMPARISONS
per cent	*number*
<0.05	41
0.05–0.09	20
0.10–0.14	9
0.15–0.24	10
0.25–0.49	15
0.62–1.40	4
0.00–1.40	99

Of the 29 individual comparisons that differ by more than 0.14 per cent P_2O_5, 23 (79 per cent) are in the direction of lower results with continuous agitation. Among the comparisons which show such differences, 22 (76 per cent) occur in the determinations on the samples of ammoniated superphosphate (Nos. 1 and 2), fused alpha phosphate (No. 6), phosphate rock-magnesium silicate glass (No. 9), and phosphate rock (No. 10). With 2 exceptions, all of the differences in excess of 0.24 per cent P_2O_5 occur in the results for these samples. These samples contain more than 1 per cent citrate-insoluble P_2O_5. On the other hand, the samples of sintered alpha phosphate (No. 7), calcined alpha phosphate (No. 8), and basic slag (Nos. 10 and 11) also contain more than 1 per cent citrate-insoluble P_2O_5, yet the differences between the 30 individual comparisons on these materials exceed 0.10 per cent P_2O_5 in only 6 instances.

For the 14 individual samples (Table 3), the average results of all the

collaborators show lower values with continuous agitation in 12 instances. The greatest differences (0.11–0.48 per cent P_2O_5) in the average results, all in the direction of lower values for insoluble P_2O_5 with continuous agitation, occur in the comparisons on the ammoniated superphosphates, fused alpha phosphate, and phosphate rock.

Separate statistical analyses of the data for each sample were made by standard analysis of variance procedures, and the differences required for significance at the 5 per cent level were determined. The statistically significant differences are indicated by asterisks in the last column of Table 3.

Among the 99 comparisons (Table 3), only 39 show significant differences between the results for citrate-insoluble P_2O_5 by the two methods of agitation. Of these 39 comparisons, 33 (85 per cent) show differences significantly in favor of lower values for citrate-insoluble P_2O_5 by continuous agitation, and 16 of these significant differences occur in the 29 comparisons on the samples of ammoniated superphosphate, fused alpha phosphate, and phosphate rock.

The average results on the 13 individual samples analyzed by more than one collaborator (Table 3) show statistically significant differences in 9 instances, all in favor of lower values by continuous agitation. It will be noted, however, that these differences exceed 0.10 per cent P_2O_5 in the case of only the ammoniated superphosphates, the fused alpha phosphate, and the phosphate rock.

MacIntire, Marshall, and Meyer (11) state that better reproducibility of values for citrate-insoluble P_2O_5 are obtained with their apparatus (continuous end-over-end rotation in a constant-temperature chamber) than with shaking at 5-minute intervals as prescribed in the official method. While the data of Table 3 are not conclusive, they indicate a decided trend in that direction. With both procedures the greatest variations in the replicate determinations occur in the results on the phosphate rock-magnesium silicate glass (Sample 9). Several of the collaborators reported much difficulty in the analysis of this sample. Hill *et al.* (8) state that this type of material shows a marked tendency to cake when it is added to neutral ammonium citrate solution and that it is necessary to shake the flask vigorously immediately after addition of the sample to the solvent.

As previously mentioned, Elmore (Collaborator 4) reported that with the MacIntire-Marshall-Meyer apparatus markedly lower results for insoluble P_2O_5 in certain samples were obtained when the citrate solution was preheated to 65°C. in a water bath, than when the solution was preheated in the digestion chamber itself. This was attributed to the greater time required for the temperature of the chamber to return to 65°C. in the latter case. Although no special study was made of this factor, comparison of the results of Collaborators 1, 3, 4, 5, and 7, who preheated the solution in a water bath, with those of Collaborators 6, 8, and 9, who preheated the solution in the digestion chamber, gives no definite indication that this variation in technique contributed to the differences in the

values obtained. It is important, nevertheless, that the operation be performed with the least possible disturbance of the chamber temperature.

L. J. Hardin and W. H. MacIntire have discussed "The Effect of Open vs. Closed Flasks in the Digestion of Phosphate Fertilizers with Neutral Ammonium Citrate" in a private communication to the Associate Referee. They write as follows:

"The effect of the retention of the NH_3 generated within the system was tested through digestions of duplicate charges of a 'calcined alpha phosphate' under two conditions, *i.e.*, (1) with the flask stoppered tightly during the period of shaking and digestion, and (2) the stopper removed after each shaking and then placed loosely during the intervals of the quiescent digestion. After 60-minute digestions the *p*H values of the respective filtrates were measured and the citrate-insoluble P_2O_5 contents determined."

CLOSED DIGESTION[a]			OPEN DIGESTION[b]	
INTERMITTENT SHAKING		CONTINUOUS SHAKING	INTERMITTENT SHAKING	
INSOLUBLE P_2O_5	*p*H OF FILTRATE	INSOLUBLE P_2O_5	INSOLUBLE P_2O_5	*p*H OF FILTRATE
per cent		*per cent*	*per cent*	
2.95	8.05	2.75	2.90	8.05
2.90	8.10	2.70	2.85	8.05

[a] Solvent brought to 65°C., sample introduced, and flask kept tightly stoppered throughout digestion and shakings.
[b] Stopper removed after each shaking and then replaced loosely until next shaking.

"The insoluble P_2O_5 values are in close agreement, whether the flask was tightly stoppered throughout or closed loosely between shakings to allow escape of some of the generated NH_3. A similar agreement was true of the final *p*H values. When the digestions were agitated continuously in the constant temperature cabinet, the citrate-insoluble values were somewhat lower than those obtained by means of the intermittent manual shaking."

Official Method vs. Other Methods of Agitation.—In Table 4 the average results for citrate-insoluble P_2O_5 obtained by shaking at 5-minute intervals (Method I, official) are compared, respectively, with those obtained by continuous end-over-end rotation in the MacIntire-Marshall-Meyer apparatus (Method II), continuous shaking in the horizontal plane in the Ross-Kershaw apparatus (Method III), and continuous stirring (Method IV). The differences required for significance at the 5 per cent level were computed in the same manner as for the data in Table 3. The following tabulation shows the number of comparisons and the average differences between the results by Method I and the other methods, for all the collaborators and all the samples. The data indicate that the results obtained by continuous shaking in the Ross-Kershaw apparatus agree somewhat more closely with those by the official method than do the results obtained either by continuous end-over-end rotation or by continuous stirring.

METHOD I VS.	COMPARISONS			
	TOTAL		SHOWING SIGNIFICANT DIFFERENCES	
	NUMBER	AVERAGE DIFFERENCE[a]	NUMBER	AVERAGE DIFFERENCE[a]
		per cent		*per cent*
Method II	27	0.08	13	0.16
Method III	27	0.04	13	0.04
Method IV	40	0.08	20	0.16

[a] The differences are in the direction of higher results for citrate-insoluble P_2O_5 by Method I.

CONCLUSIONS

Continuous agitation during the citrate digestion tends to give lower values for citrate-insoluble P_2O_5 than does shaking at 5-minute intervals as prescribed in the official method. The differences between the results by the two methods of agitation tend to be larger in the case of samples that are relatively high in citrate-insoluble P_2O_5. In general, however, these differences are much smaller than the differences in the results obtained on a given sample by different analysts using the same method of agitation. Variation in the method of continuous agitation does not appear to be an important factor.

From the analyst's standpoint, it appears that continuous agitation in an apparatus provided with means for automatic temperature control has the advantage over manual periodic agitation in that it conserves time, requires less attention, and tends to improve the reproducibility of the results.

The results of the present study, together with those of previous investigations, indicate that mechanical continuous agitation during the citrate digestion is worthy of adoption by this Association as an alternative to the present official procedure of manual intermittent agitation.

B. EFFECT OF SULFATE ON DETERMINATION OF P_2O_5 BY THE VOLUMETRIC METHOD

The interference of sulfate in the volumetric determination of P_2O_5, whereby high results are obtained when the ammonium phosphomolybdate precipitation is made at elevated temperatures, has been investigated by a number of workers (2, 3, 5, 14, 15, 16, 19). The subject was studied collaboratively by W. H. Ross, the Associate Referee on Phosphoric Acid, and the results were reported at the 1928 and 1929 meetings of this Association (15, 16). On the basis of this study the Association adopted two procedures for the volumetric determination of P_2O_5 (1, pp. 22–23) applicable, respectively, in the presence and the absence of sulfates. The first of these procedures involves precipitation of the phosphomolybdate at 25–30°C. with constant stirring or shaking for 30 minutes, while the second

specifies precipitation at 45–50°C. and the maintenance of the precipitate at such temperature for 30 minutes with occasional stirring.

The fact that most fertilizers contain considerable sulfate usually restricts the analyst to the first of these procedures in the volumetric determination of P_2O_5 in such materials. As this necessitates the use of a stirring or shaking apparatus which may not be readily available to the analyst the provision of a volumetric procedure not requiring the use of such apparatus, yet applicable to the analysis of sulfate-containing materials, seems desirable. Furthermore, some analysts prefer, for various reasons, to precipitate the phosphomolybdate at 45–50°C. instead of 25–30°.

In 1907, Richardson (14) recommended that barium chloride be added immediately after completion of the acid digestion of the sample, in order to precipitate the sulfate and to eliminate its subsequent interference in the volumetric determination of P_2O_5 by the hot precipitation procedure. Breckenridge (3) recommended a similar modification but with the use of barium nitrate instead of the chloride. Also, elimination of sulfate interference by precipitation as the barium salt has been studied briefly by Shuey (19). Recently, C. C. Howes and A. T. Blackwell[3] have reported that very satisfactory results in the volumetric determination of P_2O_5 in sulfate-containing fertilizers by hot (45–50°) precipitation of the phosphomolybdate are obtained if the sulfate is first removed by means of 10 per cent barium chloride solution.

The present report summarizes the results secured in a collaborative study of the volumetric method for P_2O_5, with special reference to elimination of sulfate interference in the precipitation of the phosphomolybdate at 45–50°.

Two ordinary superphosphates, designated as Sample 13 and Sample 14 and made, respectively, from Florida pebble and Tennessee brown-rock phosphates, were submitted to the collaborators. These were well-cured, run-of-pile, commercial materials. Gravimetric determinations of P_2O_5 and SO_3 gave the following results:

SAMPLE	P_2O_5	SO_3
	per cent	*per cent*
13	20.77	32.06
14	20.40	31.87

COLLABORATORS' DIRECTIONS FOR ANALYSIS

1. Determine total P_2O_5 in each of the samples as directed in *Methods of Analysis, A.O.A.C.*, 6th ed., 1945, volumetric method, pp. 22–23, sec. **2.10** and **2.12(a)**, and sec. **2.10** and **2.12(b)**. Prepare the solutions of the samples as directed in sec. **2.8(a)** or in sec. **2.8(b)**, pp. 21–22. (Certain of the collaborators were instructed to

[3] The Davison Chemical Corporation, Baltimore, Md. Private communications.

make the determinations on solutions prepared by method **2.8(a)**, others by method **2,8(b)**, and two by both methods.)

2. Repeat the determinations on solutions prepared as follows: Proceed as directed in sec. **2.8(a)** or **2.8(b)** to the point where digestion of the sample is completed, then add 20 ml of 10 per cent barium chloride solution and boil for a few minutes. Cool the solution and proceed with the determinations as before.

3. As it is desired to subject the results to statistical analysis, it is requested that for each sample the determinations be made simultaneously on solutions prepared in triplicate with and without addition of barium chloride. This will require a total of twelve determinations on each sample, *i.e.*, three determinations under each of the four sets of conditions as to use of barium chloride and as to temperature at which the phosphomolybdate is precipitated. It is necessary that the results of each determination be reported individually.

4. Your comments and observations concerning this investigation are requested, especially regarding the effect of precipitation temperature on the ease of filtering and washing the phosphomolybdate.

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RESULTS OF ANALYSIS

Table 5 summarizes the collaborators' results (averages of triplicate analyses) for total P_2O_5 in the samples, as determined by the volumetric procedures with: (1) precipitation of the phosphomolybdate at 25–30°C. and constant stirring or shaking for 30 minutes, without prior removal of sulfate (official); (2) the same, with prior removal of sulfate; (3) precipitation of the phosphomolybdate at 45–50°C. and occasional stirring during the 30-minute period of heating at such temperature, without prior removal of sulfate; and (4) the same, with prior removal of sulfate.

TABLE 5.—*Results for total P_2O_5 in superphosphate as affected by removal of sulfate prior to precipitation of ammonium phosphomolybdate at 25–30°C. and 45–50°C. in the volumetric procedure*

COLLABORATOR	SULFATE PRESENT			SULFATE REMOVED			COL. VI MINUS COL. II
	25–30°C.[a]	45–50°C.[b]	COL. III MINUS COL. II	25–30°C.[a]	45–50°C.[b]	COL. VI MINUS COL. V	
I	*per cent* II	*per cent* III	*per cent* IV	*per cent* V	*per cent* VI	*per cent* VII	*per cent* VIII
			A. Solution of Sample by Method **2.8(a)**[c]				
			SAMPLE 13				
1	20.86	21.08	0.22	20.80	20.93	0.13	0.07
4	20.95	21.32	0.37	20.87	20.92	0.05	−0.03
6	20.88	21.75	0.87	20.78	21.07	0.29	0.19
7	20.73	21.18	0.45	20.68	20.87	0.19	0.14
8	21.00	21.60	0.60	21.00	20.90	−0.10	−0.10
10	21.30	21.87	0.57	21.07	21.30	0.23	0.00
13	21.08	20.98	−0.10	20.48	20.47	−0.01	−0.61
15	20.91	21.41	0.50	20.95	20.79	−0.16	−0.12
16	20.93	20.92	−0.01	20.81	20.83	0.02	−0.10
18	20.87	21.34	0.47	20.92	21.08	0.16	0.21
19	20.65	21.09	0.44	20.67	20.90	0.23	0.25
20	20.83	20.83	0.00	20.77	20.77	0.00	−0.06
Av.	20.92	21.28	0.36	20.82	20.90	0.08	−0.02
Min.	20.65	20.83	−0.10	20.48	20.47	−0.16	−0.61
Max.	21.30	21.87	0.87	21.07	21.30	0.29	0.25
			SAMPLE 14				
1	20.46	20.58	0.12	20.42	20.46	0.04	0.00
4	20.45	20.65	0.20	20.42	20.47	0.05	0.02
6	20.30	21.14	0.84	20.21	20.41	0.20	0.11
7	20.21	20.57	0.36	20.18	20.37	0.19	0.16
8	20.40	21.08	0.68	20.40	20.40	0.00	0.00
10	20.73	21.50	0.77	20.73	20.90	0.17	0.17
13	20.97	21.00	0.03	20.42	20.58	0.16	−0.39
15	20.41	20.86	0.45	20.31	20.40	0.09	−0.01
16	20.61	20.48	−0.13	20.44	20.51	0.07	−0.10
18	20.43	20.84	0.41	20.43	20.53	0.10	0.10
19	20.14	20.32	0.18	20.13	20.20	0.07	0.06
20	20.40	20.47	0.07	20.40	20.40	0.00	0.00
Av.	20.46	20.79	0.33	20.37	20.47	0.10	0.01
Min.	20.14	20.32	−0.13	20.13	20.20	0.00	−0.39
Max.	20.97	21.50	0.84	20.73	20.90	0.20	0.17

[a] With constant stirring or shaking.
[b] With occasional stirring.
[c] 30 ml. HNO_3 and 3–5 ml. HCl.
[d] 15–30 ml. HCl and 3–10 ml. HNO_3.
[e] Samples 13 and 14, solution method **2.8(a)**.
[f] Samples 13 and 14, solution method **2.8(b)**.
[g] Sample 13, solution methods **2.8(a)** and **2.8(b)**.
[h] Sample 14, solution methods **2.8(a)** and **2.8(b)**.
[i] All samples and all solution methods.

TABLE 5.—(*continued*)

COLLABORATOR	SULFATE PRESENT			SULFATE REMOVED			COL. VI MINUS COL. II
	25–30°C.[a]	45–50°C.[b]	COL. III MINUS COL. II	25–30°C.[a]	45–50°C.[b]	COL. VI MINUS COL. V	
I	*per cent* II	*per cent* III	*per cent* IV	*per cent* V	*per cent* VI	*per cent* VII	*per cent* VIII
			B. Solution of Sample by Method 2.8(b)[d]				
			SAMPLE 13				
2	21.04	21.90	0.86	20.76	20.87	0.11	−0.17
3	20.81	21.12	0.31	20.71	20.81	0.10	0.00
5	20.72	21.09	0.37	20.25	20.61	0.36	−0.11
6	21.02	21.60	0.58	20.99	20.81	−0.18	−0.21
9	20.69	20.87	0.18	20.68	20.73	0.05	0.04
11	20.76	20.84	0.08	20.76	20.66	−0.10	−0.10
12	20.98	21.81	0.83	20.73	20.95	0.22	−0.03
14	21.54	21.84	0.30	21.55	21.51	−0.04	−0.03
17	20.80	20.96	0.16	20.39	20.84	0.45	0.04
18	20.99	21.40	0.41	20.82	21.01	0.19	0.02
21	21.01	21.10	0.09	20.96	20.97	0.01	−0.04
Av.	20.94	21.32	0.38	20.78	20.89	0.11	−0.05
Min.	20.69	20.84	0.08	20.25	20.61	−0.18	−0.21
Max.	21.54	21.90	0.86	21.55	21.51	0.45	0.04
			SAMPLE 14				
2	20.49	21.31	0.82	20.33	20.44	0.11	−0.05
3	20.26	20.56	0.30	20.24	20.24	0.00	−0.02
5	20.26	20.70	0.44	20.04	20.10	0.06	−0.16
6	20.52	21.02	0.50	20.47	20.27	−0.20	−0.25
9	20.19	20.42	0.23	20.26	20.28	0.02	0.09
11	20.33	20.48	0.15	20.22	20.24	0.02	−0.09
12	20.46	21.19	0.73	20.24	20.39	0.15	−0.07
14	20.79	20.96	0.17	20.91	20.84	−0.07	0.05
17	20.20	20.66	0.46	19.82	20.42	0.60	0.22
18	20.47	20.85	0.38	20.37	20.40	0.03	−0.07
21	20.47	20.58	0.11	20.32	20.36	0.04	−0.11
Av.	20.40	20.79	0.39	20.29	20.36	0.07	−0.04
Min.	20.19	20.42	0.11	19.82	20.10	−0.20	−0.25
Max.	20.79	21.31	0.82	20.91	20.84	0.60	0.22
			C. Grand Averages				
e	20.69	21.03	0.34	20.59	20.68	0.09	−0.01
f	20.67	21.05	0.38	20.53	20.62	0.09	−0.05
g	20.93	21.30	0.37	20.80	20.89	0.09	−0.04
h	20.43	20.79	0.36	20.33	20.41	0.08	−0.02
i	20.68	21.04	0.36	20.56	20.65	0.09	−0.03

COMMENTS OF COLLABORATORS

H. C. Batton.—It is my opinion that there is little, if anything, to choose between cold precipitation of ammonium phosphomolybdate without prior precipitation of sulfates, and precipitation at 45–50°C. after sulfates have been removed. In fact, for a period of almost 15 years, I have been determining total phosphate by sulfate removal and hot precipitation on presumably identical portions of samples which have been analyzed by another laboratory by cold precipitation without removal of sulfates. I estimate that, during that time, such comparative analyses have been made on about 2,000 samples, with very few instances in which significant differences occurred. In fact, only two of all those samples had to be sent to a referee for final settlement.

Charles Bollfrass.—As was expected, the samples precipitated at the higher temperature yielded larger particles which came down more rapidly and were more easily filtered and washed. However, the precipitates obtained at 25–30°C. were entirely manageable and satisfactory from the standpoint of filtering and washing ease. Elimination of the sulfate did not seem to affect the particle size of the precipitate. Commenting without knowledge of the true analyses, it would seem that either precipitation at 25–30°C. without prior removal of the sulfate or precipitation at 45–50°C. with prior removal of the sulfate, yields reproducible although slightly divergent results. In addition to the information requested, experiments were run to determine the effect of the rate of barium chloride addition. These results indicated that the speed of addition had little or no effect on the per cent total P_2O_5 determination at either temperature.

N. S. Chapman.—Filtering and washing was not facilitated by precipitating the phosphomolybdate at 45–50°C.

Alice Dunn.—No difference was noted in the ease of filtering and washing the phosphomolybdate in any of the precipitations. We use suction filters with paper pulp mats. A small error is introduced in the sulfate precipitation method by the displacing effect of the barium sulfate. However, it appears this error would change the P_2O_5 result only about 0.03–0.04 per cent.

S. J. Few and H. S. Montague.—No difficulty in filtering when sulfates were precipitated.

Roland Gilbert.—No differences were noticed in filtration and washing the yellow precipitate, nor were any difficulties encountered under any of the conditions. The yellow precipitate was filtered on Whatman No. 7 paper.

P. A. Lang and Ruth D. Tosh.—At 45–50°C. the solutions filtered more rapidly than at 25–30°C. with and without removal of sulfate. This, however, is of no real help, as we filter by gravity using a long-stem fluted funnel and a No. 40, 11 cm. Whatman filter paper which filters rapidly at both of the above temperature ranges.

E. E. Leslie.—At the temperature of 45–50°C. the "yellows" tend to cling to the Phillips beakers, making the washings more difficult. Also, they go through the filter pads more easily than at the lower temperature.

H. L. Marshall.—The solution, diluted with 50 ml. of water, was treated with barium chloride and boiled for 3 minutes. No effect was noticeable on the filtration of the phosphomolybdate. We use Moore-Shimer filters (Whatman No. 42 paper).

Wm. McAllister.—The temperature of precipitation did not seem to influence, to a very great degree, the amount of creeping of the precipitate.

W. A. Morgan.—The yellow precipitates were filtered in Shimer tubes through packed filter paper pulp (Whatman No. 1). Precipitates obtained at 45–50°C. seemed to filter more rapidly.

L. F. Rader, Jr.—Little or no difference in rate of filtering and ease of washing the phosphomolybdate precipitates was observed. More complete removal of the sulfate appears to be obtained by adding the barium chloride directly to the hot acid

digestate than by diluting the digestate before adding the chloride. The amounts of sulfate remaining in solutions after treatment by adding barium chloride directly to the hot digestates were determined with the following results:

SAMPLE	TOTAL SO_3	SO_3 REMAINING AFTER $BaCl_2$ TREATMENT OF SOLUTION PREPARED BY METHOD			
		2.8(a)[a]		2.8(b)[b]	
		IN SOLUTION	PORTION OF TOTAL	IN SOLUTION	PORTION OF TOTAL
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
13	32.06	1.00	3.1	0.23	0.7
14	31.87	1.51	4.7	0.57	1.8

[a] 30 ml. HNO_3 and 3–5 ml. HCl.
[b] 15–30 ml. HCl and 3–10 ml. HNO_3.

C. J. Rogers.—No significant difference was observed in the ease of filtering or washing of precipitates by the different methods.

P. McG. Shuey.—Results are slightly lower when barium chloride is added both by the hot and cold precipitation methods, despite the fact that the precipitated barium sulfate is separated by filtration. It appears, therefore, that the barium sulfate formed has the tendency to drag down some P_2O_5. The regular official method appears more accurate.

INTERPRETATION OF RESULTS

With very few exceptions the data of the 46 comparisons given in Table 5, columns II and III, agree with those of previous investigators in showing that, in the presence of sulfate, precipitation of the phosphomolybdate at 45–50°C. leads to considerably higher values for P_2O_5 than does precipitation at 25–30°. Removal of the sulfate prior to hot precipitation of the phosphomolybdate usually brings the values into much closer agreement (compare columns IV and VIII). Under these conditions the values obtained by hot precipitation are actually lower than those by cold precipitation in slightly more than half of the 46 comparisons (column VIII).

With cold precipitation of the phosphomolybdate the values obtained when sulfate is removed (column V) are usually lower than those obtained when sulfate is not removed (column II). Likewise, in the collaborative studies conducted by Ross (15, 16) it was found that with cold precipitation the results on sulfate-free samples were nearly always lower than those on the same samples to which sulfate had been added. This would seem to indicate some interference by sulfate even when the precipitation is made at room temperature.

It will be noted that when sulfate is removed the values obtained with hot precipitation (column VI) are usually higher than those obtained with cold precipitation (column V). A similar trend in results has been reported by Ross (15, 16) on sulfate-free samples under like conditions of temperature.

With removal of sulfate the trend appears to be toward somewhat

higher values for P_2O_5 when solution of the sample is effected by method **2.8(a)** (30 ml HNO_3 and 3–5 ml HCl) as compared with solution by method **2.8(b)** (15–30 ml HCl and 3–10 ml HNO_3), whether the phosphomolybdate is precipitated at 25–30° (column V) or 45–50° (column VI). For the same sample and precipitation temperature, however, the average difference in all results by the two methods of solution is small and is within the range of permissible variance.

Statistical analysis, by means of the standard analysis of variance procedure, was made of the combined results of each collaborator. Variations among the results of the replicate determinations by each collaborator were ignored, as these were generally very small in comparison with the differences among the average results obtained by the individual collaborators.

The results of this analysis indicate that the values obtained by precipitating the phosphomolybdate at 25–30° without first removing the sulfate are not significantly different at the 5 per cent level from those obtained when the sulfate is removed and the precipitation is then made at 45–50°. Likewise, removal of the sulfate does not affect the values significantly when the subsequent precipitation is made at 25–30°. With precipitation at 45–50°, on the other hand, removal of the sulfate results in much lower values, and the difference is significant at the 0.1 per cent level. Under comparable conditions, the values are not affected significantly by the method of dissolving the sample.

CONCLUSIONS

In the volumetric determination of P_2O_5 in sulfate-containing fertilizers, removal of the sulfate as the barium salt followed by precipitation of the phosphomolybdate at 45–50° with occasional stirring gives results which are generally in good agreement with those obtained by the official method in which the sulfate is not removed but the precipitation is made at 25–30° with constant stirring or shaking. Removal of the sulfate followed by hot precipitation of the phosphomolybdate has the advantage that it does not require the use of a stirring or shaking apparatus—equipment which may not be readily available to the analyst. The results of the present study indicate that this procedure is worthy of adoption by this Association as an alternative method for the determination of P_2O_5.

ACKNOWLEDGMENT

The Associate Referee wishes to express his appreciation of the splendid cooperation given by the collaborators and their respective organizations in the investigations covered by this report.

RECOMMENDATIONS*

It is recommended—

(1) That the first six sentences of *Methods of Analysis, A.O.A.C.*, 1945,

* For report of Subcommittee A and action by the Association, see *This Journal*, 31, 42 (1948).

p. 24, sec. **2.16(a)**, acidulated samples, lines 1–11, be changed to read:

"After removing water-soluble P_2O_5, **2.13**, transfer the filter and residue, within a period not to exceed 1 hour, to 200 or 250 ml flask containing 100 ml NH_4 citrate soln previously heated to 65°. Close flask tightly with a smooth rubber stopper, shake vigorously until filter paper is reduced to pulp, relieve pressure by momentarily removing stopper, and proceed by one of the following methods: (1) Loosely stopper flask to prevent evaporation, place in water bath regulated to maintain contents of flask at exactly 65°, keep level of H_2O in bath above that of citrate soln in flask, and shake every 5 min.; (2) continuously agitate contents of stoppered flask by means of apparatus equipped to maintain contents of flask at exactly 65°. At expiration of exactly 1 hour from time filter and residue were introduced, remove flask from bath or apparatus and immediately filter contents as rapidly as possible through Whatman filter paper No. 5 or other paper of equal speed and retentiveness."

(2) That the present paragraph of *Methods of Analysis, A.O.A.C.*, 1945, p. 23, sec. **2.11**, preparation of solution, be designated as **(a)** and be terminated with the words "suitable solvent," and that a second paragraph be added to read:

"**(b)** *Not applicable in preparation of solns by sulfuric acid digestion.*—Proceed as directed under **2.8(a)**, **(b)**, or **(c)**, preferably **(a)** when these acids are a suitable solvent, to point where acid digestion of sample is completed. Add 25 ml of 10% $BaCl_2$ soln to the hot digestate, boil ca 2 min, and continue as directed under **2.8.**"

(3) That sec. **2.12** of *Methods of Analysis, A.O.A.C.*, 1945, p. 23, be changed as follows:

(1) At the beginning of sec. **2.12(a)** add the sentence, "Prepare soln of sample as directed under **2.11(a).**"

(2) In sec. **2.12(b)** delete the phrase, "Not applicable to superphosphate and other fertilizers that contain sulfates (5)," and begin the section with the new sentence, "Prepare soln of sample as directed under **2.11(b).**"

(3) Add a new section to read:

"**(c)** *Not applicable to superphosphate and other fertilizers that contain sulfate or to solns prepared with the aid of sulfuric acid (5).*—Prepare soln of sample as directed under **2.11(a).** Proceed as directed under **(b).**"

(4) That the other phosphate investigations recommended in the reports of the Referee on Fertilizers (6) and the Associate Referee on Phosphoric Acid (10) presented at the preceding meeting of this Association (October 14–16, 1946) be undertaken.

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REPORT ON MOISTURE IN FERTILIZERS

By W. L. HILL (Bureau of Plant Industry, Soils, and Agricultural Engineering, Beltsville, Md.), *Associate Referee*

With his report on Moisture in Fertilizers in 1946 Dr. Ross closed his work on moisture. Death intervened before the report was presented for publication and the manuscript was not found among the deceased's papers. The single recommendation, it is recalled, provided for a collaborative study of the present A.O.A.C. method, the air-flow method and the vacuum-drying method for determining moisture. A paper read by Dr. Ross at the 1944 meeting reviewed his moisture work and discussed the performance of the air-flow and vacuum-drying methods on ammonium nitrate and other fertilizer materials, but did not describe the procedures for the methods. This paper and one on the air-flow method appeared in the November issue of the Association's *Journal*, Vol. 30, 1947.

In view of the publication status of previous work the consensus of opinion last year was that the recommended collaborative work should not be undertaken this year. At the same time the Referee on Fertilizers suggested that, if possible, exploratory work be done on the use of ether as an extractant for moisture and on the use of low-boiling azeotropes. It has not been possible to conduct the suggested work this year. Since the delay of one year has not lessened the need for a collaborative study of methods, the recommendation of last year is retained and the procedures proposed for the air-flow and vacuum-drying methods are attached as a part of this report.

RECOMMENDATION*

It is recommended that a collaborative study be made comparing the

* For report of Subcommittee A and action by the Association, see *This Journal*, **31**, 42 (1948).

present A.O.A.C. method with the air-flow and vacuum-drying methods for determining free moisture in fertilizer materials and mixed fertilizers.

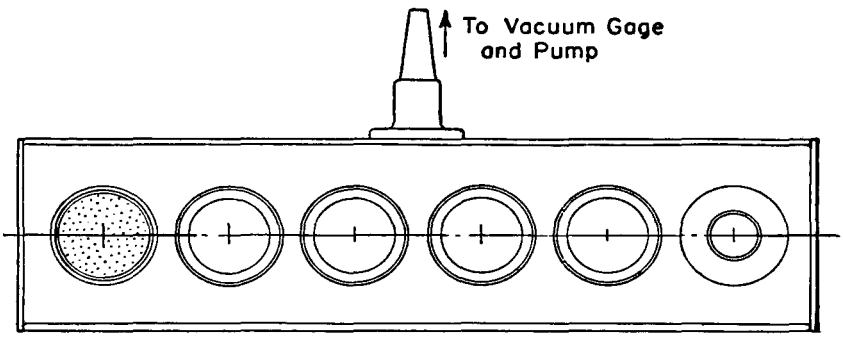
FREE MOISTURE IN FERTILIZERS

Air-flow Method

APPARATUS

(A) *Manifold Assembly.*—A metal box (Fig. 1) $10\frac{1}{2} \times 2\frac{1}{2} \times 1\frac{1}{4}$ inches in size is equipped with a $\frac{1}{4}$-inch nipple centrally located on one side for attachment to a vacuum line, and 6 $1\frac{1}{4}$-inch tapered stopper seats evenly spaced along the top to accommodate No. 6, one-hole, rubber stoppers. A $1\frac{1}{4}$-inch length of light metal tubing, $\frac{1}{2}$ inch in diameter, extends through each rubber stopper to a height of $\frac{1}{4}$ inch above the surface for the purpose of centering a fritted glass crucible over the hole in the stopper. Since the crucible is held in place by suction, it is necessary to grind a smooth surface on the lower edge of each fritted glass crucible and on the surface of the stopper in order to insure an air-tight connection between the edge of the crucible and the stopper when air is being drawn through the sample in the crucible.

(B) *Crucibles.*—Pyrex glass, approximately $1\frac{3}{4}$ inches tall, $1\frac{3}{16}$ inches in diameter and having a $\frac{7}{8}$-inch fine-porosity fritted glass plate. Individual crucibles of a set should all have approximately the same porosity. A matched set may be obtained by selecting several that pass a given quantity of air at constant pressure in approximately the same length of time.



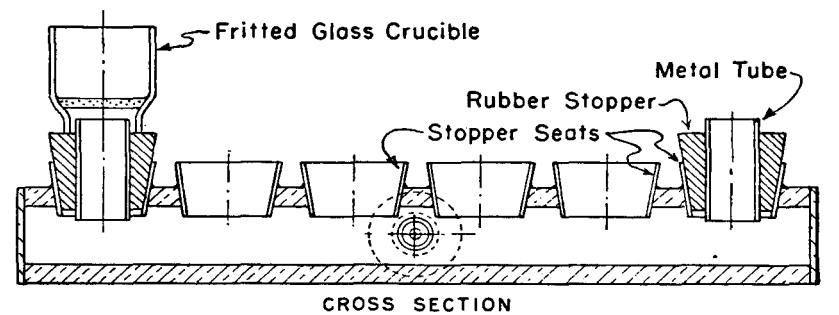


FIG. 1.—Manifold for Use in Determining Moisture by the Air-flow Method

(C) *Vacuum Gage.*—A standard instrument for insertion in the rubber vacuum line between the source of suction and the manifold.

(D) *Constant Temperature Oven.*—A standard laboratory oven, preferably of the type vented so that incoming air passes directly over the heating coils.

DETERMINATION

Weigh 2 g of prepared sample, **2.2,** in a tared, fritted glass crucible. (Extremely hygroscopic or damp materials should be weighed out of difference in covered crucibles.) Place crucible on manifold in the oven at 60°. Aspirate for 2 hours under 15 in. of vacuum. Cool in desiccator, for 30 min. and reweigh. Calculate percentage loss in weight.

Vacuum-drying Method

DETERMINATION

Place 4 g of prepared sample, **2.2,** in a short-type, tared weighing dish of a size not less than 2 in. in diameter. (Extremely hygroscopic or damp materials should be weighed out of difference in covered dishes.) Place in a vacuum desiccator over anhydrous magnesium perchlorate for 16 hours under not less than 25 in. of vacuum. Reweigh and calculate percentage loss in weight.

NOTE: A drying period of 16 hrs. represents over-night drying. The average type of fertilizer will release its free moisture in 3–6 hrs. However, over-night drying insures best results, especially on very damp materials and on samples containing high amounts of adsorbed water.

REPORT ON NITROGEN IN FERTILIZERS

By M. P. ETHEREDGE (Mississippi State Chemical Laboratory, State College, Mississippi), *Associate Referee*

Last year, A. L. Prince pointed out in his report to the Association (*This Journal,* **30,** 228, 1947) the importance of better agreements on the determination of ammonium nitrate. He was somewhat disappointed that so few of his collaborators had tried the Devarda procedure. Therefore, it was recommended that further collaborative work be done on methods of determining nitrogen in ammonium nitrate, with special reference to the Devarda alloy method.

It was further called to the attention of the Association that fertilizers with high nitrates and chlorides were difficult to check, and quite often the nitrogen content was reported as being far lower than the theoretical amount present.

With this in mind, three samples were selected for analysis. Sample No. 1 was a pure ammonium nitrate. Sample No. 2 was a commercial grade of ammonium nitrate. Sample No. 3 contained a large amount of sodium nitrate and potassium chloride.

Samples Nos. 1 and 2 were to be analyzed by the Devarda procedure, distilled with magnesium oxide and, also, with a small amount of sodium hydroxide. Collaborators were asked to use a Corning bulb of the type No. 2020 or No. 2040. This latter bulb, the Iowa type, was included be-

cause some laboratories use it. It has been the experience of the Associate Referee over a period of years that this bulb will produce a higher and less consistent blank than the other type. Collaborators were asked to use a blank.

Owing to the rapidly growing interest in the formaldehyde-titration procedure, collaborators were later asked to use this procedure for the two samples of ammonium nitrate which were sent out for study.

Collaborators were asked to determine nitrogen in the mixed fertilizer, sample No. 3, by the Kjeldahl method, **2.27**. Also enclosed was a copy of the procedure suggested to the Association by Mr. Shuey last fall. Later, the collaborators were asked to try the method of Dyer and Hamence, [*Analyst*, **63**, 866 (1938)].

The samples were sent to twenty-six collaborators. The Associate Referee is grateful to those reporting their results in time to make this report possible. Also, we are indebted to J. W. MacKay, Chief Chemist, North American Cyanamid, Niagara Falls, Canada, for furnishing both samples of the ammonium nitrate. The list of the collaborators follows:

R. L. Willis and A. C. Wark, New Jersey Exp. Sta., New Brunswick
D. M. Salter, Ohio Department of Agriculture, Columbus
C. A. Butt and W. H. Banks, Int. Min. & Chem. Co., East Point, Georgia
L. J. Hardin and H. S. Johnson, Jr., Tenn. Agr. Exp. Sta., Knoxville
R. C. Koch and J. B. Hulsey, Swift & Co. Fert. Works, Hammond, Indiana
J. S. Kuzmeski and A. F. Spelman, Agr. Exp. Sta., Amherst, Massachusetts
Gordon Hart, Asst. State Chemist, Tallahassee, Florida
A. N. Lineweaver and C. T. McCloud, Royster Guano Co., Norfolk, Va.
C. V. Marshall, Dept. of Agriculture, Ottawa, Canada
Ralph D. Miller, Chief Chemist, Spencer Chem. Co., Pittsburg, Kansas
D. B. Bates, Smith-Douglass Co., Norfolk, Virginia
O. W. Ford and R. H. Hedrick, Agri. Exp. Sta., Lafayette, Indiana
C. O. Willits, In Charge Analytical Section, Eastern Reg. Lab., Philadelphia
P. R. Bidez, Alabama Dept. Agr., Auburn
W. A. Morgan, Ammonia Dept., Du Pont, Wilmington
J. W. MacKay, North American Cyanamid, Niagara Falls, Canada
M. M. Phillippe, Clemson Agr. College, Clemson, South Carolina
C. B. Jacobs, Davison Chem. Corp., Baltimore
H. R. Allen, Kentucky Agr. Exp. Sta., Lexington
C. Reynolds Clark, State Chemist, Atlanta, Georgia
Guy S. Mitchell, Chief Chemist, Lion Oil Co., El Dorado, Arkansas
John B. Smith, Chemist, Rhode Island Exp. Sta., Kingston
Philip McG. Shuey, Savannah, Georgia
C. O. Hurst and Archie G. McKee, Mississippi State Chem. Laboratory
Geo. C. Bollinger, Am. Agr. Chem. Co., Baltimore

The results on the three samples are given in Tables 1, 2, and 3.

PROCEDURE OF REPORTING

Results were not calculated to dry basis. The reasoning on this was that the variations on moisture determinations are too great. Where more

TABLE 1.—*Sample No. 1. Total nitrogen in pure ammonium nitrate*

ANALYST	DEVARDA METHOD	MgO DISTILLATION	NaOH DISTILLATION	FORMALDEHYDE TITRATION	MOISTURE
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
1	34.80	34.70	35.14		0.39
2	34.91	34.82	34.80		0.45
3	34.79	34.88	34.94	34.70	0.13
4	34.67	34.86	34.70	34.79	0.02
5	34.63	34.60	34.40	34.77	0.50
6	34.41	34.24	34.35	34.28	0.20
7	34.58	34.60	34.56	34.80	0.27
8	34.83	35.00	34.68		0.18
9	35.09	34.72	34.70	35.06	
10	34.46	34.85	34.70	34.94	0.00
11	34.82	34.76	34.79		
12	35.26	34.99	35.22	34.96	0.51
13	34.77	34.71	34.75		0.10
14	34.76	34.60	34.60		
15	34.89	34.98	35.02	34.94	0.05
16	34.34	34.50	34.62	34.38	
17	34.97	34.67	34.70		
18	34.48	34.50	34.56		0.18
19	34.83	34.88	34.92	34.89	0.28
20	30.40	34.40		34.78	3.31
21				34.96	0.10
22	34.57	34.48	34.72	34.67	0.26
23	34.89	34.84	34.78	34.66	0.21
24	34.90	34.85	34.90	34.92	0.13
25	34.81	34.87	34.90	34.86	0.05

than one bulb was used, merely the Corning No. 2020 was reported. This seems to be the most used bulb. One collaborator used the Davisson bulb, and two used the Iowa bulb, Corning No. 2040.

Owing to a late start this year, it seemed best to defer work on bulbs and traps. The Goessman trap is not necessary if one has a long condensing surface. The Davisson bulb may offer an advantage over the Corning No. 2020 in the Devarda procedure where there is a large blank. A paper describing a new connecting bulb[1] was presented at the meeting which may give us a basis for future work on this score.

DISCUSSION OF RESULTS

Sample No. 1:

This sample was of C. P. grade, and it theoretically contained 34.98% nitrogen (dry basis). If we glance at the results by the Devarda procedure, we see one result very low and two results slightly high. Over half of the other results approach this theoretical amount.

There were no absurd low or high results by the MgO distillation pro-

[1] C. O. Willits, H. J. John, and L. R. Ross, *This Journal*, page 432.

TABLE 2.—*Sample No. 2. Total nitrogen in commercial ammonium nitrate*

ANALYST	DEVARDA METHOD	MgO DISTILLATION	NaOH DISTILLATION	FORMALDEHYDE TITRATION	MOISTURE
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
1	33.80	33.96	33.98		0.45
2	33.70	33.64	33.98		0.56
3	33.94	34.06	34.06	33.66	0.30
4	33.55	33.76	33.72	33.78	0.14
5	33.80	33.53	33.53	33.67	0.68
6	33.08	33.06	33.15	32.96	0.87
7	33.52	33.52	33.60	33.84	0.00
8	34.00	34.00	33.80		0.26
9	33.83	33.56	33.58	33.85	
10	33.58	33.97	33.84	34.01	0.00
11	33.87	33.77	33.69		
12	34.50	34.00	34.36	34.04	0.51
13	33.78	33.66	33.62		0.25
14	33.66	33.60	33.50		
15	33.86	33.80	33.78	34.00	0.17
16	33.32	33.48	33.43	33.56	
17	33.97	33.73	33.90		
18	33.80	33.88	33.76		0.28
19	33.80	33.76	33.84	33.87	0.41
20	29.90	33.00		33.86	2.73
21				34.09	0.03
22	33.58	33.76	33.44	33.64	0.43
23	33.62	33.71	33.66	33.42	0.40
24	33.88	33.81	33.89	33.90	0.29
25	33.84	33.81	33.89	33.75	0.22

cedure. There were two high results when using sodium hydroxide for distillation. The approach to the theoretical amount was about the same order as by the Devarda procedure. Of course, the ammoniacal nitrogen was multiplied by two.

It is regrettable that all did not try the formaldehyde titration method, since this probably offers the simplest possible method for the determination of nitrogen in ammonium nitrate. A cursory examination of the results submitted will show that this procedure seems to give equally as good results as the other methods. This procedure is described in a paper which was presented at the last meeting entitled "Method for Rapid Determination of Total Nitrogen in Ammonium Nitrate Fertilizer."[2]

Sample No. 2:

This sample theoretically contained 33.85% nitrogen. There was one very low result and two high results by the Devarda procedure. A majority of the analysts approached the theoretical amount, or were slightly over.

[2] Ralph D. Miller, *This Journal*, page 373.

TABLE 3.—*Sample No. 3. Total nitrogen in mixed fertilizer (high chloride nitrate)*

ANALYST	KJELDAHL (2.27)	SHUEY METHOD	DYER HAMENCE	KJELDAHL (DOUBLE SALICYLIC)
	per cent	*per cent*	*per cent*	*per cent*
1	10.50	10.86		
2	10.20			
3	10.80			
4	10.52			
5	10.70			
6	10.55			
7	10.70	10.60		
9	10.03	9.58		
11	10.45			
12	10.78			
13	10.62			
14	10.83			
15	10.74	10.75		
17	10.58			
18	10.47			
19	10.45			
20	10.40			
22	10.68			
23	10.61	10.97		
24	10.75	10.74	10.86	10.86
25	10.82	10.84		

The nitrogen obtained by doubling the ammoniacal nitrogen produced by distillation with magnesium oxide and sodium hydroxide compares favorably with that obtained by the Devarda procedure. There are very few high results; but several failed to obtain the theoretical amount. Here, again, the formaldehyde titration shows up quite well.

Sample No. 3:

This sample was prepared in the Mississippi State Chemical Laboratory. The theoretical composition was 11.0–3.7–10.8. The nitrogen was from nitrate of soda and the potassium from potassium chloride.

No one obtained the theoretical amount of nitrogen by the Kjeldahl procedure **(2.27)**. It is unfortunate that so few tried out the Shuey method, and the procedure suggested by Dyer and Hamence. One collaborator obtained 10.86 by this latter procedure, and he also obtained this amount when doubling the amount of salicylic in method **2.27.** Another analyst obtained 10.97 by the Shuey method.

In Mississippi a 4% tolerance on the total value is allowed before a manufacturer is penalized. This type of material can certainly be determined within a 4% tolerance. However, many States are not so liberal; therefore, it is necessary to find a more exacting procedure.

OTHER METHODS

One analyst used the Ulsch method as revised by Street and obtained 34.84 and 33.85 per cent, respectively, on the two ammonium nitrate samples. This is probably best known as the Reduced Iron Method.

Another analyst used the Ferrous Sulfate-Zinc-Soda Method and obtained 34.92 and 33.76, respectively.

One analyst used Arnd alloy. He obtained 34.60, *vs.* his 34.80 with Devarda alloy, on sample No. 1; and he obtained 33.50, *vs.* 34.60 on sample No. 2. It has always been found in the Mississippi State Chemical Laboratory that the Arnd alloy gives lower results than the Devarda alloy.

Several collaborators reported results by the Devarda method on sample No. 3. These results were not given. They would apply to this particular mixture; however, not in general to this type of mixture.

COMMENTS BY COLLABORATORS

Analyst 1 believes that the Reduced Iron Method still has a place in our *Book of Methods.*

Analyst 7 gives a warning to the dangers of using selenium as a catalyst.

Analyst 9 prefers method **2.28** to **2.27.**

Analyst 12 thinks that either the magnesium oxide distallation, or the formaldehyde titration, offers a simpler approach to the determination of nitrogen in ammonium nitrate.

Analyst 13 gives analyses and a detailed discussion of bulbs. Out of deference to his paper which was presented to the Association, we shall omit the discussion here.

Analyst 15 brings out a good point. He suggests that the limitations should be worked out on method **2.27** for high nitrate-chloride mixtures.

Analyst 16 found no difference by Devarda procedure using a Goessman trap. He also points out the necessity of calcining the magnesium oxide, and the importance of using a small amount of sodium hydroxide for ammoniacal distillation of ammonium nitrate. He further warns us of the necessity of having conditions just right for the formaldehyde titration.

Analyst 19 prefers to allow his Devarda determinations to stand overnight before distilling.

Analyst 20 thinks the formaldehyde procedure is very simple.

Analyst 23 also thinks the formaldehyde procedure offers possibilities; however, he suggests using a smaller amount of sample.

CONCLUSIONS AND RECOMMENDATIONS*

All four methods, when properly handled, show possibilities for approaching the theoretical amount of total nitrogen in ammonium nitrate. The formaldehyde-titration procedure seems to be the simplest. There are apparently enough data available to set it up as a tentative method. However, more work and comparison are recommended before adopting it as official.

More work should be done on the nitrate-chloride mixtures. Working with dry samples, keeping our size of sample low, and increasing our

* For report of Subcommittee A and action by the Association, see *This Journal*, 31, 42 (1948).

salicylic, a nearer approach to the theoretical amount of nitrogen is possible.

No report was given on magnesium and manganese, or on acid- and base-forming quality.

REPORT ON POTASH

By O. W. Ford (Purdue University Agricultural Experiment Station, Lafayette, Ind.), *Associate Referee*

In accordance with the recommendations of the Association that work on the details of the method for the determination of potash in commercial fertilizers be continued (*This Journal*, **30**, 228, 1947), referee work was conducted this year by collaboration. A copy of the proposed work and a set of fertilizer samples were sent to each chemist who had expressed a willingness to collaborate. This report summarizes the results of the fourteen chemists who found time to do the work and report to the Associate Referee.

Collaborative work on potash in fertilizers was directed to studies of the effect of increasing the concentrations of ethyl alcohol and formula 30 alcohol, and of saturating with potassium chloroplatinate the acid-alcohol from ethyl and formula 30 alcohols at different concentrations.

OUTLINE OF COLLABORATIVE WORK ON POTASH IN 1947

Suggestions to Collaborators

If collaborators cannot do all of the work outlined, they should complete individual sections. In reporting results, the report should include the kind of alcohol used, the amount used for each determination, and the concentration in terms of per cent by volume. Record all individual determinations as well as the average. Report by August 15, 1947. Contact the Associate Referee in case of doubt as to directions. Report all results by washing out the K_2PtCl_6 and weighing back. All should remember that the present official method calls for 80% ethyl alcohol by volume and when used the temperature of it should not exceed 30°C.

Samples selected for this work are a composite of several manufacturers' samples and should analyze approximately as follows: Sample number 4, analysis 0-9-27; sample number 5, analysis 3-9-18; sample number 6, analysis 0-0-55; sample number 8, analysis 4-12-8. All collaborators are urged to prepare a composite solution from each sample for all determinations.

PROCEDURE

Section I (80% ethyl alcohol and/or 80% formula 30 alcohol).—

A. Make six determinations of potash on samples 4, 5, 6, and 8 by the official method using 80% by volume ethyl alcohol and/or 80% formula 30 alcohol.

B. Repeat A, except that the acidified 80% ethyl alcohol or 80% formula 30 alcohol is previously saturated with K_2PtCl_6.

Section II (85% ethyl alcohol and/or 85% formula 30 alcohol).—

A. Repeat section I, A, except that 85% ethyl alcohol or 85% formula 30 alcohol be used.

B. Repeat A, except that the acidified 85% ethyl alcohol or 85% compound 30 alcohol is saturated with K_2PtCl_6.

Section III (90% ethyl alcohol and/or 90% formula 30 alcohol).—

A. Repeat section I, A, except that 90% alcohol ethyl or 90% formula 30 alcohol be used.

B. Repeat A, except that the acidified 90% ethyl alcohol or 90% compound 30 alcohol is saturated with K_2PtCl_6.

Section IV (95% ethyl alcohol and/or 95% formula 30 alcohol).—

A. Repeat section I, A, except that 95% ethyl alcohol or 95% formula 30 alcohol be used.

B. Repeat A, except that the acidified 95% ethyl alcohol or 95% compound 30 alcohol saturated with K_2PtCl_6 be used.

COMMENTS ON RESULTS

The results of the chemists who collaborated on the potash work appear in Tables 1 and 2. Tables 3 and 4 show the average values obtained by the use of the two alcohols used alone and with the corresponding acid-alcohols saturated with K_2PtCl_6 at the varying concentrations.

From the list of collaborators in Tables 1 and 2 it will be noted that the commercial chemists all used formula 30 alcohol and the control chemists used ethyl alcohol for this work. Only one chemist, collaborator 14, found time to use both alcohols in the studies. This was to be expected from past years' reports, as the commercial chemist cannot obtain ethyl alcohol tax-free for this work and because of the difference in cost, they usually use formula 30. Many commercial chemists have been using formula 30 for years even though the method was developed using ethyl alcohol. This work could be justified because a comparison of the two alcohols is needed.

1. With 80% formula 30 alcohol in 26 of 28 cases more potash was obtained when the acid-alcohol was saturated with K_2PtCl_6 (Table 2). The average increase in potash for the four samples was 0.18% with differences ranging from 0.09% to 0.27%. In most cases the greater the K_2O content the greater the increase.

2. With 85% formula 30 alcohol in 23 of 24 cases more potash was obtained by saturation of the acid-alcohol with K_2PtCl_6. The average increase was 0.12% and differences ranged from 0.08% to 0.20%.

3. With 90% formula 30 alcohol, in 18 of 24 cases more potash was obtained by saturation of the acid-alcohol with K_2PtCl_6. The average increase was only 0.06% with differences ranging from 0.02% to 0.11%.

4. With 95% formula 30 alcohol, in 32 of 36 cases more potash was obtained by saturation of the acid-alcohol with K_2PtCl_6. The average increase was 0.05% and differences ranged from 0.04% to 0.06%. As the concentration of the alcohol was increased, the amount of increase of potash by saturation of the acid-alcohol became less, which indicates that saturation of the acid-alcohol has a great effect on the amount of potash obtained at the lower concentrations of alcohol. This becomes less as the alcohol concentration is increased due to the smaller solubility of K_2PtCl_6 in the more concentrated alcohol.

TABLE 1.—*Effect of concentration of ethyl alcohol and acid-alcohol and saturation of the acid-alcohol with K_2PtCl_6 on the determination of potash in fertilizers*

ANALYST NO.	NO. OF ANALYSES	80% BY VOLUME		85% BY VOLUME		90% BY VOLUME		95% BY VOLUME	
		NOT SATURATED	SATURATED WITH K_2PtCl_6	NOT SATURATED	SATURATED WITH K_2PtCl_6	NOT SATURATED	SATURATED WITH K_2PtCl_6	NOT SATURATED	SATURATED WITH K_2PtCl_6
SAMPLE #4 0-9-27 ANALYSIS									
6	3	26.48	26.79	26.59	27.03	26.80	27.04	26.98	27.04
7	6	26.94	26.95	26.85	27.15	26.85	27.06	27.20	27.13
11	3	26.69	26.74			26.76	26.90		
12	6	26.63	27.15	26.76	27.25	26.97	27.26	27.15	27.26
14	6	27.27	27.64	28.00	28.20	27.88	28.15	28.23	28.32
Average		26.82	27.05	27.05	27.40	27.05	27.28	27.39	27.44
Av. increase due to saturation			+0.23		+0.35		+0.23		+0.05
SAMPLE #5 3-9-18 ANALYSIS									
6	3	18.27	18.69	18.37	18.85	18.55	18.87	18.65	18.93
7	6	18.26	18.45	18.40	18.58	18.46	18.55	18.16	17.90
11	3	18.70	18.82			18.80	18.92		
12	6	18.50	18.81	18.79	18.83	18.87	18.87	18.88	18.91
14	6	18.51	18.82	18.70	18.70	18.64	18.64	18.80	18.94
Average		18.45	18.72	18.57	18.74	18.66	18.77	18.62	18.67
Av. increase due to saturation			+0.27		+0.17		+0.11		+0.05

TABLE 1.—(*continued*)

SAMPLE #6 0-0-55 ANALYSIS

ANALYST NO.	NO. OF ANALYSES	80% BY VOLUME		85% BY VOLUME		90% BY VOLUME		95% BY VOLUME	
		NOT SATURATED	SATURATED WITH K_2PtCl_6	NOT SATURATED	SATURATED WITH K_2PtCl_6	NOT SATURATED	SATURATED WITH K_2PtCl_6	NOT SATURATED	SATURATED WITH K_2PtCl_6
6	3	58.99	59.04	59.09	59.53	59.11	59.55	59.24	59.58
7	6	59.17	59.58	60.07	60.24	59.10	59.14	59.48	59.66
11	3	59.03	59.07			59.29	59.31		
12	6	57.02	57.52	57.11	57.55	57.32	57.59	57.45	57.53
14	6	57.70	58.40	57.80	58.38	57.84	58.37	57.75	57.87
Average		58.38	58.72	58.52	58.92	58.53	58.79	58.48	58.66
Av. increase due to saturation			+0.34		+0.40		+0.26		+0.18

SAMPLE #8 4-12-8 ANALYSIS

6	3	7.81	8.03	7.85	8.12	7.84	8.17	7.88	8.19
7	6	8.06	8.13	8.21	8.38	8.38	8.51	8.53	8.61
11	3	7.74	7.82			7.84	7.86		
12	6	7.86	8.02	7.95	8.02	8.03	8.03	8.03	8.03
14	6	8.35	8.76	8.54	8.86	8.52	8.65	8.45	8.57
Average		7.96	8.15	8.14	8.35	8.12	8.24	8.22	8.35
Av. increase due to saturation			+0.19		+0.21		+0.12		+0.13

TABLE 2.—*Effect of concentration of formula 30 alcohol and acid-alcohol and saturation of the acid-alcohol with K_2PtCl_6 on the determination of potash in fertilizers*

ANALYST NO.	NO. OF ANALYSIS	80% BY VOLUME		85% BY VOLUME		90% BY VOLUME		95% BY VOLUME	
		NOT SATURATED	SATURATED WITH K_2PtCl_6	NOT SATURATED	SATURATED WITH K_2PtCl_6	NOT SATURATED	SATURATED WITH K_2PtCl_6	NOT SATURATED	SATURATED WITH K_2PtCl_6
SAMPLE #4 0-9-27 ANALYSIS									
1	6	26.92	27.00	26.94	26.93	27.14	27.07	27.09	27.08
2	6	26.59	26.83					27.00	27.09
3	6	26.64	26.68	26.62	26.76	26.70	26.71	26.73	26.75
4	6	26.71	26.92	27.00	27.11	26.96	27.12	27.05	27.16
5	6	26.88	26.89	26.89	26.89	26.92	26.92	26.94	26.96
8	6							26.89	26.97
9	6	26.55	27.12	26.89	27.06	26.91	27.06	27.00	27.09
10	6							26.34	26.46
14	6	27.75	27.89	27.88	28.03	27.84	27.88	27.96	27.95
Average		26.86	21.07	27.03	27.12	27.08	27.13	27.00	27.06
Av. increase due to saturation			+0.21		+0.09		+0.05		+0.06
SAMPLE #5 3-9-18 ANALYSIS									
1	6	18.38	18.39	18.55	18.55	18.63	18.63	18.62	18.66
2	6	18.41	18.60					18.64	18.66
3	6	18.40	18.51	18.49	18.49	18.51	18.53	18.57	18.59
4	6	18.47	18.59	18.64	18.69	18.62	18.66	18.70	18.74
5	6	18.56	18.59	18.56	18.65	18.70	18.73	18.79	18.79
8	6							18.88	18.97
9	6	18.59	18.88	18.67	18.95	18.76	18.94	18.74	18.79
10	6							18.16	18.31
14	6	17.69	17.93	17.89	17.95	17.82	17.96	17.82	17.87
Average		18.35	18.49	18.47	18.55	18.34	18.41	18.55	18.60
Av. increase due to saturation			+0.14		+0.08		+0.07		+0.05

TABLE 2.—(*continued*)

SAMPLE #6 0-0-55 ANALYSIS

ANALYST NO.	NO. OF ANALYSES	80% BY VOLUME		85% BY VOLUME		90% BY VOLUME		95% BY VOLUME	
		NOT SATURATED	SATURATED WITH K_2PtCl_6	NOT SATURATED	SATURATED WITH K_2PtCl_6	NOT SATURATED	SATURATED WITH K_2PtCl_6	NOT SATURATED	SATURATED WITH K_2PtCl_6
1	6	58.14	58.32	58.26	58.39	58.54	58.62	58.60	58.65
2	6	56.92	56.94					57.40	57.50
3	6	59.31	59.20	59.22	59.36	59.39	59.35	59.49	59.56
4	6	57.13	57.40	57.33	57.42	57.26	57.41	57.49	57.53
5	6	57.67	57.90	57.83	57.80	57.79	57.80	57.78	57.79
8	6							57.00	57.06
9	6	58.96	59.92	59.13	59.82	59.18	59.54	59.56	59.59
10	6							58.90	59.12
14	6	56.95	57.30	57.19	57.35	57.13	57.22	57.28	57.28
Average		57.87	58.14	58.16	58.36	58.21	58.32	57.05	57.11
Av. increase due to saturation			+0.27		+0.20		+0.11		+0.06

SAMPLE #8 4-12-18 ANALYSIS

1	6	8.28	8.30	8.30	8.36	8.33	8.32	8.32	8.37
2	6	7.80	7.85					8.03	8.08
3	6	7.97	7.96	7.96	8.00	8.10	8.07	8.12	8.11
4	6	7.78	7.99	7.86	7.93	7.90	7.94	7.93	7.96
5	6	7.88	7.93	7.95	8.09	8.08	8.07	8.06	8.16
8	6							8.11	8.16
9	6	7.94	8.24	8.00	8.29	8.06	8.25	8.08	8.15
10	6							7.77	7.86
14	6	8.55	8.60	8.54	8.61	8.73	8.62	9.33	9.24
Average		8.03	8.12	8.10	8.21	8.20	8.22	8.26	8.30
Av. increase due to saturation			+0.09		+0.11		+0.22		+0.04

TABLE 3.—*Ethyl alcohol and acid-alcohol saturated with K_2PtCl_2 vs. formula 30 alcohol and acid-alcohol saturated with K_2PtCl_2 in the determination of potash in fertilizer at 80, 85, 90, and 95% by volume concentrations*

ALCOHOL CONCENTRATION	TYPE OF ALCOHOL	SAMPLE NUMBER			
		4	5	6	8
80	Ethyl	27.10	18.72	59.68	8.15
80	Formula 30	27.07	18.49	58.14	8.12
85	Ethyl	27.40	18.63	58.92	8.35
85	Formula 30	27.12	18.55	58.36	8.21
90	Ethyl	27.37	18.73	58.75	8.34
90	Formula 30	27.13	18.41	58.32	8.22
95	Ethyl	27.32	18.72	57.24	8.25
95	Formula 30	27.06	18.60	57.11	8.30

5. Slightly larger increases of potash were obtained by the saturation of the acid-alcohols of ethyl alcohol at the different concentrations than with formula 30 alcohol.

6. In most cases both formula 30 and ethyl alcohol gave higher potash values with an increase in alcohol concentration (Tables 3 and 4). The greatest discrepancy was with sample #6. This was a mixture of potash salts and evidently subject to segregation so that if all the determinations were not made from a composite solution as indicated in the outline of the work, erratic results would be obtained. This is about the only way one could account for the great variation in potash values reported by the collaborators for this sample. Here again, however, most collaborators ob-

TABLE 4.—*Ethyl alcohol and acid-alcohol not saturated with K_2PtCl_2 vs. compound 30 alcohol and acid-alcohol not saturated with K_2PtCl_2 in the determination of potash in fertilizer at 80, 85, 90, and 95% by volume concentration*

ALCOHOL CONCENTRATION	TYPE OF ALCOHOL	SAMPLE NUMBER			
		4	5	6	8
80	Ethyl	26.82	18.45	59.32	7.96
80	Formula 30	26.86	18.35	57.87	8.03
85	Ethyl	27.05	18.47	58.52	8.14
85	Formula 30	27.03	18.47	58.16	8.10
90	Ethyl	27.12	18.63	58.44	8.19
90	Formula 30	27.08	18.34	58.21	8.20
95	Ethyl	27.06	18.66	57.09	8.15
95	Formula 30	27.00	18.55	57.05	8.26

tained more potash both by saturation of the acid-alcohol and by use of an increased alcohol concentration.

7. In general about 0.1% more potash was obtained by using ethyl alcohol than by using formula 30 alcohol. This was true whether the acid-alcohol was saturated with K_2PtCl_6 or used without saturation.

Since approximately the same amount of potash can be obtained by saturation of acid-alcohol or by increasing the concentration of the alcohol, it would seem that the choice of conditions would most logically depend

TABLE 5.—*Kind of alcohol and part of the equipment used in 1947 collaborative potash work*

ANALYST NO.	KIND OF ALCOHOL	PER CENT ALCOHOL BY VOLUME	VOLUME OF ALCOHOL	KIND OF DISH CAPACITY	TYPE OF FILTER, TEMPERATURE OF ALCOHOL
1	Formula 30	80, 85, 90, 95	150 ml	Platinum 75 ml	Asbestos Gooch 20–21° C.
2	Formula 30	80, 95	125–150 ml		
3	Formula 30	80, 85, 90, 95			
4	Formula 30	80, 85, 90, 95			
5	Formula 30	80, 85, 90, 95	150 ml	Silica and porcelain 90 ml	20–25° C.
6	Ethyl	80, 85, 90, 95			
7	Ethyl	80, 85, 90, 95			
8	Formula 30	95			
9	Formula 30	80, 85, 90, 95	95–105 ml		
10	Formula 30	95	115–120 ml		
11	Ethyl	80, 90			
12	Ethyl	80, 85, 90, 95			
13	Ethyl	80, 85, 90, 95			
14	Ethyl	80, 85, 90, 95	125 ml	Platinum 125 ml	Sintered Pyrex M. 20–25° C.
	Formula 30	80, 85, 90, 95	125 ml	Platinum 125 ml	Sintered Pyrex M. 20–25° C.

on the ease and convenience of laboratory manipulation. In most laboratories the use of more concentrated alcohol is more convenient than the saturation of the acid-alcohol with K_2PtCl_6.

Ewan, Ford, and Schall (1) and Schall and Ford (2) have shown that increases in the potash values obtained by increasing the alcohol concentration and by saturation of the acid-alcohol with K_2PtCl_6 are true potash values within the limits of experimental error. Therefore, the Associate Referee recommends that 95% ethyl alcohol and acid-alcohol or 95% formula 30 alcohol and acid-alcohol without saturation of the acid-alcohol with K_2PtCl_6 replace 80% ethyl alcohol and acid-alcohol for the determination of potash in commercial fertilizers. The Associate Referee (from a Commercial Solvent Corporation pamphlet) understands that formula 30

is a mixture of 100 gallons of ethyl alcohol and 10 gallons of pure methyl alcohol.

LIST OF COLLABORATORS

(1) Austin, W. R., and Buford, Madelane, Armour Fertilizer Works, Nashville, Tenn.
(2) Bates, D. B., Smith-Douglass Company, Inc., Norfolk, Va.
(3) Batton, H. C., Swift and Company, Norfolk, Va.
(4) Bollinger, G. C., Trimble, C. E., Charlton, R. C., The American Agricultural Chemical Company, New York City, N. Y.
(5) Byers, C. R., and Vaugh, H., Armour Fertilizer Works, Carteret, N. J.
(6) Etheredge, M. P., Mississippi State Chemical Laboratory, State College, Miss.
(7) Fudge, J. F., Ogier, T. L., and co-workers, Agricultural and Mechanical College of Texas, College Station, Tex.
(8) Lineweaver, A. N., F. S. Royster Guano Company, Norfolk, Va.
(9) Powell, R. O., and Moxon, H. L., Virginia-Carolina Chemical Corporation, Richmond, Va.
(10) Shuey, P. Mc., Shuey & Company, Savannah, Ga.
(11) Smith, C. Tyson, and Kuzmeski, J. W., Agricultural Experiment Station, Amherst, Mass.
(12) Smith, R. M., Agricultural Department, Chemical Division, Tallahassee, Fla.
(13) Willis, R. L., Rutgers University, Agricultural Experiment Station, New Brunswick, N. J.
(14) Schall, E. D., Purdue University, Agricultural Experiment Station, Lafayette, Ind.

COMMENTS OF COLLABORATORS

(1) We favor the control of temperatures even though it means some additional trouble and work, since judging from previous years work on this collaborative work, and regular work too, that weaker alcohols and normal summer temperatures found in many laboratories do give high solubility of potash in a great many instances coming under our observation.

We prefer the selection of 90% or 85% alcohol as giving on the average the more satisfactory results, as compared with 80% which we think is too low for average routine work, while at times the 95% strength does not give as clean a precipitate as the preferred strengths do give.

(2) We use a muffle furnace for burning down our potash and found that by increasing our time in the furnace the back weight, after washing out the K_2PtCl_6, was kept at a minimum. We feel that the back weight found is due to the incomplete burning off and subsequent burning of the potash.

There was a definite amount of residue.

(5) Use factor weight 1.938 grams in 250 and 25 ml aliquots equivalent to .1938 grams sample instead of 2.5 grams in 250 ml.

(10) From .09% to 0.22% K_2O increase of potash obtained by the saturation of the acidified formula 30 alcohol.

(12) In saturating the alcohols this year, I found a slight reduction or reducing effect on the potassium-chloro-platinate as evidenced by the precipitate turning brown and the solution taking on a very deep, brownish color which effect was more pronounced with the weaker alcohols. This is the first time that I have noticed this effect on saturating alcohols and I am wondering if the alcohols we are getting today are different in any way than in former years. However, when the alcohol is filtered and used in the tests, it seems to make no apparent difference. I much favor the use

of 95% alcohol when possible. Where this is not possible, it seems to me that alcohol of better than 80% should be recommended, even though saturated.

(14) Favor the use of more concentrated alcohol than the present 80% and for ease and accuracy of laboratory manipulation omit the saturation of the acid-alcohol with K_2PtCl_6.

ACKNOWLEDGMENT

The thanks of the Associate Referee are extended to F. W. Quackenbush and E. D. Schall of the Agricultural Chemistry Department, Purdue University, for valuable suggestions and criticisms in the development of the investigations covered by this report. In addition, thanks are extended to the other collaborators for their cooperation.

RECOMMENDATIONS*

It is recommended—

(1) That the 80% alcohol used in the determination of potash in fertilizers be changed to 95% ethyl alcohol or 95% formula 30 alcohol.

(2) That section **2.40 (d)** of the *Methods of Analysis* be changed to read: **(d)** *Acid-alcohol.*—Mix 200 ml of 95% ethyl alcohol, or 95% formula 30 alcohol, with 20 ml of concentrated HCl and cool to room temperature (official, first action).

(3) That all references to 80% alcohol in section **2.42 (a)** of the *Methods of Analysis* (1945) be changed to 95% ethyl alcohol or 95% formula 30 alcohol (official, first action).

LITERATURE CITED

(1) EWAN, M. A., FORD, O. W., and SCHALL, E. D., *Anal. Chem.* (in press).
(2) SCHALL, E. D., and FORD, O. W., *This Journal*, page 397.

No reports were given on sulfur, copper and zinc, or boron in fertilizers. For report on boron under Plants, see page 284.

REPORT ON CEREAL FOODS

By V. E. MUNSEY (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Referee*

The Associate Referee on soy bean flour has submitted no report. He has, however, kept in contact with the Committee on Seed and Meal Analyses of the American Oil Chemists Society and submitted their report on the analysis of soy bean flour. This committee has investigated the methods of analysis for soy bean flours for moisture and volatile matter, oil, ash, protein, and crude fiber. Its findings are published in *J. Am. Oil Chem. Soc.* **24,** 303 (1947). Their study covers the three types of soy flour, *i.e.*, high fat, low fat, and defatted flours. The method for moisture specifies 5 g at 130°C. for 2 hours, in a forced draft oven, while the present tenta-

* For report of Subcommittee A and the action of the Association, see *This Journal*, **31,** 42 (1948).

tive method dries 2 g at 130° for 1 hour in an air oven. This additional hour at 130° approaches very closely the conditions of the official air oven method for feeds (2 hours at 135°) which includes soy bean meal. The method for oil is essentially the same as the present tentative method, with the exception that the extraction period is specified for 5 hours and weight of sample at ca 2 g for full fat flour and 5 g for low or defatted flour. The method for ash is the same as the official method for feed. This requires ignition of 2 g sample in previously heated muffle furnace at 600°C. and maintained at this temperature ($\pm$15°C.) for 2 hours with automatic pyrometer control, whereas the present tentative method ignites at 550°C. until a gray ash results. Crude fiber is the same as the present tentative method. The nitrogen varies from the present tentative method in that the amount of K_2SO_4 or Na_2SO_4 is specified at 10 g and the catalyst limited to HgO or its equivalent in metallic Hg and no use of $Na_2S_2O_3$ solution. The Associate Referee emphasizes the advantage of the adoption of the same methods by the A.O.C.S. and the A.O.A.C.

RECOMMENDATIONS*

It is recommended—

(1) That both procedures proposed by the Associate Referee for the determination of phosphorus in cereals and cereal products be adopted official (first action) and the study be continued.

(2) That the dry ashing method for iron **20.9–20.12,** inclusive, be made official (final action) for enriched macaroni products, degerminated, bolted and whole corn meals, and that the study be discontinued.

(3) That the wet ashing method for iron (*This Journal,* **30,** 71, 1947) be made official (final action) and that study be discontinued.

(4) That the studies on determination of starch in raw and cooked cereals be continued.

(5) That the tentative method for the determination of fat acidity in grain, flour, corn meal, and whole wheat flour **(20.18–20.21,** inclusive) be further studied and also that the relationship of acidity to unsoundness be studied.

(6) That the Associate Referee continue his work reported this year on the application of the method for reducing and non-reducing sugars in flour **20.28–20.30,** inclusive, to the determination of sugars in bread and other bakery products, with special consideration to the article on this subject published by R. M. Sandstedt and G. C. Fleming (*This Journal,* **30,** 550–52).

(7) That the tentative method for benzoyl peroxide in flour, **20.53,** be continued for rye flour, and that the method proposed by the Associate Referee replace it for wheat flour.

(8) That work be continued on methods for determination of available CO_2 in self-rising flour containing added $CaCO_3$.

* For report of Subcommittee D and action of the Association, see *This Journal,* **31,** 58 (1948).

(9) That the method for the determination of lactose in bread (*This Journal*, **24**, 630) be further studied.

(10) That the determination of milk fat in bread, **20.86**, be further studied.

(11) That the methods for the determination of proteolytic activity of flour be continued.

(12) **(a)** That the method for moisture in soy flour, **20.77**, be changed to read—"Moisture—*see* **20.2** or **20.4**, with the exception that a 5 g sample be dried 130° for 2 hours." **(b)** That the method for ash, **20.78**, be changed to read—"Ash—*see* **27.9**." **(c)** That the method for nitrogen, **20.79**, be changed to read—"Proceed as directed under **2.26**, using 10 g K_2SO_4 or Na_2SO_4 and 0.7 g of HgO or its equivalent in Hg with the additional option of using sodium alizarin sulfonate." **(d)** That the method for oil, **20.82**, be changed to read "See **31.67** except that ca 2 g full-fat flour or 5 g low or defatted soy flour be extracted for 5 hours" and that the study be continued on these methods.

(13) That studies be made on the detection and determination of soy bean flour in cereal products.

(14) That the method proposed by the Associate Referee for determination of the amount of added inorganic material in phosphated and self-rising flour be further studied.

(15) That the method referred to in *This Journal*, **25**, 83–84, for the determination of unsaponifiable matter and sterols in noodles be studied to determine their applicability to other foods containing eggs.

(16) That studies of methods for the determination of albumin in noodles and other farinaceous egg-containing products be continued.

(17) That the method for the determination of total solids, **20.84 (b)**, for raisin bread and bread containing raisins and fruits adopted as official first action *This Journal*, **30**, 72 (1947) be adopted as official (final action), and that study on the determination of moisture, fat, crude fiber, ash, and protein in bakery products be continued.

(18) That the study on the determination of moisture in all flour-like products containing sodium bicarbonate as one of its constitutents be continued.

(19) That the study on the determination of bromates in flour be continued along the lines suggested in this year's report of the Associate Referee.

(20) That the study on the determination of apparent viscosity measurements of flour be discontinued.

No reports were given on starch in raw and cooked cereals, or on fat acidity in grain, flour, corn meal, and whole wheat flour.

REPORT ON BENZOIC ACID IN FLOUR

By V. E. MUNSEY (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

The present tentative method for the determination of benzoic acid in flour (*Methods of Analysis*, sec. **20.53**, p. 253) is long, inconvenient, and variable recoveries of benzoic acid result by this steam distillation procedure. A direct extraction of flour acidified with various amounts each of HCl and H_2SO_4 indicate organic solvents can be used. The same flour bleached with benzoyl peroxide gave the following recoveries on extraction of the HCl acidified flour with four solvents, *i.e.*, petroleum ether 20 per cent, benzene 31 per cent, ether 88 per cent, and isopropyl ether 89 per cent. Results on known amounts of benzoic acid added to flour showed a 94, 94, and 103 per cent recovery with ether, and 93 per cent with isopropyl ether. However, the recoveries for added known amounts of benzoyl peroxide were variable, depending on the degree of conversion of the peroxide to benzoic acid by the flour. Reducing substances were added to the flour before acidification with HCl to effect conversion of any benzoyl peroxide not completely changed to benzoic acid by the flour. A freshly prepared mixture of flour and a benzoyl peroxide bleaching agent was analyzed by adding ferrum reductum, hydroquinone, hydroxylamine, sodium hydrosulfite, and stannous chloride in approximately equivalent amounts to the flour before acidification with HCl. The hydroquinone, hydroxylamine, and sodium hydrosulfite gave the same results as the control, indicating no reduction of the peroxide. The stannous chloride gave a 63 per cent conversion but caused interference in the procedure. Ferrum reductum gave 100 per cent recovery and appeared to be an ideal reducing agent without any interference. A few commercial flours bleached with benzoyl peroxide gave 92–95 per cent as much benzoic acid without treatment with ferrum reductum. The small amount of interfering pigments extracted from flour was reduced somewhat by the use of carbon (Nuchar W) without loss of benzoic acid. Benzoic acid in acetone added to ether and analyzed showed 101 and 96 per cent recovery without carbon, and 97 and 95 per cent with addition of carbon. The satisfactory application of the above principles led to the submission of a tentative procedure to 12 collaborators. The method in detail is given in *This Journal*, **31**, 80 (1948), under "Changes in Methods of Analysis."

Three samples of flour were sent to each collaborator. Sample No. 1 had no added benzoyl peroxide. Sample Nos. 2 and 3 had about 8.7 and 17.4 mg of benzoic acid per 100 g of flour, respectively, based on calculation from an analysis of the benzoyl peroxide bleaching agent. The collaborators were asked to report both the qualitative and quantitative results. The results from eight collaborators are given in Table 1.

TABLE 1.—*Benzoic acid in flour*

COLLABORATOR	SAMPLE NO. 1	SAMPLE NO. 2	SAMPLE NO. 3
	p.p.m.	*p.p.m.*	*p.p.m.*
1	—	6.3	13.5
2	5.6	11.0	13.1
3	—	10.6	10.4
4	2	5	10
5	negative	positive	positive
6	0.1	8	10
7	0	11.2	20.0
		10.4	19.2
8	0.5	7.6	15.6
		8.2	15.8

In general these results do not indicate satisfactory quantitative measurements. They do indicate the degree of bleaching in accordance with the amount added and definitely indicate a flour with normal commercial bleach with benzoyl peroxide. Sample 3 contained benzoyl peroxide in the amount commonly used commercially, while sample 2 is considerably below usual practice. The qualitative results were correct except for one collaborator who thought the faint color in the untreated sample No. 1 might represent a little benzoic acid. Collaborator 4 reports 100 per cent recovery of known added amounts of benzoic acid to flour by this method. While the quantitative collaborative results did not measure up to that expected by the Associate Referee, the qualitative results indicate the method is satisfactory for the detection of benzoyl peroxide-treated flour. Further work should be done on the quantitative measurement.

The Associate Referee wishes to gratefully acknowledge the assistance of the following collaborators:

R. L. Gray, Lucidol Division, Novadel-Agene Corp., Buffalo, N. Y.
K. L. Fortmann, Novadel-Agene Corp., Newark, N. J.
W. L. Rainey, Commander Larabee Milling Co., Minneapolis, Minn.
J. J. Winston, Jacobs Products Laboratory, Inc., New York, N. Y.
R. C. Koehn, General Mills, Inc., Minneapolis, Minn.
F. J. McNall and M. A. Braun, of Food and Drug Administration, Dept. of Agriculture.

It is recommended* that the tentative method for benzoyl peroxide in flour, **20.53,** be continued for rye flour and the title changed to "Benzoic Acid in Rye Flour"; and that the proposed method replace it for wheat flour.

* For report of Subcommittee D and action of the Association, see *This Journal*, 31, 58 (1948).

REPORT ON SUGAR DETERMINATIONS IN BAKED PRODUCTS

By NILES H. WALKER (National Biscuit Company, New York, N. Y.), *Associate Referee**

The common sugars which occur in bakery products are dextrose, levulose, lactose, maltose, and sucrose. It is difficult to determine actual percentages of the various sugars in mixtures which often occur. Approximations can be made by special methods. In lieu of identity, it usually suffices to determine the quantities of reducing sugars, and of non-reducing sugars which hydrolyze and produce reducing sugars. Since the greater amount of reducing sugars in most bakery products is hexoses, *i.e.*, invert sugar from hydrolyzed sucrose, and dextrose and levulose from syrups, honey, and fruits, it seems reasonable to calculate and report reducing sugars as invert sugar unless identities are definitely known or established by special determinations. Practically all of the non-reducing sugar content, which is readily inverted to produce invert sugar by the usual procedures, is sucrose. It may be calculated as such without introducing any appreciable quantitative error.

The tentative method for the determination of sugars in baked products, **20.103** (1)[1] requires the extraction of sugars with boiling 50 per cent alcohol, evaporation of alcohol from an aliquot of the extract, taking up with water, clarification with neutral lead acetate, and deleading the solution before determinations can be carried out. This requires appreciable time and in many cases clarification is unsatisfactory. Water extraction with acid tungstate clarification has been found satisfactory for the determination of the maltose value (2, 3, 4) of flour. Methods for the determination of sucrose (5) after inversion by the acid added for clarifica-

TABLE 1.—*Per cent sugars**—*Method* **20.95, 20.103**

SAMPLE	SUGARS		
	REDUCING AS INVERT	SUCROSE	TOTAL
Wheat flour (12% moisture basis)	.20	1.08	1.28
Bread (38% moisture basis)	2.28	.24	2.52
Soda crackers	.97	.37	1.34
No. 1 (Sweet cracker)	4.31	18.39	22.70
No. 2 (Sweet cracker)	4.74	18.17	22.91
No. 3 (Sweet cracker)	7.18	15.65	22.83
No. 4 Honey Graham cracker	6.75	15.28	22.03
No. 5 Graham cracker	3.81	16.37	20.18

* All results in duplicate.

* Present address, Port Chester, N. Y., Arnold Bakers, Inc.

[1] NOTE.—References in bold face figures are all to sections in *Methods of Analysis*, 6th Ed., 1945.

tion, and the determination of the original reducing sugars, (6) by the ferricyanide procedure on water extractions from flour after clarification with acid tungstate, have also proved satisfactory.

In accordance with the recommendation of the Association, a study was started on the application of the method for determining reducing and non-reducing sugars in flour, **20.29,** to the determination of sugars in other baked products. Sugar determinations were made on wheat flour, white bread, and six varieties of crackers by the tentative method **20.95, 20.103** given for the determination of sugars in baked products, and adaptations of the official method, **20.28–20.30,** for the determination of reducing and non-reducing sugars in flour.

Table 1 gives results obtained by tentative method **20.95, 20.103.** Sugars were determined by the Munson-Walker general method. Cuprous oxide was weighed and the invert sugar equivalent taken from Table **44.11.**

Table 2 gives results obtained by adaptations of the official method **20.28–20.30** for the determination of reducing and non-reducing sugars in flour. Reagents used were the same as listed in **20.28.** Reducing sugars were calculated as invert. The weight of sample used was limited by the amount of sugar it contained. Adjustments were made to meet volume

TABLE 2.—*Per cent sugars*—Method* **20.28–20.30**

SAMPLE	SUGARS		
	REDUCING AS INVERT	SUCROSE	TOTAL
Wheat flour (12% moisture basis) 5.675 gm.	.12	1.60	1.72
Bread (38% moisture basis) 2.8375 gm. (6% moisture basis)	2.05	.25	2.30
Soda cracker 5.675 gm.	.93	.48	1.41
No. 1 (Sweet cracker) .7094 gm.	3.94	19.60	23.54
No. 2 (Sweet cracker) .7094 gm.	4.36	19.20	23.56
No. 3 (Sweet cracker) .7094 gm.	6.96	16.76	23.72
No. 4 Honey Graham cracker .7094 gm.	5.90	17.12	23.02
No. 5 Graham cracker .7094 gm.	3.80	17.36	21.16
Wheat flour (12% moisture basis) 5 gm. in 100 ml. vol.	.12	1.62	1.74
Bread (38% moisture basis) 5 gm. (6% moisture basis) in 100 ml. vol.	2.09	.25	2.34
Soda cracker 5 gm. in 100 ml. vol.	.99	.41	1.40
No. 1 (Sweet cracker) 1.25 gm. in 100 ml. vol.	3.82	20.08	23.90
No. 2 (Sweet cracker) 1.25 gm. in 100 ml. vol.	4.27	19.47	23.74
No. 3 (Sweet cracker) 1.25 gm. in 100 ml. vol.	6.93	16.25	23.18
No. 4 Honey Graham cracker 1.25 gm. in 100 ml. vol.	5.90	17.00	22.90
No. 5 Graham cracker 1.25 gm. in 100 ml. vol.	3.48	16.92	20.40

* All results in duplicate.

requirements where the amount of alcohol required to wet the charge was reduced. Charges made to volume and extracted in volumetric flasks were treated as nearly as possible as directed for the wetting and extraction of charges in Erlenmeyer flasks. Corrections were made for space occupied by insoluble solids in volumetric flasks.

Table 3 gives results of calculated sugar contents of three varieties made from weight and analysis of ingredients.

TABLE 3.—*Per cent sugars calculated from weight and analyses of ingredients*

SAMPLE	SUGARS		
	REDUCING AS INVERT	SUCROSE	TOTAL
No. 1 (Sweet cracker)	4.20	18.30	22.50
No. 2 (Sweet cracker)	4.18	18.50	22.68
No. 3 (Sweet cracker)	7.05	15.80	22.85

DISCUSSION

In general, results obtained by method **20.28–20.30**, seem slightly high especially for the crackers with the higher sugar contents.

Clarifications of extracts from most of the crackers were not very satisfactory with either neutral lead acetate or acid tungstate.

Although results obtained on extracts made as directed in **20.29** agreed within reasonable tolerances with those made in volumetric flasks (Table 2), it seems simpler and more practical to make the extractions in volumetric flasks where volumes can be more accurately measured.

Acknowledgment is made to Mr. E. K. Spotts (National Biscuit Company, N. Y.), who collaborated with the author in this work.

It is recommended* that further work be conducted in an effort to adapt method **20.28, 20.29, 20.30** to the determination of sugars in baked products.

LITERATURE CITED

(1) *Methods of Analysis, A.O.A.C.*, Sixth Edition, 1945.
(2) RUMSEY, American Institute of Baking Bulletin 8 (1922).
(3) BLISH, M. J., *This Journal*, **16**, 497 (1933).
(4) BLISH and SANDSTEDT, *Cereal Chemistry*, **10**, 189 (1933).
(5) SANDSTEDT, R. M. *Cereal Chemistry*, **14**, 767 (1937).
(6) SANDSTEDT, R. M. *This Journal*, **22**, 535 (1939).

No report was given on carbon dioxide in self-rising flour.

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* For report of Subcommittee D and action of the Association, see *This Journal*, **31**, 58 (1948).

REPORT ON PHOSPHATED FLOUR

By FRANK H. COLLINS (Food and Drug Administration, Cincinnati, Ohio), *Associate Referee*

In accordance with the recommendations of Committee D (*This Journal*, **30**: 57) a study was made of the determination of added inorganic materials in phosphated flour.

A modification of the carbon tetrachloride sedimentation method of Gustafson (*This Journal*, **19**: 82, 1936) has given satisfactory recovery of monocalcium phosphate added to one type of flour. By use of separatory funnels and large volumes of carbon tetrachloride, the determination may be made in less than one day of elapsed time. It is essential that the sediment be drawn from the separators with a minimum of solution. This can be accomplished by turning the stop-cock quickly from side to side. The carbon tetrachloride may be reused several times, after filtration, before it is necessary to distill. In this case the weighed filter paper and sediment should be washed with fresh carbon tetrachloride.

METHOD FOR PHOSPHATES IN PHOSPHATED FLOUR

Transfer 20 g of flour to a dry 250 ml separatory funnel, add 200 ml carbon tetrachloride, shake well, let stand 15 min. Draw off sediment with a minimum of soln (2–5 ml) into a dry 125 ml separatory funnel. Again shake 250 ml separatory funnel and draw off as before, after standing 1 hour. Nearly fill the 125 ml separatory funnel, containing sediment, with carbon tetrachloride, shake well, stand 2 hours, draw off sediment with a minimum of soln into a weighed, air-dry, filter paper. Dry at room temperature to constant weight (about 2 hours) and weigh.

COLLABORATIVE STUDY

In order to test the reliability of this method in the hands of other chemists, it was submitted to limited collaborative study. For this purpose straight grade soft winter wheat flour was phosphated, Sample No. 1 containing 0.18 per cent monocalcium phosphate and Sample No. 2, 0.66 per cent.

TABLE 1.—*Collaborative results*

ANALYST	BLANK (UNPHOSPHATED)	SAMPLE NO. 1 (.18%)	SAMPLE NO. 2 (.66%)
F. H. Collins	0.01	0.18	0.66
	.01	.18	.67
O. S. Keener		.22	.66
		.23	.70
F. J. McNall		.20	.65
		.18	.66

RECOMMENDATION*

It is recommended that study be continued on various types and grades of flour with definite amounts of added monocalcium phosphate.

REPORT ON BROMATES IN WHEAT FLOUR[1]

By W. F. GEDDES (Division of Agricultural Biochemistry, University of Minnesota Agricultural Experiment Station, St. Paul, Minn.), *Associate Referee*

The establishment, in 1941, of definitions and standards of identity for bromated flour, enriched bromated flour, and bromated whole wheat flour,[2] effective January 1, 1942, under the provisions of the Federal Food, Drug, and Cosmetic Act of 1938, permitting the presence of not more than 75 p.p.m. of potassium bromate in these bromated products, created a need for an accurate and convenient method for the determination of this ingredient in wheat flour. At its annual meeting in 1943, the Association of Official Agricultural Chemists took cognizance of this need by appointing an Associate Referee on the determination of bromates in flour.[3]

The ideal method for use in the administration of the standards for bromated flour should meet the following requirements:

(1) It should be simple and capable of giving accurate, reproducible results for small amounts of potassium bromate (5 to 75 p.p.m.) in the presence of other oxidizing agents that might possibly be illegally added as flour improvers.

(2) It must determine bromates to the exclusion of bromine, chlorine, and iodine naturally present in flour.

(3) It must be applicable in the presence of bromine-containing residues which result from fumigation with methyl bromide.

To these could be added a further specification that the method should be capable of determining bromate whether it is added to the milled product as a finely divided solid, or as a solution in which the wheat is soaked prior to milling or which is sprayed on clean middlings. However, on the basis of available information, treatment with solutions of potassium bromate has only been applied to a limited extent and is confined to the production of bromated whole wheat flour. As this product represents a relatively small percentage of the total bromated flour produced, it did not appear advisable, at the present stage of the work, to consider the special problems involved in determining bromate added in this manner.

From a survey of the literature it was apparent that the available methods held little promise of meeting all the requirements which have been

* For report of Subcommittee D and action by the Association, see *This Journal*, 31, 59 (1948).
[1] Paper No. 2359, Scientific Journal Series, Minnesota Agricultural Experiment Station.
[2] *Federal Register*, 6 (63), 1734 (1941).
[3] *This Journal*, 27, 16 (1944).

enumerated. Methods for the micro-determination of bromine in biological materials are not applicable, as they would determine native bromine and bromine containing residues from methyl bromide fumigation. However, several procedures for the determination of bromates in flour have been described and, as a first approach to the problem, Brown[4] made a comparative study of them by analyzing flours containing known quantities of potassium bromate. The most satisfactory method for eliminating the interfering influence of other sources of bromine in the flour involves removal of the added bromate by carbon tetrachloride flotation. The most satisfactory results were obtained by a modification of the Kuhlman[5] method in which this is accomplished through the use of a brass centrifuge tube with a removable base; after separation, the bromate is determined by iodometric titration. This procedure, designated in the present paper as "the direct method," is not applicable in the presence of other oxidizing improvers and a "complete method," involving a combination of standard procedures, was developed which is specific for bromate in the presence of persulfates and iodates (also chlorates, which do not act as flour improvers). In the complete method, the solution of the improvers (separated from the flour by flotation) is boiled to destroy persulfates and then treated with sulfurous acid to reduce bromates and iodates to halides. After elimination of the iodide by boiling with sodium nitrite and sulfuric acid, the bromide is determined by the Kolthoff and Yutzy[6] method, which is applicable in the presence of chlorides.

The direct method gave mean recoveries ranging between 94 per cent and 102 per cent when applied to straight grade, clear, and whole-wheat flours containing from 5 to 80 p.p.m. of potassium bromate. Similar recoveries were obtained by the complete method with straight grade flours containing these levels of potassium bromate, either added alone or in conjunction with potassium persulfate, potassium iodate, and potassium chlorate. As bromated straight grade flours gave quite satisfactory results by both methods, the two procedures were subjected to a limited series of collaborative tests in 1946. The experience gained from these studies led to slight modifications and the revised methods are given below.

COMPLETE METHOD FOR THE DETERMINATION OF BROMATES IN WHEAT FLOUR

(Applicable in the presence of potassium persulfate and potassium iodate)

APPARATUS

Centrifuge, International type C, equipped with head to carry holders for centrifuge tubes, 1.30″×4.2″.

Centrifuge tubes, 2 special brass centrifuge tubes with screw-cap and removable base.

Burettes: one 5 ml (micro), one 50 ml.

[4] Brown, R. E. The Determination of Bromates in Wheat Flours Containing Various Oxidizing Improvers, M.S. Thesis, University of Minnesota, June 1946.

[5] Kuhlman, J., *Z. Untersuch. Lebensm.* **68**, 375 (1934). Original not seen; C.A. **29**, 1890 (1935); Chem Listy, **28**, 241 (1934). Original not seen; C.A. **29**, 236 (1935).

[6] Kolthoff, I. M., and Yutzy, H., *Ind. Eng. Chem., Anal. Ed.*, **9**, 75 (1937).

Erlenmeyer flasks: 250 ml.
Funnels: glass, 7.5 cm.

REAGENTS

(1) *Ammonium molybdate soln*, 2.5%.
(2) *Carbon tetrachloride*, technical.
(3) *Potassium iodide*, C.P.
(4) *Sodium bicarbonate*, C.P.
(5) *Sodium formate soln*, 50%.
(6) *Sodium dihydrogen phosphate, monohydrate* C.P.
(7) *Sodium hypochlorite soln*, 1 *N* in approx. 0.1 *N* NaOH. Pass 71 g of chlorine into a cold soln containing 88 g of sodium hydroxide in 1500 ml distilled water (keep the alkali soln cool by means of an ice-salt mixture). After addition of the chlorine, dilute the soln to 2 liters. The alkalinity should be tested at intervals by destroying the hypochlorite in 1 ml of the soln with 2 ml of 3% hydrogen peroxide soln, diluting to 10 ml and titrating with 0.1 *N* hydrochloric acid soln. The titration volume should be between 0.8 and 1.2 ml. This soln is stable for several weeks if stored in a dark bottle at ice-box temperature. (If desired, a smaller quantity of this solution may be prepared.)
(8) *Sodium nitrite soln*, 0.5 *M*.
(9) *Sodium thiosulfate soln*, 0.005 *N*. Prepare from a stock 0.1 *N* soln and standardize just before use, preferably against potassium bromate.
(10) *Starch indicator, 0.5% soln.* Triturate one g of soluble starch and 5 mg of mercuric iodide (preservative) in a small quantity of cold water and pour slowly into about 150 ml of boiling water with constant stirring. Boil until the soln is clear. Cool, and dilute to approximately 200 ml.
(11) *Sulfuric acid solution*, 1 *N*.
(12) *Sulfurous acid solution*, 5%.
(13) *Sulfuric acid*, 12 *N*.

PROCEDURE

The base of the brass centrifuge tube is tightened by placing in position on the base plate, gripping the body with a pliers and applying moderate force. Pour about 10 ml of carbon tetrachloride into the tube followed by 20 g of flour, then nearly fill with carbon tetrachloride. To guard against trapping an excessive amount of air in the tube it is advisable to agitate the flour mass with a small glass rod while adding the second portion of carbon tetrachloride, taking care to rinse down the glass rod. Screw on the cap of tube and shake vigorously for one minute to insure suspension of the flour and wetting of all bromate particles. Centrifuge at 2400 r.p.m. for two minutes. No centrifuge tube holder is necessary, since the flange provides for suspension of the tube without the use of an auxiliary holder. Hold the tube over a suitable receptacle for the excess carbon tetrachloride and screw off the base. As the threads release the base it is moved to the side and the excess carbon tetrachloride flows from the body of the tube. The particles of bromate are packed firmly in the base of the tube and are not disturbed by motion of the liquid created by removing the base. The flour remains in the tube in the form of a plug. Remove most of the carbon tetrachloride from the cup-like base by means of a cotton swab and allow the remainder to evaporate at room temperature (see Note 1). Transfer the residue to a 250 ml Erlenmeyer flask by holding the cup over a 7.5 cm funnel, and wash out the bromate with about 30 ml of water. Wash and dry the base and attach it to the body of the tube. Resuspend the flour in carbon tetrachloride and repeat the flotation separation, removing the excess carbon tetrachloride and transferring the bromate to the same Erlenmeyer flask in the manner described above.

Boil the soln of oxidizing improvers for 3 min. to destroy any persulfates that

may be present. Add 5 ml of 1 *N* sulfuric acid soln and 2 ml of 5% sulfurous acid soln. Allow to stand 1 min. and boil briefly to expel excess sulfur dioxide. Add 0.25 ml of 0.5 *M* sodium nitrite soln and boil for 2 min. after the disappearance of the iodine color (see Note 2). Cool the soln (preferably in an ice bath) and add one g of sodium bicarbonate (see Notes 3 and 4). Add one g of sodium dihydrogen phosphate followed by 5 ml of the sodium hypochlorite soln (1.0 *N* in 0.1 *N* NaOH), and heat just to boiling. After about one min., add 5 ml of the 50% sodium formate soln and boil for 2 min. After cooling to room temperature, dilute to about 150 ml and add 0.5 g of potassium iodide, one drop of 2.5% ammonium molybdate soln, 10 ml of 12 *N* sulfuric acid, and titrate immediately with 0.005 *N* sodium thiosulfate soln.

Carry out a blank determination on the reagents, substituting 10 ml of distilled water for the test soln and carry out the procedure as outlined above starting with the addition of sulfuric and sulfurous acid.

Calculate the results in terms of p.p.m. potassium bromate in the sample as follows:

Potassium bromate (p.p.m.) = Normality of $Na_2S_2O_3 \times$ (titration volume − blank ×) 1392.

DIRECT METHOD FOR THE DETERMINATION OF BROMATES IN WHEAT FLOUR

(Not applicable in the presence of other oxidizing flour improvers)

PROCEDURE

Separate the bromate and other improvers which may be present by the double flotation procedure described in the complete method. To the aqueous bromate soln, add 0.5 g potassium iodide, one drop of 2.5% ammonium molybdate soln, 10 ml of 12 *N* sulfuric acid soln, and titrate with 0.005 *N* thiosulfate.

Calculate the results as follows:

Potassium bromate (ppm) = Normality ($Na_2S_2O_3$) × titration volume × 1392.

Notes

(1) Evaporation of residual carbon tetrachloride is hastened by rotating the cuplike base between thumb and forefinger and breathing gently on the liquid.

(2) When boiling the soln after the addition of the 0.5 *M* sodium nitrite soln, care must be taken to shake the flask continuously. A 3-min. boiling time is sufficient if the volume of the soln is kept small (ca 20 ml), but a longer time is required if the volume is appreciably increased. With samples containing only $KBrO_3$, no iodine coloration will be observed. Boiling here serves to destroy excess nitrous acid.

(3) It is important to cool the soln after the nitrite treatment before proceeding with the hypochlorite oxidation to avoid low results. It seems plausible that the sodium hypochlorite, being somewhat unstable, may be decomposed before the oxidation of the bromide is complete.

(4) The use of indicators in the neutralization makes for low recoveries.

(5) With samples containing from 10 to 75 p.p.m. potassium bromate, the titration volumes will vary from approximately 1 to 12 ml and burettes should be selected accordingly. With samples containing small quantities of added bromate, flotation separations should be made on two subsamples and the material combined in order to secure a larger titer. (In this case, the factor 696 is used in calculating the results.)

COLLABORATIVE STUDY OF REVISED METHODS

Preparation of Samples and Instructions to Collaborators

In 1947, nine chemists employed the revised methods in analyzing four samples, lettered A to D inclusive (Table 1). Samples A, B, and C

TABLE 1.—*Results obtained by each collaborator in the determination of potassium bromate in straight grade flour*

ANALYST NO.	DIRECT METHOD				COMPLETE METHOD			
	MIN.	MAX.	MEAN	MEAN RECOVERY	MIN.	MAX.	MEAN	MEAN RECOVERY
	p.p.m.	*p.p.m.*	*p.p.m.*	*Per cent*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*Per cent*
SAMPLE A (containing 5.0 p.p.m. of potassium bromate)								
1	4.8	5.2	5.0	100	4.4	5.2	4.8	96
2	4.5	5.7	5.1	103	3.7	4.2	4.0	81
3	4.4	5.3	5.0	100	2.4	3.2	3.0	61
4	4.5	5.2	4.8	96	3.9	4.8	4.3	86
5	3.9	4.0	3.9	78	3.2	5.3	4.4	88
6	4.3	5.2	4.8	96	0.8	2.0	1.5	30
7	4.1	5.2	4.6	91	4.1	10.8	6.5	130
8	3.5	4.2	3.8	76	4.0	4.5	4.2	84
9	4.9	5.7	5.2[1]	104	2.2	4.3	3.7	74
All	3.5	5.7	4.7	94	0.8	10.8	4.0	81
SAMPLE B (containing 40 p.p.m. of potassium bromate)								
1	34.9	39.2	37.7	94	36.0	40.0	37.9	95
2	36.7	40.1	38.5	96	34.0	39.6	37.6	94
3	35.8	37.9	37.0	92	29.4	32.7	30.8	77
4	39.3	43.3	40.6	101	35.4	41.3	37.7	94
5	36.7	39.0	38.2	96	20.7	32.4	28.2	71
6	36.2	41.5	39.2	98	16.3	23.4	20.1	50
7	37.2	42.5	39.2	98	17.4	23.0	20.2	50
8	40.4	44.5	43.0	108	32.7	36.2	34.3	86
9			40.3[2]	101	27.6	39.6	35.6	89
All	35.8	44.5	39.2	98	16.3	41.3	31.4	78
SAMPLE C (containing 75 p.p.m. of potassium bromate)								
1	71.7	72.4	72.0	96	68.2	74.6	70.8	94
2	74.2	76.9	75.6	101	69.0	74.2	70.7	94
3	70.3	76.1	72.4	96	64.7	67.9	66.4	88
4	68.5	77.1	73.6	98	64.0	74.2	69.7	93
5	69.0	73.1	71.5	95	49.4	67.1	58.8	78
6	73.1	78.1	76.5	102	42.5	54.6	49.7	66
7	66.1	81.8	72.4	96	27.8	51.5	34.8	46
8	76.5	79.3	78.1	104	68.2	70.3	68.9	92
9	73.1	78.0	75.2	100	76.2	78.9	77.6	104
All	66.1	81.8	74.1	99	27.8	78.9	64.0	85
SAMPLE D (containing 40 p.p.m. potassium bromate, 40 p.p.m. potassium persulfate, and 20 p.p.m. potassium iodate)								
1					36.6	41.8	39.5	99
2					35.8	40.5	37.8	95
3					49.1	52.4	50.7	127
4					60.7	67.8	64.1	160
5					30.6	36.9	34.6	86
6					20.9	27.4	24.6	61
7					21.6	26.1	23.8	60
8					64.0	65.4	64.5	161
9					39.3	44.5	42.2[2]	106
All					20.9	26.1	43.7	109

[1] Mean based on 3 determinations.
[2] Mean based on 2 determinations.

contained 5, 40, and 75 p.p.m. respectively, of potassium bromate, while sample D contained 40 p.p.m. potassium bromate, 40 p.p.m. potassium persulfate, and 20 p.p.m. potassium iodate. In preparing the samples, analytical reagent grade chemicals ground to pass a standard U. S. 100-mesh sieve, and an unenriched, straight grade, hard red spring wheat flour (13.0% protein, 0.47% ash, at 12.8% moisture), which had been bleached with benzoyl peroxide, were employed. A "Master Mix" containing 1000 p.p.m. of potassium bromate was first prepared by making successive additions of flour and bromate to a small MacClellan batch mixer to give a total weight of 3600 g, and mixing for one hour at 5 r.p.m. This master mix was then diluted with the appropriate quantities of flour to give the desired bromate levels in samples A, B, and C, each sample being again mixed for one hour. In preparing sample D, the other oxidizing improvers were added to the flour used for diluting the master mix. Approximately 300 grams of each sample were supplied the analysts to provide sufficient material for preliminary determinations.

The collaborators were requested to analyze samples A, B, and C in quadruplicate by both the direct and complete procedures, and sample D by the complete procedure. In order that a valid measure of the experimental error might be obtained, it was requested that, after experience had been gained with the methods, quadruplicate tests should be made and reported without elimination of any of the results, unless it was definitely known that accidental errors had occurred. Two pairs of the special brass centrifuge tubes were constructed and circulated among the analysts.

Results and Discussion:

The results of the individual analysts for each sample and the means for all collaborators are summarized in Table 1. To ascertain the experimental errors, the replicate values for each sample, excepting those for analyst No. 9, who regarded certain of his replicates as in serious error and suggested that they be eliminated in computing his mean values, were subjected to an analysis of variance. The variance due to the differences between collaborators was significant for each sample by both methods and only the means and standard errors are shown in Table 2.

For the direct method, the recovery of the added bromate by the individual analysts varied from 76 to 103 per cent for sample A, from 92 to 108 per cent for sample B, and from 95 to 104 per cent for sample C; the mean recoveries for all analysts combined were 94, 98, and 99 per cent for the three respective samples. If the low recoveries of 76 and 78 per cent of analysts 5 and 6, for sample A, are eliminated the mean recovery for this sample is increased to 99 per cent. These average results are eminently satisfactory for a microdetermination of this nature. The average reproducibility of the results within laboratories is measured by the standard errors for a single determination shown in Table 2. When these errors are expressed as percentages of the corresponding mean quantities of bromate

TABLE 2.—*Means and standard errors for analysts 1 to 8 inclusive. Direct vs. complete methods for determination of potassium bromate in wheat flour*

SAMPLE	BROMATE ADDED	DIRECT METHOD				COMPLETE METHOD			
		BROMATE FOUND	RECOVERY	STANDARD	ERROR[1]	FOUND	RECOVERY	STANDARD	ERROR[1]
	p.p.m.	*p.p.m.*	*per cent*	*p.p.m.*	*per cent*	*p.p.m.*	*per cent*	*p.p.m.*	*per cent*
A	5.0	4.6	93	0.39	8.5	4.1	82	1.14	27.9
B	40.0	39.2	98	1.83	4.7	30.9	77	2.81	9.1
C	75.0	74.0	99	3.24	4.4	61.2	82	5.82	9.5
D	40.0	—	—	—	—	42.4	106	2.32	5.5

[1] Standard errors given are those computed for a single determination. The percentage values are the standard errors of a single determination expressed as percentages of the corresponding means.

found, they may be employed for a direct comparison of the reproducibility of these determinations with those of others for which similar studies have been made. In a recent statistical study by Anderson[7] of the results of collaborative determinations of several constituents of wheat flour on samples sent out by the National Check Sample Committee of the American Association of Cereal Chemists, it was found that the interlaboratory errors of moisture, protein, and ash determinations were in the order of ± 1 per cent, whereas the errors for constituents present in microquantities, such as thiamine, niacin, and iron, ranged between 9 and 13 per cent. The interlaboratory errors of 4.4 to 8.5 per cent for potassium bromate determined by the direct method compare very favorably with the latter group, especially since the analysts participating in the vitamin and iron determinations had previous experience with the methods.

The results by the complete method, however, are quite unsatisfactory. The mean recoveries for all analysts were 81, 78, 85, and 109 per cent for samples A to D, respectively, and the recoveries obtained by individual analysts varied from a low of 30 per cent for Sample A to a high of 161 per cent for Sample D. The interlaboratory errors also greatly exceeded those for the direct method. Nevertheless, several analysts secured quite satisfactory recoveries, and further study is warranted in an effort to trace the sources of the error.

The complete method is not required if only bromates and persulfates, or bromates and iodates are present. In the former case, it is only necessary to boil the solution of the improvers which are removed from the sample by the flotation procedure and titrate in the manner employed in the direct method. In the latter case, bromates and iodates may be titrated in the same solution by the iodometric titration described by Kolthoff and Hume.[8] In this procedure the iodate is first titrated in the presence of a phthalate buffer between *p*H 4 and 5, after which the bromate is titrated upon the addition of an adequate amount of hydrochloric

[7] Anderson, J. A., *Transactions Am. Assoc. Cereal Chem.*, **5**, 178 (1947).
[8] Kolthoff, I. M., and Hume, D. N., *Ind. Eng. Chem. Anal. Ed.*, **15**, 174 (1943).

acid and a few drops of a molybdate solution as a catalyst. Alternatively, the bromates and iodates could be reduced to iodide and bromide with sodium nitrite and sulfuric acid and the iodide eliminated as iodine by boiling with sodium nitrite, as described in the complete method. By suitable qualitative tests for the presence of oxidizing improvers other than potassium bromate, simpler analytical procedures could be applied which might give more accurate and reproducible results than the complete method.

LIST OF COLLABORATORS[9]

1. A. W. Alcock, Purity Flour Mills, Limited, Winnipeg, Manitoba.
2. J. A. Anderson, Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg, Manitoba.
3. Ray Brown, International Milling Co., Minneapolis, Minn.
4. Rowland Clark, W. E. Long Co., Chicago, Ill.
5. John E. Despaul, General Chemistry Branch, Quartermaster Food and Container Institute for the Armed Forces, Chicago, Ill.
6. F. C. Hildebrand, General Mills Inc., Minneapolis, Minn.
7. W. L. Rainey, Commander Larabee Milling Company, Minneapolis, Minn.
8. M. O. Schultze, Division of Agricultural Biochemistry, University of Minnesota, St. Paul, Minn.
9. V. A. Stenger, The Dow Chemical Company, Midland, Michigan.

COMMENTS OF COLLABORATORS

(*1*) The flotation procedure is the weakest point of the methods. There is no absolute assurance that mechanical loss of bromate during the opening of the centrifuge cups can be entirely avoided. Drilling a well in the bottom of the conical portion of the cup might assist in avoiding this difficulty.

(*2*) The special brass centrifuge tubes are very unsatisfactory for separating bromate or other flour improvers from the sample. It was very difficult to obtain a clean separation with these tubes, especially with tube No. 1, which invariably left deposits of flour in the lower portion of the tube. When flour is present in the sample being titrated, the end point is, of course, obscured. We have had much better success with regular Pyrex glass conical centrifuge tubes, which are less expensive, and easier and faster to use.

(*3*) In two trials on Sample D by the complete method values of 51.6 and 52.2 p.p.m. of potassium bromate were obtained with only one flotation, as compared with a mean value of 50.7 for quadruplicate determinations based on the double flotation procedure described. As a better recovery was obtained with a single flotation separation, a further study of the method to eliminate the second flotation is indicated.

(*4*) In some cases, difficulty was encountered in obtaining a compact mass of flour and it is suggested that an extra minute of centrifuging would aid in obtaining a clearer extract.

(*5*) The direct method was fairly easily carried out and the end points in the titrations were fairly good. There may be danger of losing bromate when removing the excess of carbon tetrachloride from the base of the tube by means of a cotton swab, and it is suggested that additional remarks on this manipulation be included in the description of the method.

(*6*) The use of 12 *N* sulphuric acid is not quite strong enough to secure reproducible results. Where more acid was used, better checks were obtained.

[9] The numbers assigned to the collaborators in Table 1 bear no relation to the alphabetical arrangement in this list.

(7) It is believed that sufficient fat is extracted from the flour by the carbon tetrachloride to cause difficulty in the quantitative transfer of the potassium bromate from the cup-like base due to gumminess of the mass after evaporation of the carbon tetrachloride.

(8) A sticky deposit in the base of the centrifuge tubes was present after evaporating the carbon tetrachloride, and hot water was used to wash out the tubes to avoid loss of any potassium bromate which might remain undissolved.

(9) The procedure should include cooling the boiled solution prior to the addition of sulfurous acid.

(10) With the experience gained in completing this group of determinations, it is believed that better checks would be obtained if the work were repeated.

(11) The results are not as precise as would be desirable but the methods are probably suitable for indicating whether or not the bromate additions are within the desired range.

(12) In the notes, it is stated that the use of indicators in the neutralization makes for low recoveries. Would there be any advantage in carrying through a separate sample containing indicators for the purpose of checking and adjusting the alkalinity or acidity of the sample? If so, what should the reaction be at the various stages of the procedure?[10] The end points in the complete procedure were very poor.

RECOMMENDATIONS*

It is recommended—

(1) That an improved design of the special brass centrifuge tube with separable base be compared with conical Pyrex centrifuge tubes for the determination of potassium bromate in flours by modifications of the direct method.

(2) That no further collaborative studies by the complete method be undertaken until the possibility of developing simpler procedures for the determination of bromate in the presence of one or more additional improvers is investigated.

(3) That qualitative tests on the detection of various oxidizing improvers added to flours, both singly and in combination, be studied.

(4) That methods be outlined for the determination of bromate in solutions containing this improver in the presence of potassium persulfate and potassium iodate, respectively, and that these methods be tested collaboratively with solutions containing known quantities of these improvers.

ACKNOWLEDGMENT

The thanks of the Associate Referee are extended to Ray Brown, International Milling Company, for preparing the samples, assembling and studying the data submitted by the collaborators. His thanks are also extended to the other collaborators for their cooperation.

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[10] It would be of no apparent advantage to carry a separate sample through the procedure for the purpose of adjusting the reaction, since sodium bicarbonate is added in excess. The amount of the excess is relatively unimportant, since the solution is buffered with a large excess of sodium dihydrogen phosphate.

* For report of Subcommittee D and action by the Association, see *This Journal*, 31, 59 (1948).

REPORT ON PHOSPHORUS IN CEREAL PRODUCTS

By V. E. MUNSEY (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

The Official and Tentative Methods of Analysis, Sixth Edition, does not contain a method for the determination of phosphorus in either the Cereal Foods or the Grain and Stock Feed chapters. Two adaptations of official methods from the Plant chapter have been applied to various cereal products with satisfaction by the Associate Referee. Twelve collaborators were invited to analyze four samples, namely, bread, oatmeal, Pablum (an infant cereal food), and self-rising flour, by two new procedures. Samples in Procedure No. 1 are ashed after mixing with magnesium nitrate solution, while Procedure No. 2 requires addition of sodium carbonate, followed by ashing as in the official method for ash in flour. The ash is dissolved by acid and the determination completed in essentially the same manner in both procedures by proceeding as for the determination of phosphorus under Fertilizers, sec. **2.12**, page 23. The details of the methods are given in *This Journal*, **31**, 79, (1948), under "Changes in Methods of Analysis."

The results reported by 12 collaborators are given in Table 1, as follows:

TABLE 1.—*Phosphorus in oatmeal, bread, pablum, and self-rising flour*

COLLABORATOR		BREAD	OATMEAL	PABLUM	SELF-RISING FLOUR
		per cent	*per cent*	*per cent*	*per cent*
1	Procedure I	0.21	0.48	0.65	0.53
		0.20	0.48	0.66	0.54
	Procedure II	0.20	0.48	0.65	0.53
		0.20	0.48	0.65	0.53
2	Procedure I	0.18	0.44	0.67	0.51
		0.19	0.46	0.66	0.50
	Procedure II	0.14	0.45	0.68	0.50
		0.14	0.44	0.66	0.50
3	Procedure I	0.21	0.54	0.78	0.61
	Procedure II	0.19	0.57	0.75	0.58
4	Procedure I	0.15	0.46	0.68	0.53
		0.15	0.46	0.67	—
	Procedure II	0.14	0.46	0.67	0.52
		0.15	0.45	0.68	0.53
5	Procedure I	0.15	0.51	0.67	0.53
	Procedure II	0.15	0.45	0.66	0.52
6	Procedure I	0.16	0.48	0.67	0.54
	Procedure II	0.16	0.48	0.67	0.53

TABLE 1.—(*continued*)

COLLABORATOR		BREAD	OATMEAL	PABLUM	SELF-RISING FLOUR
		per cent	*per cent*	*per cent*	*per cent*
7	Procedure I	0.16	0.47	0.69	0.53
		0.16	0.47	0.68	0.54
	Procedure II	0.16	0.48	0.68	0.53
		0.16	0.48	0.69	0.53
8	Procedure I	0.19	0.50	0.69	0.50
		0.12	0.42	0.66	0.45
		0.15	0.47	0.66	0.51
	Procedure II	0.14	0.45	0.66	0.46
		0.19	0.45	0.65	0.45
		0.16	0.44	0.67	0.45
9	Procedure I	0.160	0.446	0.659	0.533
		0.156	0.442	0.663	—
		0.157	0.478	0.689	0.515
		0.161	0.475	0.695	0.520
		0.163	0.480	0.681	0.521
		0.161	0.479	0.686	0.520
	Procedure II	0.147	0.464	0.676	0.520
		0.156	0.458	0.676	0.524
		0.147	0.468	0.676	0.520
		0.152	0.459	0.672	0.520
		0.151	0.471	0.689	0.523
		0.152	0.472	0.692	0.521
10	Procedure I	0.13	0.44	0.63	0.51
		0.13	0.44	0.64	0.49
	Procedure II	0.13	0.44	0.65	0.49
		0.13	0.44	0.65	0.49
11	Procedure I	0.16	0.47	0.69	0.49
		0.16	0.47	0.69	0.51
	Procedure II	0.16	0.46	0.69	0.50
		0.17	0.47	0.69	0.50
12*	Procedure I	0.14	0.45	0.65	0.51
		0.14	0.45	0.66	0.51
	Procedure II	0.14	0.45	0.66	0.51
		0.14	0.45	0.66	0.51
		Procedure I			
	Min.	0.13	0.44	0.64	0.49
	Max.	0.21	0.54	0.78	0.61
	Av.	0.17	0.47	0.68	0.52
		Procedure II			
	Min.	0.13	0.44	0.65	0.45
	Max.	0.20	0.57	0.75	0.58
	Av.	0.16	0.47	0.67	0.51

* Results received after averages were made.

Collaborator 2 uses for routine analysis of flour, phosphated and self-rising flour, a method essentially that published by H. R. Allen and Lelah Gault, *This Journal*, **30**, 136 (1947), Method C. This method specifies nitric-perchloric acid digestion, requires a relatively short time of about 2½ hours and offers smaller chance for error than the two procedures submitted for collaboration. His results (% P) obtained by this method follow:

Bread	*Oatmeal*	*Pablum*	*Self-rising Flour*
0.14	0.46	0.67	0.52
0.14	0.46	0.67	0.52

Collaborator 9 reports on the use of a colorimetric method, with slight modification, of the one described by Kitson and Mellon in *Ind. Eng. Chem., Anal. Ed.*, **16**, 379, 1944. Their method is less time-consuming than the procedures submitted, and while the results obtained were slightly lower, the differences were not considered significant. His results (% P) by this procedure follow:

Bread	*Oatmeal*	*Pablum*	*Self-rising flour*
0.148	0.460	0.668	0.520
0.144	0.451	0.652	0.495
0.140	0.450	0.655	0.503
0.136	0.455	0.663	0.510
0.143	0.450	0.660	0.505

Collaborator 8 submitted results obtained by a method regularly used by them, although details of the method were not given. The results (% P) are:

Bread	*Oatmeal*	*Pablum*	*Self-rising flour*
0.14	0.45	0.65	0.50
0.14	0.46	0.64	0.51

Collaborator 11 obtained the following results by Procedure II with the exception that the samples were ashed as in the official method for ash in flour, that is, without a fixative (% P):

Bread	*Oatmeal*	*Pablum*	*Self-rising flour*
0.16	0.47	0.69	0.55
0.16	0.47	0.69	0.55

Collaborator 6 points out the possibility of getting high results, especially with small amounts of P_2O_5, an account of deterioration of the molybdate solution. He recommends the molybdate solution, *Methods of Analysis*, **26.46 (a)** p. 398, which is stable and does not give high results. Results (% P) obtained with this solution follow:

Bread	*Oatmeal*	*Pablum*	*Self-rising flour*
0.16	0.48	0.67	0.53
0.16	0.48	0.68	0.53

The variations and the averages by the two procedures submitted for collaborative study are essentially the same on all four samples. No difficulties were experienced. These procedures are an adaptation of methods already official for other products. The collaborative results indicate both procedures are satisfactory for cereal products. The results obtained by the other procedures referred to, in regular use by some collaborators, are also in close agreement. The advantages of a nitric-perchloric acid digestion are not of sufficient magnitude to overcome the difficulty and danger of its application to warrant a collaborative study.

The assistance of the collaborators is gratefully acknowledged:

J. J. Winston, Jacobs Cereal Products Laboratory, New York City
E. R. Winterlee, Victor Chemical Works, Chicago Heights, Ill.
F. H. Luckmann, The Best Foods, Inc., Bayonne, N. J.
L. C. Andrews, Department of Health, New Orleans, La.
T. W. Schilb, Monsanto Chemical Co., St. Louis, Mo.
G. W. Kirby, The Fleischmann Laboratory, New York City
H. C. Randall, Rumford Chemical Works, Rumford, R. I.
J. F. Armstrong, M. J. Gnagy, Daniel Banes, and H. W. Conroy—all of the Food & Drug Administration.

It is recommended* that both procedures be adopted as official, first action, for cereals and cereal products and that the study be continued.

REPORT ON MILK SOLIDS AND BUTTERFAT IN BREAD

By V. E. Munsey (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

Further attempts have been made to apply the method in sec. **20.122**, p. 266, *Methods of Analysis*, to eliminate extraction of the fat. In a modification of this procedure, the saponified bakery product was acidified with sulfuric acid and steam distilled. The distillate was treated for application to the column as discussed in the paper by Ramsey & Patterson (*This Journal*, **28**, 644 (1945). There was found a relatively large percentage of several acids formed from the action of strong potassium hydroxide on the starch and sugar so that the separation of butyric acid on the silicic acid column was complicated to such an extent that the effort required in preparation for the column did not seem to offer a practical procedure.

Another approach was to extract the fat from bread with and without

* For report of Subcommittee D and action by the Association, see *This Journal*, 31, 58 (1948).

added butterfat in accordance with tentative method, sec **20.86,** p. 261, up to the point where the distillation is carried out as on a Reichert-Meissl determination. Instead of that distillation, the sample was steam distilled and the distillate prepared for separation on a silicic acid column according to the above reference. The breads containing no butterfat contained 0—0.9 mg butyric acid per g of fat, whereas the fat from a milk bread contained 15.2 mg butyric acid per 1 g of fat. This procedure gives a means for the direct measurement of butyric acid and may prove to be more desirable than the present procedure using the Reichert-Meissl technic. Further work is necessary and possibly collaborative results. No work was done this year on the determination of lactose for the calculation of nonfat milk solids in bread.

It is recommended* that study be continued on (1) the determination of lactose in bread and (2) on the determination of milk fat in bread.

No report was given on the following subjects: proteolytic activity of flour; soybean flour; soybean flour (immunological tests); noodles; baked products (moisture, ash, protein, fat, and crude fiber); moisture in self-rising flour and pancake, waffle, and doughnut flours; apparent viscosity measurement; iron in cornmeal and macaroni products.

REPORT ON BAKING POWDER AND BAKING CHEMICALS

By V. E. MUNSEY (Food and Drug Administration, Federal Security Agency, Washington D. C.), *Referee*

The gasometric method, official, for residual CO_2 (sec. **17.8,** p. 210), does not give the results expected, and on one type of baking powder the results are high to such an extent that the method does not seem applicable. The Associate Referee reports collaborative results this year, which confirm this statement and also presents an improved method to replace the official method.

RECOMMENDATIONS†

The Referee concurs in the following recommendation of the Associate Referee on Baking Powder:

It is recommended—

(1) That the present official method for determination of residual carbon dioxide in baking powders be dropped (first action), for the following reasons:

(a) The wording and description of the method are susceptible to too many interpretations.

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* For report of Subcommittee D and action by the Association, see *This Journal,* **31,** 58 (1948).
† For the report of Subcommittee D and action by the Association, see *This Journal,* **31,** 59 (1948).

(b) The manner of heating and treating the sample does not yield a true residual because all of the available CO_2 is not driven off.
(c) The method is not applicable to all types of baking powder, particularly the pyrophosphate types.

(2) That the modified Quartermaster Corps method as described in the report of the Associate Referee, this year, be adopted as a tentative method, adding the words "or electricity" after the phrase "The bath should be heated with gas."

(3) That the modified Q. M. C. method referred to in recommendation (2) be subjected to further study wherein the single evaporation to dryness is carried out in a moisture oven instead of on a water bath and at temperatures ranging from 70 to 100°C.

(4) That the modified Q. M. C. method referred to in recommendation (2) be also studied using a neutral saturated sodium chloride solution as medium instead of distilled water.

(5) That the expression "or subtract 17.8 from 17.6" in method **17.9** Available Carbon dioxide—official, be deleted, and that the tentative available CO_2 be determined by subtraction of the tentative residual CO_2 from the total CO_2, official **17.6.**

(6) That an investigation be made on modifying the present A.O.A.C. gravimetric method, **17.2** and **17.3,** by changing from H_2SO_4 and KOH absorption bulbs to the use of "Caroxite" or "Ascarite and Anhydrone."

REPORT ON RESIDUAL CARBON DIOXIDE IN BAKING POWDERS

By J. E. TATAR (Standard Brands, Inc., Chicago, Ill.), *Associate Referee*

The last report[1] of collaborative work on the determination of residual carbon dioxide in baking powders indicated that the official method, (*Methods of Analysis*, 6th ed., 1945, page 210, sec. **17.8**), needed study with special attention to the time and method of heating in the treatment of the sample.

A collaborative study was instituted involving the services of eleven analysts representing manufacturers of baking powder and leavening chemicals, also research and Federal laboratories. In this work the official method was compared with the method described in the Quartermaster Corps Tentative Spec. C.Q.D. #326, May 24, 1946, and also a modification of the latter method wherein the sample is subjected to a double evaporation instead of the single evaporation outlined.

The three methods were tried on three baking powders which were marked A, B, and C.

A.—S.A.S.—Phosphate Type with starch only as a filler.

B.—S.A.S.—Phosphate Type with the same percentages of S.A.S.,

[1] *This Journal*, **29**, 259 (1946).

phosphate, and sodium bicarbonate as powder A, except that part of the starch was replaced with calcium carbonate.

C.—Pyrophosphate-Mono Calcium Phosphate Type baking powder.

FORMULAS OF THE BAKING POWDERS SUBMITTED

		per cent
Sample Marked "A":		
	Sodium Aluminum Sulphate	24.00
	Mono Calcium Phosphate	12.40
	Sodium Bicarbonate	31.20
	Cornstarch	32.40
Sample Marked "B":		
	Sodium Aluminum Sulphate	24.00
	Mono Calcium Phosphate	12.40
	Sodium Bicarbonate	31.20
	Cornstarch	16.23
	Calcium Carbonate	16.17
Sample Marked "C":		
	Sodium Acid Pyro Phosphate	20.00
	Mono Calcium Phosphate	20.00
	Sodium Bicarbonate	28.57
	Cornstarch	31.43

METHODS

Official A.O.A.C. Method

Methods of Analysis, 6th Edition (1945), page 210, sec. **17.8**; or 5th Edition (1940), page 188.

Quartermaster Corps Method

Quartermaster Corps Tentative Specifications C.Q.D. #326, May 24, 1946.

Residual Carbon Dioxide.—Place 1.7 g of baking powder in a clean, dry, 250 ml wide-mouthed soxhlet extraction flask.* Add 20 ml distilled water. Evaporate to dryness on a steam bath in which the boiling water is kept at a constant level of 2 inches below the top of the bath. The flask must be set into an opening 3 inches in diameter. After 2 hours remove from the steam bath, add 10 ml distilled water and let stand until the flask is at room temperature. Determine carbon dioxide present with Chittick apparatus as described in *Methods of Analysis of the A.O.A.C.*, Fifth Edition, page 186, using correction factors given in Table 24. To obtain the available carbon dioxide subtract the residual carbon dioxide from the total carbon dioxide.

(The determination of the carbon dioxide referred to above in the 5th Edition of the A.O.A.C. *Methods of Analysis*, page 186, is the same as that found in the 6th Edition, pages 208–209.)

Modified Quartermaster Corps Method.—Place 1.7 g of baking powder in a clean, dry, 250 ml wide mouthed soxhlet extraction flask.* Add 20 ml distilled water. Evaporate to dryness on a steam bath in which the boiling water is kept at a con-

* The 250 ml. wide-mouthed soxhlet extraction flask specified in the two Q.M.C. methods is the same as the decomposition flask used in the A.O.A.C. Official Gasometric Method.

TABLE 1.—*Results of collaborators on sample A in per cent carbon dioxide*

COLLABORATOR	OFFICIAL A.O.A.C. METHOD	DEVIATION FROM THE MEAN	Q.M.C. METHOD	DEVIATION FROM THE MEAN	MODIFIED Q.M.C. METHOD	DEVIATION FROM THE MEAN
1	1.23	.30	.88	.17	.32	.15
	1.05	.12	.81	.10	.36	.11
	1.27	.34	.84	.13	.47	.00
2	.605	.32	.344	.37	.203	.27
	.605	.32	.443	.27	.304	.17
	.705	.22	.443	.27	.304	.17
3	.782	.15	.677	.03	.581	.11
	.703	.23	.657	.05	.678	.20
	.703	.23	.657	.05	.678	.20
4	1.36	.43	.67	.04	.52	.05
	1.30	.37	.72	.01	.52	.05
	1.30	.37	.77	.06	.57	.10
5	.98	.05	1.15	.44	.41	.06
	.98	.05	1.10	.39	.62	.15
	1.04	.11	1.15	.44	.62	.15
6	.89	.04	.55	.16	.41	.06
	.76	.17	.55	.16	.47	.00
	.97	.04	.61	.10	.41	.06
			.56	.15		
7*	2.00	1.07	3.20	2.49	2.80	1.70
	2.10	1.17	2.20	1.49	2.40	1.30
	1.70	0.77	2.60	1.89	2.00	0.90
8	.93	.00	.95	.24	.75	.28
	1.00	.07	.90	.19	.66	.19
	1.05	.12	1.06	.35	.61	.14
9	.87	.06	.57	.14	.41	.06
	.77	.16	.57	.14	.46	.01
	.87	.06	.62	.09	.41	.06
10	.90	.03	.59	.12	.40	.07
	.90	.03	.50	.21	.50	.03
	.70	.23	.61	.10	.45	.02
	.99	.06	.62	.09	.48	.01
	1.07	.14	.67	.04	.34	.13
	.97	.04	.67	.04	.48	.01
	.58	.35				
	.78	.15				
	.77	.16				
11	.97	.04	.81	.10	.40	.07
	1.02	.09	.71	.00	.40	.07
	1.08	.15	.76	.05	.40	.07
Mean	.93		.71		.47	
Ave. Dev.		.16		.15		.10
Max. Var.	.78		.81		.55	

* Results for Collaborator #7 not included in computation of the mean or the average deviation, because the deviations from the mean of these results are greater than 4 times the average deviation.

TABLE 2.—*Results of collaborators on sample B in per cent carbon dioxide*

COLLABORATOR	OFFICIAL A.O.A.C. METHOD	DEVIATION FROM THE MEAN	Q.M.C. METHOD	DEVIATION FROM THE MEAN	MODIFIED Q.M.C. METHOD	DEVIATION FROM THE MEAN
1	7.23	.04	7.18	.32	6.77	.14
	7.30	.03	7.18	.32	6.88	.25
	7.43	.16	7.06	.20	6.87	.24
2	7.00	.27	6.64	.22	6.24	.39
	6.95	.32	6.49	.37	6.34	.29
	7.06	.21	6.59	.27	6.39	.24
3	7.23	.04	7.18	.32	6.88	.25
	7.23	.04	7.06	.20	6.98	.35
	7.13	.14	7.16	.30	6.93	.30
4	7.66	.39	6.79	.07	6.76	.13
	7.77	.50	6.79	.07	6.76	.13
	7.77	.50	6.69	.17	6.76	.13
5	7.73	.45	7.79	.93	6.89	.26
	7.52	.25	7.79	.93	6.99	.36
	7.52	.25	7.73	.87	6.88	.25
6	7.18	.09	7.02	.16	6.86	.23
	7.17	.10	7.07	.21	6.89	.26
	7.13	.14	7.21	.35	6.76	.13
			6.99	.13		
7	7.40	.13	6.90	.04	6.70	.07
	7.70	.43	6.90	.04	6.70	.07
	8.00	.73	7.20	.34	6.70	.07
8	7.44	.17	7.36	.50	7.02	.39
	7.46	.19	7.27	.41	6.93	.30
	7.55	.28	7.31	.45	6.80	.17
9	7.32	.05	6.94	.08	6.62	.01
	7.21	.06	6.84	.02	6.72	.09
	7.21	.06	6.94	.08	6.67	.04
10	6.60	.67	5.84	1.02	6.06	.57
	6.60	.67	5.87	.99	6.16	.47
	6.79	.48	5.88	.98	5.95	.68
	7.08	.19	6.41	.45	5.84	.79
	7.13	.14	5.96	.90	5.60	1.03
	7.01	.21	6.13	.73	5.87	.76
	6.74	.53	6.13	.73		
	6.73	.54				
11	7.49	.22	6.87	.01	6.94	.31
	7.54	.27	6.79	.07	6.92	.29
	7.24	.03	6.80	.06	6.97	.34
Mean	7.27		6.86		6.63	
Ave. Dev.		.23		.37		.27
Max. Var.	1.40		1.95		1.42	

stant level of 2 inches below the top of the bath. The flask must be set into an opening 3 inches in diameter. After 2 hours add 20 ml distilled water and evaporate to dryness in like manner. Add 10 ml distilled water and let stand until the flask is at room temperature. Determine carbon dioxide present with Chittick apparatus as described in *Methods of Analysis of the A.O.A.C.*, 6th Edition, pages 208–209, using correction factor in Table **44.30.**

The final carbon dioxide in all three methods was determined in the same way employing the gasometric method described on pages 208–209, secs. **17.4–17.6,** inclusive, using 10 ml of hydrochloric acid (1+2) instead of the sulphuric acid (1+5) specified.

Because several of the collaborators raised questions as to interpretation of the directions in the Q. M. C. Method, the Referee and Associate Referee thought it advisable to submit a more detailed version of the method, which follows:

QUARTERMASTER CORPS METHOD FOR DETERMINATION OF RESIDUAL CARBON DIOXIDE IN BAKING POWDERS

(Rewritten Version)

Place 1.7 g of baking powder in a clean, dry, 250 ml wide mouthed soxhlet extraction flask, such as is used in the Offiical A.O.A.C. Gasometric Method. Add 20 ml distilled water. Put the flask *on the cover* of a water bath (single or multiple) in which the boiling water is kept at a constant level of 2 inches below the top of the bath. The water in the bath should be boiling vigorously all through the determination and the bath should be heated with gas. The opening in the cover of the bath into which the flask is set must be 3 inches in diameter. This prevents the flask from touching the water and keeps it a definite distance above it. Evaporate the contents of the flask to *dryness.* (By dryness is meant that there is no visible evidence of moisture in the residue or the inside surface of the flask.) If the set-up is functioning properly, this should take from 1½ to 2 hours. In any case, leave the flask on the water bath for a total of 2 hours. If the sample is not completely dry in 2 hours something is wrong with the set-up. After the sample is completely dry, add 10 ml distilled water and let stand until the flask is at room temperature. This will take about 1 hour.

Determine carbon dioxide present with Chittick apparatus as described in *Methods of Analysis*, 5th Edition, page 186, using correction factors in Table 24, or 6th Edition 1945, page 208–209, and using correction factors in Table **44.30.**

The residue in the flask at this point is in such a condition that it is difficult to shake out the carbon dioxide. Shake the flask vigorously until further shaking produces no increment in the reading.

The following collaborators contributed their time and effort in this work:

Barackman, R. A., and Winterle, E. R., Victor Chemical Works, Chicago Heights, Ill.
Bryan, C. S., and Randall, H. C., Rumford Chemical Works, Rumford, R. I.
Despaul, J. E., Quartermaster Food & Container Institute, Chicago, Ill.
Dick, Ludwig, Standard Brands, Inc., Chicago, Ill.
Holch, R. D., Jaques Manufacturing Co., Chicago, Ill.
Honzak, Ruth, General Foods Corporation, Chicago, Ill.
Miller, G. E., Fleischmann Laboratories, New York, N. Y.
Morck, R. A., R. B. Davis Co., Hoboken, N. J.
Munsey, V. E., Food & Drug Administration, Washington, D. C.

TABLE 3.—*Results of collaborators on sample C in per cent carbon dioxide*

COLLABORATOR	OFFICIAL A.O.A.C. METHOD	DEVIATION FROM THE MEAN	Q.M.C. METHOD	DEVIATION FROM THE MEAN	MODIFIED Q.M.C. METHOD	DEVIATION FROM THE MEAN
1	1.22	.28	.36	.02	.00	.17
	1.31	.37	.55	.21	.05	.12
	1.28	.34	.45	.11	.29	.12
2	.655	.28	.295	.04	.101	.07
	.504	.44	.344	.00	.101	.07
	.705	.23	.197	.14	.203	.03
3	.782	.14	.249	.09	.194	.02
	.733	.21	.249	.09	.194	.02
	.782	.16	.348	.01	.290	.12
4	1.04	.10	.31	.03	.16	.01
	1.04	.10	.36	.02	.16	.01
	.99	.05	.31	.03	.16	.01
5	.99	.05	.94*	.60*	.41	.24
	1.05	.11	.94*	.60*	.52	.35
	1.05	.11	.94*	.60*	.41	.24
6	.77	.17	.18	.16	.03	.14
	.85	.09	.25	.09	.10	.07
	.82	.12	.39	.05	.07	.10
			.20	.14		
7	3.30*	2.36*	.50	.16	.30	.13
	3.30*	2.36*	.50	.16	.50	.33
	2.10*	1.16*	1.00*	.66*	.30	.13
8	.58	.36	.25	.09	.15	.02
	.81	.13	.31	.03	.22	.05
	.52	.42	.43	.09	.20	.03
9	1.59	.65	.31	.03	.00	.17
	1.48	.54	.31	.03	.10	.07
	1.53	.59	.26	.08	.05	.12
					.05	.12
					.10	.07
					.05	.12
10	1.20	.26	.54	.20	.17	.00
	.92	.02	.47	.13	.05	.12
	1.40	.46	.23	.11	.02	.15
	.61	.33	.40	.06	.19	.02
	.35	.59	.35	.01	.13	.04
	.81	.13	.34	.00	.13	.04
	.63	.31	.32	.02	.10	.07
	.68	.26	.31	.03	.19	.02
	.96	.02	.48	.14	.19	.02
			.38	.04	.15	.02
			.48	.14	.10	.07
					.20	.03
11	.92	.02	.30	.04	.20	.03
	1.07	.13	.30	.04	.25	.08
	1.17	.23	.35	.01	.20	.03
Mean	.94		.34		.17	
Ave. Dev.		.25		.08		.09
Max. Var.	1.24		.37		.52	

* Results not included in computation of the mean or the average deviation.

Schilb, T. W., and McKim, Elizabeth, Monsanto Chemical Co., St. Louis, Mo.
Stephenson, N. R., Standard Brands Limited, Toronto, Canada.

(The order in which these collaborators are listed does not conform to the numbers used in the tables of results.)

COMMENTS OF COLLABORATORS

Barackman, R. A., and Winterle, E. R.—"We prefer the Quartermaster Corps method for residual CO_2 determinations. The Q. M. C. method, however, does not take into consideration that drying of the sample may be carried too far. The caked residue which is obtained may be thought of as like the crust of a baked product whereas the interior should be simulated if possible. Some tests which we carried out indicated that if a watch-glass is placed on the mouth of the decomposition flask during the two hour heating period on a boiling water bath, the residue will remain as a gel if starch is present. We offer this suggestion for future work."

Schilb, T. A., and McKim, Elizabeth.—"We were unable to get satisfactory checks on the official A.O.A.C. We feel that this is because it is impossible to regulate the time of actual boiling of the sample with a sufficiently high degree of accuracy."

Morck, R. A.—"We have used the Q.M.C. procedure some here in our laboratory and like it very much. While the results are usually lower than those obtained by the A.O.A.C. method, we have found them more consistent. We saw no advantage in the modified procedure over the regular Q.M.C. procedure and of course it does require a great deal more time."

Bryan, C. S., and Randall, H. C.—"We prefer the Quartermaster Corps method to the other two methods for the following reasons:

1. The instructions are more concise than those for the A.O.A.C. method.
2. The A.O.A.C. method, although giving results in the shortest elapsed time, requires more of the attention and time of the operator. For instance, at the point where the method calls for bringing the sample to a quick boil and boiling for one minute, much flame adjusting must be made, for if brought too rapidly to a boil the sample foams up despite the addition of 3 drops of capryl alcohol.
3. The modified Quartermaster Corps method seems to us to be unnecessarily prolonged and to give, in the case of two of the samples, especially sample C, residual CO_2's which seem to us to be very low."

Holch, R. D.—"Evaporation to dryness is a severe condition which is not encountered during actual baking procedure. Evaporation to dryness consumes much more time than the A.O.A.C. method and the reproducibility is not improved."

Stephenson, N. R.—"The official A.O.A.C. method gave higher values than either of the Q.M.C. methods. However, this procedure yields more variable results. A considerable error is introduced into each method unless the Chittick flask is shaken until there is no further increment in the reading of the gas burette."

DISCUSSION

The maximum variances and average deviations of the results in Table 1 on powder A indicate that there is not much difference in precision between the official and Q.M.C. methods. The modified Q.M.C. results have a slight edge in precision, although the precision of all three methods was satisfactory for a determination of this kind. Note that one collaborator had trouble with all three methods on powder A.

The results in Table 2 on powder B are not very good with any of the

three methods. This may be due to the calcium carbonate in the formula which brings the results up to such a relatively high level that if the error is partially factorial this would account for the greater maximum variances and average deviations. Calcium carbonate reacts with the acids in the baking powder yielding higher availables by as much as 1.00 per cent in powder B over powder A. Does this mean that powder A is compounded on the acid side and does not really have an excess of soda? At any rate the presence of the calcium carbonate further complicates this determination because it introduces additional reactions.

The results in Table 3 on powder C demonstrate that both Q.M.C. methods are superior to the official method and the precision is probably as good as we ever hope to attain in a determination of this type. Collaborator #7 had trouble with the official method on this powder while #5 had trouble with the Q.M.C. method. On the whole it appears that the precision of the two Q.M.C. methods is at least as good as that of the official method on powders A and B and definitely superior on powder C. It is certainly a disappointment to see that the simple and thoroughly descriptive instructions given in the Q.M.C. methods (particularly the rewritten version) did not produce better agreement in results than the official method on powders A and B. The wording and description of the official method is certainly not up to the standard set by the A.O.A.C. Every analyst has his own interpretation of a "metal drying cell" and the most critical point in the determination "boiling for 1 minute" is very difficult to control and standardize.

Note that there is a difference in the level of the results on all three powders by all three methods, the greatest difference being on powder C where the mean by the official method is 0.94 per cent and 0.34 per cent by the Q.M.C. method. This difference in level is of course due to the difference in time and method of heating. Two of the collaborators comment that the evaporation to dryness in the Q.M.C. method is too severe and is a condition which does not simulate actual baking. This objective of trying to simulate baking conditions with a method of this kind has some merit, but we must remember that distilled water as a medium for the reaction certainly does not even approximate dough conditions where you have a large mass of neutral or slightly acid materials and in the case of cake dough a saturated sugar solution. Also that in dough any carbon dioxide generated by the baking powder and retained in the dough will leaven whereas in distilled water solutions the carbon dioxide generated must be completely driven off or it will reflect in the residual and yield a lower available carbon dioxide.

The amount of carbon dioxide retained in the residual solution is dependent on the following factors:

1. *p*H of the residual salts.
2. Amount and degree of gelatinization of the starch in the residual solution.
3. Concentration of soluble salts.

To obtain a true available CO_2 all the retained CO_2 which is in solution must be driven off. To my knowledge this can only be accomplished in one of two ways.

1. Evaporation to dryness.
2. By using a saturated solution of neutral sodium chloride or sodium sulphate as the reaction medium.

Evaporation to dryness is the basis of both of the Q.M.C. methods and it does bring the results down to lower levels which, in my opinion, are closer to the true values than the official method. Incidentally the modified Q.M.C. method is practically identical to the Official British method at present, the only difference being that the final determination of the CO_2 is done in an alkalimeter.

To those who think that evaporation to dryness is too severe, it may be pointed out that the use of a saturated sodium chloride solution instead of distilled water in the official A.O.A.C. method will bring the results down to the same level as those obtained with a single evaporation to dryness.

Gelatinization of the starch is another factor which makes it very difficult to drive out the available CO_2. Therefore some work should be done with the Q.M.C. method where evaporation to dryness is carried out at a temperature which is below the point at which starch gelatinizes. The gelatinized starch also causes trouble when the residual CO_2 is shaken out on the Chittick apparatus.

RECOMMENDATIONS*

It is recommended—

(1) That the present official method for determination of residual carbon dioxide in baking powders be dropped for the following reasons:

(a) The wording and description of the method are susceptible to too many interpretations.
(b) The manner of heating and treating the sample does not yield a true residual because all of the available CO_2 is not driven off.
(c) The method is not applicable to all types of baking powder, particularly the pyrophosphate types.

(2) That the Quartermaster Corps method (Rewritten Version) be adopted as a tentative method, adding the words "or electricity" after the phrase "The bath should be heated with gas."

(3) That the Q.M.C. method be subjected to further study wherein the single evaporation to dryness is carried out in a moisture oven instead of on a water bath and at temperatures ranging from 70 to 100°C.

(4) That the Q.M.C. method be also studied using a neutral saturated sodium chloride solution as a medium instead of distilled water.

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* For report of Subcommittee D and action by the Association, see *This Journal*, 31, 59 (1948).

ACKNOWLEDGMENT

The Associate Referee wishes to extend his thanks to all of the collaborators for their cooperation, and to Mr. Ludwig Dick (Standard Brands Inc., Chicago, Ill.) for his valuable suggestions and criticisms in this work.

No reports were given on disinfectants, or on leathers and tanning materials.

REPORT ON PLANTS

By ELROY J. MILLER (Michigan Agricultural Experiment Station, East Lansing, Mich.) *Referee*

During the past year efforts have been made not only to continue the studies already in progress but to initiate new ones; however, not as much was accomplished in either respect as was originally hoped for.

Miss Lillian I. Butler, Associate Referee on Copper and Cobalt, submitted no report on either subject and asked to be relieved of this assignment.

Dr. J. T. Sullivan, Associate Referee on Carbohydrates, submitted no report on his subject. Sometime ago he asked to be relieved of this assignment, because the press of other duties prevented him from doing justice to it, and it is with regret that his request is acceded to at this time. The title of the subject has been changed to "Sugar."

Dr. J. S. McHargue, Associate Referee on Iodine and Boron, will present a report on boron but not on iodine.

Dr. E. J. Benne, Associate Referee on Carotene and Zinc, will report on the latter but not the former.

No report on sampling will be presented.

Steps are being taken to initiate studies on methods for determining the following constituents of plants: Cobalt, copper, fluorine, lead, sugar, starch, sodium, potassium, and different forms of nitrogen. A number of qualified analytical chemists have signified their willingness to serve as Associate Referees; however, the only definite assignment which has been made to date is that of Dorothy R. Waldron of the Michigan Agricultural Experiment Station, who has agreed to serve in this capacity for sodium. Other assignments will be made as soon as possible and when completed the Secretary will be notified of the appointees. It is hoped that the new appointments can be included in this report before it is published.

RECOMMENDATIONS*

It is recommended—

(1) That the recommendations made by the Associate Referees in their reports on Boron and Zinc, respectively, be accepted.

(2) That the following Associate Referees be appointed:

* For the report of Subcommittee A and action by the Association, see *This Journal*, 31, 43 (1948).

Boron and Iodine—L. K. Wood, University of Kentucky, Agricultural Experiment Station, Lexington, Ky.

Carotene and Zinc—Erwin J. Benne, Dept. of Agricultural Chemistry, Mich. State College, East Lansing, Mich.

Cellulose and Lignin—Gordon H. Ellis, U. S. Plant, Soil, and Nutrition Laboratory, Ithaca, N. Y.

Cobalt and Copper—Kenneth C. Beeson, U. S. Plant, Soil, and Nutrition Laboratory, Ithaca, N. Y.

Pectin—C. O. Willets, Eastern Regional Research Laboratory, Chestnut Hill Station, Philadelphia, Pa.

Sampling—Elroy J. Miller, Department of Agricultural Chemistry, Mich. State College, East Lansing, Mich.

Sodium—Ray L. Shirley, Dept. of Agricultural Chemistry, Mich. State College, East Lansing, Mich.

Starch—Carroll L. Hoffpauir, Southern Regional Research Laboratory, New Orleans 19, La.

Sugar—Kenneth T. Williams, Western Regional Research Laboratory, Albany 6, Calif.

REPORT ON BORON IN PLANTS

By CALVIN M. AUSTIN and J. S. MCHARGUE (*Associate Referee*), Kentucky Agricultural Experiment Station, Lexington, Ky.

This report is an outline of a new colorimetric method for the determination of total boron in plant material. The reagent used is p-nitrobenzeneazo-1,8-dihydroxynaphthalene-3,6-disulfonic acid. This compound will be referred to as "Chromotrope-B." Feigl has reported the use of this compound as a spot test for boron.

The procedure is similar to the quinalizarin method, the chief difference being in the ignition of the sample. The ignition is made on air dry samples at 600°C. after the addition of an alkali.

Details of the method and the results obtained are described in a paper presented at this meeting, entitled "The Determination of Total Boron in Plant Material with 'Chromotrope-B'."[1]

PROCEDURE

Ignite 0.2 to 0.5 g of air-dried, 60-mesh sample in a platinum dish after the addition of barium hydroxide. The dish should be placed in a cool muffle furnace and the temperature raised to 600°C. The total time in the furnace should be two hours. After ignition extract the boron from the ash with 5.0 or 10.0 ml of 70% acetic acid. Add 10.0 ml of a .005% soln of "Chromotrope-B" in concentrated, sulfuric acid (reagent grade) to a 1.0 ml aliquot of the extract. Allow at least 30 min. for full color development and determine the per cent transmission spectrophotometrically at 620 mμ against a reference soln containing 1.0 ml of 70% acetic acid and 10.0 ml of

[1] Calvin M. Austin and J. D. McHargue, *This Journal*, page 427.

the reagent soln. The boron content of the solution is then found from a standard curve determined by treating known amounts of boron in the same way.

RESULTS

The results of analyses of several plant samples and a synthetic sample containing 97.2 p.p.m. of boron as anhydrous $Na_2B_4O_7$ are shown in Table 1.

TABLE 1.—*Results*

SAMPLE	BORON ADDED	NUMBER OF DETERMINATIONS	MEAN BORON FOUND	AVERAGE DEVIATION OF A SINGLE OBSERVATION	RECOVERY
	p.p.m.		*p.p.m.*	*p.p.m.*	*per cent*
Synthetic	97.2	9	92.7	1.1	95.4
Bean Seed		6	21.8	.4	
White Pine Needles (N)		5	38.0	1.1	
White Pine Needles (D)		5	42.6	1.1	
Wild Cherry Leaves (1)		4	17.1	.2	
Wild Cherry Leaves (2)		3	22.9	.4	
Kelp		6	314.3	2.8	

These results seem to justify further study. It is planned to continue this work particularly in regard to the analysis of biological material, soils, and fertilizers.

RECOMMENDATIONS*

It is recommended that the method be studied independently by others and that collaborative work be undertaken for next year.

REPORT ON ZINC IN PLANTS†

By RAY L. SHIRLEY, DOROTHY R. WALDRON, ELVA D. JONES, and ERWIN J. BENNE, *Associate Referee* (Michigan Agricultural Experiment Station, East Lansing, Mich.)

During the 1945 revision of *Methods of Analysis, A.O.A.C.* (1), the dithizone method for determining zinc in plant materials replaced the former H_2S-ferrocyanide procedure in the Plant Chapter. The dithizone method depends upon the combination of zinc with dithizone (diphenylthiocarbazone) in an ammoniacal solution of controlled reaction to form a red compound. This compound and some excess dithizone are removed from the aqueous solution by extracting it with carbon tetrachloride, and the zinc present is evaluated photometrically by a mixed-color procedure. Interference by copper is prevented by removing it as the dithizonate from

* For report of Subcommittee A and action by the Association, see *This Journal*, 31, 43 (1948).

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acidified solution, and diethyldithiocarbamate is added to prevent cations other than zinc from forming dithizonates when the solution is made ammoniacal.

This method was published in its present form by Cowling and Miller (2). It was submitted for collaborative study by Cowling, who served as Associate Referee on zinc in plants from 1940 to 1941. Results from this study were reported at the meeting of the Association in 1940 and published in the Journal in 1941 (3). On the basis of these results Cowling recommended that the Association accept the method as a tentative one and that the study of it be continued. These recommendations were accepted; however, Cowling resigned as Associate Referee before acting upon his second recommendation.

The present Associate Referee was appointed in 1942 to succeed Cowling, but while the War was in progress other activities necessitated that an investigation of methods for determining zinc be postponed. During the past year, however, such an investigation was begun, and the results obtained to date are presented in this report.

EXPERIMENTAL AND RESULTS

It was decided to investigate the tentative A.O.A.C. method first, and later to study other procedures in an effort to find, or if possible to devise, one possessing additional advantages. To date the following aspects of the subject have been studied:

1. The spectral properties of carbon tetrachloride solutions of dithizone and zinc dithizonate, individually, and together as used in the mixed-color method.
2. The effects of daylight upon the spectral properties of these solutions and means of protecting them against such effects.
3. The following points in the mixed-color procedure:
   (a) Amount of dithizone reagent necessary when the concentration of zinc is high.
   (b) Standardization of the rinsing process with carbon tetrachloride after extraction of the aqueous solution with dithizone reagent.
   (c) Effects of varying the amount of solution B.
   (d) Effects of using a stock solution of carbamate reagent which was stored in a refrigerator.
4. (a) The practicability of removing excess dithizone from the carbon tetrachloride solutions of zinc dithizonate and using a one-color instead of a mixed-color procedure.
   (b) Determination of the accuracy with which known quantities of zinc added to extracts of plant ash, plus the zinc present in the extract, could be evaluated by use of this procedure.
5. Reproducibility of results by the mixed-color and one-color procedures when used by four different analysts in an intra-laboratory study.

Each of the above sections will be treated separately in the sequence given.

It was thought that a study of the spectral properties of the components of the mixed-color system should help to afford a clearer understanding of, and possibly lead to improvements in, the dithizone method. Consequently, spectral transmission curves were prepared, from 400 to 725 millimicrons, for carbon tetrachloride solutions of: (A) Dithizone reagent

diluted 1 plus 4, (B) Zinc dithizonate and excess dithizone obtained by carrying 20 micrograms of zinc through the entire mixed-color procedure, and (C) Zinc dithizonate prepared by freeing a solution similar to (B) of excess dithizone with dilute aqueous ammonium hydroxide. A Cenco-Sheard Spectrophotelometer was used for this purpose. The results obtained are presented graphically in Figure 1.

H. R. Kraybill, one of Cowling's collaborators (3), called attention to the

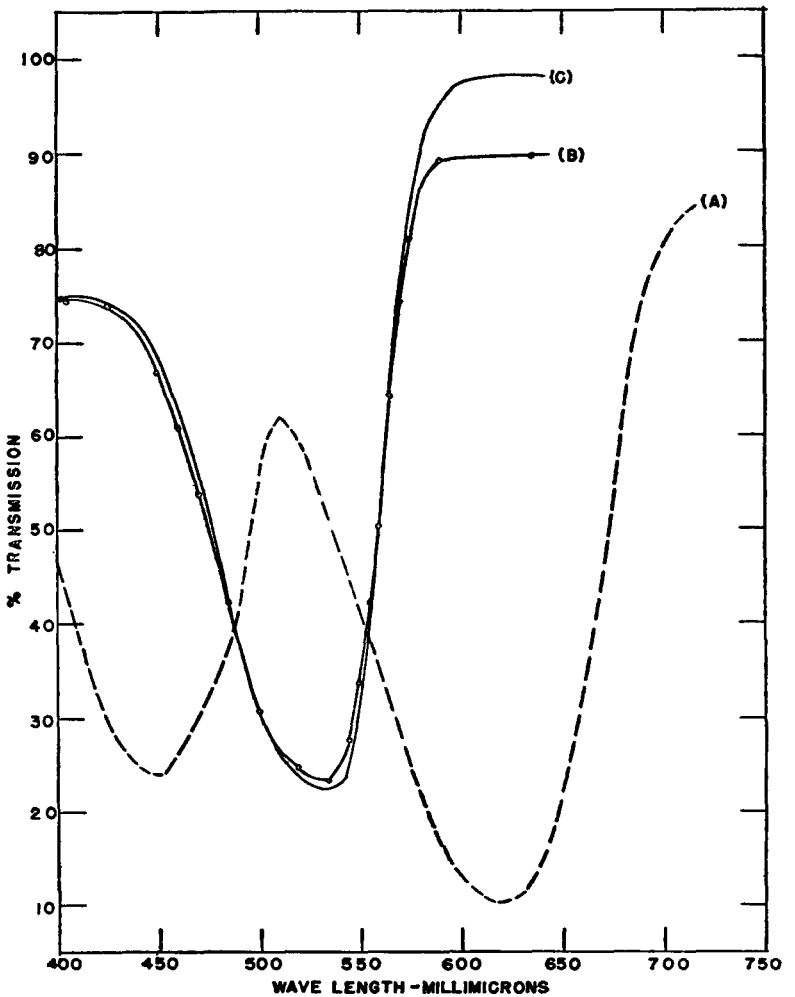

FIG. 1.—Spectral transmission curves of carbon tetrachloride solutions of (A) Dithizone reagent, (B) Zinc dithizonate and excess dithizone reagent, and (C) Zinc dithizonate.

tendency of the colors to fade when the carbon tetrachloride solutions of dithizone and zinc dithizonate involved in the mixed-color procedure were exposed to even ordinary diffused sunlight. He further observed that if electric light supplied the only illumination, fading did not occur, but rather the colors of the solutions tended to intensify during the first two hours under these conditions. When the solutions were maintained in darkness no changes in their color intensities were discernible in forty-eight hours.

During the early part of this investigation the authors corroborated Kraybill's observations. It occurred to them that the use of amber, or low-actinic, glassware might effectively protect the solutions against daylight. Accordingly separatory funnels, bottles, volumetric flasks, and pipettes made of such glass were obtained and used throughout the remainder of the investigation. Repeated trials showed this practice to be quite effective in stabilizing the optical densities of the carbon tetrachloride solutions. Given below are representative data showing the differences in the per cent of light transmitted, before and after a 3-hour exposure to daylight, by solutions of dithizone and zinc dithizonate which were carried through the mixed-color procedure, and maintained, in colorless or amber glassware:

KIND OF GLASSWARE USED	MICROGRAMS OF ZINC PRESENT	LIGHT TRANSMITTED	
		BEFORE EXPOSURE	AFTER EXPOSURE
		per cent	*per cent*
Colorless	0	67.0	82.0
Colorless	15	40.0	47.0
Colorless	20*	85.5	96.0
Amber	0	74.5	74.7
Amber	15	43.0	43.0
Amber	20*	87.3	87.5

* These solutions were diluted 10 times more than the others and per cent of light transmitted was determined with a Cenco-Sheard Spectrophotelometer. In all other instances throughout the investigation a Cenco-Sheard-Sanford Photelometer, equipped with the light filter recommended by Cowling and Miller (2), was used for this purpose.

During extraction of the ash extract with dithizone reagent an excess of this reagent must be present, and the presence of an excess is indicated by an orange or yellow-orange coloration of the aqueous phase after the phases have separated. Such a color is difficult to see when separatory funnels made of amber or low actinic glass are being used; hence, another means of judging whether an excess was present became necessary. The authors found that the use of 15 ml. of dithizone reagent assured an excess for all of the plant materials which they sampled and analyzed according to the recommended procedure. Therefore, they adopted the practice of using this amount of reagent in all cases where the concentration of zinc was suspected of being high.

Likewise, in both the first and second extractions, rinsing with carbon tetrachloride is judged to be complete on the basis of the green coloration acquired by the carbon tetrachloride layer. Even with colorless glass vessels the authors found this to be a somewhat unreliable criterion, and with amber or low actinic glassware a revised rinsing technique was required. Repeated use showed the following procedure to be effective and reliable in both the first and second extractions: After making the first separation in the usual manner, add 2–3 ml. of carbon tetrachloride to the contents of the separatory funnel. Allow the layers to separate and draw off the lower layer; in the first extraction into the second separatory funnel; in the second into the container for waste carbon tetrachloride. Repeat once. Then add 3–5 ml. of carbon tetrachloride and shake vigorously for 15 seconds. Allow the layers to separate and draw off the lower layer as before. Repeat once.

Dithizone is very soluble in aqueous ammonia and presumably would be less readily extracted by carbon tetrachloride from solutions in which the concentration of ammonia is high. Hence, it was thought that differences in the amounts of solution B used might be partly responsible for variations in percentages of light transmitted by the carbon tetrachloride extracts. To determine if such were the case, varying amounts of solution B, and sufficient distilled water to make a total volume of 55 ml. were added to the usual quantities of 0.02 *N* HCl and dithizone reagent in each of three separatory funnels, and the remainder of the procedure was completed in the usual way. The volumes of solution B added and the percentages of light transmitted are given below:

Ml. of solution B added	*Per cent of light transmitted*
48	80.0
50	79.5
52	80.5

It is obvious from these results that significant variations in the percentages of light transmitted by the carbon tetrachloride extracts are not due to slight differences in the amounts of solution B used.

In an effort to eliminate variations in light transmission values that might accompany separate preparations of carbamate reagent for each series of determinations, a study was undertaken to ascertain how long a stock solution of this reagent could be stored and still be used safely. It was found that such a solution had no perceptible effect on the reproducibility of light transmission values for at least a month, if stored in the refrigerator when not in use. Hence, the practice of preparing a liter of this solution at a time, storing it in the refrigerator, and withdrawing required amounts immediately before use, was adopted.

It was thought that differences in the amount of dithizone removed from the aqueous phase in the final extraction might be responsible for the variations in the percentages of light transmitted by solutions containing

the same amounts of zinc. If such were the case, it appeared that a one-color procedure, in which the excess dithizone was removed by extracting the carbon tetrachloride solutions with dilute ammonium hydroxide, should improve the reproducibility of transmission values. Cowling and Miller (2) discussed such a procedure, but they believed that when lead was present high values for zinc would result. However, they gave no data on this point; hence, it seemed of value to determine the effects of added lead. Accordingly similar solutions with and without lead were carried through the mixed-color procedure as usual. The aqueous layer was removed by use of suction, 50 ml. of 0.01 *N* NH_4OH were added to each separatory funnel, and the contents were shaken vigorously for 30 seconds. After the layers had separated, the carbon tetrachloride solutions were drawn off, aliquots were diluted as usual and the per cent of light transmitted by each was determined. Amounts of zinc and lead added and typical results obtained are summarized below:

Micrograms present		*Per cent of light transmitted*
Zn	*Pb*	
0	0	75.2
0	0	77.5
0	0	76.0
0	25	74.9
0	25	76.1
0	25	76.5
10	0	50.5
10	25	48.0
20	0	30.5
20	0	31.2
20	25	30.5
20	25	31.0

These data show no greater variations in results when added lead was present than when it was absent. Therefore, the procedure was tested further by applying it to the determination of zinc in extracts of plant ash.

A reference curve relating concentration of zinc and light transmission was prepared in the usual way by carrying known amounts of zinc through the entire procedure and determining the per cent of light transmitted by each carbon tetrachloride solution with the photoelectric colorimeter. Extracts of the ash from samples of plant materials were prepared as directed by the A.O.A.C. method, and aliquots were analyzed for zinc by the one-color procedure. Other aliquots were similarly analyzed after addition of known quantities of zinc. Representative results obtained, together with similar data pertaining to the mixed-color procedure reproduced from Cowling and Miller (2) for comparison, are given below.

Judged on the percentages of zinc recovered the two procedures appeared to be entirely comparable; hence, both were subjected to an intra-laboratory study participated in by four analysts. All were experienced analysts; however, analyst C had had no previous experience with

NATURE OF SAMPLE	ADDED[1]	MICROGRAMS OF ZINC		PER CENT OF TOTAL RECOVERED
		TOTAL PRESENT	DETERMINED[2]	
Lettuce leaves	0	—	11.88	100.0
	1.20	13.08	13.02	99.5
	2.40	14.28	13.85	97.0
Alfalfa leaf meal	0	—	8.25	100.0
	4.81	13.06	13.00	99.5
	9.62	17.87	17.58	98.4
	14.43	22.68	23.53	103.7
Cowling and Miller—				
Dried grapes	0	—	11.4	100.0
	10	21.4	21.9	102.3
	20	31.4	31.3	99.7
	40	51.4	51.2	99.6
Dried asparagus	0	—	13.6	100.0
	10	23.6	23.4	99.2
	20	33.6	33.5	99.7
	40	53.6	53.6	100.0

[1] Added to the lettuce, grape, and asparagus samples prior to ashing and to aliquots of the extract of ash from alfalfa meal immediately before analyzing.
[2] Averages of results from 2 or more determinations.

either procedure. Each participant prepared, and used, his own reference curves relating concentration of zinc and light transmission. The data from which these curves were constructed are shown below as an indication of the comparative reproducibility of results with known quantities of zinc when the two procedures were used by different analysts.

MICROGRAMS OF ZINC	PER CENT OF LIGHT TRANSMITTED							
	MIXED-COLOR PROCEDURE, ANALYST—				ONE-COLOR PROCEDURE, ANALYST—			
	A	B	C	D	A	B	C	D
0	74.5	70.0	71.2	72.1	83.0	81.0	79.1	79.8
10	51.6	48.0	48.7	47.6	50.0	48.1	48.8	48.5
15	41.5	39.9	42.2	40.6	39.0	36.9	—	38.0
20	33.9	32.1	32.0	33.7	31.9	30.1	30.1	30.0
25	28.9	28.0	27.1	29.7	26.0	24.1	25.2	24.0
30	24.0	23.1	23.3	25.0	19.9	19.3	21.0	20.0

Fortunately it was possible to obtain for this study portions of the three samples analyzed by Cowling and his collaborators (3). Extracts of each were prepared, and all four participants analyzed aliquots by both procedures. Results obtained, together with those obtained by Cowling and his collaborators with the mixed-color procedure (3) to permit comparison of reproducibility, follow:

ANALYST	P.P.M. OF ZINC DETERMINED[1]					
	MIXED-COLOR PROCEDURE SAMPLE NO.[2]			ONE-COLOR PROCEDURE SAMPLE NO.[2]		
	1	2	3	1	2	3
A	27.8	23.4	46.6	22.4	24.1	46.4
B	27.4	24.1	45.0	22.5	21.9	45.3
C	25.5	19.8	42.7	23.1	24.1	46.7
D	26.7	21.5	46.2	22.0	22.9	46.5
Av.	26.9	22.2	45.1	22.5	23.3	46.2
Cowling's collaborators—						
No. 1	26.4	22.3	49.3			
No. 2	24.6	19.7	49.5			
No. 3	21.0	17.8	45.8			
No. 4	26.5	23.5	42.5			
Cowling—	25.3	22.3	46.9			
Av.	24.8	21.1	46.8			

[1] Averages of results from 2 or more determinations.
[2] Sample 1 was alfalfa leaf meal, 2 was parsnip roots, and 3 was spinach leaves.

Agreement of results by the two procedures when used by different analysts was quite satisfactory; moreover, the values for zinc agreed well with those obtained by Cowling and his collaborators. Unfortunately, the extracts used with the mixed-color procedure became exhausted, and others had to be prepared for use with the one-color procedure. Hence, errors attendant with the ashing and extracting processes are probably reflected in the differences in results obtained for a given sample by the two procedures. This appears to be especially true of sample 1.

DISCUSSION

Cowling and Miller (2) published a spectral transmission curve for a carbon tetrachloride solution of zinc dithizonate. However, they gave none for dithizone alone nor for a mixture of dithizone and zinc dithizonate as used in the mixed color procedure; therefore, the curves shown in Figure 1 appeared to be of sufficient interest to include in this report.

Curve (C) for zinc dithizonate is practically identical with that of Cowling and Miller. It corroborates their choice of a light filter, which transmitted light most strongly near 535 millimicrons, for use in a photoelectric colorimeter. The authors used a wave length setting of 530 millicrons on a Cenco-Sheard Spectrophotelometer and obtained excellent reproducibility of results with carbon tetrachloride solutions of zinc dithizonate of the same concentration.

It will be noted that curve (A) for dithizone reagent exhibits two minima and one maximum. Fortunately the latter coincides closely with the region of strong absorption by zinc dithizonate. This shows that by taking transmission readings in this region for evaluating the concentration of

zinc dithizonate, much of the effect of excess dithizone is eliminated. Curve (B) for a mixture of dithizone and zinc dithizonate further illustrates this point. It shows that the excess dithizone caused only slight digression from curve (C) except in the region of strongest absorption, and should have, therefore, but little effect on readings taken between 530 and 540 millimicrons.

This investigation yielded the following points of information which should be helpful in the determination of zinc as the dithizonate:

1. Unless daylight can be effectively excluded, use of amber or low actinic glassware should aid in obtaining reliable results.

2. The procedure described for rinsing the aqueous solutions with carbon tetrachloride following extraction with dithizone reagent is more convenient and reliable than that now included in the tentative method.

3. Withdrawal of required amounts of carbamate reagent from a properly stored stock solution immediately before use is a more convenient and efficient practice than preparing a fresh solution of this reagent each time the procedure is used.

4. The intra-laboratory study conducted by the authors showed that the results obtained with the one-color procedure by different analysts were as consistent as those obtained for the same samples with the mixed-color procedure. The one-color procedure gave somewhat more consistent values for blank corrections and low concentrations of zinc than it was possible to obtain with the mixed-color procedure. It should be pointed out, however, that the phasic separations common to both procedures appear to be responsible for experimental variations which could not be entirely overcome by exercise of the most rigid care in manipulation.

It is recommended* that the study be continued.

LITERATURE CITED

(1) *Methods of Analysis, A.O.A.C.*, 6th Edition, 1945.
(2) *Ind. Eng. Chem., Anal. Ed.*, **13**: 145 (1941).
(3) *This Journal*, **24**: 520 (1941).

No report was given on sampling, iodine, carbohydrates, copper and cobalt, or carotene, in plants.

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* For report of Subcommittee A and action by the Association, see *This Journal*, **31**, 43 (1948).

MONDAY—AFTERNOON SESSION

REPORT ON PROCESSED VEGETABLE PRODUCTS

By V. B. BONNEY (Food and Drug Administration, Federal Security Agency, Washington D. C.), *Referee*

RECOMMENDATIONS*

It is recommended—

(1) That studies of methods for quality be continued.

(2) That studies be continued on determination of moisture in dried vegetables.

(3) That studies for methods for determination of enzymes in frozen vegetables be continued.

No reports were given on quality factors, moisture in dried vegetables, or catalase in frozen vegetables.

No reports were given on fill of container methods, or on coffee and tea.

REPORT ON COLORING MATTERS IN FOODS

By C. F. JABLONSKI (Food and Drug Administration, Federal Security Agency, New York 14, N. Y.), *Referee*

The Committee of the A.O.A.C. requested the Referee to continue the collaborative study for the detection of small amounts of tartrazine FD&C Yellow #5 in alimentary paste products. The method proposed (published in the "Method of Anlaysis" A.O.A.C., 6th Ed. Chapter 20, **125,** page 268) has already been studied collaboratively; however, a slight modification was deemed essential in order to insure better results. With this consideration in view, the Referee sent out a number of alimentary paste samples of five (5) subdivisions each to various collaborators, with instructions to follow the method as published, but use only 0.1 ml. of saturated aqueous hydrazine sulfate solution, instead of the stated 1.0 ml (third line from bottom of paragraph). The composition of the samples was as follows:

No. 1, *Egg Noodles* containing 3 p.p.m. of FD&C Yellow No. 5
No. 2, *Egg Noodles*, free from any added color
No. 3, *Macaroni*, containing 3 p.p.m. of FD&C Yellow No. 5
No. 4, *Macaroni*, free from any added color
No. 5, *Macaroni* containing 2 p.p.m. of FD&C Yellow No. 5

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* For the report of Subcommittee C and action by the Association, see *This Journal*, 31, 49 (1948).

The report of the collaborators is as follows:

N. E. Freeman Atlanta Station	Sample No. 1—color—present 2—color—absent 3—color—present (strong) 4—color—doubtful 5—color—present
W. S. Cox Atlanta Station	Sample No. 1—color—present 2—color—absent 3—color—present (strong) 4—color—absent 5—color—present
M. C. Harrigan (Miss) Boston Station	Sample No. 1—color—positive 2—color—negative 3—color—positive less than in 1 4—color—negative 5—color—positive less than in 3
M. Matluck Boston Station	Sample No. 1—color—positive 2—color—negative 3—color—positive 4—color—negative 5—color—positive
C. S. Purcell Boston Station	Sample No. 1—color—positive 2—color—negative 3—color—positive 4—color—negative 5—color—positive
A. L. Suslam Boston Station	Sample No. 1—color—positive 2—color—negative 3—color—positive 4—color—negative 5—color—doubtfully positive
H. I. Macomber Baltimore Station	Sample No. 1—color—positive 2—color—negative 3—color—faintly positive 4—color—negative 5—color—positive
S. M. Walden Baltimore Station	Sample No. 1—color—positive 2—color—negative 3—color—positive (faint) 4—color—negative 5—color—positive
J. L. Hogan New York Station	Sample No. 1—color—present 2—color—absent 3—color—present 4—color—absent 5—color—present

M. L. Offutt (Miss) New York Station	Sample No. 1—color—positive 2—color—negative 3—color—positive 4—color—negative 5—color—positive
F. C. Minsker Philadelphia Station	Sample No. 1—color—positive 2—color—negative 3—color—positive 4—color—negative 5—color—positive
H. M. Boggs Philadelphia Station	Sample No. 1—color—positive 2—color—negative 3—color—positive 4—color—negative 5—color—positive
R. T. Marwin Conn. Agricultural Experiment	Sample No. 1—color—positive 2—color—negative 3—color—positive 4—color—negative 5—color—positive
J. J. Winston Jacobs Cereal Products Labor, New York	Sample No. 1—color—positive 2—color—negative 3—color—positive 4—color—negative 5—color—positive
J. M. Bisogno (Miss) Jacobs Cereal Products Labor, New York	Sample No. 1—color—positive 2—color—negative 3—color—positive 4—color—negative 5—color—positive

COMMENTS BY THE COLLABORATORS

N. E. Freeman: Filtration was slow but clear and no precipitate was formed in the coupling test.

W. S. Cox: No difficulties were encountered in the coupling test.

M. C. Harrigan: Suggest a pancreatic digestion of the alimentary paste for larger recovery of color.

M. Matluck: Samples 1, 3, and 5 gave a definite yellow color on wool, whereas samples 2 and 4 gave only a straw color. However, the usual spotting reagents gave no clear differentiation between positive and negative samples.

C. S. Purcell: No comments.

A. L. Suslam: No comments.

H. I. Macomber: No comments.

S. M. Walden: In general the dyeing tests were all less conclusive than the coupling. None of the solutions were clear after the additions of the precipitants with subsequent centrifuging.

J. L. Hogan: I experienced no particular difficulties with those determinations. In samples 1, 3, and 5 the pieces of wool took on a decided yellow color, while in 2 and 4 the cloth remained almost colorless. In the former cases, the spot tests resembled those of standard tartrazine of similar intensity. While the color produced

by the coupling test is not very deep yet there seems to be sufficient color developed to differentiate these from the uncolored samples.

M. L. Offutt: Coupling test very definite in showing the presence of color. Method worked well throughout.

F. C. Minsker: The method as described was used and very good results obtained.

H. M. Boggs: Some trouble with the coupling was experienced on the first attempt, but definite though fairly light pinks were produced in the second attempt.

R. T. Marwin: No comments.

J. J. Winston: Samples 1 and 2 were examined twice by the recommended procedure with a slight modification the second time. After the protein matter was precipitated by the specified reagents, the flask was placed overnight in the refrigerator. The clear solution was then further examined according to the prescribed procedure and the results indicated that more of the protein matter was removed due to freezing This resulted in a better dyeing and coupling test for sample #1.

J. M. Bisogno: There was little difficulty in detecting the added color. The coupling reactions particularly were very good.

DISCUSSION

The results reported by the collaborators indicate that by the application of the method as published in the *Methods of Analysis*, 6th Ed., with the incorporation of a slight change, the presence of very small amounts of tartrazine can definitely be established. It is gratifying to state that every one of the coworkers has been able to detect the added coloring matter, although the majority were probably unfamiliar with the method. Some collaborators report more or less cloudiness of the solution preparatory to dyeing. It is the Referee's observation that this condition which has been generally prevalent during the warm season will in no way hinder the dyeing and stripping operation. However, if time permits, it is advantageous to place the solution in the refrigerator overnight for complete clarification. This suggestion has been offered by collaborator W.

To sum up results: Tartrazine FD&C Yellow No. 5 can be detected positively by the use of the described method in 200 gm. of material containing as low as 2 p.p.m. of dye.

The Referee desires further to report considerable progress in the problem of a quantitative separation and estimation of Tartrazine FD&C Yellow No. 5 and Sunset Yellow FCF, FD&C Yellow No. 6.

A method for distinguishing between Oil Orange SS, FD&C Orange No. 2, and Oil Red XO, FD&C Red No. 32 was studied.

Oil Orange SS and Oil Red XO are both oil soluble azo dyes, differing in their respective chemical structure by only a methyl group. Both dyes respond similarly towards acids, alkalies, and reducing agents. The dyed fabric is also of similar shade. It was, therefore, impossible up to now to distinguish between these colors in the usual manner. However, experiments disclosed that under certain definite conditions Orange SS is precipitated as a brick red flaky precipitate, while Oil Red XO under similar condition remains in solution.

The mode of procedure is as follows: Solutions required—formaldehyde 39%; pyridine, U.S.P.

Prepare a dye solution of approximately 0.001% in ethanol.

Color solution 0.001%	2	ml.
Water	16	ml.
Pyridine	0.15	ml.
Formaldehyde	0.20	ml.

Place in the order named above into a 50 ml Erlenmeyer flask. In presence of Orange SS the solution becomes slightly opalescent in approximately 5 minutes and later (in about 30 minutes) a brick red precipitate is apparent. Oil Red XO treated similarly remains in orange solution.

In presence of both dyes (in above concentration) the precipitation of Orange SS will not be apparent before 12 or more hours, depending upon the percentage of Oil Orange SS present in the mixture. However, if the dye concentration should be 0.005% or over the precipitation of Orange SS takes place immediately, but Oil Red XO will also partially precipitate after approximately 30 minutes. It is, therefore, desirable to keep the color concentration between 0.001–0.002%.

RECOMMENDATIONS*

The Referee therefore recommends—

(1) That the rapid method of detection of small amounts of Tartrazine FD&C yellow No. 5 (as published in the *Book of Methods*, VI) with the proposed slight modification be made official (final action).

(2) That investigational work be continued on the quantitative separation of FD&C Yellow No. 5 (Tartrazine) and FD&C Yellow No. 6 (Sunset Yellow FCF).

(3) That investigational work be undertaken to separate and determine quantitatively FD&C Green No. 2 (Light Green SF Yellowish), FD&C Green No. 3 (Fast Green FCF), and FD&C Blue No. 1 (Brilliant Blue FCF).

(4) That investigational work be undertaken to separate and estimate quantitatively FD&C Yellow No. 3 (Yellow AB), FD&C Yellow No. 4 (Yellow OB), FD&C Orange 2 (Orange SS), and FD&C Red No. 32 (Oil Red XO).

(5) That collaborative work on analytical methods for coal-tar colors certifiable for use in foods be conducted.

REPORT ON DAIRY PRODUCTS

By GUY G. FRARY (State Chemical Laboratory, Vermillion, S. Dak.), *Referee*

The following recommendations† are made as Referee on Dairy Products.

(1) That the Sander-Sager phosphatase test, as reported by the Associate Referee, be made official, first action, for detection of incomplete pasteurization of fluid milk and cream, cheddar type cheeses, and soft unripened cheeses.

* For report of Subcommittee C and action by the Association, see *This Journal*, 31, 49 (1948).
† For report of Committee C and action by the Association, see *This Journal*, 31, 50 (1948).

(2) That the Sanders-Sager phosphatase test, as reported by the Associate Referee, be adopted as tentative for detection of incomplete pasteurization of types of cheeses other than those named in (1), ice cream mix, sherbet mix, milk drinks, butter, buttermilks, goats' milk, cheese whey, and concentrated milk products; and that the method be studied collaboratively during the coming year.

(3) That the methods for phosphatase tests set out in **22.43–22.57,** inclusive, be dropped.

(4) That the rapid method for moisture in cheese, employing the higher temperature of 130°, be adopted as tentative and that study of methods for sampling, fat, and moisture in cheese be continued.

(5) That the method of preparation of butter samples by means of mechanical stirring be adopted as official, first action.

(6) That the present official method for preparation of butter samples be reworded as recommended, with some condensation.

(7) That the present tentative method for mechanical preparation of butter samples be dropped.

(8) That application of shaking machines in the official method be studied collaboratively under one familiar with such application.

(9) That studies be continued on methods for detection of reconstituted milk.

(10) That studies be continued on ash in milk and in evaporated milk.

(11) That studies be continued on chlorine in milk.

(12) That studies with collaborative work be made of the method for determination of the acidity of milk.

(13) That an associate referee on fat in homogenized milk be appointed and studies be undertaken to develop an accurate method.

(14) That the method for fat in ice cream (**22.149**) be editorially changed by adding, after "60°" at the end of the second sentence, the following: "for 20 min. with occasional shaking"; and by changing the third sentence in the method to read as follows: "cool and proceed as directed under **22.25**, beginning with 'Add 10 ml alcohol and mix well.'"

(15) That statements be incorporated in the methods for added water in milk which employ the Zeiss immersion refractometer, which will give the comparable readings obtained under the same conditions by the Bausch and Lomb instrument: **(22.28 (a), 22.29,** and **22.30).**

(16) That study of the serum methods for added water in milk **(22.28, 22.29,** and **22.30)** be continued.

(17) That the sediment test **22.40, 22.41,** and **22.42,** be dropped, and that the A.P.H.A. method be adopted as tentative.[1]

(18) That the cryoscopic method for added water in milk, **22.33,** (p. 316) be editorially corrected by inserting the word "depression" after "point" line 6, p. 316; and by deleting "depression" in line 9 and in 3rd line from end of method, on the same page.

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[1] The details of the method are given in *This Journal*, 31, 93 (1948).

REPORT ON SAMPLING, FAT, AND MOISTURE IN CHEESE

By WILLIAM HORWITZ (*Associate Referee*) and LILA KNUDSEN (U. S. Food and Drug Administration, Minneapolis, 1, Minnesota, and Washington 25, D. C.)

The previous report of the Associate Referee[1] presented an outline of an experiment designed to determine the moisture distribution in small cheeses of the Cheddar variety. Eight cheeses in this series have been analyzed for moisture; more work on cheeses of other sizes is contemplated.

The modified fat method presented in the previous report, has been subjected to collaborative study. Three types of cheeses were used in this work: a process American, a rindless Cheddar, and a daisy Cheddar. The cheese was frozen[2] at 0°F and a portion from each cheese was shredded while still frozen.[3] About one quart of the shredded material was placed in a two quart jar and the contents were thoroughly mixed by rolling and shaking. The shredded samples were then allowed to stand at 0°F for about a week for "tempering" and they were then remixed. Small portions from the two quart jars were transferred to ten four ounce screw cap "cheese jars" in rotation until all of the original material was distributed. In this way some of the top, middle, and bottom portions of the cheese in the original container was present in each of the small sample jars. The small samples were mixed, capped, and packed with dry ice for shipment. Each collaborator received two jars of cheese from each of the three cheeses. The first five jars from the process American cheese were given the sample number 1; the last five jars, the sample number 2. The first five jars from the rindless Cheddar were given the sample number 3; the last five jars, the sample number 4. The first five jars from the daisy Cheddar were given the sample number 5; the last five jars, the sample number 6. The first collaborator received the first and sixth jar from each cheese; the second, the second and seventh; etc. Thus each of the five collaborators received six samples, each consecutive set of two numbers being duplicates. Duplicate samples were sent to the collaborators rather than requesting them to run duplicates from a single sample in order to obtain information regarding the homogeneity of the original shredded material.

The collaborators were instructed in detail regarding further remixing of the samples before analysis and were given a definite order for removing portions for analysis so that manipulative details up to the point of weighing the analytical portions would be fairly consistent among the analysts. Four determinations were performed on each sample: Moisture by two methods—I, the official method, and II, 1.25 hours in the forced draft oven at 130°C; and fat by two methods—III, direct weighing of sample

[1] *This Journal*, 30, 421 (1947).
[2] This temperature was lower than necessary but a 20–30°F. cold room was not available.
[3] The Griscer Grater (Griscer Industries, Fort Wayne, Ind.) was used for shredding.

TABLE 1.—*Individual results on collaborative cheese samples*

(Results in parentheses are reruns and are not included in the calculations)

COLLABORATOR	SAMPLE	MOISTURE		FAT		FAT D.B.†	
		METHOD I	II	III	IV	III	IV
A	1	39.19	39.08	31.24	31.02	51.37	51.01
	2	38.91	39.13	31.15	30.90	50.99	50.58
B	1	39.04	39.28	30.83	30.89	50.57	50.67
	2	39.08	39.11	30.99	30.87	50.87	50.67
C	1	39.02	39.08	31.08	31.22	50.97	51.20
	2	38.96	38.77	30.86	31.01	50.56	50.80
	1	(38.79)	(38.71)				
	2	(39.01)	(39.03)				
D*	1	39.02	38.78	30.53	30.10	50.07	49.36
	2	39.29	38.98	29.86	29.92	49.18	49.28
E	1	39.02	39.08	31.11	31.37	51.02	51.44
	2	38.86	39.28	31.24	31.25	51.10	51.11
A	3	36.03	36.14	35.27	35.20	55.14	55.03
	4	35.99	36.48	35.26	35.24	55.08	55.05
	3		(36.59)				
	4		(36.31)				
B	3	36.04	36.29	35.08	34.96	54.85	54.66
	4	36.04	36.29	34.82	34.89	54.44	54.55
C	3	35.74	35.80	35.35	35.25	55.01	54.86
	4	35.58	36.07	35.29	35.12	54.78	54.42
	3	(35.41)	(35.43)				
	4	(35.52)	(35.49)				
D*	3	36.34	36.18	34.32	34.12	53.91	53.60
	4	36.18	36.15	33.92	33.96	53.15	53.21
E	3	36.09	36.37	35.40	35.30	55.39	55.23
	4	36.25	36.33	35.36	35.19	55.47	55.20
A	5	36.40	36.66	32.35	32.18	50.86	50.60
	6	36.43	36.82	32.38	32.14	50.94	50.55
	5	(36.71)					
B	5	36.72	37.04	31.91	31.86	50.43	50.35
	6	36.69	36.80	31.95	31.77	50.47	50.18
C	5	37.02	36.49	32.40	32.13	51.44	51.02
	6	36.00	35.98	32.06	32.23	50.09	50.36
	5	(35.70)	(35.71)				
	6	(36.04)	(36.65)				
D*	5	36.88	36.51	31.28	30.90	49.56	48.95
	6	36.86	36.56	31.69	31.35	50.19	49.65
E	5	36.37	36.75	32.29	32.15	50.75	50.53
	6	36.46	36.77	32.39	32.45	50.98	51.07
	5				(32.33)		(50.81)

* Apparently a constant error is present in the fat of this collaborator's determinations.
† Calculated on results for moisture by Method (I).

into and digestion in the Mojonnier tube; and IV, the official method. The methods used are as follows:

MOISTURE

I. *Official Method:* Sec. **22.124.**

II. *Modified Method:* Weigh 2–3 g of prepared sample into moisture dishes with tight-fitting covers. Partially dry on the steam bath with lids removed and then insert in a forced draft oven which has come to equilibrium at 130 ± 1°C. Dry for 1.25 hours (with covers entirely off), cover tightly, remove from oven, cool, and weigh.

FAT

III. *Modified Method:* Weigh 1 g prepared sample into a tared (with stopper) Mojonnier tube using a rounded blade spatula. Introduce the cheese so that no particles are left on the neck of the tube and so that the cheese is deposited as close to the lower neck as possible. Stopper and reweigh. Shake the particles into the bulb with a sharp tap on the palm of the hand. Add 10 ml NH_4OH (1 + 9) from a pipet, washing down the sides of the tube. Place in a hot water bath (70–80°C) and shake frequently until the casein is well softened. Cool slightly, neutralize with HCl using litmus as indicator, and add 10 ml more of HCl. Mix and heat in a boiling water bath until the casein is digested and the liquid has darkened (15–40 minutes). Shake occasionally to wash down any particles which may have remained in the upper part of the tube. When digestion is complete, cool and extract fat according to **22.130.**

IV. *Official Method:* Sec. **22.130.** Report moisture, fat, and fat on the dry basis (using moisture by the official method).

RESULTS AND DISCUSSION

Table 1 presents the individual results obtained by the collaborators. Results in parentheses are reruns which are not included in the calculations of averages and standard deviations. Table 2 summarizes the data and compares it with the averages and standard deviations found by Ferris[4] in his comparison of the official fat method with a method similar to that above except for the use of a special "Ferris tube" instead of the Mojonnier tube. The data are also compared with the results of Lepper and Hart[5] on jar samples of cheese run for moisture and fat by the official methods.

An analysis of the collaborative results can be best shown graphically. Since primary interest lies in the variation from sample to sample and the variation between laboratories, we must use some method of putting the results from the three types of cheeses on the same basis so that they may be compared. Accordingly, a general average or mean was calculated for all results by the official A.O.A.C. moisture method on samples 1 and 2, and differences were obtained between each result and that general average. The same thing was done for samples 3 and 4, and then for samples 5 and 6. Since these differences are all of the same relative magnitude, they can be plotted together on the same scale and grouped by collaborator as shown on the left hand side of Figure 1 for the official moisture method. The right hand side of Figure 1 shows the same thing for the modified

[4] L. W. Ferris, U. S. Food and Drug Administration, Buffalo, New York, unpublished study.
[5] H. A. Lepper and L. Hart, *This Journal*, **16**, 584 (1933), Table 2.

moisture method; and Figure 2 treats similarly the modified method for fat and the official method for fat.

TABLE 2.—*Summary of averages and standard deviations of collaborative cheese samples in this and previous studies*

This study: 5 collaborators, 10 determinations

	PROCESS NO. 1 & NO. 2	RINDLESS NO. 3 & NO. 4	CHEDDAR NO. 5 & NO. 6	AVERAGE STANDARD DEVIATION FOR METHODS*
Moisture I—Official:				
Average	39.04	36.03	36.58	
Std. Deviation	.13	.23	.31	.23
Moisture II—130°, 1.25 hours:				
Average	39.06	36.21	36.64	
Std. Deviation	.17	.19	.28	.22
Fat III—Modified:				
Average	30.89	35.01	32.07	
Std. Deviation	.42	.51	.37	.44
Fat IV—Official:				
Average	30.86	34.92	31.92	
Std. Deviation	.48	.48	.47	.48

Results reported by Ferris.[4]

	Sample 1		*Sample 2*	
	Official	*Ferris Tube*	*Official*	*Ferris Tube*
Fat, Average	27.69 (7, 16)†	27.73 (7, 14)	28.89 (5, 11)	28.88 (5, 10)
Std. Deviation	0.43	0.40	0.33	0.35

Results reported by Lepper and Hart.[5] (Table 2, Jar Samples)

Moisture, Average	36.22 (11, 27)†	Fat, Average	30.37 (11, 28)
Std. Deviation	0.25	Std. Deviation	0.40

* Obtained by taking the square root of the average of sums of squares of individual standard deviations.

† Figures in parentheses denote, respectively, the number of collaborators and total number of determinations.

From two figures following it can be seen that the variation between the results from samples of the same cheese at one laboratory is negligible, compared to the variation from one laboratory to another. This indicates a homogeneous shredded sample of each cheese, and that any difference between results of different collaborators is not due to a difference between the samples sent to them. This has also been shown by a statistical technique called analysis of variance, from which were obtained the two types

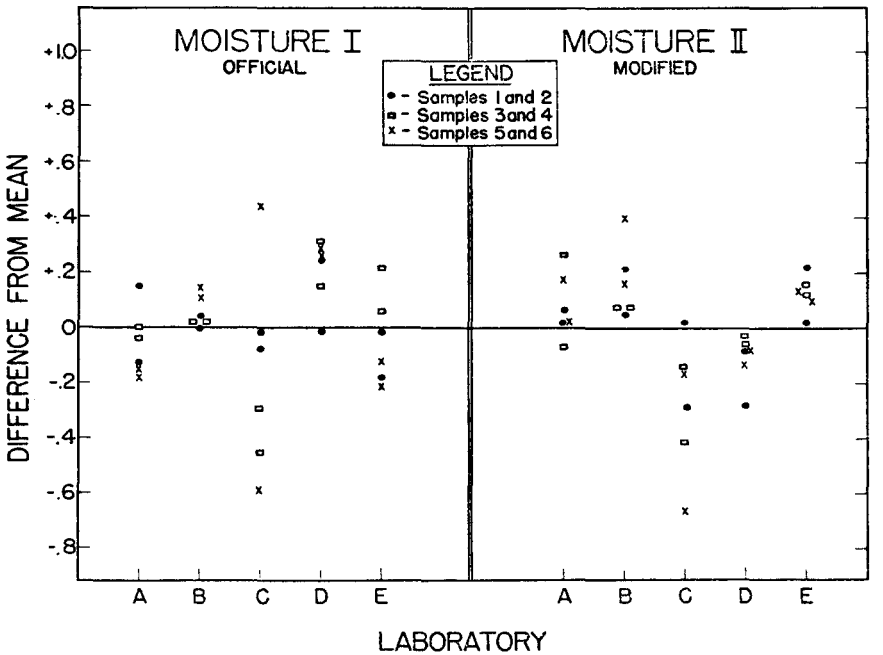

FIG. 1.—Differences of individual moisture determinations from the average determination for each cheese.

For moisture methods I and II, the over-all standard deviations are 0.23 and 0.22, respectively, and the standard deviations within stations (average standard deviation for any one station) are 0.21 and 0.16, respectively.

of standard deviations shown on each figure—the over-all standard deviation on a particular method and the standard deviation within the stations. These two standard deviations indicate respectively how closely the method can be checked at any laboratory, and how closely it can be checked at the same laboratory.

An interesting observation can be made from the charts; each collaborator's results have about the same position on both methods for each constituent, for instance, collaborator C is low on both moisture methods; collaborator B is high on both moisture methods; collaborator B is average on both fat methods; collaborator D is low on both, etc.

The over-all standard deviations of the official moisture and the modified moisture method are not significantly different (.231 and .222, respectively). The same is true of the standard deviations for the modified fat method and the official fat method (.437 and .477, respectively). These are about the same as those found by Ferris[4] and Lepper and Hart.[5] The average moisture by the modified method is slightly higher than that obtained by the official method. The difference, about 0.1 per cent, is so small

that the modified method can be used as a rapid screening moisture test. It is therefore recommended as such. The average fat by the modified method is slightly higher, by about 0.1 per cent, than by the official method. This may be attributed to the elimination of the fat transfer from beaker to flask which is required in the official method, as well as to the impossibility of fat loss from spattering in the modified method. As one collaborator commented, "The uncertainty in the official method due to loss in boiling and possibly in transferring makes method III a desirable improvement."

Practically all of the collaborators performed this work in hot, humid weather. One mentioned that moisture condensed on the spatula during the preliminary mixing, but when these samples were rerun the moisture values were usually lower than those obtained on the original determination. It would appear desirable to repeat this collaborative work under more favorable conditions in the fall or early winter and to extend it to other varieties of cheese.

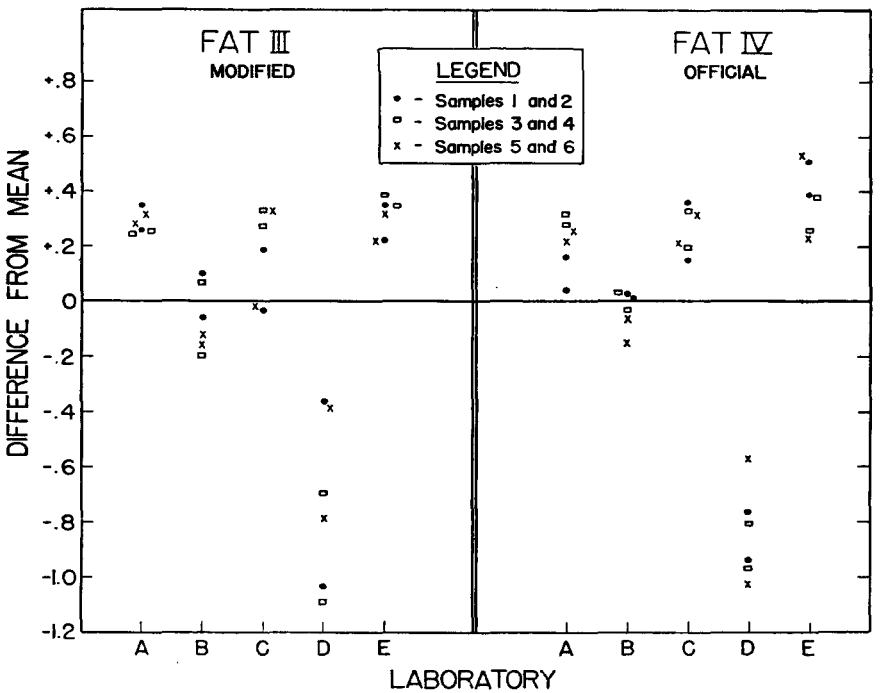

FIG. 2.—Differences of individual fat determinations from the average determination for each cheese.

For fat methods III and IV the over-all standard deviations are 0.44 and 0.48, respectively, and the standard deviations within stations (average standard deviation for any one station) are 0.19 and 0.13, respectively.

Three of the five collaborators reported loss of some fat determinations by the official method because of violent bumping. From the limited experience of the associate referee, it would appear that the required use of sand in the official fat method actually favors bumping rather than diminishes it. The statement in this method ". . . neutralize with HCl, using litmus as indicator" is ambiguous. Should litmus in the form of solid, solution, or paper be used? We may even go further and inquire whether such a strict neutralization is required or whether it can not be assumed that 1 ml of HCl (0.012 moles) is roughly equivalent to 1 ml of NH_4OH (0.015 moles) especially when a ten-fold excess of HCl is finally present.

SOFT CHEESES

It has been brought to the attention of the Associate Referee that the present official method for the preparation of cheese samples (**22.123**) is not applicable to the soft cheeses. Preparation of samples of cottage, creamed cottage, and cream cheeses is important both for composition and for phosphatase determinations. Such a study will be inaugurated next year.

ACKNOWLEDGMENT

Grateful acknowledgment is due to the following collaborators (all of the U. S. Food and Drug Administration): Sidney Williams, Minneapolis; John H. Bornmann, Chicago; F. J. McNall, Cincinnati; and N. Aubrey Carson, St. Louis.

RECOMMENDATIONS*

It is recommended—

(1) That the forced draft oven method for moisture in cheese be adopted as tentative.

(2) That study of methods for sampling, fat, and moisture in cheese be continued.

(3) That studies of methods for preparation of samples of soft cheeses be undertaken.

No report was given on ash in milk and evaporated milk.

REPORT ON THE PHOSPHATASE TEST IN PASTEURIZATION OF DAIRY PRODUCTS

By GEORGE P. SANDERS (Division of Dairy Research Laboratories, Bureau of Dairy Industry, Agricultural Research Administration, Department of Agriculture, Washington 25, D. C.), *Associate Referee*

A description of the Sanders-Sager modification of the phosphatase test applied to Cheddar cheese and fluid milk was published in 1946 (1). The

* For report of Committee C and action of the Association, see *This Journal*, **31**, 50 (1948).

modified test was developed as a result of urgent requests from members of the cheese industry and public health officials to investigate the possibility of developing a reliable method for testing cheese to determine accurately whether or not the milk used in making the cheese had been adequately pasteurized. The details of the method proposed are the result of exhaustive research on available tests for pasteurization and on the chemistry of the milk phosphatase enzyme and the quantitative measurement of its activity.

While the research work was under way and in process of publication, collaborative work was done by Dr. F. W. Gilcreas, Associate Referee on the phosphatase test for hard cheese, and by William Horwitz, Associate Referee on the phosphatase test for soft cheese. In Dr. Gilcreas' work (2), both a modified Kay-Graham and the Sanders-Sager methods were investigated collaboratively, with tests on a large number of samples. The results showed, as Sanders and Sager also had found, that the modified Kay-Graham procedure could not be adapted satisfactorily as an index of pasteurization in testing cheese. Gilcreas found that the Sanders-Sager method provides a reliable estimate of the inactivation of the enzyme by heat, and thus of the degree of heat treatment of the milk from which hard cheese was made. He recommended that it be adopted as a tentative method.

Horwitz (3) likewise found that the Sanders-Sager method could be applied successfully in testing soft cheeses. Further, he referred to the possibility of applying to dairy products the method proposed for blood serum by Bessey *et al.* (4), in which *p*-nitrophenyl phosphate (colorless) is used as the substrate and the enzyme hydrolyzes it to *p*-nitrophenol (yellow); he suggested that this procedure be investigated.

During the progress of the development work by Sanders and Sager, in which the successful application of the method to Cheddar cheese and to fluid milk was demonstrated, it became apparent that the enzyme remains active in all dairy products that have not been pasteurized, and therefore that the quantitative measurement of its activity could be applied to all dairy products, with, however, suitable adjustments of the reagents to compensate for the different buffering capacities of the various products. Accordingly, the Referee on Dairy Products recommended (5) that the Associate Referee study the development of a unified method applicable to various dairy products.

The following is a description of the laboratory method of Sanders and Sager for testing various dairy products to determine the adequacy of pasteurization. It includes those modifications needed to produce uniformly quantitative phosphatase values under fixed, standard conditions in applying the test to various common varieties and kinds of cheese and to fluid milk, cream, ice cream mix, sherbet mix, chocolate drink, butter, sweet buttermilk, cultured buttermilk, fermented milk drinks, goats'

milk, and cheese whey. The modifications for different products are based on the results of extensive research in the laboratories of the Bureau of Dairy Industry, involving large numbers of tests on the various products, subjected to known heat treatments; also on collaboration and communications with the National Cheese Institute and many of its member firms and individuals, with Directors of Research in numerous dairy products firms, with public health laboratories in various States and cities, with research workers in several Agricultural Experiment Stations, and with Dr. F. W. Gilcreas and William Horwitz.

PHOSPHATASE TEST FOR PASTEURIZATION

REAGENTS

1. *Buffers:*

a. *Barium borate-hydroxide buffer.*—Dissolve 25.0 g of C. P. barium hydroxide [$Ba(OH)_2 \cdot 8H_2O$—fresh, not deteriorated] in distilled water and dilute to 500 ml. In another flask or cylinder dissolve 11.0 g of C. P. boric acid (H_3BO_3) and dilute to 500 ml. Warm each to 50°C, mix the two together, stir, cool to approximately 20°C, filter, and stopper the filtrate tightly (*p*H 10.6).

The buffer thus prepared is designated as the 25-11 buffer, the figures indicating the grams per liter of each of the respective reagents. Modifications in the quantities of these two reagents, necessary in preparing the appropriate buffers for testing various products, are indicated in Tables 1 and 2.

b. *Color development buffer.*—Dissolve 6.0 g of sodium metaborate ($NaBO_2$)[1] and 20 g of sodium chloride in water and dilute to 1 liter with water (*p*H 9.8).

c. *Color dilution buffer.*—Dilute 100 ml of color development buffer **1-b** to 1 liter with water.

d. *Standard borax buffer*, 0.01-molar, for checking *p*H meter, *p*H 9.18 at 25°C.[2] Dissolve 0.9603 g of pure borax (Bureau of Standards Sample 187) in distilled water (distilled recently or freshly boiled and cooled) and dilute to 250 ml. Keep stoppered tightly.

2. *Buffer substrates:*

a. *For evaluating pasteurization.*—Dissolve 0.10 g of phenol-free crystalline disodium phenyl phosphate[3] in 100 ml of the appropriate (Tables 1 and 2) barium borate-hydroxide buffer **1 a.**

b. *For quantitative results with raw milk and raw-milk products.*—Dissolve 0.20 g of the phenol-free crystalline disodium phenyl phosphate in 100 ml of the appropriate (Tables 1 and 2) barium borate-hydroxide buffer **1-a.**

3. *Protein precipitants:*

(a) *Zinc-copper precipitant for milk.* Dissolve 3.0 g of zinc sulfate ($ZnSO_4 \cdot 7H_2O$) and 0.6 g of copper sulfate ($CuSO_4 \cdot 5H_2O$) in water and dilute to 100 ml with water. The precipitant thus prepared is designated as the 3.0–0.6 precipitant.

b. *Zinc-copper precipitant for unripened cheese.* Dissolve 6.0 g of zinc sulfate and 0.1 g of copper sulfate in water and dilute to 100 ml with water. This precipitant is designated as the 6.0–0.1 precipitant.

c. *Zinc precipitant for ripened cheese and for butter.* Dissolve 6.0 g of zinc sulfate in water and dilute to 100 ml with water. This precipitant is designated as the 6.0 precipitant.

The quantities of the respective reagents to use in preparing the precipitants for

[1] Obtainable from Amend Drug and Chemical Company, Inc., 117 East 24th Street, New York 10, N.Y.

[2] All *p*H values reported herein were determined at 25° C or corrected to that temperature.

[3] Obtainable, relatively pure, from Applied Research Institute, 2 East 23rd Street, New York 10, N.Y.

TABLE 1.—*Phosphatase test modifications for different kinds of cheese and cheese of different ages*

KIND OF CHEESE	AGE OR EXTENT OF CURING; OTHER DETAILS	BUFFER FOR OPT. *pH* (9.85–10.20)	PRECIPITANT	CRITERION, EXPERIMENTAL, PHENOL EQUIVALENT[a]
				mmg/0.25 g.
Cheddar, granular, stirred curd, hard cheese	<1 wk.	25-11[b]	6.0-0.1[c]	3
	1 wk.–1.5 mo.	25-11	6.0[d]	3
	1.5–4 mo.	26-11	6.0	3
	>4 mo.	27-11	6.0	3
Washed curd, soaked curd, Colby	<1 wk.	25-11	6.0-0.1	3
	1 wk.–2 mo.	25-11	6.0	3
	>2mo.	26-11	6.0	3
Swiss, Gruyère	<1 wk.	25-11	6.0-0.1	3
	1 wk.–1 mo.	25-11	6.0	3
	1–3 mo.	26-11	6.0	3
	>3 mo.	27-11	6.0	3
Brick, Muenster	<1 wk.	25-11	6.0-0.1	3
	1 wk.–1 mo.	25-11	6.0	3
	1–2 mo.	25-11	6.0	3
	>2 mo.	26-11	6.0	3
Edam, Gouda	<1 wk.	25-11	6.0-0.1	3
	1 wk.–2 mo.	25-11	6.0	3
	2–4 mo.	26-11	6.0	3
	>4 mo.	27-11	6.0	3
Blue mold, blue	<1 wk.	25-11	6.0-0.1	3
	1 wk.–1 mo.	26-11	6.0	3
	1–4.5 mo.	27-11	6.0	3
	>4.5 mo.	28-11	6.0	3
Camembert, Limburger	<1 wk.	25-11	6.0-0.1	4
	1 wk.–1 mo.	25-11	6.0	4
	1–2 mo.	26-11	6.0	4
	>2 mo.	27-11	6.0	4
Monterey	<1 wk.	25-11	6.0-0.1	3
	1 wk.–2 mo.	25-11	6.0	3
	>2 mo.	26-11	6.0	3
High moisture Jack	<1 wk.	25-11	6.0-0.1	3
	1 wk.–2.5 mo.	25-11	6.0	3
	>2.5 mo.	26-11	6.0	3

[a] Values higher than those shown indicate under-pasteurization.
[b] Grams $Ba(OH)_2 \cdot 8H_2O$ and H_3BO_3, respectively, per liter.
[c] Grams $ZnSO_4 \cdot 7H_2O$ and $CuSO_4 \cdot 5H_2O$, respectively, per 100 ml.
[d] Grams $ZnSO_4 \cdot 7H_2O$ per 100 ml.
[e] See also more sensitive modification in text (p. 316), alternative.
[f] Eight parts of 25-11 buffer plus 2 parts of water.

TABLE 1.—(*continued*)

KIND OF CHEESE	AGE OR EXTENT OF CURING; OTHER DETAILS	BUFFER FOR OPT. *pH* (9.85–10.20)	PRECIPITANT	CRITERION, EXPERIMENTAL, PHENOL EQUIVALENT[a]
				mmg/0.25 g.
Provolone, pasta filata	<1 wk.	25-11	6.0-0.1	3
	1 wk.–1 mo.	25-11	6.0	3
	1–3 mo.	26-11	6.0	3
	>3 mo.	27-11	6.0	3
Parmesan, reggiano, monte, modena, Romano, asiago old	<1 wk.	25-11	6.0-0.1	3
	1 wk.–2 mo.	26-11	6.0	3
	2–6 mo.	27-11	6.0	3
	6 mo.–1 yr.	28-11	6.0	3
	>1 yr.	29-11	6.0	3
Asiago fresh	Same as Cheddar			
Asiago medium	<1 wk.	25-11	6.0-0.1	3
	1 wk.–1 mo.	25-11	6.0	3
	1–3 mo.	26-11	6.0	3
	>3 mo.	27-11	6.0	3
Gorgonzola	Same as blue			
Cottage,[e] cook cheese, koch kaese	Dry	25-11	6.0-0.1	1
	Moist	25-11 (8+2)[f]	4.5-0.1	1
Cream cheese		25-11 (7+3)	4.5-0.1	3
Semi-soft cheese	<1 wk.	25-11	6.0-0.1	3
	1 wk.–1 mo.	25-11	6.0	3
	>1 mo.	26-11	6.0	3
Soft ripened cheese	<1 wk.	25-11	6.0-0.1	4
	1 wk.–1 mo.	25-11	6.0	4
	>1 mo.	26-11	6.0	4
Nokkelost, kuminost, sage cheese	<1 wk.	25-11	6.0-0.1	3
	1 wk.–1.5 mo.	25-11	6.0	3
	1.5–4 mo.	26-11	6.0	3
	>4 mo.	27-11	6.0	3
Pasteurized process, past. proc. pimiento, past. proc. with fruits, meats, etc.	Soft, mild	25-11	6.0	3
	Medium firm	26-11	6.0	3
	Firm, sharp (incl. Swiss, Gruyère)	27-11	6.0	3
Past. proc. cheese foods; past. proc.	Same as pasteurized			

TABLE 1.—(*continued*)

KIND OF CHEESE	AGE OR EXTENT OF CURING; OTHER DETAILS	BUFFER FOR OPT. *p*H (9.85–10.20)	PRECIPITANT	CRITERION, EXPERIMENTAL, PHENOL EQUIVALENT[a]
				mmg/0.25 g.
cheese foods with fruits, meats, etc.	process			
Past. proc. cheese spreads; past. proc. cheese spreads with fruits, meats, etc.	Soft, high moisture, incl. cream spreads	25-11	6.0	3
	Less soft, incl. blue	26-11	6.0	3
Cold pack, club; cold pack cheese foods; cold pack cheese foods with fruits, meats, etc.	Mild to medium flavored, soft	26-11	6.0	3
	Sharp, firm	27-11	6.0	3

testing other products, not mentioned under "Protein precipitants" above, are indicated in Tables 1 and 2.

4. *BQC* or *2,6-dibromoquinonechloroimine solution (Gibbs' reagent)*.—Dissolve 40 mg of *BQC*[4] powder in 10 ml of absolute ethyl or methyl alcohol and transfer to a dark-colored dropper bottle. This reagent remains stable for at least a month if kept in the ice tray of a refrigerator. Do not use it after it begins to turn brown.

5. *Other reagents:*

a. *Copper sulfate, 0.05 per cent, for standards.*—Dissolve 0.05 g of copper sulfate in water and dilute to 100 ml.

b. *Butyl alcohol.*—Specify n-butyl alcohol, boiling point 116–118°C. To adjust the *p*H, mix 50 ml of the color development buffer **1-b** with a liter of the alcohol.

6. *Phenol standards:*

a. *Stock soln.*—Weigh accurately 1.0 g of pure phenol, transfer to a liter volumetric flask, dilute to a liter with water, and mix. One ml contains 1 mg of phenol. Use this stock soln to prepare standard solns. It is stable for several months in the refrigerator.

b. *Preparation of standards.*—Dilute 10.0 ml of the stock soln **6-a** to a liter with water, and mix. One ml contains 10 micrograms (0.00001 g; 10 mmg, or 10 units) of phenol. Use this standard soln to prepare more dilute standard solns; *e.g.*, dilute 5, 10, 30, and 50 ml to 100 ml with water to prepare standard solns containing 0.5, 1.0, 3.0, and 5.0 mmg or units of phenol per ml, respectively. Keep standard solns in the refrigerator.

In a similar manner, prepare from the stock soln as many more concentrated standard solns as may be needed, containing, for example, 20, 30, and 40 units per ml.

Measure appropriate quantities of the phenol standard solns into a series of tubes (preferably graduated at 5.0 and 10.0 ml) to provide a suitable range of standards as needed, containing 0 (control or blank), 0.5, 1.0, 3.0, 5.0, 10.0, etc., to 30 or 40 units.

[4] Obtainable from Applied Research Institute.

To increase the brightness of the blue color and improve the stability of the standards, add 1.0 ml of 0.05% copper sulfate soln **5-a** to each.

Add 5.0 ml of color dilution buffer **1-c** and add water to bring the volume to 10.0 ml. Add 4 drops (0.08 ml) of BQC, mix, and allow to develop for 30 min. at room temperature. If the butyl alcohol extraction method is to be used in the test, extract the standards as described under "Conducting the test."

Read the color intensities with a photometer, subtract the value of the blank from the value of each phenol standard, and prepare a standard curve (straight line). When the standards are to be used for visual comparisons they should be stored in a refrigerator.

TABLE 2.—*Phosphatase test modifications for various dairy products other than cheese*

PRODUCT	QUANTITY OF SAMPLE	BUFFER FOR OPTIMAL *p*H (9.85–10.20)	PRECIPITANT	CRITERION, EXPERIMENTAL, PHENOL EQUIVALENT[a]
				mmg
Milk:				
Fresh	1 ml.	25-11[b] (5+5)[c]	3.0-0.6[d]	2/0.5 ml.
Old or slightly sour	1 ml.	25-11	6.0[e]	2/0.5 ml.
Cream:				
Fresh	1 ml. or 1 g.	25-11 (5+5)	3.0-0.6	2/0.5 ml. or 0.5 g.
Old or slightly sour	1 ml. or 1 g.	25-11 (8+2)	4.5	2/0.5 ml. or 0.5 g.
Ice cream mix	1 ml.	25-11 (8+2)	4.5-0.1	2/0.5 ml.
Sherbet mix	1 ml.	25-11 (5+5)	3.0-0.6	2/0.5 ml.
Chocolate drink	1 ml.	25-11 (8+2)	4.5-0.1	2/0.5 ml.
Butter	1 g.	18-8	6.0	2/0.5 g.
Sweet buttermilk	1 ml.	25-11 (5+5)	3.0-0.6	2/0.5 ml.
Cultured buttermilk and fermented drinks:				
Medium acid	1 ml.	25-11	6.0	2/0.5 ml.
Very acid, *p*H <4.5	1 ml.	26-11	6.0	2/0.5 ml.
Goats' milk[f]	3 ml.	27-11	7.5-0.1	1/1.5 ml.
Cheese whey	1 ml.	25-11 (5+5)	3.0-0.6	2/0.5 ml.

[a] Values higher than those shown indicate under-pasteurization.
[b] Grams $Ba(OH)_2 \cdot 8H_2O$ and H_3BO_3, respectively, per liter.
[c] Five parts of 25-11 buffer plus 5 parts of water.
[d] Grams $ZnSO_4 \cdot 7H_2O$ and $CuSO_4 \cdot 5H_2O$, respectively, per 100 ml.
[e] Grams $ZnSO_4 \cdot 7H_2O$ per 100 ml.
[f] Four-hour incubation period; use 7.0 ml of filtrate and add 3.0 ml. of color development buffer 1-b.

PHOTOMETRIC DETERMINATION

To read the color in aqueous soln, use a filter with maximum light transmission in the region of 610 mμ wave length.

To read the color in butyl alcohol, extract the color as described above and centrifuge the sample for 5 min. to break the emulsion and to remove the moisture suspended in the alcohol layer. A Babcock centrifuge can be adapted for this purpose by making special tube holders as follows: Slice a section ¼ inch thick from a rubber stopper of suitable diameter to fit into the bottom of the centrifuge cup. Glue together two cork stoppers of appropriate diameter, bore through the center a hole of

proper size to hold the tube snugly, and insert the double cork section into the cup. After centrifuging, remove nearly all of the butyl alcohol by means of a pipet with a rubber bulb on the top end. Filter the alcohol into the photometer cell and read with a filter with maximum light transmission in the region of 650 mμ wave length.

If more than approximately 4 ml of butyl alcohol is required for the photometer used, conduct the test in a larger tube and extract the color, in both the test and the standards, with the necessary quantity of butyl alcohol rather than with 5 ml specified above.

SAMPLING

1. Milk and other fluid products.—Mix the product well, pour several ml into a small tube, stopper the tube, and keep it in a refrigerator.

2. Hard cheese. Take a sample from the interior with a *clean* Roquefort trier, place in a small tube, stopper the tube, and keep it in a refrigerator.

3. Soft and semi-soft ripened cheese.—Harden the cheese by chilling it in the freezing chamber of a refrigerator. Taking special precautions to avoid contaminating the sample with phosphatase that may be present on the surface, use either of the following methods for sampling:

(a) Cut a portion from the end of the loaf or from the side of the cheese, extending in at least 2 inches if possible or to a point somewhat beyond the center in the case of a small cheese. Cut a slit ¼ to ½ inch deep at least halfway around the portion and midway between the top and bottom. Break the portion into two parts, pulling it apart so that it breaks on a line with the slit, being careful not to contaminate the freshly exposed, broken surface. Remove the sample from the freshly exposed surface at or near the center of the cheese.

(b) Remove the surface of the area to be sampled—*e.g.*, the end and the adjacent sides—with a clean knife or spatula, to a depth of ¼ inch. Clean the instrument and hands with hot water and phenol-free soap and wipe them dry. Remove the freshly exposed surface to a similar or greater depth, and repeat the cleaning. Then take the sample from the center of the freshly exposed area, preferably at or near the center of the cheese in the case of a small cheese.

4. Process cheese, spreads, butter, and other non-fluid products.—Take the sample from beneath the surface with a clean knife or spatula.

5. Ice cream and sherbet.—Melt the portion removed and allow it to remain melted for an hour or longer before testing, testing it as a fluid product.

Avoid the use of samples contaminated with mold.

6. Preservation.—If a preservative is necessary, for liquid products add 1 to 3 per cent of chloroform; for solid products put 1 to 3 ml of chloroform in the container, cover with a plug of cotton, insert sample and stopper container tightly. *Label preserved samples: "Poison, preservative added."*

CONDUCTING THE TEST

The chemical principles involved in the detection and measurement of milk phosphatase activity are the same for all dairy products. Some modifications, described below, have been found necessary for different dairy products, because of their differences in physical properties, compositions, and especially buffering capacities.

Cheese:

Step 1. Weigh, on a *clean* balance pan or watch glass, a 0.50-g sample (preferably in duplicate) and place in a culture tube 16 or 18×150 mm. Similarly, weigh another sample and place in a tube as a control or blank. If the cheese is sticky, weigh the sample on a piece of wax paper about 1×1 inch and insert the paper with the sample into the tube. Macerate the blank and the test with a glass rod about 8×180 mm.

Step 2. Add *to the blank* 1.0 ml of the appropriate (Table 1) barium buffer **1-a** (without substrate added), macerate with the rod, leave the rod in the tube, heat for

about a minute to at least 85°C (185°F) in a beaker of boiling water with the beaker covered so that the entire tube is heated to approximately 85°C, cool to room temperature, and macerate again with the rod.

Step 3. Add *to the test* 1.0 ml of barium buffer substrate **2-a** or **2-b** (Table 1) and macerate.

From this point, treat the blank and the test in a similar manner.

Add 9.0 ml of the appropriate barium buffer substrate **2-a** or **2-b** (total, 10.0 ml added), and mix. The rod may be left in the tube during incubation; if it is removed at this point, cut a piece of filter paper ca 1 ×1 inch, wrap and hold it tightly around the rod, rotate the rod while withdrawing it from within the tube so as to wipe the rod clean, insert the paper with the adhering fat into the tube, and stopper the tube.

Step 4. Incubate in a water bath at 37–38°C for 1 hour, mixing or shaking the contents occasionally.

Step 5. Place in a beaker of boiling water for nearly a minute, heating to approximately 85°C (use a thermometer in another tube containing the same volume of liquid), and cool to room temperature.

Step 6. Pipet in 1.0 ml of the zinc precipitant **3-c** for ripened cheese, or the zinc-copper precipitant **3-b** for unripened cheese, and mix thoroughly (*p*H of mixture, 9.0–9.1).

Step 7. Filter (5-cm funnel, 9-cm Whatman No. 42 or No. 2 paper recommended) and collect 5.0 ml of filtrate in a tube, preferably graduated at 5.0 and 10.0 ml.

Step 8. Add 5.0 ml of color development buffer **1-b** (*p*H of mixture, 9.3–9.4).

Step 9. Add 4 drops[5] of BQC, mix, and allow the color to develop for 30 min. at room temperature.

Step 10. Determine the intensity of blue color by either of two methods:

a. *With a photometer:* Read the color intensity of the blank and that of the test, subtract the reading of the blank from that of the test, and convert the result into phenol equivalents by reference to the standard curve described under "Phenol standards." The butyl alcohol extraction method ordinarily is unnecessary when using a photometer.

b. *With visual standards:* For quantitative results in borderline instances, *e.g.*, tests yielding 0.5 to 5 units of color, extract with butyl alcohol **5-b.** Add 5.0 ml of the alcohol and invert the tube slowly several times, centrifuge if necessary to increase the clearness of the alcohol layer, and compare the blue color with the colors of standards in the alcohol.

With samples yielding more than 5 units, compare the colors in aqueous tests with those of aqueous standards.

Step 11. *Dilution method for quantitative results:* In tests that are observed to be strongly positive during color development—*e.g.*, 20 units or more—in which 4 drops of BQC may be much less than sufficient to combine with all of the phenol, pipet an appropriate proportion of the contents into another tube, make up to 10.0 ml with color dilution buffer **1-c**, and add 2 drops more of BQC in the case of an unripened product or 4 drops in the case of a ripened product. With each test, dilute and treat the blank in the corresponding manner. Dilute each strongly positive test thus until the final color is within the range of the visual standards or photometer. Allow 30 min. for color development after the last addition of BQC, and make the reading at the end of the 30-min. period. To correct, multiply by 2 for a 5 +5 dilution, by 10 for a 1 +9 dilution, and by 50 for a 1 +9 followed by a 2 +8 dilution.

Alternatively, to reduce the amount of yellow off color, add 2 instead of 4 drops of BQC after each dilution, and allow the color to develop. Then test the completeness of color development by adding a third drop. Repeat the dilution procedure until the addition of an extra drop does not cause any further increase in the amount of blue color.

[5] For merely detecting under-pasteurization in testing unripened cheese, 2 drops are sufficient, provided the visual standards likewise are prepared with 2 drops.

Step 12. *Calculation and evaluation of result:* When using 0.5 g of solid sample and adding a total of 11.0 ml of liquid, multiply the value of the reading by 1.1 to convert it to units of color or phenol equivalents per 0.25 g of cheese. The result, if desired, may be converted to phenol equivalents per 1 g by multiplying by 4.4. Evaluate the result by comparing it with the criteria of pasteurization in Table 1.

Milk and other fluid products:

Step 1. Pipet a 1.0-ml sample (preferably in duplicate) into a tube and pipet 1.0 ml into another tube as a control or blank. In testing cream, the sample may be weighed (1.0 g) if desired; in testing goats' milk, pipet in a 3.0-ml sample (Table 2).

Step 2. Heat the *blank* to the temperature indicated under "Cheese: Step 2," and cool to room temperature. From this point, treat the blank and the test in a similar manner.

Step 3. Add 10.0 ml of barium buffer substrate **2-a** or **2-b** (Table 2), stopper the tube, and mix.

Steps 4 to 11, inclusive. Follow the directions given for the corresponding steps under "Cheese" above, substituting the appropriate precipitant (Table 2) in Step 6, and, for merely detecting under-pasteurization, using 2 rather than 4 drops of BQC in Step 9.

Step 12. *Calculation and evaluation of result:* When using 1.0 ml of fluid sample and adding 11.0 ml of liquid (total liquid 12.0 ml, 5.0 ml of filtrate used), multiply the value of the reading by 1.2 to convert it to phenol equivalents per 0.5 ml of sample. If desired, the result may be converted to phenol equivalents per 1 ml by multiplying by 2.4. Evaluate the result by comparing it with the criteria of pasteurization in Table 2.

Butter:

Step 1. Weigh, on a piece of wax paper about 1×1 inch on a balance, a 1.0-g sample (preferably in duplicate) and insert the paper with the sample into the tube. Similarly, weigh another sample and place in a tube as a control or blank.

Step 2. Heat the *blank* to the temperature indicated under "Cheese: Step 2," and cool to room temperature. From this point, treat the blank and the test in a similar manner.

Step 3. Add 10.0 ml of barium buffer substrate **2-a** or **2-b** (prepared with 18-8 barium buffer, Table 2), stopper the tube, and mix.

Steps 4 to 11, inclusive. Follow the directions given for the corresponding steps under "Cheese" above, mixing the contents frequently and thoroughly during incubation, substituting the appropriate zinc precipitant (Table 2) in Step 6, and for merely detecting under-pasteurization, using 2 rather than 4 drops of BQC in Step 9.

Step 12. *Calculation and evaluation of result:* When using 1.0 g of butter and adding 11.0 ml of liquid, multiply the value of the reading by 1.1 to convert the result of phenol equivalents per 0.5 g of butter. Evaluate the result by comparing it with the criterion of pasteurization in Table 2.

MODIFICATIONS FOR DIFFERENT CHEESES AND OTHER DAIRY PRODUCTS

Different dairy products, and different kinds of cheese and cheeses of different ages, have different buffering capacities, and therefore some of them require modification of concentrations of the reagents. The modifications of the barium buffer needed to produce optimal *p*H conditions during incubation (9.85–10.20), and of the precipitant to yield uniformly clear filtrates and to minimize interference during color development under optimal *p*H conditions (9.3–9.4), are specified in Tables 1 and 2.

With some samples, especially cheese samples of unknown history, slight deviations from the optimal *p*H range may occur, but such deviations do not very materially affect the results. For example, *p*H values as low as 9.6 or as high as 10.35 during incubation have been found to result in an average decrease of not more than 20 per cent below the maximum in the quantity of phenol liberated. The use of the 25-11 buffer substrate with samples for which the 27-11 buffer substrate is specified yields *p*H values not lower than 9.8.

A trace of cloudiness in the filtrate, following the use of the precipitant as prescribed, indicates that the concentration of barium hydroxide in the buffer was not sufficiently great, *i.e.*, that the buffer substrate was not sufficiently alkaline. For example, the 25-11 buffer diluted 5+5 with water, for use with fresh milk, may yield a cloudy filtrate if used with old milk having a *p*H below approximately 6.0, or with milk that has soured, and the test should be repeated with a more concentrated buffer and precipitant. Likewise, the 25-11 buffer, for use with unripened cheese, may yield a cloudy filtrate if used with ripened cheese, indicating that the concentration of the buffer used was not sufficient. Increasing the concentration of zinc sulfate in the precipitant also eliminates turbidity of the filtrate.

In testing cheese of unknown history or age, information as to the percentage of solids, especially the nonfat solids, is useful as an indication of the correct buffer to use; cheese with a relatively high percentage of nonfat solids generally requires the use of a relatively concentrated buffer to adjust the *p*H of the mixture correctly.

For precise quantitative results on unknown samples, adjust the *p*H to 10.0–10.05 for the incubation.

Cottage cheese curd is heated in the presence of considerable acid during manufacture, and therefore its phosphatase values are comparatively low. Alternatively, to increase the sensitivity of the test on cottage cheese, apply the following modifications: Use a 1.0-g sample, 27-11 buffer substrate, 2-hour incubation, 6.0-0.1 precipitant, and a pasteurization criterion of 2 units per 0.5 g.

Phosphatase activity is much less in goats' milk than in cows' milk. The details of a modification designed to increase the sensitivity of the test applied to goats' milk are specified in Table 2.

To test concentrated milk products, reconstitute the product with water to its original concentration of milk solids and test in the manner specified for the original product.

To test for the presence of microbial phosphatase, which is indicated by blue color in the blank prepared as directed above, repeat the determination, adding 1 ml of the appropriate barium buffer (without substrate) to the blank and heat it for 5 min. in boiling water in a covered beaker. If the blank treated thus is negative, it indicates that the blue color in the original blank was due to microbial phosphatase, *i.e.*, a "false positive" sample.

PRECAUTIONS

The length of time that the crystalline disodium phenyl phosphate and the BQC powder will remain stable can be increased greatly by keeping them in the freezing chamber of a refrigerator.

The glassware, stoppers, and sampling tools should be scrupulously clean, and it is desirable to soak them in hot, running water after cleaning.

The bottles containing solid barium hydroxide and the barium buffer must be kept stoppered tightly to prevent absorption of carbon dioxide.

Phenolic contamination from plastic closures on reagent bottles has been encountered, and therefore the use of plastic closures should be avoided. Rubber stoppers should not be used in flasks in which butyl alcohol is stored. Glass or cork stoppers should be used.

DISCUSSION

In this work phosphatase activity caused by microorganisms has not been found in any fresh or reasonably fresh products. It has been encountered in some samples of old butter and old cream, on the surfaces of some soft and semi-soft ripened cheeses, and in several specific cultures of microorganisms. Such activity, in all samples tested to date, is indicated by blue color in the controls or blanks heated as described above. This seems to indicate, as others have pointed out earlier in tests on a few strains of microorganisms, that microbial phosphatases require a higher temperature for inactivation than milk phosphatase, and provides a means for tentatively differentiating between the two types of phosphatases.

The application of the method of Bessey *et al.* (4) for blood serum was investigated. It was found that the substrate, *p*-nitrophenyl phosphate, decomposes relatively rapidly under the influence of heat when the controls and tests are heated after incubation, whereas disodium phenyl phosphate undergoes relatively little hydrolysis when heated. Moreover, in tests prepared with known concentrations of raw milk in pasteurized milk, the photometric readings of the yellow color at 400 mμ with *p*-nitrophenyl phosphate were considerably smaller than those of the blue color at 610 mμ with disodium phenyl phosphate. The color produced with the latter substrate was much more distinct and the results much easier to interpret precisely. Whereas the enzymic activity in a concentration of 0.1 per cent of raw milk added to pasteurized milk could be detected visually by means of the Sanders-Sager method, and a concentration of 0.05 per cent could be detected with a photoelectric colorimeter, precise detection could not be made of quantities smaller than approximately 1 per cent by means of the method of Bessey *et al.*

The method of Bray and King (6), and also the modification described by Huggins and Talalay (7), involving the hydrolysis of phenolphthalein phosphate (colorless) yielding alkaline phenolphthalein (pink), were also investigated. However, even less precision was obtained with phenolphthalein phosphate as a substrate. The rate of hydrolysis of this substrate by the enzyme is too slow for a practical test for dairy products, and the pink color fades rather rapidly. For precise, quantitative determinations, both of these substrates were found less suitable than disodium phenyl phosphate.

All of the available time of the Associate Referee during the year has been devoted to research necessary for the development of the method and incidental cooperation with firms and individuals in the dairy industry desiring to use the method, and formal collaborative work has not been undertaken. Collaborative work on the use of the method for various dairy products should be done during the coming year.

RECOMMENDATIONS*

It is recommended—

(1) That, on the basis of the successful results reported on the test applied to Cheddar cheese and fluid dairy products by the Associate Referee, and the favorable results of collaborative work reported on the test applied to hard cheese by Gilcreas and applied to the soft cheeses by Horwitz, the Sanders-Sager phosphatase test be made an official method for testing fluid milk and cream, Cheddar type cheeses, and the soft unripened cheeses, as an index of the adequacy of pasteurization.

(2) That the test be made a tentative method for testing the various other dairy products for which a pasteurization test may be needed, including the other types of cheese, ice cream mix, sherbet mix, chocolate drink, butter, sweet buttermilk, cultured buttermilk, fermented milk drinks, goats' milk, cheese whey, and concentrated milk products; also that further collaborative work be done thereon during the coming year.

(3) That the present phosphatase test for pasterurization, **22.43–22.57,** inclusive, be dropped.

REFERENCES

(1) Sanders, G. P., and Sager, O. S., *J. Dairy Sci.*, **29,** 737 (1946).
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(4) Bessey, O. A., Lowry, O. H., and Brock, M. J., *J. Biol. Chem.*, **164,** 321 (1946).
(5) Frary, Guy G., *This Journal*, **30,** 418 (1947).
(6) Bray, J., and King, E. J., *J. Path. and Bact.*, **55,** 315 (1943).
(7) Huggins, C., and Talalay, P., *J. Biol. Chem.*, **159,** 399 (1945).

No reports were given on frozen desserts (gelatine and gums), frozen desserts (composition), chlorine in milk, or acidity of milk.

REPORT ON THE PREPARATION OF BUTTER SAMPLES

By H. J. Meuron (Food and Drug Administration, San Francisco, California), *Associate Referee*

This report deals with a continuation of the investigation initiated by the predecessor Associate Referee.[1] He devised a plan for comparing and evaluating two rapid procedures for preparation of butter and oleomargarine samples as well as for testing a proposed rewording of the present official method. He subjected the plan of investigation to preliminary trial in the hands of collaborators in this laboratory. The results obtained indicated that this approach to the problem would yield information from which reasonable conclusions could probably be reached if more extended data were similarly obtained.

* For report of Subcommittee C and action by the Association, see *This Journal*, **31,** 50 (1948).
[1] Vorhes, Frank A., *This Journal*, **29,** 119 (1946).

Accordingly the writer has solicited aid from as many sources as had expressed active interest. He has been fortunate in obtaining the cooperation of nine collaborators from eight different laboratories, which, incidentally, happen to be well distributed as to geographical location and size.

Instructions to collaborators were the same as those summarized in the previous report and in addition included the following:

Comments of collaborators are solicited for the purpose of obtaining a consensus of opinion as to:

(1) Whether the proposed rewording of the official method[2] represents as accurately as is practicable the actual technique employed (or that which should be employed) under present wording, except for specified temperature limits.

(2) Which of the rapid methods[3] (if either) is more convenient and rapid.

(3) Which of the rapid methods[3] (if either) is preferred for reasons other than convenience, rapidity, effectiveness and reliability; for example, in which procedure has the collaborator greater confidence solely as a result of using it.

The following constructive excerpts, from comments received, provide a basis for at least partial conclusion as to the consensus of opinion sought. The remarks of collaborators follow the order in which their results appear in Table 1, the form of which is the same as that employed in the tabulation presented in the previous report.

COMMENTS BY COLLABORATORS[4]

C. E. Hynds, Food Laboratory, N. Y. State Department of Agriculture & Markets, Albany, New York.

The proposed wording [of the official method] seems to accurately describe the technique used except the words "shake vigorously . . . until sample cools." It seems more likely that "shake vigorously at intervals . . . until sample cools" would be more appropriate. Method III seems more convenient and faster and the mixer is easier to clean. I prefer Method III.

H. S. Mitchell (VCM), Swift & Co., Union Stock Yards, Chicago, Ill.

Prefer Method III because of ease of mixing sample. Found that Methods I and II require from 15 to 30 minutes hand shaking for the preparation of the sample.

George E. Keppel, Minneapolis Station.

Method I represents accurately the technique used in following the present official method. I shake the jars intermittently until the contents cool to a creamy or "semi-solid" consistency. In addition, most of the analysts here give the butter an additional mixing for about 10 seconds after the jar is opened, using a long-handled spoon, and withdrawing portions for analysis with the same spoon.

I believe Method III is more convenient than Method II, in that the mixing blade is easier to clean between subs, and it is easier to incorporate butter adhering

[2] Method I, contained in previous report, *This Journal*, 29, 125 (1946).
[3] Method II—Present tentative method—*Methods of Analysis*, 1945, 333, sec. 22. 109-10. Method III—*This Journal*, 29, 125 (1946).
[4] With only 2 exceptions, collaborators were all in Food and Drug Administration.

to the sides of the jar. Both methods are about equally rapid. Further, I believe Method III is more effective and reliable. I had never used either Method II or III before this, and noticed that in Method II, the paddles on our stirrer did not fit well in the pint Mason jars, being apparently intended for a taller jar. Therefore, I had to tilt the jar considerably to get at the butter under the jar shoulders, and the lower corners could not be reached. I am not certain that either Methods II or III offer much saving of time over the official method. In this laboratory as a general rule, we start analysis of butter almost as soon as it arrives. If the sample can be allowed to stand overnight, Method III has a distinct advantage. However, if analysis must be started at once, it requires almost as long to temper the butter evenly to 23°C. as it does to warm it to 35° (official method).

Albert L. Weber, New York Station:

Method I is practically the same as that followed here. E-Z seal glass top Mason jars and International shaking machines have been used for some time and found to be satisfactory for mixing the samples. Shaking machines are essential when a large number of samples must be analyzed within 24 hours after collection. No material differences in results have been noted when comparing the shaking machine with hand shaking.

As to Methods II or III, neither shows much advantage in convenience or speed over the other. We feel that Method II may give better mixing. Both of these methods are too slow for our mass production needs. The time taken to soften the butter by Methods II and III is longer than that required by the official method, particularly if the butter is hard frozen initially [and shaking machines are used].

Meyer Matluck, Boston Station:

The third from the last sentence in Method I, "when optimum fluidity is attained . . . " is not clear. In actual practice, even though the sample has cooled to a "homogeneous semi-liquid of the consistency of thick cream," it may still flow down the side of the jar even though a layer does remain which obscures ready location of the surface of the sample. The deletion of the phrase " . . . no longer flows down the side of the jar" would, I believe, eliminate confusion without changing essential technique.

Assuming an average room temperature of 25°C., the duration of shaking required to go from optimum fluidity to a semi-liquid is in the neighborhood of an hour or more. On a hot summer day, room temperature of 28°, such as is frequent here, some artificial cooling, whether by shaking in a refrigerator or before an open window with an off-the-water breeze blowing in, is absolutely necessary. How can one reconcile such a situation with the admonition against employing artificial aids to cooling? The conditions described in Method I are such as to render it prohibitive from the standpoint of time consumption and physical exertion.

Methods II and III seem equally rapid and convenient. Method II, having a cover, minimizes moisture loss during preparation. Method III allows one to scrape all surfaces of the jar. Perhaps a cover could be adapted to this method. I feel that both Methods II and III are effective and reliable and far superior to the official method.

Herman O. Fallscheer, Seattle Station:

Method III is definitely more convenient and somewhat more rapid than Method II, and is also easier on the nerves, as Method II is quite noisy and "chattery." I would place more confidence in Method III than II. The blades in Method II don't always adequately mix the butter in the corners and shoulder of the jar, particularly if some separation has taken place in heating the sample.

John F. Armstrong, Los Angeles Station:

Method III is most desirable because it is rapid, smooth in operation (less chance

of chipping pieces of glass into the sample), efficient (mixes the sample instead of boring a hole into it), and is physically easy as it eliminates laborious shaking.

Howard M. Bollinger, Los Angeles Station:

I found it impossible to get a visibly thorough mixing using Method II. After 5 minutes stirring darker yellow streaks were still observed on the interior surfaces of the jar, especially in the lower crevice where the butter was hardly disturbed regardless of the attempted thoroughness.

Our shaking machine can be adjusted so that the agitation is no greater than hand shaking and when the butter jars are placed on their sides a continuous washing action takes place without incorporation of air to make foam. I prefer Method III to Methods II and I, even when a shaking machine is used with the latter.

E. H. Zilliox, State Chemical Laboratory, Vermillion, South Dakota:

Only Methods I and III were used as the equipment for Method II was not available. Using Method I, it was necessary to shake the jars in the open doorway of a refrigerator as the room temperature was 90°F. Only enough toweling was folded around the jar to permit holding it. I do not favor Method I for it is too laborious especially when the room temperature is hovering around the century mark.

Method III is convenient and produces a very uniform sample. However, because of the chance of loss of moisture, I believe Method I should be used.

With minor changes, which are reflected in the accompanying recommendations, there is general agreement that the proposed rewording of the official method represents as accurately as is practicable the actual technique that should be employed.

Of the collaborators who commented on the comparative convenience of the two rapid methods, the majority (6) prefer Method III and the minority (2) conclude that the two methods are equally convenient, although one of the latter prefers the official method with the proviso that a shaking machine be employed. The collaborators whose results appeared in last years' report all prefer Method III.

From responses to the third question it was hoped to discover which, if either, of the rapid methods impart to the analyst the greater confidence. This would be a useful quality in a method for sample preparation in that, if supported by experimental results, it would provide the analyst some basis for deciding when the mixing had been completed. It was perhaps optimistic to expect that a definite and well grounded conclusion in this regard could be reached on initial application of a method. Nevertheless, the majority of collaborators give cogent reasons for their choice and, of these, four express greater confidence in Method III, while one comments favorably as to Method II. One collaborator cites a reason for some degree of lack of confidence in each of the rapid methods.

USE OF SHAKING MACHINES

Collaborator Weber's remarks on the use of shaking machines in the application of the official method, and the comments of others in regard to artificial aids to cooling, bear on certain general observations which were included within the instructions to collaborators, as follows:

Our experience with this study to date indicates that the reworded official method has been found mechanically difficult. On two occasions collaborators have endeavored to employ a shaking machine to obviate the extended exertion of hand shaking. In one instance the results were quite comparable to those obtained by hand shaking, but in the other instance the results failed to check by as much as 2%. The latter experience is in agreement with our own. We observed that the motion of the "International" shaking machine, when vigorous enough to effect mixing, tended to produce a foamy texture which possibly accounts for the poor results obtained. Under the circumstances, we must view the use of a shaking machine with disfavor at this time.

On one occasion a collaborator found it impossible to cool the sample while shaking at the temperature of the laboratory. He adopted the expedient of shaking it in the open doorway of a refrigerator, which proved successful. He subsequently expressed the opinion that the warmth of his hands on the jar may have prevented cooling. He suggested that a folded towel wrapped around the jar might have protected it against his body heat and permitted cooling at laboratory temperature. If other collaborators encounter such difficulty it is requested that the suggested expedient of wrapping the jar be tried. Please report such experience in full in order that consideration may be given to appropriate modification of the rewording of the method.

The writer has been unaware of any widespread use of shaking machines for this purpose. He and his associates had given the matter some consideration but the unfavorable initial results, cited above, discouraged immediate exploration of the potentialities of the modification. Moreover, our brief experience inclined us to the conclusion that in actual practice the use of shaking machines would be unduly cumbersome.

Collaborator Weber's remarks are entirely at variance with these conclusions. He speaks for a large laboratory that is accustomed to handling a heavy load of samples and states that the practice he describes has been in use by that laboratory over an extended period. Presumably the technique obviates the need for artificial aids to cooling.

This favorable experience, and the prospect of so revising the official method as to render it comparable to the rapid methods in convenience, would seem to warrant investigation with a view to developing supporting collaborative data. Pending successful outcome of such a study, however, the Associate Referee is not justified in modifying the wording of the official method in a manner to provide for mechanical shaking.

For the present this leaves the official method in the status of being not only inconvenient but, under certain climatic conditions, virtually impracticable. This situation need not necessarily be untenable, however, if either or both of the rapid methods qualify for official recognition by the Association.

COLLABORATIVE RESULTS

The nature of this investigation was such that collaborative samples could not be distributed by the Associate Referee. Each collaborator provided and prepared his own sample. Since each sample therefore represents entirely different butter, the determined per cent moisture, and, to a lesser

degree, the variations and moisture losses cannot be compared in an absolute sense, but only relatively. Futhermore, each result is influenced by factors other than those of incomplete preparation and moisture loss. Therefore it is important to recognize that only the trend of the variations and moisture losses is strictly comparable.

Table 2 is a re-presentation of the variation figures from Table 1, combined with those of the previous report and arranged for convenient comparison.

The variation within each group of 6 results by Method II is less than that within either of the two corresponding groups of 6 results by Method I, in 1 out of the 17 sets.

The variation within each group of 6 results by Method III is less than that within either of the two corresponding groups of 6 results by Method I, in 8 out of the 18 sets.

The variation within each group of 6 results by Method III is less than that for the corresponding group of 6 results by Method II, in 10 out of the 17 sets.

Comparing the variations in another way:—the maximum variation is shown by Method I in 5 out of the 17 sets; by Method II in 7 out of the 17 sets; by Method III in 4 out of the 17 sets.

By both comparisons the trend appears slightly less favorable to Method II; by one comparison the indicated trend is slightly favorable to Method I and by the other comparison, slightly favorable to Method III.

These indications, however, are based on differences of very small magnitude. The mean variation for Method I is only .056% (standard deviation .031); for Method II the mean variation is .086% (standard deviation .037); and for Method III the mean variation is .060% (standard deviation .056). To conclude that one procedure is more effective than another, in producing a uniform mixture, on the basis of differences in results of the order of three or four hundreths of a per cent is to ascribe to the error of the moisture determination itself, an insignificance probably not in accord with the facts. At any rate it can be safely concluded that the variations fail to reveal any substantial lack of effectiveness, on the part of any one of the methods, to produce a practically uniform mixture.

Table 3 re-presents the moisture loss figures from Table 1 combined with those of the previous report and arranged for convenient comparison. Some of the figures are expressed in the negative sense, indicating a calculated moisture gain. This apparent anomaly must arise from the influence of extraneous factors, such as, for example, a difference in composition of the subdivisions or the normal analytical error of the moisture determination.

The moisture losses were calculated in both of the ways described in the previous report. The two types of calculation indicate a similar trend in the majority of the sets of four results, *i.e.*, where the first calculation indicates a higher or a lower moisture loss for one method, as compared to the

TABLE 1.—*1947 collaborative results in study of methods for preparation of butter samples*

COLLABORATORS			C.E.H.	H.S.M.	G.E.K.		A.L.W.	M.M.	H.O.F.	J.F.A.	H.M.B.	E.H.E.
			% Moisture determined on subdivisions initially prepared by indicated method									
Sub.	Meth.											
A	I	Max.	15.55	16.04	16.48	16.19	18.39	16.59	16.68	16.63	16.57	16.27
		Min.	15.44	15.97	16.45	16.16	18.28	16.50	16.64	16.00	16.52	16.24
		Ave.	15.507	16.010	16.458	16.182	18.327	16.540	16.655	16.610	16.537	16.252
		Var'n	.11	.07	.03	.03	.11	.09	.04	.03	.05	.03
B	I	Max.	15.07	16.07	16.51	16.17	18.41	16.58	16.68	16.66	16.60	16.27
		Min.	15.02	15.97	16.49	16.14	18.34	16.50	16.64	16.60	16.57	16.17
		Ave.	15.034[1]	16.022	16.502	16.152	18.357	16.522	16.657	16.634	16.590	16.207
		Var'n	.05	.10	.02	.03	.07	.08	.04	.06	.03	.10
Ave. Subs A & B			15.271	16.016	16.480	16.167	18.342	16.531	16.656	16.622	16.563	16.229
C	II	Max.	15.30	16.01	16.53	16.12	18.48	16.59	16.63	16.59	16.58	
		Min.	15.16	15.85	16.43	15.97	18.35	16.51	16.61	16.53	16.50	
		Ave.	15.227	15.962	16.493	16.032	18.412	16.550	16.623	16.562	16.551	
		Var'n	.14	.16	.10	.15	.13	.08	.02	.06	.08	
		Loss	.044	.054	−.013	.135	−.070	−.019	.033	.060	.012	
D	III	Max.	15.39	16.01	16.44	16.09	18.41	16.54	16.63	16.61	16.57	16.15
		Min.	15.36	15.84	16.38	16.07	18.17	16.48	16.60	16.55	16.53	16.11
		Ave.	15.380	15.942	16.417	16.082	18.327	16.512	16.617	16.577	16.542	16.130
		Var'n	.03	.17	.06	.02	.24[2]	.06	.03	.06	.04	.04
		Loss	−.109	.074	.063	.085	.015	.019	.039	.045	.021	.099
			% Moisture determined on subdivisions reprepared by indicated method									
A	II	Max.	15.49	15.90	16.49	16.19	18.33	16.55	16.67	16.57	16.53	
		Min.	15.38	15.79	16.45	16.17	18.28	16.48	16.63	16.55	16.48	
		Ave.	15.438	15.850	16.467	16.177	18.302	16.515	16.648	16.554	16.506	
		Var'n	.11	.11	.04	.02	.05	.07	.04	.02	.05	
		Loss	.069	.160	−.009	.005	.025	.025	.007	.056	.031	
B	III	Max.	15.09	16.03	16.50	16.13	18.29	16.52	16.66	16.63	16.56	16.10
		Min.	15.02	15.91	16.49	16.11	18.19	16.46	16.64	16.59	16.53	16.05
		Ave.	15.057	15.967	16.503	16.117	18.247	16.493	16.652	16.609	16.541	16.068
		Var'n	.07	.12	.01	.02	.10	.06	.02	.04	.03	.05
		Loss	−.023	.055	−.001	.035	.110	.029	.005	.025	.049	.139

[1] Average of 5 determinations.—All others average 6.
[2] One determination was very low, the other 5 were nearly alike. If the low determination is discarded the variation is .09.

TABLE 2.—*Variations*

	1947 COLLABORATORS										1946 COLLABORATORS							
	BUTTER										BUTTER				OLEOMARGARINE			
	C.E.H.	H.S.M.	G.E.K.		A.L.W.	M.M.	H.O.F.	J.F.A.	H.M.B.	E.H.Z.	L.H.MCR.	L.A.S.	M.G.Y.	H.W.G.	L.H.MCR.	L.A.S.	M.G.Y.	H.W.G.
METHOD I	.11	.07	.03	.03	.11	.09	.04	.03	.05	.03	.05+	.05	.08	.05	.03	.02	.06	.02
	.05+	.10	.02	.03	.07	.08	.04	.06	.03	.10	.15	.05	.04	.03	.04	.09	.05	.03
METHOD II	.14	.16	.10	.15	.13	.08	.02	.06	.08	—	.07	.05	.07	.09	.06	.05	.08	.07
METHOD III	.03	.17	.06	.02	.24*	.06	.03	.06	.04	.04	.03	.07	.01	.04	.01	.07	.02	.08

Method I includes figures for—Subs A and B.
Method II includes figures for—Sub C.
Method III includes figures for—Sub D.
All figures are from a variation of 6 determinations, except those followed by a + which are from a variation of 5 determinations.
* See Note 2, Table 1.

TABLE 3.—*Moisture losses*

	1947 COLLABORATORS										1946 COLLABORATORS							
	BUTTER										BUTTER				OLEOMARGARINE			
	C.E.H.	H.S.M.	G.E.K.		A.L.W.	M.M.	H.O.F.	J.F.A.	H.M.B.	E.H.Z.	L.H.MCR.	L.A.S.	M.G.Y.	H.W.G.	L.H.MCR.	L.A.S.	M.G.Y.	H.W.G
								FIRST WAY[1]										
METHOD II	.044	.054	−.013	.135	−.070	−.019	.033	.060	.012	—	−.021	.051	.025	.147	.085	.216	.045	.062
METHOD III	−.109	.074	.063	.085	.015	.019	.039	.045	.021	.099	.034	−.021	−.024	.051	.028	.089	.037	.036
								SECOND WAY[2]										
METHOD II	.069	.160	−.009	.005	.025	.025	.007	.056	.031	—	.070	.020	.036	.038	.072	.110	.103	.045
METHOD III	−.023	.055	−.001	.035	.110	.029	.005	.025	.049	.139	.090	.000	.043	.043	.054	.035	.021	.011

[1] Average moisture by Method I on Subs A and B less moisture by Method II on Sub C, and less moisture by Method III on Sub D.
[2] Average of moisture by Method I less Method II on Sub A and average of moisture by Method I less Method III on Sub B.

other, the second calculation yields the same comparison, in 13 out of the 17 sets.

An equal or lower moisture loss is indicated for Method III, in comparison to Method II, in 20 of the 34 sets of comparisons.

The mean calculated moisture loss for Method III is .031% (standard deviation .040); that for Method II is .052% (standard deviation .055).

The trend appears favorable to Method III. The differences upon which the comparisons are based are again small, even somewhat less substantial than in the variation comparisons. The order of magnitude of the moisture losses for either method is slightly less than that of the variations. The Associate Referee believes that such order of magnitude would seldom if ever be of consequence to the considerations of the regulatory official, nor for that matter, of consequence to the interests of those whose product the regulatory official is called upon to consider. On those rare occasions when this factor need be taken into account its influence upon the matter under consideration can be gauged from the findings herein presented.

As to the rapid methods, the Associate Reeferee concludes (1) that substantial preference has been expressed in favor of Method III; (2) that, if any choice based on the experimental evidence is justified, it is in favor of Method III; (3) that the experimental evidence is of a character and extent warranting consideration of Method III for official status, and (4) that, in view of these facts, there is no compelling reason for retaining Method II.

Method III is published in detail in the preceding number of *This Journal*, **31**, 91 (1948), under "Changes in Methods of Analysis."

REWORDING OF THE OFFICIAL METHOD

The following modification of the reworded official method is intended to give effect to collaborators' suggestions, with which the Associate Referee agrees.

Soften entire sample in a closed, wide-mouth fruit canning jar (fitted with a flat metal-disc cover secured by threaded ring) by warming gradually in water bath or running water maintained at about 39°C., shaking intermittently to reincorporate any separated fat and to observe fluidity of sample as softening progresses. Optimum fluidity is attained at that point where the emulsion is still substantially intact, but the mixture moves freely on shaking, washing all surfaces and flowing freely down the sides of the jar under gravity, revealing the surface of the body of the sample almost immediately, and permitting visual observation as to whether all portions of the sample have become liquefied sufficiently to promote their incorporation in the body of the sample under influence of shaking. At a higher temperature the emulsion usually tends to separate on short standing. Avoid overheating. When optimum fluidity is attained, shake vigorously at frequent intervals until the sample cools to an homogeneous semi-liquid of the consistency of thick cream and flows only sluggishly down the sides of the jar, a layer remaining which obscures ready location of the surface of the body of the sample by inspection of the exterior. Running water, water baths, and other liquid aids to cooling may not be used but a cool

draft of air is permissible, if climatic conditions require, provided shaking is continuous, not intermittent. Weigh portion for analysis promptly.

ACKNOWLEDGMENT

Acknowledgement is gratefully extended to the collaborators whose names are given in connection with their comment.

RECOMMENDATIONS*

It is recommended—

(1) That the present official method for preparation of butter samples be reworded as described in this report.

(2) That the method for preparation of butter samples identified in this report as Method III and described above be adopted as official (first action).

(3) That the present tentative method for preparation of butter samples be dropped.

(4) That collaborative investigation of the application of shaking machines in execution of the present Official Method for preparation of butter samples be initiated under an Associate Referee familiar with such application.

No reports were given on tests for reconstituted milk or sour serum test.

REPORT ON MICROANALYTICAL METHODS FOR EXTRANEOUS MATERIALS IN FOODS AND DRUGS

By KENTON L. HARRIS (Food and Drug Administration, Federal Security Agency, Washington 25, D. C.), *Referee*

Reports of the Associate Referees for Drugs, Spices, and Miscellaneous Products; Fruit Products and Beverage Materials; Baked Products, Cereals, and Eggs; and Vegetable Products are presented herewith. Each change proposed is identified by the paragraph number in the 6th Edition if the *Methods of Analysis* to which it refers. It is recommended that these changes be accepted by the Association.

It is recommended that collaborative study be assigned and completed as rapidly as possible on the methods that warrant such additional attention. Such a program should be inaugurated during the coming year.

In the absence of a complete report by the Associate Referee for Dairy Products the Referee proposes the following changes in **42.11**:

Line 3 change "Transfer ca 100 vegetable fragments . . . " to "Transfer up to 100 vegetable fragments. . . . "

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* For report of Subcommittee C and action by the Association, see *This Journal*, 31, 50 (1948).

RECOMMENDATIONS*

It is recommended—

(1) That the method (sec. **42.32**), discussed in the report of the Associate Referee on extraneous materials in baked products, cereals, and eggs, be adopted as official.

(2) That methods for extraneous matters in fruits be subjected to collaborative studies.

(3) That methods for extraneous matters in drugs be further studied.

(4) That the changes in wording of section **42.57** (mold in tomato products) providing for adjusting the total solids in the sample by the use of the Abbé refractometer, as recommended by the Associate Referee, be made.

(5) That the changes in wording of section **42.11** (manure fragments in dairy products), recommended by the Associate Referee, be made.

REPORT ON EXTRANEOUS MATERIALS IN DRUGS, SPICES, AND MISCELLANEOUS PRODUCTS

By WILLIAM V. EISENBERG (Food and Drug Administration, Federal Security Agency, Washington, D. C.),
Associate Referee

It is recommended that all of the methods for ground spices, **42.78** through **42.84** inclusive, be reviewed in order to change the size of sample examined from 10 grams to 25 grams. The larger sample will be more representative for the detection of certain types of filth such as rodent hairs and excreta, which are often not uniformly distributed.

Our experience has shown that no physical difficulties will be encountered using the present methods for extracting 25 grams of black pepper, nutmeg, or ginger for microscopic counting of the filter papers. Cinnamon, because of its woody texture and extremely fine grind, introduces some difficulty due to the tissues floating and thus hampering the examination of the filter papers. Experimental work should be carried on next year to test the applicability of the other spice methods to change in size of sample from 10 to 25 grams.

Those methods for whole or cracked spices which presently use a 25 gram sample should also be reviewed for the purpose of changing the size of sample from 25 to 100 grams, for reasons similar to that stated above.

The spice methods and most of the microanalytical methods attempt to extract the so-called "light filth" consisting principally of insect and rodent filth simultaneously by means of one procedure. Recent experience has indicated that the defatting operation with petroleum benzine, which is often helpful in increasing the recovery of insect parts, will on the other hand decrease the recovery of rodent hairs. Duplicate analyses using

* For report of Subcommittee C and action by the Association, see *This Journal*, 31, 51 (1948).

methods **42.78** and **42.83** for whole black pepper seem to verify this premise. Further work, however, should be carried out which will include the examination of collaborative samples. Similarly, collaborative work should include other spices and methods with a view to evaluating the wisdom of using one and the same extraction for recovery of insect and rodent filth.

It is recommended—*

That methods for extraneous materials in drugs be further studied.

No report was given of extraneous materials in dairy products or nut products and confectionery.

REPORT ON EXTRANEOUS MATERIALS IN FRUIT PRODUCTS AND BEVERAGE MATERIALS

By F. ALLEN HODGES (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

Comparative studies were conducted using two methods for determining insect infestation in whole dried figs and two methods for determining insect contamination in fig paste. The methods used were (1) Macroscopic examination of 100 figs, (2) A gasoline flotation method on whole figs, (3) Fig paste prepared from 100 figs by putting the figs through a food chopper and using method **45.52** (A.O.A.C. 6th Ed. 1945), and (4) A dilution procedure of fig paste.

A. INFESTATION IN WHOLE DRIED FIGS

(1) The macroscopic method consisted of the examination of 100 figs (ca 1500 grams) by cutting open each fig and examining the interior for the presence of larvae and/or adult insects.

TABLE 1.—*Results by the macroscopic method*
(100 figs examined)

SAMPLE	A	B	C	D	E	F	G	H	AVE./100 FIGS
Larvae—2 mm and over	7	2	4	1	1	3	1	2	2.6
Larvae—less than 2 mm	6	3	3	3	1	1	0	3	2.5
Whole Insects	3	0	1	2	0	0	1	0	0.9
Larval Fragments	0	0	0	0	0	0	0	0	0.0
Larval Heads	0	0	0	0	2	1	0	0	0.4
Insect Fragments	0	0	0	0	0	0	0	0	0.0

(2) The gasoline flotation method used consisted of taking 100 figs and cutting them into halves. These halves were covered with water, heated, boiled for 10–15 minutes, and cooled to ca 50°. Ca 50–75 ml of

* For report of Subcommittee C and action by the Association, see *This Journal*, 31, 51 (1948).

gasoline was added to the container and thoroughly stirred. Water was added so that the gasoline layer came to about 1″ from the top of the container. The container was allowed to stand 20–30 minutes with occasional stirring, and the larvae and insects which rose to the gasoline layer were picked out and examined under a wide field binocular microscope and counted.

TABLE 2.—*Results by the gasoline flotation method*
(100 figs examined)

SAMPLE	A	B	C	D	E	F	G	H	AVE/100 FIGS
Larvae—2 mm and over	10	10	15	4	5	5	12	10	8.9
Larvae—less than 2 mm	20	5	8	25	19	11	20	9	14.6
Whole Insects	6	2	5	1	1	0	4	1	2.5
Larval Fragments	15	0	2	1	3	7	4	1	4.1
Larval Heads	4	1	1	0	3	0	9	5	2.9
Insect Fragments	3	2	1	0	1	0	5	0	1.5

A comparison of method (1) and method (2) shows that gasoline flotation gave a higher recovery than did the manual macroscopic method.

B. INSECT CONTAMINATION OF FIG PASTE

(3) Method **42.52.** After passing the whole figs through a food chopper eight 100 g. samples were examined by this method and results are shown in Table 3. Since the figs were ground up into a paste many of the insects and/or larvae present will be broken up into fragments rather than remain whole. For this reason a large proportion of insect heads and fragments were found.

TABLE 3.—*Examination of fig samples method 42.52*
Fig paste

SAMPLE*	A	B	C	D	E	F	G	H	AVE./100 G.
Heads	9	9	12	3	8	5	7	15	3.6
Larvae—2 mm or over	0	0	1	0	0	1	1	0	0.2
Larvae—less than 2 mm	1	2	3	0	10	1	7	1	1.3
Miscellaneous insects	0	0	1	1	1	0	0	0	0.2
Larval fragments and larval skin fragments	17	6	11	0	12	5	5	32	4.6
Miscellaneous insect fragments	19	8	12	2	1	3	3	18	3.5

* Samples A–G each represented 2–100 g. portions; sample H was of 5–100 g. portions.

(4) In the dilution procedure fig paste was prepared from 100 figs and mixed with water in the proportion 1 part paste to 3 of water. To this

mixture was added 4% formalin. The examination consisted in using 400 g. portions of the diluted material. The procedure used in (3) above was followed except that the NaOH and HCl steps were omitted. Five samples of figs were examined using 400 g. portions. Results obtained are shown in Table 4. As in the case of method (3) above, a small number of whole larvae and/or insects were recovered since the figs were ground up into a paste.

TABLE 4.—*Examination of diluted fig paste*
(400 g. portions of 1:3 diluted material examined)

SAMPLE NO.	A	B	C	D	E	AVE./100 G. ORIGINAL PASTE
Larvae—less than 2 mm	1	1	0	0	2	0.8
Larvae—2 mm or over	3	2	0	1	1	1.4
Heads	13	11	4	3	0	6.2
Miscellaneous Insects	0	0	2	0	0	0.4

A comparison of method (3) (**45.52**) and method (4) shows that the NaOH and HCl treatment in **42.52** gave a more complete separation of insects than did the simple dilution of method "(4)."

DISCUSSION

The use of the gasoline flotation method in comparison with the other methods used showed much better recovery of insects, particularly whole larvae. It also eliminates the grinding up of the figs into a paste and for this reason whole larvae and insects are detected rather than insect heads and fragments. It requires less time to examine a sample and is easier to pick out the insects.

It is recommended* that collaborative work be done on the following method.

Examination of Whole Dried Figs—(Flotation)

INSECTS

Pick out 100 figs from each subdivision of sample to be examined. Cut the figs into halves and transfer to a large container. Cover with water. Heat. Boil for 10–15 min. or until the figs are soft. Allow to cool to ca 50°. Add ca 50 ml gasoline. Allow to stand ca 20–30 min., stirring occasionally during this period. Fill the container with additional water to within ca 1 inch from top.

Examine the surface of the beakers or container under a strong light using a magnifier of ca 5×. Pick out larvae, insects, and/or insect fragments which rise into the gasoline layer. Examine the insects found, using a Greenough type microscope at a magnification of ca 20×.

* For report of Subcommittee C and action by the Association, see *This Journal*, 31, 51 (1948).

REPORT ON EXTRANEOUS MATERIALS IN VEGETABLE PRODUCTS

By FRANK R. SMITH (Food and Drug Administration, Federal Security Agency, Washington, 25 D. C.), *Associate Referee*

In the methods for extraneous materials in vegetable products only one slight revision seems necessary this year. In method **42.57** it is necessary to determine the total solids by an impractical and lengthy weighing and drying procedure. The method now reads in part, "in case of puree and paste mix H_2O to make total tomato solids of diluted product 8.37–9.37%." To obviate this necessity provision can be made to control the solids by refractometer.

This involves no change in the method, but such revision provides a practical way of securing these dilutions. It is recommended* that it be adopted for official, final action. The details of the proposed revision as it applies to the official method for molds in tomato products, section **42.57**, are given in *This Journal*, **31**, 118, 1948.

REPORT ON EXTRANEOUS MATERIALS IN BAKED PRODUCTS, CEREALS, AND EGGS

By KENTON L. HARRIS (Food and Drug Administration, Federal Security Agency, Washington 25, D. C.), *Associate Referee*

Section **42.32** concerns the separation of rodent excreta pellet fragments from whole corn meal. The precursor of this method first appeared in a U. S. Food and Drug Administration mimeograph by Helsel and Harris (1939), was published in *Cereal Chemistry*, **18**, 655 (1941), and in its present form first appeared in the *Methods of Analysis*, Ed. 6, p. 781.

From time to time collaborative samples have been prepared and sent to various analysts. The results on these samples are given in Table 1 opposite. It should be noted that there are omitted from the table several counts reported by analysts inexperienced in the microscopic identification of the extracted pellet fragments. Samples consisted of 50 gram portions of meal taken from a well-mixed batch of grossly contaminated commercial corn meal.

The results of the collaborative work show a marked agreement between the various collaborators. The method is recommended* for adoption as official.

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* For report of Subcommittee C and action of the Association, see *This Journal*, **31**, 51 (1948).

TABLE 1.—*Results from various analysts on 50 gram samples of whole corn meal examined for rodent excreta pellet fragments*

SAMPLE NUMBER	ANALYST	RODENT PELLET FRAGMENTS COUNT
M49057	KLH (4 counts)	21, 27, 27, 25
	He	24
	Wo	23
	Co	20
	ME	27
	Lo	21
M49058	KLH (2 counts)	17, 18
	Wo	15
	ME	13
	QCR	18
	QSJ	12
	Lo	20
	Bo	18
	Coh	16
	Cou	16
	Ca	17
	Pi	15
M49059	KLH (4 counts)	25, 26, 33, 21
	Sa	21
	Yo	23
	QCR	20
	QSJ	24
	Lo	39
	Bo	33
	Coh	20
	Cou	19
	Ca	17

REPORT ON FISH AND OTHER MARINE PRODUCTS

By ANDREW M. ALLISON (Food and Drug Administration, Federal Security Agency, Boston 10, Mass.), *Referee*

In accordance with recommendations of Subcommittee C (*This Journal*, **30**, 51, 1947) collaborative studies have been continued on the Modified Roese-Gottlieb method for determination of ether extract in fish. The method has been modified considerably and a report by Associate Referee Voth is being submitted. It is recommended—*

(1) That the Modified Roese-Gottlieb, or acid extract, method for fat in fish be adopted as tentative.

(2) That the work on methods of determining total solids in fish be continued (*This Journal*, **26**, 226, 1943).

* For report of Subcommittee C and action by the Association, see *This Journal*, **31**, 52 (1948).

REPORT ON ETHER EXTRACT IN FISH

By MENNO D. VOTH (Food and Drug Administration, Federal Security Agency, Boston, Mass.), *Associate Referee*

A digestion or acid extract method for the determination of fat in fish has been previously described (1).

Considerable additional work has since been done on this method. It has been improved and simplified. Specifically, the following changes have been made: 1. The charge has been increased from 5 grams to 8 grams. This 60 per cent increase helps to offset errors due to unequal fat distribution in the sample or to other causes. 2. The use of equal parts of ethyl ether and petroleum benzine has been substituted for the use of ethyl ether alone. This change eliminates the necessity of washing the ether extract and, therefore, simplifies the method. 3. The steam bath has been substituted for the hotplate or burner as a means of heating during digestion. This change eliminates all bumping, which constituted a real hazard, and also eliminates the need for specially treated sand.

The proper proportions of fish, acid, and alcohol used in the method were determined after numerous trials and are believed to be in the best possible ratio. The major difficulty encountered in the work was the residue not soluble in petroleum benzine which remained in the fat flask after drying, and which was found principally in low fat fish, such as haddock, cod, etc.

The original plan was to obtain the weight of the extracted fat by difference as is done in the Roese-Gottlieb method (2). However, it was found that fish fats extracted from low fat fish (such as haddock) by the usual fat solvents have a tendency to polymerize or solidify during heating in such a manner as to make them insoluble in petroleum benzine (or in any of the common fat solvents). This polymerization is believed to be affected by various factors. Some of these are: 1. Age of fish in storage. 2. Length of heating period after evaporation of the solvents. 3. Length of digestion period. 4. Lack of clarification of the extract (by centrifuging). Under certain conditions, this polymerization can be controlled. By taking special precautions (see Table 1), such as heating the fats for a minimum length of time in a vacuum oven or in an atmosphere of carbon dioxide, insignificant polymerized residues are obtained. It was concluded, however, that to include such precautions would make the method cumbersome. Any appreciable residue due to polymerization, which is obtained after washing with petroleum benzine, must be considered a fat, since experimental evidence shows that when certain precautions are taken, the residue obtained is insignificant. It is also to be noted that the weight of fat plus the residue remains constant within the limits of experimental error, regardless of the factors which increase or decrease the weights of those components. Some of the experimental data is shown in Table 1 below.

TABLE 1.—*Polymerized residues obtained from fat extracted from haddock flesh using the Roese-Gottlieb method*

EXTRACTED AND HEATED USING SPECIAL PRECAUTIONS			EXTRACTED AND HEATED WITHOUT USING SPECIAL PRECAUTIONS		
PER CENT FAT AND RESIDUE	PER CENT RESIDUE	PER CENT FAT BY DIFFERENCE	PER CENT FAT AND RESIDUE	PER CENT RESIDUE	PER CENT FAT BY DIFFERENCE
0.53	0.01	0.52	0.54	0.37	0.17
0.53	0.02	0.51	0.53	0.37	0.16
0.56	0.02	0.54	0.56	0.33	0.23
0.53	0.01	0.52	0.55	0.32	0.23
0.53	0.00	0.53	0.52	0.33	0.19
0.52	0.01	0.51	0.53	0.31	0.22
0.54	0.01	0.53	0.54	0.12	0.42
			0.53	0.40	0.13
			0.56	0.13	0.43
			0.53	0.27	0.26

It was also determined that 8 grams is the maximum possible charge that can be handled in the small Mojonnier tubes which are readily available. Considerable experimental work was done before this conclusion was reached. This was particularly true in low fat fish, such as haddock (see Table 2 below).

TABLE 2.—*Per cent of fat extracted from different weights of haddock by digestion*
(8 ml HCl used in each case)

Sub	1	2	3	4	5	6	7	8
Wt. of fish used (gms)	5	6	7	8	8	9	9	10
Per cent fat	0.57	0.57	0.57	0.58	0.58	0.53	0.52	0.48

For comparison, fat determinations by two other methods were made by the Referee on fish samples identical with those sent to collaborators. The first method used was that previously described (1). Ethyl ether alone is here used as the solvent. Results shown in the table below are comparable with those obtained by the revised method shown in Table 5.

Determinations were also made using the Stansby-Lemon continuous extraction method (3). As was expected, the results are a little higher than those obtained by the digestion method (see Table 3 below). In the Stansby-Lemon method, a crude extract is first obtained using acetone as the solvent. This extract is then dried and re-extracted with anhydrous ethyl ether. The slightest bit of moisture in the crude extract, solvent, or apparatus will dissolve some of the large quantity of water-soluble residue left behind during the re-extraction with the anhydrous ethyl ether. Even after taking the utmost precautions, such as a 5-hour vacuum oven drying period, a small portion of water-soluble material comes over as indicated by the brown-colored ether solution and the final dark viscous fat.

TABLE 3.—*Results on collaborative samples by two methods*
(Per cent fat)

	FRESH HADDOCK		FRESH MACKEREL		CANNED MACKEREL	
		Ave.		*Ave.*		*Ave.*
Ethyl ether extraction method (1)	0.53		3.83		11.92	
	0.55	0.54	3.89	3.86	12.03	11.98
Acetone extraction method (3)	0.83		4.27		12.52	
	0.89	0.86	4.27	4.27	12.61	12.57

A limited study was made on the effect of a longer digestion period. Under ideal conditions, the fish are adequately digested in 60 minutes or even less on the steam bath. In order to determine whether a longer period would have a tendency to reduce the yield, two types of fish were digested on the steam bath for varying periods of time. It was found that within limits longer heating periods were not detrimental (see Table 4 below).

TABLE 4.—*Effect of digestion period on the steam bath on per cent of extracted fat*

HADDOCK			CANNED MACKEREL		
SUB	DIGESTION TIME IN HOURS	PER CENT FAT	SUB	DIGESTION TIME IN HOURS	PER CENT FAT
1	1	0.52	1	1	12.10
2	1.5	0.51	2	1.5	12.02
3	2	0.52	3	2	11.97
4	3	0.53	4	4.5	12.04
5	4	0.52			

Three samples of fish consisting of fresh haddock, fresh mackerel, and canned makerel were submitted for collaborative studies. The samples of fish which were sent to the collaborators for determination of fat were prepared in the same manner as described in the Referee's previous report (1). The revised method is as follows:

CRUDE FAT—ETHER EXTRACT

PREPARATION OF SAMPLE

Prepare the sample, according to the type of pack, as directed under par. **24.2** (*Methods of Analysis, A.O.A.C.*, 1945, 359) and keep ground material in a sealed jar. If the jar has been chilled, allow the sample to come to room temperature and shake jar so that any separated liquid will be absorbed by the fish. Open jar and stir contents with spatula, thoroly contacting the sides and lid so as to incorporate any separated liquid or fat.

METHOD

Weigh into a 50 ml beaker 8 grams of the well mixed sample. Add 2 ml of HCl. Break up coagulated lumps with a stirring rod having an extra large flattened end

and continue until a homogeneous mixture is obtained. Add an additional 6 ml of HCl, mix, cover with watch-glass and heat on the steam bath for 90 min. with an occasional stirring. Cool solution and transfer to Mojonnier fat extraction tube. Rinse beaker with 7 ml of ethanol, add to extraction tube and mix. Rinse beaker with 25 ml of ethyl ether in three portions and add to extraction tube, stopper with cork or stopper of synthetic rubber unaffected by usual fat solvents, and shake tube vigorously for one min. Add 25 ml of petroleum benzine (b.p. below 60°) to extraction tube and repeat vigorous shaking. Centrifuge Mojonnier flask 20 min. at ca 600 r.p.m. and proceed as directed under par. **20.16** (*Methods of Analysis, A.O.A.C.*, 1945, 240) beginning "Draw off as much as possible of ether-fat soln."

Drying to constant weight takes ca 40 min. for fish. Long heating periods tend to increase the weight of the fat. If a centrifuge is not available, the extraction can generally be made by letting Mojonnier flasks stand until the upper liquid is practically clear, then swirling flask and again letting stand until clear. If a troublesome emulsion occurs, draw off as much of the ether-fat soln as possible after letting flask stand, add a ml or two of ethanol, swirl, and again allow mixture to separate.

DISCUSSION

The results obtained by the collaborators are given in Table 5. Good results were obtained.

TABLE 5.—*Collaborative results on fat*

(Per cent)

COLLABORATOR	DIGESTION METHOD					
	FRESH HADDOCK		FRESH MACKEREL		CANNED MACKEREL	
		Ave.		*Ave.*		*Ave.*
G. Kirsten	0.56		3.84		11.99	
	0.58	0.57	3.88	3.86	12.02	12.01
H. I. Macomber	0.55		4.03		11.92	
	0.56	0.56	4.04	4.04	11.67	11.80
S. H. Perlmutter	0.54		3.71		11.78	
	0.56	0.55	3.80	3.76	11.82	11.79
T. J. Klayder	0.56		3.62		11.95	
	0.53	0.55	3.65	3.64	11.90	11.93
F. C. Minsker	0.56		3.68		11.98	
	0.58		3.76		11.98	
	0.56	0.57	3.71	3.71	11.81	11.92
M. D. Voth	0.56		4.01		12.09	
	0.56	0.56	4.03	4.02	12.14	12.12
Mean		0.56		3.83		11.93
Ave. Deviation from Mean		0.01		0.13		0.10
Maximum Deviation from Mean		0.03		0.21		0.26

No unusual difficulties were experienced by any of the collaborators. The Associate Referee wishes to thank the collaborators for their cooperation and help.

REFERENCES

(1) *This Journal*, **29**, 46 (1946).
(2) *Methods of Analysis, A.O.A.C.*, 1945, sec. **22.25.**
(3) *This Journal*, **27**, 237 (1944).

No reports were given on gums in foods.

REPORT ON SPICES AND CONDIMENTS

By SAMUEL ALFEND (Food and Drug Administration, Federal Security Agency, St. Louis, Mo.), *Referee*

Reports were submitted ty the Associate Referees on Vinegar, Mustard, and Mayonnaise and Salad Dressings.

VINEGAR

Associate Referee Loughrey has reworded the method for permanganate oxidation number for convenience and clarity, and has subjected it to collaborative study. The results were promising. The Associate Referee recommends another collaborative test before offering it for adoption as official, and the Referee agrees. The Referee has noted the precipitation of iodine, mentioned by Garfield,[1] and the release of iodine vapor above the solution, and suggests increasing the concentration of potassium iodide to dissolve all the iodine.

No work was done on mineral acids. The qualitative method for free mineral acids proposed by Rokita and Henry is excellent, but the quantitative method proposed last year[2] has obvious theoretical deficiencies. It was proposed to run total phosphates, sulfates, chlorides or nitrates in the sample, and correct for naturally occurring salts by subtracting the amount of the pertinent radicle found in the ash. It is obvious that some of the chloride would be lost as potassium chloride on ignition, and that potassium nitrate would break down on ashing, leaving little or none in the ash. It is also apparent that in the presence of free sulfuric or phosphoric acid, the potassium carbonate which constitutes much of the ash of apple or wine vinegar would be converted to the sulfate or phosphate, and the correction for the salts would be far too large. It might prove practicable, once the mineral acid adulterant has been identified, to determine the radicle in the vinegar, and apply a salt correction based on the normal content of the particular salt in that type of vinegar. The correction would

[1] *This Journal*, 30, 446 (1947).
[2] *This Journal*, 29, 304 (1946).

be very small for distilled vinegar, and even for cider vinegar the possible error in the correction might not be important. Another approach might be the setting up of curves or nomographs relating *p*H, titratable acidity, and proportions of acetic and mineral acids.

The Associate Referee has done some preliminary work on tartaric acid. This value is of interest primarily as a means of detecting adulteration of wine vinegar. Since the ability to detect dilution with other vinegars is limited by the variation in the natural content of tartrates in wine vinegar, it is worth while to seek methods depending on the presence of a characteristic constituent of the adulterant. Ey[3] has suggested the determination of sorbitol, a characteristic component of cider, as a means of detecting the presence of cider in grape wine. This should prove equally useful in detecting cider vinegar in grape vinegar, and the possibilities should be investigated.

Loughrey has pointed out the possibility of false positive tests for caramel in old cider vinegars, by the Lichthardt test, and has recommended study of other tests now being studied in wines. He expresses doubt as to the desirability of studying the Mallory and Love quantitative method before exploring the possibilities of the other tests. The Referee agrees.

MAYONNAISE AND SALAD DRESSINGS

Associate Referee Fine has submitted the results of collaborative work demonstrating that there is no detectable loss of starch when mayonnaise is held for a month at room temperature. The Referee endorses his recommendation that the method be made official (first action), and that no further work be done on it.

Final adoption of several methods has been held up pending final action on the adoption of the method for preparation of salad dressing samples as official. It was decided that it would be well to wait for several years, to determine if experience with the methods developed any unknown "kinks." Several field stations of the Food and Drug Administration have reported difficulty in removing the fat from mayonnaise prior to acid digestion in the determination of nitrogen, and Ferris and Macomber[4] have found that this difficulty can be overcome by adding 100 ml chloroform to the sample and evaporating off the chloroform, before extracting the fat with petroleum ether. Macomber has also reported difficulty in the determination of fat in salad dressings having less than 50 per cent of fat. He pointed out that the A.O.A.C. collaborative work has been done on samples containing more than 50 per cent of fat. It is believed that these points should be cleared up, and that the methods for preparation of sample, fat, and sugars should then finally be made official.

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[3] Detection of Cider in Grape Wine. Dr. Ey, *Schweiz. Apoth. Ztg.*, **83**, 605–607 (1945). Abstracted in *J.A.Ph.A.*, **36**, Abs., p. 12 (Jan., 1947).

[4] Unpublished reports.

MUSTARD

Associate Referee Garfield has submitted collaborative results on starch in mustard flour and prepared mustard which fully justify his recommendation that the method be adopted as official and no further work be done. Garfield has also done some preliminary laboratory work on the methods for the pungent principles in mustard (volatile oil, etc.), but is not yet ready to report on this subject.

Since the methods for starch in mustard and in salad dressing are essentially the same, after the preliminary treatment of the sample, it is suggested that wording agreeable to both Associate Referees be adopted for one method applicable to both types of food.

No report on volatile oil in spices was submitted by the Associate Referee.

RECOMMENDATIONS*

It is recommended—

(1) That studies on the application to vinegar of the Mathers and the A.O.A.C. confirmatory tests be continued in comparison with the tentative Lichthardt method.

(2) That further collaborative study be made of the tentative permanganate oxidation number.

(3) That methods for the quantitative determination of free mineral acids in vinegar be further studied.

(4) That work on determination of tartrates in vinegar be continued.

(5) That methods for determining sorbitol, and the usefulness of this value in detecting cider vinegar in wine vinegar, be studied.

(6) That the tentative method for starch in salad dressing be adopted as official (first action), and that no further work be done. The wording of the method, after the preliminary preparation, should be the same as that of the method for starch in mustard.

(7) That further study be made of the tentative method for fat in salad dressings, particularly those containing less than 50 per cent fat, so that the method may be made official.

(8) That the efficiency of the preliminary removal of fat in the official method for nitrogen in mayonnaise be studied.

(9) That the tentative method for starch in prepared mustard and mustard flour, as described in the Associate Referee's report, be made official (first action). The wording of the method, after the preliminary preparation, should be the same as that of the method for starch in mayonnaise and salad dressing.

(10) That studies be made of a suitable method for determination of ash in prepared mustard.

(11) That the official method for copper-reducing substances by direct inversion **(33.40)** be dropped (final action).

* For report of Subcommittee C and action by the Association, see *This Journal*, 31, 54 (1948).

(12) That studies be made of a suitable method for determination of sugars in prepared mustard.

(13) That studies be continued on the determination of volatile oil and other pungent principles in mustard.

(14) That studies be continued on volatile oil in spices.

REPORT ON VINEGAR

By JAMES H. LOUGHREY (Food and Drug Administration, Federal Security Agency, Boston, Mass.), *Associate Referee*

Of the recommendations approved by Committee C last year (*This Journal*, **30**, 53, 1947), consideration has been given to those on caramel tests and the permanganate number. The Mallory and Love method seems to be less fitted for the detection of caramel in vinegar than are several methods used for its detection in wines and spirituous liquors. These two methods are Mathers test and the present A.O.A.C. confirmatory test **(15.39)**, both to be finally confirmed by the use of 2.4-dinitrophenylhydrazine reagent. These tests and the results of collaborative work are the subject of Valaer's report on caramel coloring (p. 178).

Several complaints have been received about false positives when the present tentative method **(33.78)**, for caramel in vinegar, is used on cider vinegars. These false tests appear to occur in old cider vinegars. The Associate Referee has obtained one such test on a cider vinegar, three years old, whose authenticity seems to be unquestioned. The two tests reported on by Valaer, on the other hand, seemed to give accurate results. Further study of the tentative method is therefore indicated.

The wording of the method for determining the permanganate oxidation number was changed slightly. The reworded method follows:

REAGENTS

(a) *Sulfuric acid soln.*—1+1.

(b) *Potassium permanganate soln—approx. 1 N.*—It is suggested that the soln be slightly stronger than 1*N*; however, standardization is not necessary. Prepare according to **43.17**, p. 807.

(c) *Sodium thiosulfate—0.5 N.*—Accurately standardized against $K_2Cr_2O_7$. (Method **43.28**, p. 809, should be modified to correspond to the stronger $Na_2S_2O_3$. It has been found that the following quantities of reagents are satisfactory—0.50 gm $K_2Cr_2O_7$, 10 gm KI, 10 ml *conc.* HCl, and 90 ml H_2O.)

(d) *Potassium iodide soln.*—Dissolve 30 g of KI in 100 ml of H_2O, and filter. Do not use unless colorless.

DETERMINATION

Adjust vinegar to 4 g/100 ml acidity as acetic acid. Steam distil 50 ml of adjusted vinegar and collect 50 ml of distillate; distillation should be regulated so that ca 45 ml remain in distilling flask when 50 ml of distillate are collected. (All-glass apparatus is preferable; if not available, cork or rubber stoppers should be covered with

Sn or Al foil). The apparatus used for determination of volatile fatty acids in fish products (**24.9**, p. 361) is very convenient for this distillation. Keep distillate and reagents at 25°C.

Transfer the 50 ml of distillate to 250 ml glass-stoppered Erlenmeyer flask. Add 10 ml of the H_2SO_4 soln and 25 ml (accurately measured) of the $KMnO_4$ soln. Hold at 25°C., preferably in water bath, for exactly one hour. Then immediately add 20 ml of the KI soln and mix well. Titrate the liberated I with the 0.5 *N* $Na_2S_2O_3$. Conduct a blank determination at the same time, using 50 ml of H_2O, 10 ml of the H_2SO_4 soln and 25 ml of the $KMnO_4$ soln.

The number of ml of the 0.5 *N* $Na_2S_2O_3$ required by the blank less the quantity used in the determination divided by two is the permanganate oxidation number of the vinegar. Report on basis of the adjusted vinegar (4% acid).

The reworded method was sent to collaborators together with four samples of vinegar, to be analyzed according to the proposed method. The composition of the four samples was as follows:

Sample A—Cider vinegar, reduced to 4% acidity.
Sample B—Distilled vinegar, reduced to 4% acidity.
Sample C—Dilute Acetic Acid (4%).
Sample D—75% Cider Vinegar, 25% Dil. Acetic Acid (Acidity of mixture, 4%).

COLLABORATORS' RESULTS

COLLABORATOR	STATION	SAMPLE A	SAMPLE B	SAMPLE C	SAMPLE D
Sidney Kahan	New York	6.10	3.02	0.00	4.56
		6.09	3.06	0.00	4.56
		Av. 6.10	Av. 3.04	Av. 0.00	Av. 4.56
F. M. Garfield	St. Louis	5.70	3.0	0.00	4.2
Robert E. O'Neill	Atlanta	6.01	2.93	0.19	—
		6.06	3.21	0.05	2.61
		Av. 6.04	Av. 3.07	Av. 0.12	
C. G. Cunningham	Boston	5.17	2.56	0.18	4.05
		5.23	2.62	0.19	4.16
		Av. 5.20	Av. 2.59	Av. 0.19	Av. 4.11
A. L. Suslam	Boston	5.26	2.85	0.03	4.01
M. D. Voth	Boston	5.38	2.83	0.06	4.24
J. H. Loughrey	Boston	5.20	2.53	0.15	4.09
		5.27	2.55	0.14	3.99
		Av. 5.24	Av. 2.54	Av. 0.15	Av. 4.04

COLLABORATORS' COMMENTS

Sidney Kahan: "Concentration of permanganate solution as given is somewhat vague. Suggest that it be made about 31 grams per liter in order to avoid having the blank titrate more than 50 ml. Also suggest that the instructions be amended to include draining the permanganate pipette for a definite length of time, as in iodine number, to secure more uniform results."

F. M. Garfield: "Amount of KI used should be increased to prevent precipitation of iodine before the titration with the thiosulfate."

The results of the collaborative work are quite promising. Although there are variations between the results obtained by the different collaborators on any one sample, the values obtained for any one type of vinegar are in the same range. The type of reaction involved and the strength of reagents necessary for the method are such that slight variations may be expected. The results on Sample D were very encouraging. This sample consisted of 75% of Sample A and 25% of Sample C. With one exception, the actual results by any one collaborator on Sample D agree very closely with the theoretical value calculated on his figures for A and C.

The Associate Referee wishes to thank the collaborators for their prompt assistance.

RECOMMENDATIONS*

It is recommended—

(1) That the method for the determination of caramel in vinegar **(33.78)** be dropped and that studies be made of other tests for caramel, including the Mathers test, and the test for caramel in wine **(15.39)**, to determine their applicability to the detection of caramel in vinegar.

(2) That the permanganate oxidation method be applied to a number of samples of distilled vinegars of known history to determine its value in differentiating this type of vinegar from a dilute acetic acid.

(3) That the methods for the quantitative determination of tartaric acid and tartrates in vinegar be further studied.

(4) That studies of the determination of tartaric acid and tartrates in vinegar be continued.

No report was given on volatile oil in spices.

REPORT ON STARCH IN PREPARED MUSTARD AND MUSTARD FLOUR

By FREDERICK M. GARFIELD (Food and Drug Administration, Federal Security Agency, St. Louis, Mo.), *Associate Referee*

The determination of added starch in prepared mustard and mustard flour has been the subject of study for a number of years. Several methods have been investigated, and the most satisfactory was found to be a modification of a procedure developed by Sullivan[1] for the estimation of starch in plant materials. The method,[2] when first subjected to collaborative study called for (1) solution of the starch (in the mustard product)

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* For report of Committee D and action of the Association, see *This Journal*, 31, 54 (1948).

[1] *This Journal*, 18, 621 (1935).

[2] J. T. Field, *Ibid.*, 24, 700 (1941) and 25, 705 (1942).

in boiling 30% calcium chloride solution; (2) precipitation of the starch from the calcium chloride solution with alcohol to remove soluble interfering reducing substances; (3) a further purification of the starch by solution in water and reprecipitation as a starch-iodine complex; (4) decomposition of the complex with alcoholic sodium hydroxide to recover the starch; (5) hydrolysis of the starch to dextrose by boiling in hydrochloric acid and (6) determination of the resulting dextrose by the Munson-Walker copper reduction method. The starch was calculated using the equation dextrose × 0.9 = starch. Collaborative results were encouraging, but recoveries were somewhat low and erratic.

Further study[3] showed that modifications in the procedure were necessary. It was demonstrated that failure to neutralize the acidity in prepared mustard caused losses as high as 5% in starch recovery. These losses occurred during the initial solution of the starch in the calcium chloride solution, apparently due to partial hydrolysis of the starch by the vinegar present in the prepared mustard. Neutralization of free acidity was made a part of the method. A correction for water introduced by the moisture

RESULTS OF COLLABORATORS

Sample 1.—Prepared Mustard

COLLABORATOR	DATE ANALYZED	STARCH FOUND	RECOVERY	DATE REANALYZED	STARCH FOUND	RECOVERY
		per cent	*per cent*		*per cent*	*per cent*
1	4/ 9/47	4.50	102.0	5/ 9/47	4.32	98.0
		4.34	98.4		4.48	101.6
2	4/16/47	4.30	97.5	5/16/47	4.58	103.9
		4.46	101.1		4.45	100.9
3	4/15/47	4.52	102.5	5/15/47	4.52	102.5
		4.34	98.4		4.39	99.5
3				5/15/47	4.30	97.5
					4.39	99.5
5	4/21/47	4.53	102.7			
		4.46	101.1			
6	4/11/47	4.45	100.9	5/ 6/47	4.32	98.0
		4.39	99.5		4.28	97.0
Averages		4.43			4.40	
Overall average		4.416% or 100.1% recovery				
Standard deviation		±0.088% of starch				
Probable error		0.059% of starch				
Range		4.58 − 4.28 = 0.30%				

[3] *Ibid.*, in press.

Sample 2.—Mustard Flour

COLLABORATOR	STARCH FOUND	RECOVERY
	grams	*per cent*
1	0.2117	99.8
	0.2153	101.5
2	0.2102	99.1
	0.2124	100.1
3	0.2196	103.5
	0.2102	99.1
4	0.2074	97.7
	0.2074	97.7
5	0.2102	99.1
	0.2137	100.7
6	0.2102	99.1
	0.2102	99.1
Average	0.2115	99.7
Standard deviation	±0.0033 g	
Probable error	0.0023 g	
Range	0.2196 − 0.2074 = 0.0122 g	

present in the prepared mustard was included for obvious reasons. Minor manipulative changes were made to permit a more convenient method of decomposing the starch-iodine complex. The method as modified then gave good recoveries of starch except for an occasional high result. These high results were due to failure to remove interfering mustard material after solution of the starch in calcium chloride. To accomplish this the method was modified to call for centrifuging the calcium chloride solution digest and filtration through cotton on this partially clarified solution. Unless removed, solid material is carried through the various steps along with the starch and is eventually broken down during the hydrochloric acid hydrolysis to substances capable of reducing Fehling's solution. As a result they are calculated as starch and lead to high recoveries.

PREPARATION OF SAMPLES AND INSTRUCTIONS TO COLLABORATORS

Sample 1.—Prepared Mustard

This sample was made by adding commercial corn starch to a commercially prepared mustard. The starch assayed 84.89% starch (ave. of 6 determinations) by direct acid hydrolysis and subsequent estimation of dextrose by the Munson-Walker method. Starch was calculated as dextrose × 0.9. As prepared, the sample contained 4.41 per cent of 100 per

cent starch. Solids and acidity determinations were made by the Associate Referee and reported to the collaborators.

Instructions were to assay the sample by the method given in *This Journal* **30,** 75 (1947) on receipt of the sample, and again in one month. The mustard was to be held at room temperature during this period.

Sample 2.—Mustard Flour

A sample of commercially prepared mustard flour and one of corn starch were sent to collaborators with instructions to weigh 2.0000 g mustard flour and 0.2500 g starch (equivalent to 0.2122 g of 100% starch) and assay by the same procedure.

COLLABORATORS' COMMENTS

H. P. Bennett: "Difficulty was encountered in the resuspension of the starch in water after the first precipitation with 95% alcohol." The following slight change was introduced. "The alcohol was decanted from the starch mass after centrifuging without filtering thru asbestos. Glass beads were added to the bottle after the water was added and vigorous shaking for a few minutes carried. out. This suspended the starch very well and the subsequent extraction of the excess iodine with alkaline alcohol was expedited."

S. D. Fine: "We had some difficulty filtering the $CaCl_2$ solution of the starch thru a pledget of cotton. McNall suggested filtering thru a thin layer of cotton in a funnel, much as in the insoluble solids determination in preserves. This worked much better than the pledget in a funnel.

DISCUSSION

Comments of both collaborators are quite pertinent. The addition of glass beads to aid in breaking up the precipitated starch, as suggested by Bennett, had been tried independently by Joiner and the Associate Referee and found to give good results.

McNall's suggestion of filtering the calcium chloride solution thru a thin layer of cotton is a good one. This filtration is generally quite slow, since the solid material is slimy and readily clogs both paper and cotton.

The results reported by the collaborators are quite good. For mustard flour the average recovery (ave. of 12 determinations) is 99.7 per cent of the starch added, and for prepared mustard (ave. 20 determinations) 100.1 per cent. The standard deviations are not excessive, especially when consideration is given to the number of manipulations required by the method. Even the range (difference between maximum and minimum values) is reasonably good, and the range coefficient (defined as the quotient of the range and the arithmetic mean) does not exceed 7 per cent for either method.

There appears to be no loss of starch in prepared mustard when the mustard is allowed to stand at room temperature for one month. This is shown by the recoveries under Sample 1.

The Associate Referee is indebted to the following members of the Food and Drug Administration field laboratory staff: H. P. Bennett,

Kansas City, Mo.; S. D. Fine and F. J. McNall, Cincinnati, Ohio; D. M. Heller and C. R. Joiner, St. Louis, Mo.

RECOMMENDATIONS*

It is recommended that the present tentative method for starch in mustard flour and prepared mustard, with slight modification of wording† be adopted as official, first action, and further work on this subject be discontinued.

REPORT ON STARCH IN MAYONNAISE AND SALAD DRESSING

By SAM D. FINE (Food and Drug Administration, Federal Security Agency, Cincinnati, Ohio), *Associate Referee*

The present tentative method[1] for the determination of starch in mayonnaise and salad dressing was subjected to further study in accordance with the recommendations of the Referee on spices and condiments. A

TABLE 1.—*Mayonnaise containing 3.92% starch.*
(Calculated from weight of air dry starch and acid hydrolysis of starch)
Prepared March 31, 1947

ANALYST	DATE OF ANALYSIS	STARCH	RECOVERY
		per cent	*per cent*
S. D. Fine	4/ 1/47	3.85	98.2
	4/ 1/47	3.88	99.0
	4/ 1/47	3.84	98.0
	5/ 5/47	4.29	109.4
	5/ 5/47	4.04	103.1
F. M. Garfield	4/ 3/47	4.07	103.8
	4/ 3/47	4.09	104.3
	5/ 6/47	4.00	102.0
	5/ 6/47	3.94	100.5
D. M. Heller	4/ 9/47	4.07	103.8
	4/ 9/47	4.17	106.4
	5/ 9/47	3.97	101.3
	5/ 9/47	3.99	101.8
H. C. Van Dame	4/ 4/47	3.71	94.6
	4/ 4/47	3.72	94.9
	5/12/47	3.80	96.9
	5/12/47	3.79	95.7
		Ave. 3.95	100.8

* For report of Subcommittee C and action by The Association, see *This Journal*, 31, 55 (1948).
† The modifications are reported in *This Journal*, 31, 108 (1948).
[1] *This Journal*, 30, 74 (1947).

previous report[2] had suggested the possibility that losses in starch originally present in mayonnaise might occur on ageing.

EXPERIMENTAL

Two different starch containing mayonnaises were prepared and submitted for collaborative study with instructions to the collaborators to determine starch immediately on receipt and again at the end of a month. The mayonnaises were held at room temperature during this month.

Tables 1 and 2 give the results obtained on the two samples.

TABLE 2.—*Mayonnaise containing 4.10% starch*
(Calculated from weight of air dry starch and acid hydrolysis of starch)
Prepared April 2, 1947

ANALYST	DATE OF ANALYSIS	STARCH	RECOVERY
		per cent	*per cent*
S. D. Fine	4/ 3/47	3.85	93.9
	4/ 3/47	3.93	95.9
	5/ 5/47	4.01	97.8
	5/ 5/47	3.95	96.3
H. D. Van Dame	4/ 4/47	3.77	92.0
	4/ 4/47	3.84	93.7
	5/12/47	3.86	94.1
	5/12/47	3.82	93.2
		Ave. 3.88	94.6

DISCUSSION

The samples submitted for study were prepared by placing a weighed amount of air dry starch in a tared beaker and adding water. The material was heated on a steam bath until a smooth paste was formed. After cooling, egg yolk, spices, sugar, oil, etc., in known amounts were added while the contents were stirred with an electrical stirrer. The final weight was obtained, added water was calculated by difference, and the percentage of starch calculated. Each batch yield was approximately two pounds. The uniformity of the mayonnaise so prepared may not be as perfect as desired or as might be obtained in commercial practice. This may account for the spread obtained in the analytical results.

Consideration must be given to the complex nature of starch in evaluating the results obtained in the quantitative determination of this substance in foods. The precision obtained in the determination of a simple inorganic compound is not possible in the determination of a complex organic material such as starch. It is the belief of the author that the present method gives about the maximum precision obtainable in the estimation of starch in mayonnaise and salad dressing.

[2] Sam D. Fine, *Ibid.*, 27, 261 (1944).

The results obtained indicate that at the end of one month there is no detectable loss of the amount of starch originally present in a starch-containing mayonnaise.

SUMMARY

There is no detectable loss of starch from starch-containing mayonnaise, when the mayonnaise is held at room temperature for a month.

The precision of the present method is sufficient for the determination of starch in mayonnaise and salad dressing.

RECOMMENDATIONS*

It is recommended—

(1) That the present tentative method be adopted as official, first action, and that the study be dropped.

REPORT ON METALS, OTHER ELEMENTS, AND RESIDUES IN FOODS

By H. J. WICHMANN (Food and Drug Administration, Federal Security Agency, Washington 25, D. C.), *Referee*

Associate Referee's reports were received on copper and DDT. A contributed paper on mercury spray residues on apples was also read at the meeting.[1] The Referee's report will, therefore, be confined to these and related subjects.

COPPER

The Associate Referee reports the experience of his laboratory (one that has probably had more experience with copper methods than many others) with the all-dithizone copper methods. His report summarizes the advantages and disadvantages of each, and concludes with a recommendation for collaborative study of two methods with the greatest sensitivity, one a "two-color" and the other one a "one-color" method. The Referee approves this recommendation.

MERCURY

The Referee became well aware of the peculiar difficulties encountered when the Laug method for mercury was applied to apples, and the implications thereof. Little progress had been made for some years on the determination of mercury until the novel and unorthodox suggestion was made of using selenium, either as a catalyst as in Kjeldahl digestions or as a fixative, the reverse of the official selenium method, in the preparation of the sample. Thus, a rather good beginning of an entirely new approach to the mercury problem has been made, and the prospects are much brighter. The Referee recommends that strenuous efforts be made to perfect the

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* For report of Subcommittee C and action by The Association, see *This Journal*, 31, 55 (1948).

[1] Freda Kunze, "Determination of Mercury on Apples," see *This Journal*, p. 438.

new method of sample preparation for all kinds of organic materials, especially as applied to spray residues. The stripping method so successful in the past on all sorts of spray residues on fruits should be applicable to mercury residues. Other things being equal, the decided decrease in the amount of organic material that must be oxidized after a stripping should make for success.

The present dithizone method, for the actual determination of mercury, might be made even more sensitive by the Greenleaf "two-color" dithizone procedure, as developed in connection with the determination of copper. The Referee hopes that interest in the determination of mercury in organic matter will be intensified and that, as a result, more rapid progress will become possible.

DDT

The collaborative results on the determination of DDT reported by the Associate Referee are fairly good, with the two colorimetric methods producing the best results, the methods based on the determination of organic chlorine, either labile or total, being somewhat disappointing. The generally high results for the total chlorine method may stem from inability to successfully separate organic chlorine from inorganic chlorine, or from the insensitivity of the Volhard titration, especially in the presence of incompletely removed natural coloring matter. The labile-chlorine method may suffer from the difficulties just mentioned and, in addition, from the removal of more than one chlorine atom from some varieties of commercial DDT (o′ p′ fraction). The chloride methods are actually in use in all sorts of investigations and, in the case of some of the newer insecticides, are all that are available at the present time. Therefore, it is not expedient to recommend tentative adoption of the color methods and exclusion of consideration for the chloride methods on the basis of these first collaborative results. The Referee approves the Associate Referee's recommendation that the chloride methods be further studied, particularly with respect to the exclusion of inorganic chlorides, and to a more sensitive method for the determination of the chlorine ion. The Referee understands that the electrometric method is good when it works, but that the instrument sometimes gets out of order for some not readily determined reasons. Some suggestions along chemical lines have already been made. The Referee hopes that these will be followed up.

There is not too much information about interferences in the DDT determination, such as those caused by other chlorinated insecticides. Furthermore, no report on the determination of DDT in canned foods is being made. The Referee believes there is no demand for hasty adoption of tentative methods for DDT at the present time, since a new edition of the A.O.A.C. *Methods of Analysis* is not due for two years. All of these reasons impel the Referee to postpone the making of recommendations for tentative adoption of DDT methods for a year, in the hope that some of

the uncertainties of the determination in canned, as well as in other, foods will be clarified by studies to be expanded during the year.

BENZENE HEXACHLORIDE

At the present moment there exists a tremendous demand for a sensitive, accurate, and rapid method for the determination of benzene hexachloride ($C_6H_6Cl_6$) existing as spray residue on foods and forage crops, as a contaminant of soils by spray residue or by deliberate addition; in fatty foods and tissues by absorption; in biological material of all kinds, and in or on foods in general. The strong suspicion that commercial benzene hexachloride (BHC), or some of its impurities, can cause unpleasant flavors in foods, directly or by starting undesirable physiological processes, has increased this demand. The infra-red method, applicable to the insecticide itself, cannot be applied to the small amounts present in spray residues, contaminated as they are with all sorts of interferences, insecticidal and non-insecticidal. The recoveries with the colorimetric method, mentioned last year as being in the process of development, have been 80 to 90 per cent, and in a few instances, 95 per cent. The losses appear to occur just prior to or during the nitration of the trichlorbenzene derived from the benzene hexachloride. Also too little is known about interferences, and this method is not ready for publication. Another method based on the measurement of certain absorption peaks in the ultra-violet absorption spectrum of 1-2-4 trichlorbenzene (derived from benzene-hexachloride isomers in greater proportion than the other two possible trichlorbenzenes) has come to the attention of the Referee.[2] It is understood that it leaves something to be desired in the way of sensitivity and accuracy, and needs further development. A biological method,[3] with the house fly as the test animal, has been used with some degree of success for the determination principally of the gamma isomer of benzene hexachloride in biological material. All of these methods need further development and study of the effect of interferences before publication. This leaves the chlorine methods as the last recourse, but any recommendation for their use can be made only with a degree of hesitation and reserve in view of the numerous other chlorine-containing insecticides, hormones, or herbicides in use or in prospect. Within the next year a benzene hexachloride method may be developed, but the appointment of an Associate Referee would now be premature.

CHLORDANE AND TOXAPHENE

No definite methods for the determination of chlordane or toxaphene, other than those based on their chlorine content, has come to the attention of the Referee. The chlorine methods, of course, lack specificity. Suggestions for methods of analysis for these products in the form of

[2] Private Communication from B. Davidow, Pharm. Div., Food and Drug Administration.
[3] Private Communication from Edwin P. Laug, Pharm. Division, Food and Drug Administration

spray residues, or in biological materials of all kinds, from the manufacturers of these products, would be much appreciated. Undoubtedly, the Association will have to give attention to these products and their residues as soon as it can, but this can hardly be done before the determinations of DDT and benzene hexachloride are out of the way.

Hexaethyltetraphosphate (HETP) and diethylparanitrophenylthiophosphate (AATP)

Two new phosphorus-containing insecticides, under these names, have come to the attention of the Referee. They are said to be very toxic to both insects and warm-blooded animals. Hexaethyltetraphosphate, known to the Germans as "Bladan," is useful for the treatment of aphids and the mite tribe of insects in general. It is generally used as an adjunct to DDT. It is readily altered and loses its effectiveness as an insecticide in 24 hours. Apparently the decomposition products (not exactly known at present) do not possess any serious degree of toxicity for warm blooded animals. While HETP may have serious effects on careless sprayer crews, it is not expected to build up toxic spray residues. Hardin and MacIntire[4] report that hexaethyltetraphosphoric, triethylphosphoric, or monoethylphosphoric acids all require long treatment with aqua regia at the boiling point before they will hydrolyze completely to orthophosphoric acid. Whether reaction with alkalies is more expedient or rapid remains to be determined. A method for the determination of HETP and its decomposition products may be possible but not easy or rapid. Since its use is not expected to result in toxic residues, the Referee believes the Association can ignore it, at least for the present.

The Referee understands that diethylparaphenylthiophosphate (AATP) will do what HETP does and be effective for the codling moth besides. Hence it may be expected to have wider application. Fortunately the manufacturers of this compound have supplied a micro method[5] for its determination simultaneously with its commercial introduction. Hence there need be no time lost in development of a method. Perhaps the Referee will be in a position to assess the extent of the commercial usage of AATP by the next meeting and be prepared to recommend collaboration on a method for its determination.

RECOMMENDATIONS*

It is recommended—

(1) That the work on the determination of cadmium, copper, mercury, and zinc be continued.

(2) That the determination of DDT in canned foods and in foods in general be studied further.

[4] *This Journal*, present number, p. 400.
[5] Unpublished; obtainable from American Cyanamide Co., Stamford, Conn.
* For report of Subcommittee C and action by The Association, see *This Journal*, 31, 52 (1948).

REPORT ON COPPER IN FOODS

By G. H. BENDIX (Continental Can Company, Inc., Chicago, Ill.), *Associate Referee*

Collaborative work on copper in foods was last conducted by Greenleaf (1) in 1945, at which time he described results obtained by a combined dithizone-carbamate method. Greenleaf recommended that the dithizone-carbamate method be adopted as tentative, but he also pointed out that serious consideration should be given to the development of an all-dithizone method. In recent years several all-dithizone methods have appeared in the literature (2, 3, 4). The earliest of these is that of Bendix and Grabenstetter, which is a single color method, *i.e.* the violet copper dithizonate is separated from the green uncombined dithizone before determining the optical density. The second method, that of Morrison and Paige, is essentially the same as that of Bendix and Grabenstetter except that the excess dithizone is not removed, the spectrophotometric measurements being made on a two-color system. The most recent publication, that of Greenleaf, is also a "two-color" method and avoids objectionable features of the two other methods just mentioned.

In his 1946 report on metals in foods Wichmann (5) commented on the advantages of the Greenleaf "two-color" method and recommended it to the Associate Referee.

The method of Bendix and Grabenstetter is subject to two criticisms: (a) The extraction of copper, being carried out at a *p*H of 2.3 and in the presence of a high concentration of dissolved salts, requires an excessively long shaking time of 10 minutes and thereby necessitates a mechanical shaker unless only a few determinations are to be made. (b) Removal of the excess dithizone by extraction with dilute ammonia subjects the violet keto copper dithizonate to conversion to the yellow enol tautomer. This fact has been pointed out by Sandell (6) and by Morrison and Paige. The latter authors avoid the second of these objections by omitting the extraction with ammonia, thereby making the method a "two-color" method. This change, although it also simplifies the laboratory manipulations, is accompanied by a very appreciable decrease in sensitivity.

The Greenleaf method avoids both of the above mentioned objections to the Bendix-Grabenstetter method and does so without a sacrifice of sensitivity which characterizes Morrison's method. This accomplishment, however, was not achieved without a sacrifice of the simplicity common to the other two methods.

Although collaborative work has not been conducted during the past year, work has been carried out in the Associate Referee's laboratories for the purpose of comparing, one with the others, the all-dithizone methods of Bendix-Grabenstetter, Morrison-Paige, and Greenleaf. Table 1 shows the results obtained.

TABLE 1.—*Comparison of all-dithizone methods for copper in foods*

SAMPLE	BENDIX-GRABENSTETTER	MORRISON-PAIGE	GREENLEAF
	p.p.m.	*p.p.m.*	*p.p.m.*
Dried Peas	34.9 35.2	31.5 32.4	30.9
Dried Corn	14.2 14.0	14.5 13.8	14.0
Chocolate Ovaltine	14.7	—*	13.8
Pea brine A (Canned)	1.09 1.09	1.10 1.10	—*
Pea brine B (Canned)	1.15 1.15	1.15 1.15	—*

* No analysis made.

DISCUSSION OF RESULTS

The results of Table 1 show no significant difference in the results obtained by the three methods and indicate that the objections to the Bendix method (conversion to enol tautomer) and the Morrison method (lack of sensitivity) are not as significant as might be expected. This fact may not be capable of duplication by other laboratories, since it must be admitted that the Associate Referee's laboratory has extensive experience with the Bendix method and may have avoided the objectionable features by carefully controlling the conditions during the extraction of excess dithizone with dilute ammonia.

The various advantages and disadvantages of the three methods are given in the summary below, from which it may be noted each method has certain desirable characteristics. The method of Greenleaf, however, appears to more nearly approach the ideal of a referee method.

A COMPARISON OF THE CHARACTERISTICS OF ALL-DITHIZONE METHODS FOR COPPER IN FOODS

Specificity.—Little difference between the three methods. All methods probably satisfactory for most food products. All methods subject to interference by excessive concentrations of bismuth and noble metals.

Sensitivity.—Morrison method appreciably inferior to those of Bendix and Greenleaf, the latter two having a very high sensitivity.

Simplicity.—Greenleaf method considerably more complicated with respect to technique and reagents required.

Theoretical Soundness.—Greenleaf method is best with the Morrison method a close second best. Bendix method open to question with respect to the one color technique.

The Associate Referee is indebted to Mr. W. C. Stammer and his associate, Mr. N. H. Strodtz, for conducting the analyses reported above and for their comments relative to the methods under consideration.

RECOMMENDATIONS*

It is recommended that the "two-color" method of Greenleaf and the "one-color" method of Bendix and Grabenstetter be submitted to collaborative study.

LITERATURE CITED

(1) GREENLEAF, C. A., *This Journal*, **28**, 269 (1945)
(2) BENDIX, G. H., and GRABENSTETTER, D., *Ind. Eng. Chem., Anal. Ed.*, **15**, 649 (1943).
(3) MORRISON, S. L. and PAIGE, H. L., *Ibid.*, **18**, 211 (1946).
(4) GREENLEAF, C. A., *This Journal*, **30**, 51 (1947).
(5) WICHMANN, H. U., *This Journal*, **30**, 451 (1947).
(6) SANDELL, E. B., "Colorimetric Determination of Traces of Metals," pp. 84, 218, *Interscience Publishers*, New York, 1944.

REPORT ON DDT IN FOODS

By R. H. CARTER (Agricultural Research Administration, Bureau of Entomology and Plant Quarantine, Beltsville, Md.), *Associate Referee*

In 1946 the Associate Referee presented a report (1) reviewing the methods which had been recommended or presented for the determination of DDT in insecticide residues and in animal products. Methods for the preparation of samples were discussed briefly. No samples were sent out for collaborative testing.

In 1947 an apple-strip solution containing a known added amount of DDT was prepared, and identical samples were submitted to a number of collaborators who had expressed a willingness to take part in this program. This solution was prepared as follows:

Samples of apples weighing approximately 2 kg. were placed in 2½-gallon wide-mouth pickle jars, 500 ml of benzene was added to each, and the jars were rotated sidewise in a tumbling machine at approximately 4 r.p.m. for 30 minutes. This method of stripping does not bruise the apples excessively, but imparts enough rolling motion with a slight abrasive action to insure thorough wetting of the surfaces and solution of the wax and DDT. At the end of the 30-minute period the machine was stopped, the samples were allowed to drain for a few minutes, and the benzene solution was decanted, the amount measured and then calculated as an aliquant of the original 500 ml. Approximately 10 liters of this strip solution was made. No DDT-free apples were available; so a number of analyses were made to determine the DDT content of this solution. To this solution was then added enough pure *p, p'*-DDT and *o, p'*-DDT in the

* For report of Subcommittee C and action by The Association, see *This Journal*, **31**, 52 (1948).

ratio of 3 to 1 to make a total content of 17.12 mg. per 500 ml; the volume also was adjusted so that each 500 ml represented 2 kg. of apples. Seventeen samples of 500 ml each were then sent out to the collaborators. The method of analysis to be used was left to the choice of the analyst. Unfortunately two samples were lost in transit.

Results of the analyses by four methods with modifications are shown in Table 1.

TABLE 1.—*Results of analyses of apple-strip solutions for DDT by different methods (DDT present, 8.6 p.p.m.)*[1]

COLLABORATOR	LABILE-CHLORINE METHOD[2]	TOTAL-CHLORINE METHOD[3]	COLORIMETRIC METHODS	
			SCHECHTER-HALLER	STIFF-CASTILLO
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*
A	—	8.7*	—	—
B	—	—	8.5	—
C	—	—	—	8.3
D	9.7	7.1	7.8	7.8
E	—	—	—	8.3
F	15.0	10.7		
G	—	8.4 8.2*		
H	14.9	12.4		—
I	—	—	—	9.1
J	—	9.9	—	8.7
K	—	10.9 8.2*	8.6	
L	10.4	11.1	—	
M	10.6	8.6	—	
N	8.8	8.3	—	8.4
Average	11.6	9.5	8.3	—

[1] Results reported are averages of 2 to 5 determinations.
[2] Collaborator N used procedure 1 B, all others used 1 A, All used Volhard titration.
[3] Collaborators A, G, and N used procedure 2 B, all others used 2 A. Asterisk (*) indicates determinations by electrometric titration; all others by Volhard titration.

A summary of the results reported by the four methods is given in Table 2.

TABLE 2.—*Summary of the results reported in analysis of collaborative apple strip solution No. 1, containing 8.6 p.p.m. of DDT*

METHOD OF ANALYSIS	NUMBER OF COLLABORATORS	DDT REPORTED (P.P.M.)		
		MINIMUM	MAXIMUM	AVERAGE
Labile chlorine	6	8.8	15.0	11.6
Total chlorine	10	7.1	12.4	9.5
Schechter-Haller colorimetric	3	7.8	8.6	8.3
Stiff-Castillo colorimetric	5	7.8	9.1	8.4

The collaborators were as follows:

Food and Drug Administration, Federal Security Agency:
Robert H. Dick, L. W. Ferris, Sam D. Fine, Curtis R. Joiner, Harold F. O'Keefe, C. D. Schiffman, Shirley M. Walden, Louis C. Wiess.

Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture:
P. E. Hubanks, L. Koblitsky, H. D. Mann, W. E. Westlake.

California State Department of Agriculture:
J. B. LaClair.

Maine Agricultural Experiment Station:
A. Stanley Getchell.

DISCUSSION

Most of the results reported by the labile-chlorine method were obtained by following the procedure (referred to as 1A) recommended by Wichmann *et al.* (2), in which 350 ml. of the benzene strip solution is treated with 10 ml of 2 *N* alcoholic potassium hydroxide, 250 ml of the benzene solution is then distilled off, the remainder of the solution is refluxed for 30 minutes, and the chloride is determined by standard procedures.

In another procedure (2) (referred to as 1B) for labile chlorine the benzene is distilled off and then 1 *N* alcoholic alkali is added and the solution is refluxed for 10 minutes. The latter procedure appears to give much better results, but even 1 *N* alcoholic alkali gives results considerably too high on some commercial samples of DDT.

The labile-chlorine method has been reported by a number of investigators as being the simplest and easiest for the determination of DDT spray residues on fruit. It is apparently in extensive use in some of the principal apple-producing regions.

Some of the determinations of total organic chlorine were made by the method recommended by Wichmann *et al.* (2). In this procedure (referred to as 2A) the benzene strip solution is refluxed with metallic sodium and a small amount of isopropanol. In another procedure (referred to as 2B) benzene is distilled off on a steam bath and the residue is then refluxed with 25 to 50 ml of isopropanol and metallic sodium. The results reported by this procedure generally appear to agree with the known amount much better than do the results by the former procedure.

Determinations of both labile chlorine and total organic chlorine are rapid and well adapted for routine work. The latter are more accurate for small samples because of the larger amount of chlorine to be determined.

High results in the analysis of the strip solution were reported in many determinations by the labile-chlorine method and to a lesser extent by the total-chlorine method.

An outstanding difficulty in the chlorine determinations is the lack of sensitivity of the indicator, so that the analysts are unable to see the end point in the Volhard back titration with thiocyanate. This is especially

true in colored solutions. Electrometric titration apparatus obviates this difficulty, but trouble has been reported by some operators in using such apparatus.

The colorimetric methods developed by Schechter *et al.* as recommended by Wichmann *et al.* (2), and by those of Stiff and Castillo as recommended by Claborn (3), appeared to give consistent results in the analysis of the standard strip solution. Measurements of the color developed were reported as being made by neutral wedge photometers, photoelectric photometers equipped with filters, and spectrophotometers. These methods are of course subject to some interferences, but in general they can be regarded as confirmatory for the presence of DDT.

RECOMMENDATIONS*

(1) It is recommended that a thorough study of the labile-chlorine method be made to determine the optimum conditions, time, temperature, strength of alcoholic alkali, and other factors, including the kinds of agricultural products, such as fruit, vegetables, forage crops, animal products, and soil, to which it is applicable.

(2) It is recommended that a study of the total-chlorine method be continued to determine optimum conditions, such as time, amount of benzene to be left in the flask during refluxing, and other factors, and of the kinds of agricultural products to which it is applicable.

(3) It is recommended that an investigation be made of other methods for the determination of small amounts of chloride.

(4) It is recommended that an investigation be made to determine other insecticide residues that may interfere with the Schechter-Haller and the Stiff-Castillo colorimetric procedures.

LITERATURE CITED

(1) CARTER, R. H., *This Journal*, **30**, 456 (1947).
(2) WICHMANN, H. J., PATTERSON, W. I., CLIFFORD, P. A., KLEIN, A. K., and CLABORN, H. V., *Ibid.*, **29**, 188 (1946).
(3) CLABORN, H. V., *Ibid.*, **29**, 330 (1946).

No reports were given on cadmium, zinc, mercury, or DDT in canned foods.

REPORT ON OILS, FATS, AND WAXES

By J. FITELSON (Food and Drug Administration, Federal Security Agency, New York, N. Y.), *Referee*

The Associate Referees have made some progress in their studies, but no formal reports will be submitted. The chromatographic technique for purification of unsaponifiable matter[1] has been studied as an alternative

* For report of Subcommittee C and action by The Association, see *This Journal*, 31, 51 (1948).
[1] N. D. Sylvester, A. N. Ainsworth, and E. B. Hughes, *Analyst*, 70, 295 (1945).

method to the somewhat lengthy official S.P.A. washing procedure **(31.40)**. Results show very close agreement between the two methods, and collaborative studies are planned for the coming year.

The tentative modified Bellier Test **(31.47–31.48)** has been applied to mixtures of peanut oil and other edible vegetable oils. Preliminary results indicate that an approximately quantitative relationship exists between the turbidity temperature and the peanut oil content. The Associate Referee plans to continue studies in this field.

The recently appointed Associate Referee on antioxidants has reviewed the extensive literature on this subject and has also initiated a survey of the field to ascertain the nature of the most commonly used antioxidants. In view of the large number of compounds used for this purpose, it is planned to restrict the initial studies to the most widely used antioxidants.

RECOMMENDATIONS*

It is recommended:

(1) That the official S.P.A. method for unsaponifiable matter (sec. **31.40**) be made official, final action.

(2) That the chromatographic purification of the unsaponifiable matter (1) be studied collaboratively.

(3) That the F.A.C. method for unsaponifiable matter (**31.37–31.39**) be deleted (final action).

(4) That the official, first action, method for squalene **(31.41–31.43)** be made official, final action.

(5) That studies on methods for determining the stability of fats be discontinued for the present.

(6) That studies on methods for the estimation of peanut oil be continued.

(7) That studies on methods for the determination of antioxodants in oils be continued.

No reports were given on unsaponifiable matters, peanut oil, stability of fats, or antioxidants; see Referee report on oils, fats, and waxes.

REPORT ON SOILS AND LIMING MATERIALS

By W. H. MacIntire (University of Tennessee Agricultural Experiment Station, Knoxville), *Referee*

The Referee has functioned mainly in suggestive capacity in obtaining the digests and contributions from the Associate Referees whose reports will be tendered individually. The one exception is the work stipulated in recommendation (5), "That the analytical technique previously pro-

* For report of Subcommittee C and action of the Association, see *This Journal*, 31, 53 (1948).

posed for ignition of charge and distillation of fluorine therefrom be studied collaboratively." (*This Journal*, **30**, 43).

A study of that technique resulted in findings to the effect that the prescribed extended calcination of the charge of soil and calcium peroxide at 900°C. is unnecessary and its deletion will be recommended. It was found also that substitution of calcium hydroxide for calcium peroxide is not admissible in calcinations at 900°C., unless the proportion of the hydroxide to soil be at least 10 to 1. The substitution of calcium hydroxide in 1 to 1 proportion was found effective, however, provided the prefatory calcinations of the charges of soil plus calcium hydroxide are conducted at 500°C. for periods in the range of from 5 to 60 minutes.

RECOMMENDATIONS*

It is recommended—

Soils:

(1) That the studies on the "combination dithizone-spectrographic method," and on the polarographic procedure for the determination of zinc in soils, be continued.

(2) That the study of the determination of copper in soils be continued.

(3) That the utilization of carmin as an indicator in the determination of boron content of soils be studied further, and that p-nitrobenzeneazo-1, 8-dihydroxynaphthalene-3, 6-disulfonic acid, or "chromotrope-B," be studied as a reagent suitable for the determination of boron on soils.

(4) That further studies of *p*H in soils of the arid and semi-arid regions be based upon soil systems of moisture content representative of air-dry soil.

(5) That the analytical technique previously proposed for ignition of charge and distillation of fluorine therefrom be studied collaboratively.

(6) That the present wording "at 900° for 30 minutes," under Fluorine (p. 13, section **1.32,** line 3) be made to read "at 500° for 30 minutes."

(7) That a study be made as to the adequacy of $Ca(OH)_2$ as a fixative for fluorine in soil charges of 1 to 1 proportion with calcination at 500°C. in 5 to 60 minute periods.

(8) That the direct distillation of *unignited* soil with H_2SO_4 at 165°C., with sequential distillation of an aliquot at 135°C., be studied collaboratively.

(9) That the "2-point" titration procedure for the determination of exchangeable H in soils be studied further, in relation to liming practice.

(10) That the survey and comparisons of methods for the determination of phosphorus (a) that fraction in "available" state and (b) the proportions of organic-inorganic forms therein (*This Journal*, **30**, 43), be continued.

(11) That the survey and comparisons of methods for the determination of "exchangeable" K in soils (*This Journal*, **30**, 44), be continued.

* For report of Subcommittee A and action of the Association, see *This Journal*, 31, 43 (1948).

Liming materials:

(12) That the direct titration against bromcresol green (Method II), *This Journal*, **30**, 297 (1947) be adopted as optional for the determination of the neutralization value of blast furnace slags.

(13) That the tentative procedures in "Liming Materials" be annotated by the statement, "without correction for sulfide content."

(14) That correction for sulfide sulfur content be studied further.

REPORT ON ZINC AND COPPER IN SOILS

By Lewis H. Rogers (Department of Soils, Florida Agricultural Experiment Station, Gainesville, Fla.), *Associate Referee*

Additional experience has been obtained with the dithizone-spectrographic procedure for the determination of zinc extracted from soils by various extractants. Details are presented below.

The procedure of Sandell[1] has been adapted for use with soil extracts. Reagents are prepared essentially as given by Sandell and also in section **12.24,** page 123, *Methods of Analysis*, sixth edition. In addition a standard cadmium solution containing 200 gamma cadmium per ml is prepared; further dilutions are prepared daily. Also a cadmium-dithizonate standard in CCl_4 containing 1 gamma cadmium per ml is prepared.

The soils are extracted with ammonium acetate or other extracting agent in the conventional manner. The leachate is evaporated to dryness, organic matter destroyed with 30% hydrogen peroxide, the residue taken up in a minimum of dilute hydrochloric acid, the solution diluted to 30 ml and an aliquot transferred to a 60 ml separatory funnel. Five ml of 10% sodium citrate solution are added, several drops of purified phenol red indicator solution introduced, and the solution titrated with dilute (1:3) ammonium hydroxide to about *p*H 8, and an excess of 0.5 ml is added. This is extracted with successive portions of 0.01% dithizone in carbon tetrachloride until no further color change is obtained with the dithizone, then one additional extraction is made. Two ml of standard cadmium dithizonate solution are added to the extracted dithizonates, evaporated to dryness on a hot plate at low temperature while air is blown into the beaker to hasten evaporation. The residue is taken up with a minimum of redistilled chloroform and transferred with a micro dropper to a graphite electrode which is heated in a small metal block resting on a hot plate. Graphite electrodes, $\frac{1}{4}$in.$\times$1 in., with a shallow crater drilled in the lower electrode and upper electrodes pointed in a pencil sharpener, are used.

Spectrograms were taken on a quartz spectrograph with a slit width of 40 microns. The image of the D.C. arc was focused on the collimator with

[1] "Colorimetric Determination of Traces of Metals," Interscience Publishers, p. 458 (1944).

a lens located at the slit of the spectrograph. A rotating step sector with 1:1.5 ratio was used for plate calibration. An iron spectrum was made on each plate with the step sector running; preliminary and final plate calibration curves were plotted as described by Churchill.[2] Background and line intensities were determined from the plate calibration curves in the conventional manner. Eastman process plates, developed in D-19 at 70°F. for 3 minutes and fixed in F-10 were used. Plates were evaluated with a non-recording microphotometer. An analytical working curve was prepared from several independent determinations of known amounts of zinc, with constant amounts of cadmium as internal standard. Working curves were prepared using the zinc line at 3282 A. with the cadmium line at 3261 A. as internal standard.

Preliminary work has shown that with this procedure it is feasible to determine copper and zinc simultaneouly, since copper is concentrated by dithizone under the same conditions as zinc and gives suitable spectrum lines which can be conveniently measured simultaneously with zinc.

It is recommended* that the study be continued.

PRELIMINARY REPORT UPON THE COLLABORATIVE WORK ON THE EXCHANGEABLE POTASSIUM IN SOILS

By IVAN E. MILES (North Carolina Department of Agriculture, Raleigh, N. C.), *Associate Referee*

Realizing that there was some variance of opinion in regards to methods ordinarily used in determining exchangeable potassium, the officials of *A.O.A.C.* have this year initiated some work in an effort to secure a more complete picture of this situation. Several leading laboratories of the country were circularized as to their willingness to assist with a collaborative study of exchangeable potassium. Every worker contacted very graciously agreed to assist.

Subsequently, soils samples with extreme variance in texture, reaction, organic matter, and exchangeable potassium were collected. Two soils came from Arizona, one from New York, one from Tennessee, one from the Piedmont area of North Carolina, and one from the Coastal Plain area of North Carolina.

These soils were air-dried, screened, and mixed thoroughly, and identical samples sent to all collaborators: Brown, Irving G., in Reitemier's Laboratory, U.S.D.A. Caster, A. B., in McGeorge's Laboratory, University of Arizona. Capp, L. C., in Fudge's Laboratory, Texas Agricultural and Mechanical College. Lee, C. K., in Bray's Laboratory, University of Illinois. Mehlich, A., in Research Laboratory, N. C. State College. Piland, Rodney, in Research Service Laboratory, N. C. State College. York, E. T.,

[2] *Ind. Eng. Chem., Anal. Ed.*, 16, 653 (1944).

* For the report of Subcommittee A and action of The Association, see *This Journal*, 31, 43 (1948).

TABLE 1.—*Potassium collaborative results*

All Data Reported as m.e. of Potassium per 100 grams of Soil

SOILS	COLLABORATORS												
	A[1]	B[1]	C[1]	D[1]	E[2]	F[3]	G[4]	H[5]	C-1[6]	AVE.	C-2[7]	E-1[8]	AVE.
1	1.432	1.055	1.064	1.210	1.270	1.019	1.043	1.469	1.120	1.187	1.025	1.440	1.233
2	3.829	3.727	4.161	3.480	3.380	3.599	3.696	4.636	4.110	3.846	3.080	3.960	3.520
3	.247	.120	.173	.155	.161	.117	.131	.246	.169	.169	.165	.190	.178
4	.205	.106	.128	.180	.147	.069	.109	.221	.118	.143	.133	.117	.125
5	.196	.061	.095	.107	.124	.043	.071	.198	.091	.110	.097	.114	.106
6	.286	.142	.176	.179	.197	.067	.146	.263	.190	.183	.165	.156	.161
Ave.	1.033	.869	.966	.885	.880	.819	.866	1.172	.966		.778	.996	

[1] Ammonium acetate—Volumetric—Potassium Permanganate—U.S.D.A. Circular 757 (1947).
[2] Ammonium acetate—Volumetric—Ceric Sulphate—*Ind. Eng. Chem. Anal. Ed.*, 10, 652 (1938).
[3] Ammonium acetate—Gravimetric—A.O.A.C. and *Ind. Eng. Chem., Anal. Ed.*, 9, 136 (1937).
[4] Ammonium acetate—Colorimetric—Nitroso-R-Salt—*Soil Science Soc. Proc.*, 9, 56–60 (1944).
[5] Ammonium acetate—Colorimetric—Nitroso-R-Salt—*Soil Science*, Vol. 59, 25–37 (1945).
[6] Ammonium acetate—Turbidimetric—(After removing excess ammonium acetate) Mimeograph Directions, Ill. Agri. Exp. Sta. (1942).
[7] Sodium nitrate—Turbidimetric—Mimeograph Directions, Ill. Agri. Exp. Sta. (1942).
[8] Barium-chloride—Triethanol amine *N*—*Soil Science Soc. Proc.*, Vol. 9 56–60 (1944).

in Peech's Laboratory, Cornell University, New York. Robinson, Brooks, in MacIntire's and Shaw's Laboratory, University of Tennessee.

The collaborators were requested to use whatever method they normally use, but if the method used is other than the ammonium acetate method, to use the ammonium acetate method also for comparative purposes.

The methods used by the collaborators can be divided into two general categories, namely: 1, extraction and 2, leaching. In the first method, extraction was made with ammonium acetate and the K precipitated as sodium cobalti-nitrite in all cases. Most collaborators used an extracting procedure similar to that described in U.S.D.A. Circular 757. Two investigators used a leaching technique. After precipitating with sodium cobalti-nitrite, four investigators determined K volumetrically with potassium permanganate, whereas one investigator used ceric sulphate procedure. Two investigators determined the K colorimetrically, using nitroso-R-salt, and in one case the K was determined turbidimetrically. One investigator precipitated the K with sodium cobalti-nitrite and determined the K gravimetrically.

From the results obtained (see Table 1) it would seem that any one of the several methods used might be satisfactory in the high range, but there is very considerable variance in the lower or critical range. This would seem to indicate that further work is needed on methods.

Therefore, the writer would recommend* that the work be continued, enlarging it to include other workers. Furthermore, it now seems advisable to have all collaborators use both a leaching and extracting method, and determine K by a volumetric, colorimetric, turbidimetric, or flame photometry procedure.

The writer gratefully acknowledges and appreciates the fine work done, and the spirit of helpful cooperation manifested by all collaborators throughout the study.

No reports were given on hydrogen-ion concentration of soils, boron and fluorine, exchangeable calcium and magnesium, exchangeable hydrogen, or phosphorus in soils. For report on boron under "Plants," see p. 284.

REPORT ON INSECTICIDES AND FUNGICIDES

By J. J. T. GRAHAM, Chemist (Production and Marketing Administration, Livestock Branch, Beltsville, Md.), *Referee*

Consideration of methods of analysis for insecticides, fungicides, and other economic poisons during 1947 was limited to a study of those applicable to rodenticides, products containing DDT, and a preliminary investigation of methods for pyrethrins in pyrethrum flowers. Because of

* For report of Subcommittee A and action by the Association, see *This Journal*, 31, 43 (1948).

pressure of their official duties, the Associate Referees on fluorine and nicotine were unable to study methods for determination of these ingredients. The General Referee has examined the reports of the Associate Referees on rodenticides and DDT and concurs in their recommendations.

METHODS FOR THE DETERMINATION OF PYRETHRINS IN PYRETHRUM FLOWERS

Considerable disagreement with methods for the determination of pyrethrins in pyrethrum flowers has arisen between growers of pyrethrum and the purchasers of the flowers in this country, because results for pyrethrin content obtained in the country of origin have differed materially from those obtained in some laboratories in this country. This difference is principally due to the fact that the pyrethrum growers made their analyses by the official mercury reduction method, while the analyses made for the purchasers were in most instances made by the Seil method.[1]

In order to improve this situation, the Imperial Institute of Great Britain sent representatives to this country, in the latter part of 1946, to solicit cooperation of American chemists in a world-wide study of methods for determination of pyrethrins, with a view to adoption of a uniform procedure for this determination.

As a result of this solicitation, the Chemical Analysis Committee of the National Association of Insecticide and Disinfectant Manufacturers promised the cooperation of that organization; and your Referee promised to solicit the help of official chemists.

The Consultative Committee on Insecticide Materials of Vegetable Origin, of the Imperial Institute, planned the investigation, and it appeared desirable to conduct a preliminary study on variations of the methods in order to decide on the best procedures to be followed in the final tests. A number of modifications of the mercury reduction method were suggested and studied.

The Referee assisted in this preliminary work and further assisted by reviewing and commenting on the revised procedures. A recent letter from Dr. G. T. Bray of the Imperial Institute states that their committee has decided to study the mercury reduction method, the Seil method, and the Ripert method. The latter method was included because it is the principal method used in France.

It is not yet possible to state when the samples will be ready for distribution. However, those chemists who have signified their desire to assist in the work will be kept informed of developments in connection with the investigation. If there are other members of the Association who desire to volunteer their assistance in this work, the Referee will be glad to have their names.

[1] *Soap*, **10**, 89 (1934).

REPORT ON RODENTICIDES

By J. W. ELMORE, (State Department of Agriculture, Sacramento, Calif.), *Associate Referee*

Methods of analysis of rodenticides containing 1080 (sodium fluoroacetate) and Antu (alpha naphthyl thiourea) have been under consideration.

Reactions specific for 1080 have not come to the writer's attention. Total fluorine can be determined in this compound by fusion with sodium peroxide in a Parr bomb followed by precipitation of lead chlorofluoride, as described in the official procedure for fluorine in insecticides. (*Methods of Analysis*, Sixth Edition, page 58 et seq., sec. **6.18, 6.19**). Analysis of a sample of technical 1080 by this method, gave 93.75 per cent. Since determination of total fluorine in grain poisoned with 1080 involves ashing with lime or some other fixative, it was thought possible that some fluorine might be lost during ignition, due to a reaction similar to that which occurs on heating calcium acetate giving acetone and calcium carbonate:

$$(CH_3COO)_2Ca = CH_3COCH_3 + CaCO_3$$

In the case of sodium fluoroacetate, this might be:

$$2\ CH_2FCOONa = CH_2FCOCH_2F + Na_2CO_3$$

However, no evidence of this reaction has been observed. Possibly, fluorine is replaced by the hydroxyl group with the formation of glycollic acid and sodium fluoride similarly to the reaction which takes place when monochloracetic acid is heated with water or alkalies. (*Richter Organic Chemistry*, 1944 Edition, Vol. 1, page 334). Analysis of the above sample of 1080 by drying with lime hydrate slurry and distillation of the ash with perchloric acid gave 93.70 per cent of 1080, of which compares well with results by Parr bomb.

Grains poisoned with 1080 may be analyzed for total fluorine by the general method for fluorine in foods (*Methods of Analysis*, Sixth Edition, page 445, sections **29.24–29.28** incl.) and the result calculated to sodium fluoroacetate. Results so obtained are somewhat high because of the fluorine originally present in the grain, or possibly due to contamination with sodium fluosilicate used for repelling rats and mice in warehouses. Since baits may contain as little as 0.05 per cent of 1080 (corresponding to 0.0095 per cent of fluorine), a considerable error may thus be introduced. Results of analyses of corn and wheat listed by Clifford (*This Journal*, **28**, 278, 1945) indicate about 1 part per million fluorine is to be expected in grains; but local samples analyzed by the writer were considerably higher than this. Further work should be done along this line.

Total combined fluorine in 1080 poisoned grain may be determined on the finely ground sample by weighing 4 grams into a platinum dish and

continuing the analysis as directed in the general method (section **29.26 (a)**) beginning "add 25 ml of the $Ca(OH)_2$ suspension."

The solubility of Antu (alpha naphthyl thiourea) in several solvents was determined.

Approximate solubility of Antu, grams per 100 ml solution

SOLVENT	25°C.	100°C.
Acetone	2.43	—
Acetonitrile	1.18	—
Chloroform	0.63	—
1,4-Dioxane	6	30
Ethyl alcohol	0.70	—
Ethyl ether	0.18	—
Petroleum ether	0.001	—
Triethylene glycol	8.6	16
Water	0.006	—

The pure compound contains nitrogen (13.86 per cent) and sulphur (15.84 per cent). Examination of commercial products received to date has shown a higher percentage of Antu calculated from sulphur than from nitrogen. Representative analyses by J. B. LaClair of this laboratory are as follows:

	Antu calculated from nitrogen	*Antu calculated from sulphur*
Sample A	94.44	103.07
Sample B	97.04	100.55

In routine examination of samples of Antu for purity, it would therefore seem preferable to determine total nitrogen rather than total sulphur.

The insolubility of Antu in water renders the application of common reactions of thiourea difficult. Attempts were made to carry out the oxidation method of St. Skramovsky (*Chemical Abstracts*, **38**, 2288) in some other solvent, but without success.

Examinations of prepared Antu rat poisons have been carried out in the following manner:

Weigh an amount of sample equivalent to approximtely 0.2 gm Antu and transfer to a 250 ml Erlenmeyer flask. Add 50 ml acetone and digest or reflux for 15 min. Filter and wash with acetone. Transfer filtrate to a 500 ml Kjeldahl flask. Place on steam bath and evaporate acetone with an air stream. Determine total nitrogen in the residue by Kjeldahl method and calculate to alpha naphthyl thiourea, using the factor: 7.215 times percentage nitrogen equals percentage Antu.

If much greasy material is present, the sample may be digested with a little petroleum ether, filtered on a dry Gooch crucible and washed with petroleum ether. The residue is then heated to 100°C. to remove petro-

leum ether and the analysis carried out as indicated above beginning "transfer to a 250 ml Erlenmeyer flask."

In case the Antu has been incorporated in material insoluble in acetone, such as starches or sugars, it is advisable first to digest with about 25 m of water, after which 150 to 200 ml of acetone are added and the analysis continued as above beginning "digest or reflux for 15 minutes."

It is recommended* that more specific methods be sought for determination of 1080 and Antu in rodenticides.

REPORT ON DDT

By ELMER E. FLECK (Agricultural Research Administration, Bureau of Entomology and Plant Quarantine, Beltsville, Md.), *Associate Referee*

During the past year several new methods and modifications of existing methods for the determination of DDT have been reported. A new colorimetric method for the determination of *p,p'*-DDT has been reported by Bradbury, Higgons, and Stoneman (1). This method is based upon the wine-red color developed by heating *p,p'*-DDT, diethyl sulfate, hydroquinone, and concentrated sulfuric acid. These authors found no interference from *o,p'*-DDT or TDE, but the dehydrochloro derivatives do interfere if present in larger amounts than 5 per cent. The method is sensitive to 0.1 mg of DDT, and the over-all accuracy was 1 per cent. This method is recommended by the authors for use in determining the *p,p'*-DDT content of technical DDT. It has the advantage of being simple and rapid in operation.

Iling and Stephenson (2) have published two variations in the production of color from nitrated DDT. One method provides for the reduction of the nitro groups using hydroxylamine to give an orange color, and the other produces a plum color by the addition of 5 per cent of potassium hydroxide in acetone-methyl alcohol solution. The effect of the compounds usually encountered in DDT other than the *p,p'*-isomer was not discussed.

A new approach to the problem of determining the *p,p'*-DDT content of technical DDT, using solubility methods, has been made by Thorp (3). Small increments of technical DDT were added to a given volume of solvent, and the mixture was then shaken at 25°C. until equilibrium conditions were established. The total amount of DDT added after each increment was then plotted as an abscissa, the amount in solution at each stage of the addition was plotted as an ordinate. As long as all the DDT dissolves, a straight line is obtained. As the solution becomes saturated with any one component, the slope of the line is changed. A similar change in slope occurs as the solution becomes saturated with each component of

* For report of Subcommittee A and action of the Association, see *This Journal*, 31, 42 (1948).

the technical DDT. When the solution is saturated with respect to all components, a line parallel to the abscissa is obtained. The resulting series of straight lines is projected to the ordinate. From the ratio of the lengths of the segments of the ordinate so obtained it is possible to calculate the percentage of each component present in the DDT. The method can be used to determine TDE as well as *o,p'*-DDT. This method works best in cases where the components are only moderately soluble in the solvent.

LaClair (4) has extended the methods for determining the *p,p'*-DDT content of insecticidal mixtures based on the difference in the rate of dehydrochlorination of *p,p'*-DDT and *o,p'*-DDT. Dehydrochlorination was carried out at 25°C. in order to avoid the dehydrochlorination of *o-p'*-DDT and the hydrolysis of the trichloromethyl group to the corresponding acid.

This is in line with the work of Wain and Martin (5). who showed that refluxing *p,p'*-DDT with 1 *N* alcoholic potassium hydroxide for 30 minutes gave 1.04 mole of chloride ion. They were able to isolate small amounts of 2,2,bis (*p*-chlorophenyl) acetic acid from the reaction mixtures. With 0.1 *N* alkali at 23°C. the quantitative amount of chloride ion was obtained when the reaction was allowed to run for 30 to 60 minutes.

The tentative A.O.A.C. method for the determination of *p,p'*-DDT (6) requires 0.1 *N* alcoholic caustic under reflux conditions for 15 minutes. The results from a collaborative study of this method (7) gave an over-all average of 1.00 mole of chloride ion, but variations on individual runs indicate that some hydrolysis of the trichloromeythyl group may have occurred. It therefore seems advisable to conduct this determination at 25°C.

In last year's report (8) the recommendation was made that the method for the determination of total chlorine in DDT be subjected to further collaborative study, with pure *p,p'*-DDT as the test sample. The results of this study, given in Table 1, show that the method is satisfactory.

W. A. Kirklin and W. W. Haden suggested that the method (1) be modified by use of isopropanol instead of benzene as the diluent. The weighed sample is placed in a 250-ml volumetric flask, 10 ml of benzene is added, and when solution is effected it is made to volume with 99-per cent isopropanol. A 25-ml aliquot is then used for the determination of total chlorine. This modification eliminates the time-consuming step of evaporating the benzene from the aliquot before adding the isopropanol. The results in Table 1 which are indicated by asterisks (*) show this method to be as accurate as the original method. The use of benzene in the extraction of dusts in methods (2) and (3) should be retained to insure complete extraction of the DDT.

The tentative methods for the analysis of insecticidal preparations containing DDT adopted last year (8) did not provide for the analysis of aqueous emulsions. Therefore, methods used in the Bureau of Entomology

TABLE 1.—*Collaborative results on p,p'-DDT and 10 per cent DDT aqueous emulsions*

COLLABORATOR	p,p'-DDT	10 PER CENT DDT EMULSION
	PER CENT CHLORINE	PER CENT DDT FOUND
1	50.56	9.99
	49.78	10.04
	Av. 50.17	10.02
2	49.71	9.96*
	49.78	9.86*
	Av. 49.75	9.91
3	50.16	9.92
	49.86	10.03
	Av. 50.01	9.98
4	50.0	10.18*
	50.1	10.02*
	50.1	10.19*
	50.1	10.20*
	Av. 50.05	10.15
5	49.64	9.62
	49.47	9.36
	49.64	9.94
		9.94
	Av. 49.57	9.71
6	49.95*	
	49.85*	
	50.2*	
	49.9*	
	50.3*	
	50.0*	
	Av. 50.05	
General Average	49.93	9.95

* Isopropanol used as solvent.

and Plant Quarantine by R. H. Carter and Anita C. Hazen, and in the Production and Marketing Administration, U. S. Department of Agriculture, by C. G. Donovan, were combined for collaborative testing this year. The combined method originally specified the use of absolute alcohol as the solvent for breaking the emulsion. Later 99-per cent isopropanol was

found to have definite advantages in removing water from the sample, as the constant-boiling mixture contains 12.3 per cent by weight of water instead of the 7.7 per cent when ethyl alcohol is used. Since the use of absolute alcohol would require the addition of another reagent to the procedure, the use of isopropanol is recommended. No other change was made in this method.

J. B. LaClair suggests that the last traces of solvent be removed in a current of air after the flask has been cooled to room temperature.

The results of collaborative tests on a 10 per cent DDT emulsion are shown in Table 1. Isopropanol was used in some of the determinations, and no significant difference in the results could be detected.

The following method is to be added to those methods adopted in last year's report:

METHODS FOR DETERMINATION OF TOTAL CHLORINE IN EMULSIONS CONTAINING DDT, SOLVENT, EMULSIFYING AGENT, AND WATER

Weigh a quantity of well-mixed sample containing about 0.75 g of DDT in a tared weighing bottle. Wash into a 100-ml volumetric flask and make to volume with 99-percent isopropanol. Transfer a 10-ml aliquot to a 250–500 ml standard tapered Erlenmeyer flask. Place on a steam bath, and expel the isopropanol and water in a current of air. Remove last traces of solvent and water from the cooled flask. If drops of water still remain, add 10 ml of isopropyanol and repeat the evaporation. Proceed as directed in method (6) *This Journal*, **30**, 65 (1947), line 2, beginning "Add 25 ml of 99% isopropanol. . . . "

The emulsion sent out for analysis was made by mixing equal weights of water and emulsion concentrate. The concentrate contained 20 parts of technical DDT, 21 parts of Deobase (deodorized kerosene), 26 parts of xylene, 26 parts of Velsicol AR-50 (alkylated naphthalenes), and 7 parts of Triton X-100 (an aralkyl polyether alcohol). All parts were by weight.

Grateful appreciation is expressed to the following collaborators who participated in this study:

J. B. LaClair, California Bureau of Chemistry
C. G. Donovan, Production and Marketing Administration, U.S.D.A.
W. A. Kirklin and W. W. Haden, Hercules Powder Co.
R. H. Carter and Anita C. Hazen, Bureau of Entomology and Plant Quarantine, U.S.D.A.

RECOMMENDATIONS*

It is recommended:

(1) That A.O.A.C. method **6.151** for *p,p'*-DDT be amended as follows: Delete "Reflux alcohol for 15 minutes," and substitute "Place in a water bath at 25°C. for 30 to 60 minutes." Delete " . . . cool to room temperature," and join the remainder of this sentence to the following one.

(2) That method (1), *This Journal*, **30**, 64 (1947), be retained as a tentative method for the determination of total chlorine in DDT, with the following modifications: Delete " . . . and make to volume . . . " thru "Add 25 ml of 99-per cent isopropanol and . . . , " substituting therefor "add 10 ml thiophene-free benzene, dissolve the sample, and then make to

* For report of Subcommittee A and action of the Association, see *This Journal*, **31**, 42 (1948).

volume with 99-per cent isopropanol. Transfer a 25 ml aliquot to a 250–500 ml standard tapered Erlenmeyer flask. Add . . . ."

That editorial changes be made in methods (2) and (3) to provide for the evaporation of benzene and in (4) to provide for the addition of isopropanol.

(3) That the method given above for the determination of DDT in aqueous emulsions in the absence of other organic chlorine-containing compounds be adopted as tentative.

REFERENCES

(1) BRADBURY, F. R., HIGGONS, D. J., and STONEMAN, J. P., *J. Soc. Chem. Ind.*, **66**, 65 (1947).
(2) ILING, E. T., and STEPHENSON, W. H., *Analyst*, **71**, 310 (1946).
(3) THORP, D., *J. Soc. Chem. Ind.*, **65**, 414 (1947).
(4) LACLAIR, J. B., *Anal. Chem.*, **18**, 763 (1946).
(5) WAIN, R. L., and MARTIN, A. E., *Analyst*, **72**, 1 (1947); *Nature*, **159**, 68 (1947).
(6) *Methods of Analysis, A.O.A.C.*, 1945, 86, sec. **6.151.**
(7) FLECK, E. E., *This Journal*, **28**, 585 (1945).
(8) ——, *Ibid.*, **30**, 319 (1947).

No report was given on fluorine compounds, or on nicotine and nornicotine.

ANNOUNCEMENTS

REFEREE ASSIGNMENTS, CHANGES, AND APPOINTMENTS

James F. Guymon, Agricultural Experiment Station, College of Agriculture, Davis, Calif., Associate Referee on Methyl Alcohol (Miscellaneous Drugs), and on Methanol (Alcoholic Beverages).

William Horwitz, Food and Drug Administration, Minneapolis, Minn., Associate Referee on Phosphatase Test in Dairy Products, in place of George P. Sanders.

Joseph H. Cohen, Chemical Foods Corp., Woburn, Mass., Associate Referee on Gelatine and Gelatine Desserts (Constituents).

R. P. Smith, National Biscuit Company, 449 W. 14th St., New York, N. Y. Associate Referee on Baked Products (Moisture, Ash, Protein, Fat, Crude Fiber, and sugar), to succeed N. H. Walker.

Sidney Kahan, Food and Drug Administration, New York, N. Y., Associate Referee on Glycerol, Vanillin, and Coumarin, in Vanilla and Imitation Vanilla.

Sidney Williams, Food and Drug Administration, Cincinnati, Ohio, Associate Referee on Moisture in Self-rising Flour, and in Pancake, Waffle, and Doughnut Mixes.

CORRECTIONS IN FEBRUARY *Journal*

In the list of Referees, the address of H. C. Heim, given as San Francisco, 2, Calif., should be corrected to School of Pharmacy, University of Colorado, Boulder, Colo.; that of S. Gottleib should be changed to Food and Drug Administration, Washington 25, D. C.

The name of Fred Hillig should be inserted under Dairy Products (Decomposition in Foods), in place of K. L. Harris.

In the method for Indole in Shrimp, Oysters, and Crabmeat, given on page 96, in paragraph on *Color Reagent*, "0.92 ml Phosphoric acid" should read "92 ml."

In par. (6) on page 81, changes in method for soybean flour, next to last line, "see 31.07," etc. the section should be "**31.67.**"

CONTRIBUTED PAPERS

METHOD FOR RAPID DETERMINATION OF TOTAL NITROGEN IN AMMONIUM NITRATE FERTILIZER*

By RALPH D. MILLER (Spencer Chemical Co., Jayhawk Works, Pittsburg, Kans.)

The method presented here to the Association of Official Agricultural Chemists is Spencer Chemical Company's Standard Procedure N-6 for the determination of total nitrogen in ammonium nitrate fertilizer. This method is specific for ammonium salts and should not be confused with methods for mixed fertilizers. The method mentioned here has been thoroughly tried and tested through the years of war as well as many years previous to the war. The need of such a method has been amply demonstrated by the wide variation of results from different laboratories on the same product. The method described herein is not a new method, but rather a revision of one which has been widely used for many years. The advantage of the method now presented over methods previously available in literature is that it is more reliable for ammonium nitrate solutions and ammonium nitrate fertilizer.

There has been a great deal of work in the past year or so by the late Dr. W. H. Ross[1] of the U. S. Dept. of Agriculture, which was presented in a report on "Nitrogen," by A. L. Prince,[2] New Jersey Agricultural Experiment Station, New Brunswick, New Jersey. In this report, Prince gave conclusive evidence that there is definite need for a more uniform method for determining nitrogen in ammonium nitrate fertilizers. This information is further substantiated by reports received at Spencer Chemical Company from several State laboratories on total nitrogen content of products shipped from this Company.

THE ORIGINAL METHOD

Accurately weigh ca 1 gram sample and transfer to a 250 ml Erlenmeyer flask. Dilute to ca 100 ml with distilled water. Add 25 ml of neutral 20% formaldehyde and heat to 60°C. Cool to 30° and titrate with 0.1 *N* sodium hydroxide using 5 drops of phenolphthalein as indicator. Continue titration until pink remains for 30 seconds.

HISTORY OF THE METHOD

This original method is the standard accepted method for shipments made by contractors to the Army and Navy as covered by the joint Army-Navy Specification 50-11-59F and 51A23, respectively. It was used almost exclusively for this purpose during and since World War II. It is also the

* Presented at The Annual Meeting of the Association of Official Agricultural Chemists held at Washington, D. C. October 20–22, 1947.

[1] Retired.

[2] *This Journal*, **30**, 228 (1947).

accepted method for inspection of ammonium nitrate manufactured by the Ordnance Department in the fertilizer program at present. These specifications and analytical procedures for the government's fertilizer program were established by the Tennessee Valley Authority, and are also used by the T.V.A. in their own plants. The estimated output of the government operated plants in this program amounts to 72,000 tons per month of ammonium nitrate fertilizer. Another significant figure is the 240,000 tons of ammonium nitrate shipped by the Jayhawk Ordnance Works alone during the war years. This material has had duplicate analyses made on each lot by this method, which amounts to about fifty-five thousand analyses. The fact remains that this is only a small part of the actual number of this type of analysis which has been made to date on ammonium nitrate. (When consideration is given to this information, the argument in favor of this basic method's thorough reliability is greatly strengthened.)

REVISION OF METHOD

During the operation of the Jayhawk Ordnance Works for the Government, the specifications and methods of analysis for ammonium nitrate were set up and run to conform with U. S. Army Specification PXS-898, dated November 24, 1942 (Picatinny Arsenal). This practice was required at that time because the Ordnance Department was the sole user of the products manufactured at this facility. Since the conversion to private operation in June, 1946, this method has been reviewed as a part of a general program to obtain the maximum degree of efficiency in control methods without loss of accuracy. As is noted in the original procedure, the U. S. Ordnance specification calls for a weighed sample to be treated with formaldehyde (HCHO), heated to 60°C. and cooled to 30°C. before titrating with a standard sodium hydroxide solution. The equation:

$$6HCHO+4NH_4NO_3+4NaOH\longrightarrow C_6H_{12}N_4+4NaNO_3+10H_2O$$

Observation of this method indicated that approximately 33 per cent of the total determination time was consumed in the sample heating and cooling step. Therefore, the following series of tests were made at the laboratories of Spencer Chemical Company on ammonium nitrate over a period of two months and covering seventy-six separate determinations. The comparative samples of nitrate were treated by the original method, except that one pair of samples was heated and cooled, after the addition of formaldehyde and before titration, while the other pair of samples was not heated and was titrated at room temperature.

COMPARISON OF HEATED AND UNHEATED SAMPLES

The results of these analyses are completed in Table 1 of this report. Of the seventy-six determinations made during this work, the average variation was ± 0.1 per cent. Thirty-three (33) analyses were lower by the

TABLE 1.—*Variation of "unheated" results from "heated"*

NO.	%NH_4NO_3 UNHEATED	%NH_4NO_3 HEATED	VARIATION IN %	NO.	%NH_4NO_3 UNHEATED	%NH_4NO_3 HEATED	VARIATION IN %
1	83.7	83.8	−0.1	39	82.9	83.0	−0.1
2	83.7	83.6	0.1	40	83.2	83.2	0.0
3	83.7	83.8	−0.1	41	83.9	83.8	0.1
4	83.6	83.7	−0.1	42	83.6	83.6	0.0
5	83.5	83.6	−0.1	43	83.6	83.5	0.1
6	83.4	83.7	−0.3	44	83.4	83.4	0.0
7	83.9	83.7	0.2	45	83.5	83.7	−0.2
8	83.6	83.7	−0.1	46	83.5	83.7	−0.2
9	83.8	83.7	0.1	47	82.9	83.1	−0.2
10	83.5	83.6	−0.1	48	82.8	82.9	−0.1
11	83.3	83.3	0.0	49	83.6	83.3	0.3
12	83.4	83.5	−0.1	50	83.4	83.5	−0.1
13	83.0	83.3	−0.3	51	83.7	83.8	−0.1
14	83.5	83.5	0.0	52	83.6	83.4	0.2
15	83.3	83.1	0.2	53	83.4	83.4	0.0
16	83.2	83.2	0.0	54	83.5	83.5	0.0
17	83.4	83.3	0.1	55	83.8	83.8	0.0
18	83.8	83.8	0.0	56	84.0	83.9	0.1
19	83.8	83.8	0.0	57	83.8	83.7	0.1
20	83.7	83.7	0.0	58	83.6	83.5	0.1
21	83.9	83.9	0.0	59	83.7	83.4	0.3
22	83.5	83.6	−0.1	60	84.0	84.1	−0.1
23	83.6	83.6	0.0	61	83.9	83.9	0.0
24	83.5	83.5	0.0	62	85.0	85.0	0.0
25	83.8	83.8	0.0	63	85.1	84.9	0.2
26	83.8	83.9	−0.1	64	84.2	84.2	0.0
27	83.8	83.7	0.1	65	83.9	84.0	−0.1
28	83.6	83.8	−0.2	66	84.2	84.3	−0.1
29	83.7	83.7	0.0	67	84.0	84.2	−0.2
30	83.6	83.7	−0.1	68	83.7	83.6	0.1
31	83.7	83.6	0.1	69	83.9	83.8	0.1
32	83.5	83.6	−0.1	70	83.3	83.4	−0.1
33	83.5	83.6	−0.1	71	82.7	82.7	0.0
34	83.6	84.0	−0.4	72	82.4	82.7	−0.3
35	83.3	83.7	−0.4	73	82.6	82.2	0.4
36	83.4	83.7	−0.3	74	83.4	83.4	0.0
37	83.6	83.6	0.0	75	83.2	83.4	−0.2
38	83.4	83.4	0.0	76	83.7	83.9	−0.2

No. of Analyses	*Variation per cent*	*% of Total*	*Cumulative % of Total*
24	0.0 (Equal)	31.58	31.58
32	±0.1	42.11	73.69
11	±0.2	14.47	88.16
6	±0.3	7.89	
3	±0.4	3.95	
76		100.00	

unheated method, nineteen (19) were higher, and twenty-four (24) were equal. Of the seventy-six (76) analyses, thirty-two (32) determinations were ±0.1 per cent; eleven (11) determinations were ±0.2 per cent; six (6) were ±0.3 per cent; three (3) were ±0.4 per cent. Table 1 gives the number of analyses in each variation range and the per cent of the total that each represents.

Table 1 shows that 88 per cent of the determinations agree within 0.2 per cent or better, and 74 per cent with 0.1 per cent or better. From the previous experience on nitrate determinations here, it has been found that 0.2 per cent is a reasonable deviation for check samples on the same material. It would appear that the agreement between the two methods is very satisfactory for nitrate determinations since it may be expected to find as much as 1 per cent, or even greater, between two Kjeldahl analyses (using Devarda alloy) on the same material. The method under test was, of course, different from that recommended by the Army. However, in regard to the heating and cooling of these samples nothing could be found in literature giving the formaldehyde reaction with ammonium nitrate where heating and cooling of the sample was specified. On the other hand, the text, "Formaldehyde," by Walker (Reinhold Publishing Company, 1944, page 121), states: "Addition of formaldehyde to ammonium salt solution at room temperature results in the liberation of an acid. When this acid is neutralized or removed, hexamethylenetetramine is obtained. When excess alkali is added to solutions containing ammonium salts, and strong acids plus an excess of formaldehyde, the acid which was originally combined with ammonia may be accurately determined by measuring the amount of alkali consumed. A volumetric method for the determination of NH_4NO_3 is based on the reaction given,

$$6\,HCHO + 4NH_4NO_3 + 4\,NaOH \longrightarrow C_6H_{12}N_4 + 4NaNO_3 + 10\,H_2O.$$

Also in the *Journal of Chemical Education* (November, 1946, Vol. 23, No. 11, page 552), the formaldehyde reaction with NH_3 requires no heating and cooling, and from Allen's Commercial Organic Analysis (5th Ed., Vol. III, page 612), the formal titration does not call for the heating and cooling of the sample, but a titration at room temperature. From these data and from the literature cited, there is believed to be adequate evidence that the analysis of ammonium nitrate can be determined accurately by eliminating the heating and cooling of the sample after the addition of formaldehyde. This change has saved a great deal of time in Spencer laboratories, and with the addition of another slight change, good reproducible results have been obtained.

MODIFICATION OF END POINT

Instead of titration to the first pink color remaining for 30 seconds, the procedure has been changed to continue titration until one drop of .25 normal NaOH produces no perceptible color change at the point of con-

tact. This end point has been found to give better and more consistent results and has been used on all analyses cited in this proposal. It was also used during operation for the Government; therefore, this group has had a great deal of experience with its usage. To use this end point, it is necessary to standardize the sodium hydroxide against C.P. ammonium nitrate with the same end point. It is believed that the *p*H obtained with this end point corresponds to absolute completion of the reaction.

COMPARISON OF STANDARD PROCEDURE N-6 VS. KJELDAHL METHOD

To further test Standard Procedure N-6, the laboratories of Spencer Chemical Company have done a great deal of experimental work in check-

TABLE 2.—*Comparison of results by Standard Procedure N-6 and the Kjeldahl method (using Devarda alloy) for determination of total nitrogen in ammonium nitrate fertilizer*

SAMPLE NO.	STD. PRO. N-6	DEVARDA WITH TRAP KJELDAHL
	per cent	*per cent*
1	33.7	33.6
2	33.7	33.7
3	33.6	33.8
4	33.3	33.3
5	33.9	34.0
6	33.4	33.3
7	33.5	33.7
8	33.4	33.3
9	33.5	33.3
10	33.7	33.8
11	33.3	33.1
12	33.1	33.0
13	33.2	33.4
14	33.1	33.2
15	33.2	33.3

ing this procedure by the various other known analytical procedures. Since the Association of Official Agricultural Chemists at present only recognizes the Kjeldahl method (with various modifications), this was the first to be used in comparing results. It was decided to check various samples from Spencer Chemical Company's graining plants. These samples were taken from shipments and retained in sealed quart fruit jars. The results found in Table 2 are somewhat closer between the two different methods than can be normally expected between two different Kjeldahl tests run on the same sample. This fact was adequately demonstrated in the report by A. L. Prince.[3] Table 1 of his report gave some 31 determina-

[3] *Loc. cit.*

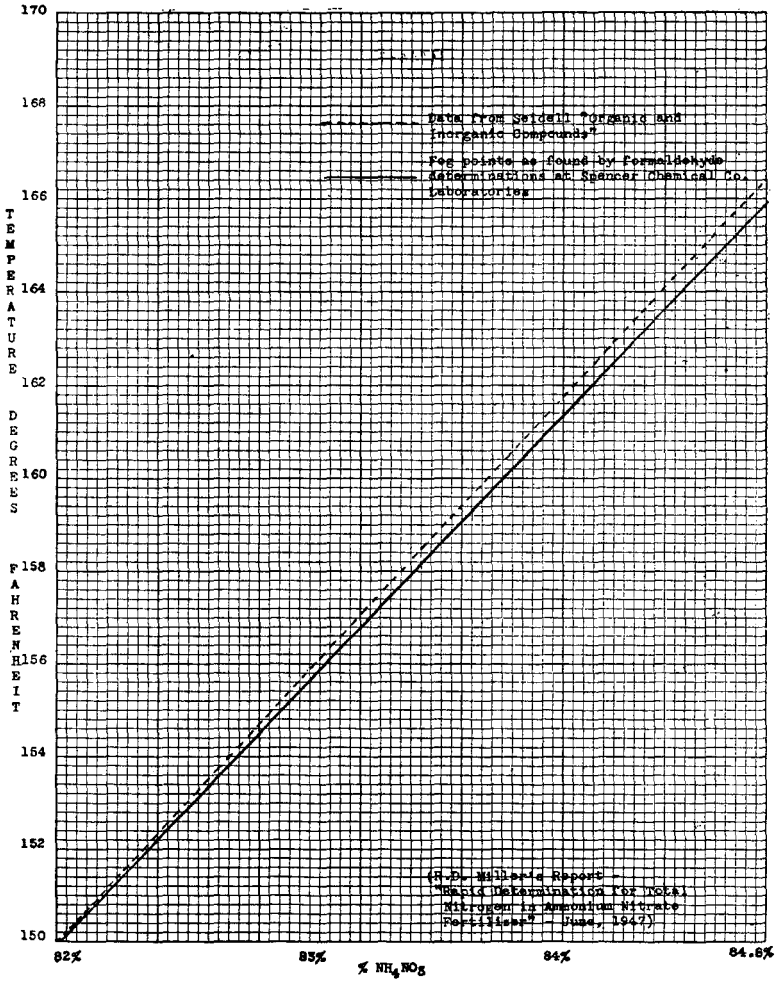
tions on Sample #1 ammonium nitrate with results ranging from 33.92 per cent to 35.18 per cent as found by the collaborators using various modifications of the Kjeldahl method.

COMPARISON OF STANDARD PROCEDURE N-6 VS. FOG POINT METHOD

The next set of comparisons was made using the Fog Point determination which is a standard control method on pure ammonium nitrate solutions made at the Jayhawk Works of Spencer Chemical Company. This determination is based on the freezing point of the concentrated NH_4NO_3 solutions manufactured at this facility. A brief description of this procedure is as follows:

The equipment used consists of a variable speed motor with a glass stirrer; this attached to a solid base to prevent excessive vibration. To the same base a centigrade or fahrenheit thermometer is suspended vertically and fastened rigidly just far enough from the stirrer to prevent breaking. A 150 ml beaker for holding the hot solution is used, and just behind it is placed a light to enable close observance of the transparency of the solution. The hot ammonium nitrate solution is poured into the 150 ml beaker, being careful that no nitrate is crystallized on the sides of the beaker. The amount of solution is about 90 ml. The stirrer is started and the speed controlled to prevent splashing of the solution from the beaker. As the temperature drops and the freezing point is approached, a scum will form on the surface of the warm liquid, which serves to speed the freezing and prevent supersaturation. The "Fog Point" is the point where the clear solution changes to opaque. A temperature reading is taken at this point.

This method was established as a quick control method for operations and has been found to be very accurate and satisfactory for calculations on blending of various solutions and mixes made in the nitrate division. This method was established only after much work had been done in the establishment of its reliability. The complete report on this work is given in a technical report of Spencer Chemical Company No. 904AN-4, by Paul E. Wachter. In order to check the fog point method, a graph was prepared, using information taken from Seidell "Solubilities of Inorganic and Organic Compounds in Water," (page 1043, Vol. II, 2nd Ed.), along with authoritative information published by N. I. Rauich, U.S.S.R. 1933. Our established Fog Point results compare very well with this information, as shown on Graph #1. This Fog Point method has proven superior in every respect for control purposes to the specific gravity determinations used previously. With this confidence in our method, it was felt that the formaldehyde (Standard Procedure N-6) method should be compared with it as a test for reliability. Table 3 shows a comparison of the fog point against the formaldehyde (Standard Procedure N-6)



method on prepared samples, using C.P. ammonium nitrate in distilled water. The samples No. 1, No. 2, and No. 3, were analyzed by the formaldehyde (Standard Procedure N-6) method in three different laboratories by three different analysts. The results are given in percentage ammonium nitrate. It should be noted, on Table 3, the good comparison between the two different methods and the excellent reproducibility of results by the Standard Procedure N-6.

CONCLUSIONS

The Standard Procedure N-6 of the Technical Department of Spencer Chemical Company has been thoroughly tested on ammonium nitrate

determinations; it is satisfactory for reproducible results and has the distinct advantage of saving time in the laboratory. It is specific for ammonium salts, and its time requirement is hardly longer than that of deter-

TABLE 3.—*Correlation of Fog Point analysis along with three separate analyses by different analysts in different laboratories using the formaldehyde method*

(Results are in percentage ammonium nitrate (NH_4NO_3)*

SAMPLE NO.	FOG POINT	NO. 1	NO. 2	NO. 3
1	—	76.64	76.58	76.60
2	77.77	77.93	77.89	78.12
3	77.81	77.57	77.88	78.10
4	79.00	78.98	—	78.86
5	79.39	79.55	79.46	79.14
6	79.52	79.33	79.56	79.39
7	79.96	79.72	79.74	79.72
8	80.14	79.92	79.83	79.92
9	80.66	80.66	80.76	80.85
10	81.18	80.91	80.93	80.77
11	81.71	81.59	81.65	81.94
12	81.88	81.91	81.74	82.04
13	81.88	81.48	81.78	81.88
14	82.23	82.27	82.09	82.03
15	82.72	82.73	82.96	82.95
16	82.84	83.03	83.01	83.00
17	83.19	83.12	83.33	83.27
18	83.49	83.33	83.35	83.63
19	83.63	—	83.78	83.43
20	83.81	84.09	83.79	83.66
21	83.90	83.80	83.91	84.10
22	84.33	84.46	84.34	84.44
23	84.60	84.47	84.42	84.38
24	84.74	84.60	84.39	84.49

* It is to be noted the good comparison between two different methods and the excellent reproducibility over results by the formaldehyde method.

mining the strength of nitric acid. The possible argument that mixed acids might have been used during neutralization is eliminated in Standard Procedure N-6 by the colorimetric tests for chlorides and sulfates; these being the salts of the only acids believed capable of entering this type of manufacture.

Standard Procedure N-6 is herewith submitted for consideration by the Association of Official Agricultural Chemists as a standard method for determination of total nitrogen in fertilizer grade ammonium nitrate, and of pure ammonium nitrate (solid or in aqueous solutions).

TOTAL NITROGEN IN AMMONIUM NITRATE FERTILIZERS

(Taken from Spencer Chemical Company's Standard Procedure N-6)

REAGENTS

Chemicals Required for Analysis:
20% Formaldehyde Soln.—Prepared from C.P. or Reagent Grade Formaldehyde.
0.25 Normal Sodium Hydroxide Soln.—Standardized against C.P. NH_4NO_3.
Phenolphthalein Indicator.
Distilled Water.
C.P. Ammonium Nitrate.

DETERMINATION

Total Nitrogen:

Place approximately 1.5 gm of sample into a previously weighed 50 ml beaker. The weight of nitrate may be obtained by reweighing the beaker on an analytical balance. The sample thus weighed is dissolved in distilled water and transferred to a 250 ml Erlenmeyer flask. Add 30 ml of 20 per cent formaldehyde (neutral to phenolphthalein) then titrate with 0.25 *N* sodium hydroxide, using phenolphthalein indicator, until the addition of one drop of NaOH produces no perceptible color change at the point of contact.

CALCULATION

$$\frac{\text{Ml NaOH} \times \text{Normality of NaOH} \times 2.802}{\text{Wt. of Sample}} = \% \text{ Nitrogen}$$

COMPOSITION AND FERTILIZER VALUE OF PHOSPHATE ROCK-MAGNESIUM SILICATE GLASSES[1]

By W. L. HILL, F. N. WARD, W. H. ARMIGER, and K. D. JACOB (Division of Fertilizer and Agricultural Lime, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, Beltsville, Maryland)

The production of soluble phosphates by heating mixtures of phosphate rock and siliceous materials, such as blast-furnace slag, calcium and magnesium silicates, magnesium salts and silica, and the like, has been proposed in patents several times during the present century (7, 9, 10, 12, 13). Until recently this type of process attracted little serious attention. Since the publication in 1943 of an article by Walthall and Bridger (11) describing experiments with the production of available phosphate in the form of a glass by fusing phosphate rock with olivine, a growing interest has been aroused in this type of product (2–4, 6, 8). Such a material

[1] Presented at the Annual Meeting of the Association of Official Agricultural Chemists held at Washington, D. C., October 20–22, 1947.

has recently been put on the market in California under the name *Thermo-Phos*, which is being used both for direct application and in mixed fertilizer (3). The fertilizer possibilities of soluble glasses have recently been emphasized by Badger and Bray (5). This laboratory has investigated samples of experimental and commercial materials produced at various places by fusing phosphate rock with olivine or serpentine with a view towards establishing the physical and chemical composition of these products and determining their nutrient values by means of greenhouse tests. The results of the study are summarized in this report.

MATERIALS

Phosphate rock-magnesium silicate glass (Thermo-Phos) now available in commercial quantities is produced by the Permanente Metals Corpora-

TABLE 1.—*Chemical analyses of phosphate-silicate glasses*

SAMPLE NO.	TYPE OF MATERIAL		P_2O_5	CaO	MgO	Al_2O_3	FeO[b]	SiO_2[c]	F
	PHOSPHATE ROCK FUSED WITH—	PRODUCTION	%	%	%	%	%	%	%
[a]2497-a	Serpentine	Large-scale	20.04	32.4[d]	17.1[d]	1.8[d]	3.5[d]	23.2[d]	1.70
[a]2463	Serpentine	Experimental	19.75	31.60	18.53	1.95	2.52	22.78	1.65
[a]2488	Olivine	Experimental	22.74	33.76	11.51	2.64	3.82	22.59	2.22
[a]2483	Olivine	Experimental	22.18	31.65	11.98	3.73	3.81	21.66	2.08
2481	Olivine	Experimental	23.61	34.43	11.96	0.59	2.59	22.97	2.21
2489	Olivine	Experimental	19.94	28.33	18.02	2.95	2.38	23.80	1.89
2527	Olivine	Experimental	22.92	35.55	17.82	1.40	1.96	19.24	1.82
—	[e]	Laboratory	13.8	47.0	—	—	—	39.2	—

[a] Material used in greenhouse experiments.
[b] Total Fe.
[c] Without regard for the effect of the presence of fluorine.
[d] Producer's analysis.
[e] A fusion of pure calcium pyrophosphate, calcium carbonate, and cristobalite.

tion, Permanente, California, by fusing a proportioned mixture of Idaho phosphate rock and serpentine at temperatures approaching 1500°C. in electric-arc furnaces formerly used for ferrosilicon production (3) and quenching the melt in a violent spray of water. The dried granular product is ground to finenesses thought to be appropriate for the market—a −6-mesh material for direct application and a −100-mesh material for use in mixed fertilizers. Samples 2497-a (Table 1) and 2497-b, received in December 1946, are materials of these finenesses, respectively. Apart from fineness the two samples were identical. Sample 2463, received in July 1946 from the same concern, is presumed to be an experimental product. Samples 2497-c and 2533 are regrinds of commercial −6-mesh materials.

The other numbered samples are experimental products. No. 2481 and 2483 were produced in the State of Washington with the use of Montana

phosphate rock and olivine. No. 2489 was prepared some years ago by Walthall and Bridger (11) of the Tennessee Valley Authority. No. 2527 was prepared in Sweden.[1] The laboratory preparation is a homogeneous glass that was prepared several years ago in connection with a phase-rule study. The basic slag (No. 2507) is an imported material that contains 16.9 per cent of total P_2O_5. The alpha phosphate (No. 2496) is the Fused Tricalcium Phosphate produced by the Tennessee Valley Authority and contains 27.68 per cent of total P_2O_5, of which 80.9 per cent was soluble in neutral ammonium citrate and 83.2 per cent in 2 per cent citric acid.

METHODS OF ANALYSIS

Chemical analysis.—The samples (−80- or −100-mesh) were analyzed with the use of standard procedures and appropriate precautions to insure complete solution of the constituent sought. Calcium, magnesium, aluminum, iron and silica were determined in solutions obtained by digesting the sample with hydrofluoric and sulfuric acids and evaporating the digestion until copious fumes of sulfuric acid appeared. Fluorine was determined by distilling the sample with perchloric acid at 125°C. at a rate of 100 ml of distillate per hour and titrating the distillate with thorium nitrate. Since the phosphate glasses do not give up fluorine readily and also vary considerably in this respect, the distillation was prolonged (up to 3 hours) until the titers of successive 50-ml distillates showed that the evolution of fluorine had ceased. Total phosphorus was determined in the solution obtained by treating the sample with aqua regia, evaporating the mixture to dryness to dehydrate the bulky gelatinous silica and dissolving the soluble salts in nitric acid. In order to minimize troublesome caking of the material, the sample was wetted with sufficient water to cover it before the acid was added.

Analyses for citrate-insoluble and citric acid-soluble phosphorus were made in accordance with the official methods for fertilizers (1). Because of the tendency of the material to cake upon the addition of reagents, it was found necessary to shake the flask vigorously immediately after the addition of the sample to the solvent. The citrate-insoluble residue was ignited with magnesium nitrate to destroy the large amount of filter paper and then treated with hydrochloric acid to dehydrate the silica.

Screen analysis.—A sample of 50 grams was used, and the sieves were shaken for 20 minutes on a Ro-Tap machine. The error from dust losses, which ranged from 0 to 1.5 per cent, was usually less than 0.5 per cent and was applied to the finest fraction.

CHEMICAL AND PHYSICAL CHARACTERISTICS OF GLASSES

Chemical composition.—Chemical analyses of eight materials are given in Table 1. Aside from the laboratory preparation of calcium-phosphate-

[1] The sample was furnished by P. Gunnar Brundell, Vargon, Sweden.

silicate glass, the phosphorus content of the materials ranges from about 19.7 to 23.6 per cent of P_2O_5, and the silica varies more or less independently of the phosphorus from about 19.2 to 23.8 per cent. The greatest variation occurs in the magnesium content (11.5 to 18.5% MgO). The observation that the small amounts of sulfur in the glasses are present as sulfide, indicating reducing conditions during fusion, is the basis for reporting the iron as ferrous oxide. Since the larger part of the fluorine of the phosphate rock remains in the finished product, these furnace materials cannot be viewed as defluorinated phosphates. The presence of fluorine makes fluorapatite a probable crystalline constituent of the product obtained when the furnace charge is allowed to crystallize during slow

TABLE 2.—*"Solubility" of phosphate-silicate glasses in neutral ammonium citrate and 2% citric acid solutions*

SAMPLE NO.	TYPE OF MATERIAL		FINENESS	TOTAL P_2O_5	FRACTION OF PHOSPHORUS EXTRACTED		
	PHOSPHATE ROCK FUSED WITH—	PRODUCTION			BY CITRATE	BY CITRIC ACID	DIFFERENCE
			mesh	*per cent*	*per cent*	*per cent*	*per cent*
[a]2497-a	Serpentine	Large-scale	− 6[b]	20.04	62.8	99.0	36.2
[a]2497-b	Serpentine	Large-scale	−100[b]	20.17	83.8	98.3	14.4
2497-c	Serpentine	Large-scale	− 80	20.04	65.3	98.0	32.7
[a]2463	Serpentine	Experimental	− 80	19.75	72.0	99.5	27.5
2533-c	Serpentine	Large-scale	− 25	19.50	76.7	98.9	22.2
[a]2488	Olivine	Experimental	− 80	22.74	84.1	87.0	2.9
[a]2483	Olivine	Experimental	− 80	22.18	78.2	93.0	14.8
2483	Olivine	Experimental	− 10	22.18	32.7	65.3	32.6
2481	Olivine	Experimental	− 80	23.61	67.5	79.0	11.5
2489	Olivine	Experimental	− 80	19.94	84.2	95.6	11.4
2527	Olivine	Experimental	−100	22.92	95.5	98.0	2.5
—	[d]	Laboratory	− 80	13.83	65.6	93.6	28.0

[a] Material used in greenhouse experiments.
[b] As ground by the producer for the market.
[c] Marketed −6-mesh material reground by the California Bureau of Chemistry for chemical-control analysis. The sample was kindly supplied by F. D. Fowler, Permanente Metals Corporation, Permanente, California.
[d] A fusion of pure calcium pyrophosphate, calcium carbonate and cristobalite.

cooling. Apatite has been identified in the crystallized glass and in poorly quenched products.

Phosphorus solubility.—The "solubilities" of several phosphate-silicate glasses in neutral ammonium citrate and 2 per cent citric acid solutions are given in Table 2. Considering first the materials with a fineness of 80 mesh or finer, the citric acid solubilities are with two exceptions (No. 2481 and 2488) well into the nineties, whereas the citrate solubilities range from about 65 to 96. A noteworthy observation (Table 2) has to do with the effect of fineness on the solubility of phosphate rock-magnesium silicate glass. The greater sensitivity of citrate solubility to fineness is further illustrated by the series of results obtained on glass No. 2497-c given in Figure 1, where results obtained on fused alpha phosphate and on im-

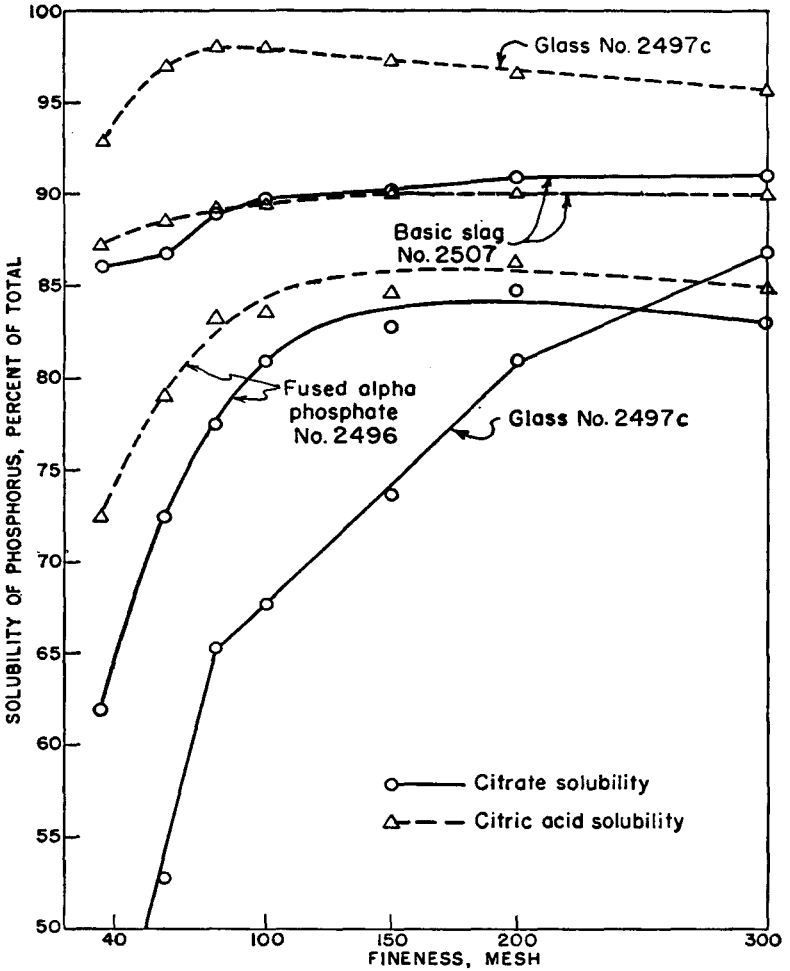


FIG. 1.—Effect of fineness on "solubility" of glass, fused alpha phosphate and basic slag.

ported basic slag are also shown for purposes of comparison. The "solubility" curves for the basic slag lie surprisingly close together. The curves for both the slag and the alpha phosphate fall between the widely separated curves for the phosphate rock-olivine glass. The effect of fineness on the "solubility" of the basic slag is negligible beyond −100 mesh, whereas the citrate solubility of the glass is apparently still increasing markedly at −300 mesh. The decrease in the citric acid solubility of the glass with increase in fineness is attributed to the tendency, already mentioned, of this material to cake under the action of acid.

Screen analyses of the fineness series of phosphate rock-olivine glass included in Figure 1, and of three other similar glasses, are shown in Table 3. Special significance attaches to the two commercially-ground materials with nominal finenesses of −6- and −100-mesh. In these materials the particle-size distribution is such that approximately 80 per cent of the one is coarser than 60 mesh whereas 80 per cent of the other is finer than 300 mesh—a condition that vitiates a comparison of the solubilities of these samples with those of laboratory grinds of similar material. For example, the commercial −100-mesh material showed a citrate solubility of 83.8

TABLE 3.—*Screen analyses of phosphate rock-magnesium silicate glasses, alpha phosphate, and basic slag*

SAMPLE NO.	MESH SIZE OF SAMPLE	PERCENTAGE OF SAMPLE PASSING SCREEN[b] OF—						
		35 MESH	60 MESH	80 MESH	100 MESH	150 MESH	200 MESH	300 MESH
[a]2497-a	− 6[c]	53.6	17.8	7.8	4.0	1.6	0.4	0.2
[a]2497-b	−100[c]	99.8	99.6	99.4	99.2	97.4	90.4	80.4
2533[d]	− 25	98.3	66.2[e]	—	49.6	38.1	28.3	11.0[f]
[a]2497-c	− 35	100.0	44.4	27.0	19.0	13.0	7.8	5.8
[a]	− 60	—	100.0	76.2	60.2	46.2	33.2	26.4
	− 80	—	—	100.0	85.2	68.6	53.4	44.6
	−100	—	—	—	100.0	91.4	70.2	51.6
[a]	−150	—	—	—	—	100.0	80.0	60.0
	−200	—	—	—	—	—	100.0	83.0
[a]2496	−100	—	—	—	100.0	80.4	50.4	35.8
2507	−100	—	—	—	100.0	93.0	80.8	57.4

[a] Material used in greenhouse experiments.
[b] Screen openings in sieve series were 420, 250, 177, 149, 104, 74 and 46 microns, respectively.
[c] As ground by the producer for the market.
[d] Marketed −6-mesh material reground by the California Bureau of Chemistry for analysis. Sample and screen analysis were kindly supplied by F. D. Fowler, Permanente Metals Corporation, Permanente, California.
[e] A 65-mesh sieve was used.
[f] A 325-mesh sieve was used.

per cent (Table 2) in comparison with 67.5 per cent for a −100-mesh sample (Fig. 1) prepared by the authors by grinding the −6-mesh miateral of practically identical chemical composition.

Optical and thermal properties.—The phosphate rock-magnesium silicate fusion products included in this study are essentially all-glass materials. The small amounts of crystalline material often present are mainly quartz, unreacted magnesium silicate and perhaps apatite. To the naked eye the glasses range in color from light green (No. 2527) through brownish grey to black. Observations made on some of the samples under the petrographic microscope[2] are given in Table 4.

All of the iron-bearing materials assume a dirty brown color when they are heated in air for some time below the temperature of complete fusion.

[2] Examinations were made by J. G. Cady, of this Bureau.

They vary considerably in ease of crystallization in the temperature range of 1000 to 1300°C. For example, No. 2463 annealed at 1200° for 4 hours contained abundant crystals, whereas No. 2483 had not begun to crystallize with 48 hours of heating at this temperature. Determined fusion temperatures lie between 1300 and 1400°C.—1320°, 1335°, 1370° and 1400° (all ±5°) for Nos. 2463, 2497, 2527, and the laboratory preparation, respectively. A few scattered observations indicate that apatite is not always the compound that first crystallizes from the melt, but it is apparently always present in materials that crystallize at temperatures some-

TABLE 4.—*Optical properties of phosphate rock-magnesium silicate glasses*[a]

SAMPLE NO.	INDEX OF REFRACTION	GENERAL APPEARANCE UNDER MICROSCOPE
2497-c	1.62	Smoky greenish-gray glass, some with opaque inclusions; rare irregular masses, almost opaque, showing faint birefringence.
2463	1.62	In general, same as No. 2497-c; rare high-index crystals, presumably pyroxene and olivine; some glass grains contain masses of lath-like crystals.
2483	1.61	Less uniform than No. 2497-c or 2463; gray, light brown, and dark reddish-brown glass containing many black inclusions with a liberal sprinkling of very small (less than 2 microns) high-index crystals.
2489	1.61	Pale brownish-gray to orange glass sprinkled with very small crystals as in No. 2483; rare mineral grains—olivine, sillimanite, quartz, etc.; trace of apatite.
2527	1.61	Clear grayish glass; rare small crystals (quartz, olivine and perhaps some apatite) imbedded in glass.

[a] Examinations were made on −80-mesh materials.

what below the melting point. The low "solubility" of poorly quenched material is, therefore, attributable to apatite formation.

Reactivity of glasses in mixtures.—Experiments are in progress to determine the behavior of phosphate rock-magnesium silicate glasses with respect to certain constituents of mixed fertilizers. The results[3] thus far obtained are briefly summarized below.

The reactivity of one glass (No. 2463) with respect to ammonium salts was tested by aspirating air through a suspension of the −80-mesh glass in saturated ammonium nitrate solution at room temperature for a period of 9 days. The glass suspension lost 208 mg. of NH_3 per gram of P_2O_5 in comparison with a loss of 115 mg from a suspension of *alpha* tricalcium phosphate (45.1% P_2O_5) under the same conditions. The behavior of the same

[3] Data obtained by E. J. Fox, of this Bureau.

glass towards superphosphate was determined by analyzing glass-superphosphate mixtures containing 20, 40, 60 and 80 per cent of superphosphate (20.8% P_2O_5, 1.46% F) at the end of storage periods of 14, 28, 56, and 84 days at 30° and 79.2 per cent relative humidity. All mixtures showed a continuous decrease in water-soluble phosphorus. Nevertheless, no progressive change in citrate-insoluble phosphorus was observed. The insoluble phosphorus at the end of the 14-day period was less than the value calculated from the phosphorus solubility of the initial ingredients by about 1 per cent of P_2O_5, and thereafter showed little change. The mixtures were dry and dusty at the end of the storage period, which indicates lack of moisture, rather than a property of the glass, as the factor that limited reversion of the soluble phosphorus. Mixtures at high moisture contents will, therefore, need to be studied in order to arrive at a definite conclusion as to the compatibility of the glass with superphosphate.

GREENHOUSE PROCEDURE

Millet was grown on three soils (— 14-mesh) in two-gallon glazed earthenware pots, namely: an Evesboro loamy sand (*p*H 4.8) from Beltsville Maryland, known to be responsive to applied phosphorus, the optimum moisture content of which was found to be 16 per cent of the oven-dry weight of soil; a Nunn,[4] alluvial clay loam soil (*p*H 8.0) from the Experimental Field at Huntley, Montana, which proved to be moderately responsive to applied phosphorus; and Superstition sand (*p*H 8.5), a calcareous soil from Yuma, Arizona, that is known to be highly responsive to applied water-soluble phosphates.

Each pot of Evesboro soil received an application of calcium hydroxide equivalent to 2240 pounds of calcium carbonate per acre, which previous tests had indicated as the lime requirement for an initial *p*H of 6.5. The lime was mixed with all the soil about 3 weeks prior to planting, and during the interim the soil was watered to the optimum moisture content and allowed to air-dry once.

Minor elements were applied in the form of a solution to the Evesboro and Superstition sand soils. The elements applied and their amounts in parts per million parts of soil were: B 1, Cu 0.5, Zn 0.1, Mn 0.1, Fe 0.5, and Mg 10.

All pots were given a basal treatment of nitrogen and potash—nitrogen at the rate of 75 pounds per acre with equal quantities as ammonium sulfate (21.20% *N*) and sodium nitrate (16.48% *N*), and potash in the form of potassium sulfate (54.06% K_2O) at the rate of 150 pounds of K_2O per acre. The phosphorus carriers were applied in the top 3-inch layer of soil by removing the requisite weight of soil from the pot, breaking up the lumps, and then mixing in the fertilizer.

The greenhouse tests, comprising four of the glasses (one at six fine-

[4] Tentative classification pending broader study of area in which it occurs.

nesses), a fused alpha phosphate and double superphosphate at two and three rates of application, were conducted in three experiments (I, II, III), each of which was designed in a manner that would permit statistical treatment of the yield data.

Experiment I. Twelve phosphate materials were used in this experiment, but only six are pertinent to this discussion. Siberian millet was grown on Evesboro soil. Four of the glasses (one at two finenesses) and double superphosphate were the phosphorus carriers; the rates of application were 50 and 100 pounds of total P_2O_5 per acre; and the design was a randomized block with four replicates. The basal treatment (mixed with all the soil) was applied 22 days prior to seeding, the phosphorus carriers two days before, and the minor elements one day before planting.

The seed (95% germination) was planted March 7, 1947, at the rate of 32 equally-spaced seeds per pot and thinned to 12 plants per pot on March 26. The over-all stand was 90 per cent on the sixth day after planting. The plants, in full head, were harvested on April 11 after a growth period of 31 days.

Experiment II. Golden millet was grown on Evesboro, Nunn, and Superstition sand soils. One glass (two finenesses), a fused alpha phosphate and double superphosphate were the phosphorus carriers, and the rates of application were 50, 100, and 200 pounds of total P_2O_5 per acre. The design (3 replicates) consisted of three randomized split-plot blocks of 39 pots each with all three soils in each block.

The basal treatment and minor elements, both in the form of solutions, were applied to the Evesboro soil and Superstition sand 7 days prior to planting, whereas the basal treatment (solution) without minor elements was applied to the Nunn soil 13 days after seeding. The phosphorus applications were made 8 days prior to seeding.

The millet was planted May 27, 1947, at the rate of 33 equally-spaced seeds per pot and thinned to 10 plants per pot on June 13. Plants were up in all pots in the late afternoon of the second day. The plants, in the boot stage, were harvested on July 8 after a growth period of 42 days.

As early as June 12 the plants growing on Superstition sand showed marked signs of nutrient deficiency—light stripes extending the full length of the leaves were readily apparent. The stripes persisted throughout the growth period and at harvest time were very marked in the plants growing in pots treated with the glass and double superphosphate. On the other hand, pots treated with fused alpha phosphate showed the striping only to a slight extent.

Experiment III. Golden Millet was grown on Superstition sand treated in the manner described for this soil in Experiment II. The dates of planting, thinning, and harvesting were also the same as in the preceding experiment. A glass at four finenesses and double superphosphate was used as phosphorus carriers at 50, 100, and 200 pound rates of application.

The pots (3 replicates) were arranged in a randomized block. Striping of the plant leaves was very pronounced in pots treated with double superphosphate and to a variable extent in pots treated with the glass.

NUTRIENT VALUE OF GLASSES

Statistical analysis of the yields of millet (Tables 5, 6, and 7) show very high significance (Table 8) to variations arising from soil differences, phosphorus carrier, fineness of carrier, and rate of application.

Evesboro Soil.—In greenhouse experiments (I and II) on acid soil the growth response of millet (Tables 5 and 6) to phosphate rock-magnesium silicate glasses ground to -80 mesh or finer was greater than that to double superphosphate in 9 of the 11 instances (Table 9). The coarse material was definitely inferior in both experiments. Statistical significance was attained in all cases at the 100- and 200-pound rates of application. The results show a trend towards lower efficiency of olivine glasses.

TABLE 5.—*Yields of millet on Evesboro soil treated with phosphate rock-magnesium silicate glasses and double superphosphate (Experiment I)*

PHOSPHORUS CARRIER			DRY WEIGHT OF PLANTS IN GRAMS PER POT AT P_2O_5 APPLICATIONS OF—			
			50 LB. PER ACRE		100 LB. PER ACRE	
SAMPLE NO.	MATERIAL	MESH SIZE	RANGE	AVERAGE[a]	RANGE	AVERAGE[a]
—	Double superphosphate	− 40	2.55–3.54	3.11[b]	2.76–4.51	3.69
2497-a	Phosphate rock-serpentine glass	− 6	1.08–1.56	1.23	1.19–2.00	1.42
2497-b	Phosphate rock-serpentine glass	−100	2.84–3.90	3.54	5.01–6.51	5.56
2463	Phosphate rock-serpentine glass	− 80	3.23–5.65	4.07	4.69–5.76	5.33
2488	Phosphate rock-olivine glass	− 80	3.00–3.60	3.39	4.40–5.70	5.16
2483	Phosphate rock-olivine glass	− 80	2.35–3.51	3.00	4.32–5.43	4.67

[a] Differences in average dry weights required for significance are 0.80 at 5% level and 1.05 at 1% level.
[b] Dry weights of plants without applied phosphorus were 0.40 to 0.51 with average of 0.44.

Nunn Soil.—The growth responses (Tables 6 and 9), in general, parallel those on Evesboro soil. The 100-mesh glass gave higher responses at the 100- and 200-pound rates of application, whereas the coarse material again was inferior to double superphosphate.

Superstition Sand.—Growth responses of millet (Tables 6 and 7) to the glass are on the average significantly lower than those to double superphosphate (Table 9). Again finely-ground material is superior to coarse material. It would appear from these results that a fineness exceeding 60-mesh is necessary for the glass to compare favorably with double superphosphate. It should be remembered in this connection, however, that the plants growing on this soil showed deficiency symptoms, which lends some uncertainty to the comparisons.

Hartsells Fine Sandy Loam and Fullerton Silt Loam.—In greenhouse experiments reported by Walthall and Bridger (11) the responses of Sudan grass to a phosphate rock-olivine glass on Hartsells fine sandy loam (*p*H

TABLE 6.—*Yields of millet on soils treated with phosphate rock-magnesium silicate glass, fused alpha phosphate, and double superphosphate (Experiment II)*

SAMPLE NO.	PHOSPHORUS CARRIER		DRY WEIGHT OF PLANTS[a] IN GRAMS PER POT AT P_2O_5 APPLICATIONS OF—					
	MATERIAL	MESH SIZE	50 LB. PER ACRE		100 LB. PER ACRE		200 LB. PER ACRE	
			RANGE	AVERAGE	RANGE	AVERAGE	RANGE	AVERAGE
			Evesboro soil[b]					
—	Double superphosphate	− 40	14.53–16.97	15.56	17.49–21.55	19.15	15.90–19.41	17.54
2497-a	Phosphate rock-serpentine glass	− 6	10.38–12.92	11.42	7.84–17.93	12.16	12.70–18.00	15.22
2497-b	Phosphate rock-serpentine glass	−100	13.79–18.28	16.20	16.52–19.65	18.44	17.14–19.65	18.51
2496	Fused alpha phosphate	−100	12.95–18.29	15.49	16.23–16.78	16.55	19.51–21.07	20.45
			Nunn soil[c]					
—	Double superphosphate	− 40	16.90–21.62	19.87	18.53–20.97	19.50	18.45–20.62	19.39
2497-a	Phosphate rock-serpentine glass	− 6	17.21–19.85	18.09	15.75–20.44	17.41	16.83–18.30	17.74
2497-b	Phosphate rock-serpentine glass	−100	15.75–18.71	17.64	19.08–23.62	21.52	18.77–21.29	20.15
2496	Fused alpha phosphate	−100	11.48–19.57	15.64	13.19–17.35	14.62	16.67–20.11	18.24
			Superstition sand[d]					
—	Double superphosphate	− 40	4.18– 8.77	5.79	6.37–10.60	8.04	6.88–16.39	11.57
2497-a	Phosphate rock-serpentine glass	− 6	0.07– 1.91	0.88	0.27– 2.18	1.45	1.24– 4.20	2.84
2497-b	Phosphate rock-serpentine glass	−100	4.52–10.13	7.93	5.77–11.00	8.90	3.40–11.85	6.76
2496	Fused alpha phosphate	−100	0.12– 0.84	0.40	0.06 1.48	0.81	0.15 · 1.84	1.02

[a] Difference in average dry weights required for significance are 0.60 at 5% level and 0.78 at 1% level.
[b] Dry weights of plants without applied phosphorus were 1.55 to 4.65 with average of 3.05.
[c] Dry weights of plants without applied phosphorus were 13.84 to 15.44 with average of 14.89.
[d] Dry weights of plants without applied phosphorus were 0.05 to 0.07 with average of 0.06.

TABLE 7.—*Yields of millet on Superstition sand treated with phosphate rock-serpentine glass of different mesh sizes and double superphosphate*[a] (*Experiment III*)

PHOSPHORUS CARRIER			DRY WEIGHT OF PLANTS[a] IN GRAMS PER POT AT P_2O_5 APPLICATIONS OF—					
SAMPLE NO.	MATERIAL	MESH SIZE	50 LB. PER ACRE		100 LB. PER ACRE		200 LB. PER ACRE	
			RANGE	AVERAGE	RANGE	AVERAGE	RANGE	AVERAGE
—	Double superphosphate	− 40	8.01–12.00	9.74	8.52–12.59	11.15	13.32–15.58	14.79
2497-c	Phosphate rock-serpentine glass	− 35	2.57– 3.54	3.07	5.22– 7.26	6.10	7.59–10.51	8.35
		− 60	3.88– 7.70	6.38	4.34–12.17	9.10	9.30–11.09	10.31
		−150	6.62– 8.45	7.74	7.76–10.57	9.42	6.20–10.58	8.91
		−300	10.05–12.76	11.07	8.31–10.42	9.69	6.07– 9.55	8.23

[a] Differences in average dry weights required for significance are 3.21 at 5% level and 4.34 at 1% level.

TABLE 8.—*Analysis of variance*

SOURCE OF VARIATION	DEGREES OF FREEDOM	MEAN SQUARE	DEGREES OF FREEDOM	MEAN SQUARE
	RANDOMIZED BLOCK EXPERIMENTS ON EVESBORO SOIL			
	EXPERIMENT I. 50- AND 100-LB. RATES		FROM EXPT. II. 50- TO 200-LB. RATES	
Total	95	—	63	—
Block	3	2.31†	3	5.11†
Phosphorus carrier[a]	11	11.72‡	3	19.08‡
Rate of application	1	24.95‡	3	18.50‡
Error	80	0.33	54	0.38
	EXPERIMENT II. SPLIT-PLOT BLOCK EXPERIMENT ON EVESBORO, NUNN, AND SUPERSTITION SAND SOILS			
	WHOLE PLOT:		SUB-PLOT:	
Total	8	—	107	—
Soil	2	1955.02‡	—	—
Replicates	2	3.79	—	—
Phosphorus carrier	—	—	3	146.10‡
Rate of Application	—	—	2	37.67‡
Rate × carrier	—	—	6	6.37‡
Rate × soil	—	—	4	3.88‡
Soil × carrier	—	—	6	36.99‡
Soil × carrier × rate	—	—	12	29.28‡
Error	4	21.84	66	0.14
	EXPERIMENT III. FINENESS SERIES ON SUPERSTITION SAND			
Total	44	—		
Block	2	2.74		
Phosphorus carrier	4	40.60‡		
Rate of application	2	25.84†		
Rate × carrier	8	10.00*		
Error	28	3.63		

[a] Of the twelve materials used only six are pertinent to the discussion in this article.
* Significant at 5% level.
† Significant at 1% level.
‡ Significant at 0.1% level.

4.9) and Fullerton silt loam (*p*H 5.3) were as good as those to double superphosphate.

GLASSES COMPARED WITH FUSED ALPHA PHOSPHATE

Fused alpha phosphate was used as a source of phosphorus for millet growing on Evesboro, Nunn, and Superstition sand soils (Table 6). The growth responses to this phosphorus carrier were in nearly all cases significantly less (Table 9) than those to glasses of comparable fineness.

SOLUBILITY OF PHOSPHORUS COMPARED WITH GROWTH RESPONSE

When the increases in growth response to the glasses in comparison with double superphosphate are set alongside the "solubility" results

TABLE 9.—*Increase in growth response of millet on soils treated with phosphate rock-magnesium silicate glasses and fused alpha phosphate in comparison with double superphosphate*

PHOSPHORUS CARRIER				INCREASE[a] IN AVERAGE DRY WEIGHT IN GRAMS PER POT AT P_2O_5 APPLICATIONS OF—		
SAMPLE NO.	MATERIAL	MESH SIZE	EXPERIMENT NO.	50 LB. PER ACRE	100 LB. PER ACRE	200 LB. PER ACRE
			Evesboro soil			
2497-a	Phosphate rock-serpentine glass	− 6	I	−1.88†	−2.27†	—
	Phosphate rock-serpentine glass		II	−4.14†	−6.99†	−2.32†
2497-b	Phosphate rock-serpentine glass	−100	I	+0.43	+1.87†	—
	Phosphate rock-serpentine glass		II	+0.64*	−0.71*	+0.97†
2463	Phosphate rock-serpentine glass	− 80	I	+0.96*	+1.64†	—
2488	Phosphate rock-olivine glass	− 80	I	+0.28	+1.47†	—
2483	Phosphate rock-olivine glass	− 80	I	−0.11	+0.98*	—
2496	Fused alpha phosphate	−100	II	−0.07	−2.60†	+2.91†
			Nunn soil			
2497-a	Phosphate rock-serpentine glass	− 6	II	−1.78†	−2.09†	−1.65†
2497-b	Phosphate rock-serpentine glass	−100	II	−2.23†	+2.02†	+0.76*
2496	Fused alpha phosphate	−100	II	−4.23†	−4.88†	−1.15†
			Superstition sand			
2497-a	Phosphate rock-serpentine glass	− 6	II	−4.91†	−6.59†	−8.73†
2497-b	Phosphate rock-serpentine glass	−100	II	+2.14†	+0.86†	−4.81†
2497-c	Phosphate rock-serpentine glass	− 35	III	−6.67†	−5.05†	−6.44†
	Phosphate rock-serpentine glass	− 60	III	−3.36*	−2.05	−4.48†
2497-c	Phosphate rock-serpentine glass	−150	III	−2.00	−1.73	−5.88†
	Phosphate rock-serpentine glass	−300	III	+1.33	−1.46	−6.56†
2496	Fused alpha phosphate	−100	II	−5.39†	−7.23†	−10.55†

[a] Plus sign indicates a yield greater than that with double superphosphate.
* Significant at 5% level.
† Significant at 1% level.

TABLE 10.—*Availability of Phosphate Rock-magnesium Silicate Glasses by "Solubility" and Growth-response Methods*

DESCRIPTION OF GLASS				SOLUBILITY[a] OF PHOSPHORUS IN—		INCREASE IN GROWTH RESPONSE[b] IN GRAMS PER POT AT RATE OF APPLICATION OF—			SOIL USED
NO.	MADE WITH	PRODUCTION	MESH SIZE	NEUTRAL AMMONIUM CITRATE	2 PER CENT CITRIC ACID	50 LB. PER ACRE	100 LB. PER ACRE	200 LB. PER ACRE	
						−1.88†	−2.27†	—	Evesboro
2497-a	Serpentine	Large-scale	− 6	63	99	−4.14†	−6.99†	−2.32†	Evesboro
						−1.78†	−2.09†	−1.65†	Nunn
						−4.91†	−6.59†	−8.73†	Superstition
						+0.43	+1.87†	—	Evesboro
2497-b	Serpentine	Large-scale	−100	84	98	+0.64	+0.71*	+0.97†	Evesboro
						−2.23†	+2.02†	+0.76*	Nunn
						+2.14†	+0.86†	−4.81†	Superstition
			− 35	43	93	−6.67†	−5.05†	−6.44†	Superstition
2497-c	Serpentine	Large-scale	− 60	53	97	−3.36*	−2.05	−4.48†	Superstition
			−150	74	97	−2.00	−1.73	−5.88†	Superstition
			−300	87	96	+1.33	−1.46	−6.56†	Superstition
2463	Serpentine	Experimental	− 80	72	100	+0.96*	+1.64	—	Evesboro
2488	Olivine	Experimental	− 80	84	87	+0.28	+1.47	—	Evesboro
2483	Olivine	Experimental	− 80	78	93	−0.11	+0.98	—	Evesboro

[a] In percentage of the total phosphorus.
[b] In comparison with that to double superphosphate at rate of 100 pounds of P_2O_5 per acre (Table 9).
* Significant at 5% level.
† Significant at 1% level.

(Table 10), one noteworthy comparison stands in bold relief. The difference between the fineness of the −6- and −100-mesh materials, which is reflected markedly in growth response, is indicated to a moderate extent by citrate solubility and is not shown at all by citric acid solubility. The trend of the results for the fineness series applied to Superstition sand at 50- and 100-pound rates indicate similar relationships. It would appear therefore that, as concerns immediate utilization of the phosphorus by plants, citric acid solubility, and perhaps also citrate solubility, overrates the availability of coarse glass.

It has been suggested that in the case of the coarse material, which probably consists of granules as they come from the quenching operation, the nature of the surface rather than its extent may be the factor that limited the utilization of this material by growing plants. Actually the −6-mesh material does consist of rods and spherical and vesicular granules, many with attached filament, with mirror-like surfaces, all of which indicates a quenched surface little disturbed by grinding. The consideration is, then, a quenched surface in comparison with a surface of fracture. The data at hand do not permit a decision on this question.

SUMMARY

Seven commercial phosphate rock-magnesium silicate glasses, including one sample of large-scale production and experimental glasses produced with the use of both serpentine and olivine, were analyzed for P_2O_5, CaO, MgO, Al_2O_3, FeO, and F, and their solubilities in neutral ammonium citrate and 2 per cent citric acid were determined.

Selected glasses were subjected to screen analysis, to a study of fineness-solubility relationships, to petrographic examination, to crystallization tests, to reactivity tests in mixtures, and to plant-response tests on Evesboro, Nunn, and Superstition sand soils in the greenhouse, with the use of fused alpha phosphate and double superphosphate as comparison materials.

The major constituents of the glasses are P_2O_5 (19.75–23.61%), SiO_2 (19.24–23.80%), CaO (28.33–35.55%), and MgO (11.51–18.53%); other important constituents are Al_2O_3 (0.59–3.73%), FeO (1.96–3.82%) and F (1.65–2.22%).

The effect of fineness on citrate solubility is much more pronounced in the case of these glasses than in the cases of basic slag and fused alpha phosphate. The citrate solubilities of a glass at finenesses of −60 and −300 mesh, respectively, were 52.8 and 86.8 per cent in comparison with 86.7 and 91.0 for a basic slag at the same finenesses. Citric acid solubility did not change markedly over this range of fineness.

The glasses vary considerably in rate of crystallization when they are annealed at temperatures below the melting point (1300 to 1400°C.). Apatite is always an important constituent of crystallized samples, and

the lowered phosphorus solubility accompanying crystallization is attributable to apatite formation.

The glass caused a marked loss of ammonia when it was suspended in an aqueous solution of ammonium nitrate. Glass-superphosphate mixtures (20 to 80% superphosphate) in indecisive tests showed continuous decreases in water-soluble phosphorus without progressive changes in citrate-insoluble phosphorus.

In greenhouse tests on Evesboro (pH = 4.8) and Nunn (pH = 8.0) soils, the growth response of millet to the glasses (-80-mesh or finer) was greater than that to double superphosphate in twelve of the twenty cases, whereas on Superstition sand (pH = 8.5) the response was usually less than that to double superphosphate. Coarse glass was inferior to finely-ground glass on all three soils, and a fineness exceeding 60 mesh is indicated as necessary for favorable comparison of the glass with double superphosphate.

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EFFECT OF ALCOHOL CONCENTRATION AND SATURATION OF THE ACID ALCOHOL WITH K_2PtCl_6 IN THE ANALYSIS OF POTASH IN FERTILIZERS*

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In the official A.O.A.C. method for determining potash in fertilizers the K_2PtCl_6 is treated with acid alcohol and washed with 80% alcohol (1). Ford and Hughes (2) showed that an increase in the concentration of the alcohol and acid alcohol resulted in an increase in the amount

* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 20–22, 1947.
[1] Journal Paper No. 317 of the Purdue University Agricultural Experiment Station.

of potash obtained in the determination of potash in fertilizers. Mitchell and Ford (3) showed that in the case of potash determined from pure potassium chloride the increases obtained with the higher alcohol concentrations were due to true potash. Ewan, Ford, and Schall (4) showed that the increases in potash obtained in analyzing mixed fertilizers when 95% alcohol was used in place of 80% alcohol were also true potash. The

TABLE 1.—*Effect of concentration of ethanol and saturation of acid alcohol with K_2PtCl_6 on K_2O values*

	85% ACID ALCOHOL +ALCOHOL			90% ACID ALCOHOL +ALCOHOL		
SAMPLE	% K_2O			% K_2O		
	High	*Low*	*Av.*	*High*	*Low*	*Av.*
A. (0-12-12)						
Unsat.	13.09	13.00	13.05*	13.19	13.12	13.16*
Sat.	13.36	13.25	13.33	13.31	13.26	13.29
B. (0-20-20)						
Unsat.	21.18	21.11	21.15	21.30	21.20	21.26
Sat.	21.70	21.51	21.58	21.42	21.40	21.41
C. (0-9-27)						
Unsat.	28.20	27.88	28.00	27.88	27.88	27.88
Sat.	28.24	28.16	28.20	28.20	28.13	28.15
D. (8-8-8)						
Unsat.	8.12	8.09	8.11	8.37	8.19	8.30
Sat.	8.56	8.45	8.50	8.42	8.34	8.37
E. (4-12-8)						
Unsat.	8.60	8.48	8.54	8.58	8.45	8.52
Sat.	9.05	8.68	8.86	8.70	8.59	8.65

* Average in each case is the result of 3 analyses.

same workers also reported a large increase of potash when the 80% acid alcohol was saturated with K_2PtCl_6 and a smaller increase when the 95% acid alcohol was saturated with K_2PtCl_6. In each case, however, the increases were found to be true potash.

The purpose of this work was to supplement that of Ewan, Ford, and Schall (4) by determining the influence of 85 and 90% ethanol with and without prior saturation with K_2PtCl_6 upon the potash values and the purity of the precipitate obtained.

To test the purity of the K_2PtCl_6 obtained, the weighed salt in each case was reduced for platinum recovery and estimation of the chloride by a Volhard titration. To facilitate the reduction the salt in each case was dissolved in hot water, transferred to a new, unscratched 250 ml. beaker, and if necessary concentrated to a volume of about 75 ml. While boiling,

2–5 ml. of 40% formic acid was added and as soon as reduction started a piece of filter paper on a glass rod was used to prevent sticking. As soon as the reduction was complete, the reduced platinum black together with the filter paper was filtered on a tared asbestos-padded Gooch, dried at 100°C., ashed in a muffle at 600°C. for about 5 hours, cooled, and weighed. The filtrate from the reduction in each case was collected, made to volume and aliquots taken for the estimation of the chloride by the Volhard titration method. The endpoint of the titration was sharpened by coagulating the precipitated silver chloride by shaking it with 1–2 ml. of nitrobenzene and filtering it off before the final titration was made.

RESULTS

The results of this work (Table 1) confirm and extend those of Ewan, Ford, and Schall (4) in showing that the increases in potash are smaller with the higher concentrations of alcohol. On the other hand, saturation of the 85% acid alcohol with K_2PtCl_6 gave larger increases than saturation of 90% acid alcohol.

TABLE 2.—*Effect of ethanol concentration and saturation of the acid alcohol on the purity of the K_2PtCl_6*

SAMPLE	PLATINUM (GRAMS)			CHLORIDE (GRAMS)		
	THEORETICAL	FOUND	DIFFERENCE	THEORETICAL	FOUND	DIFFERENCE
			85% Unsaturated			
A.	.0676	.0674*	−.0002	.0737	.0737*	.0000
B.	.1097	.1093	−.0004	.1195	.1193	−.0002
C.	.1453	.1453	.0000	.1582	.1581	−.0001
D.	.0420	.0417	−.0003	.0458	.0465	+.0007
E.	.0441	.0438	−.0003	.0483	.0485	+.0002
			85% Saturated			
A.	.0690	.0687	−.0003	.0753	.0748	−.0005
B.	.1117	.1115	−.0002	.1220	.1207	−.0013
C.	.1461	.1460	−.0001	.1582	.1579	−.0003
D.	.0447	.0452	+.0005	.0491	.0488	−.0003
E.	.0459	.0458	−.0001	.0501	.0500	−.0001
			90% Unsaturated			
A.	.0681	.0680	−.0001	.0743	.0746	+.0003
B.	.1101	.1098	−.0003	.1201	.1201	.0000
C.	.1444	.1441	−.0003	.1575	.1557	−.0018
D.	.0430	.0428	−.0002	.0469	.0473	+.0004
E.	.0442	.0449	+.0007	.0482	.0483	+.0001
			90% Saturated			
A.	.0689	.0688	−.0001	.0751	.0744	−.0007
B.	.1109	.1110	+.0001	.1210	.1204	−.0006
C.	.1459	.1459	.0000	.1590	.1572	−.0018
D.	.0434	.0432	−.0002	.0472	.0472	.0000
E.	.0448	.0442	−.0006	.0488	.0489	+.0001

* Each value is the average of 3 analyses.

The analysis of the K_2PtCl_6 residues for platinum and chloride indicate that within the limits of experimental error the potash obtained is true potash whether obtained by an increase in alcohol concentration or by saturation of the corresponding acid alcohol with K_2PtCl_6 (Table 2).

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A PROCEDURE FOR THE DETERMINATION OF THE PHOSPHORUS CONTENT OF HEXAETHYL TETRAPHOSPHATE AND OF CERTAIN OTHER ORGANIC PHOSPHATES*

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Hexaethyl tetraphosphate, $C_{12}H_{30}O_{13}P_4$, has been reported to be an ideal contact insecticide for the control of aphids and spider mites. "Known in Germany under the trade name *Bladan*, the compound is used as a contact insecticide in either liquid or dust formulations. It has been found effective as a spray in liquid dilutions ranging from 1:1000 to 1:4000 and in concentrations of from 3 to 5 per cent when incorporated with dust carriers."[1]

Several questions arose when this organic phosphate was suggested as an ideal means of insect control in the greenhouse in which plant cultures were being used to establish the fertilizer effectiveness of certain new types of phosphorus compounds—Would the spray residues register to enhance the true phosphorus content of the crops; would the contamination affect plant response through a direct intake of phosphorus into the above-ground growth, or through an uptake from the soil? An additional consideration was whether substantial inputs of the organic phosphate would persist in un-ionized form in the soil and thus provide an effective phosphorus carrier resistant to fixation. Therefore, it was necessary to determine the phosphorus content of the hexaethyl tetraphosphate and its hydrolytic behavior under laboratory treatments and after incorporation into the soil. In the initial analysis of the first sample of that compound, the digestions were those imposed in the determination of the phosphorus

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* Based upon findings obtained in a study conducted in the Cooperative Chemical Research Laboratory of the Department of Chemical Engineering, Tennessee Valley Authority, at the Agricultural Experiment Station of The University of Tennessee.
† Presented at the Annual Meeting of the Association of Official Agricultural Chemists held at Washington, D. C., October 20–22, 1947.
[1] *Ind. Eng. Chem., News Edition*, 25, 1069, April 1947.

content of metaphosphate fertilizers, since those digestions had been found adequate to bring into ortho form all of the other inorganic phosphates used in earlier experiments.[2] In exploratory pot culture trials of the fertilizer effectiveness of the first sample of hexaethyl tetraphosphate, the soil incorporations were based upon the P_2O_5 equivalences indicated by the initial analysis. Upon that basis, crop response to the hexaethyl tetraphosphate exceeded the response to the inorganic phosphates, when phosphorus input was 80 pounds of P_2O_5 per acre surface, half-depth incorporation. This apparent superiority was nullified, however, by the revelation that organically integrated phosphorus was not converted to PO_4 by the conventional prefatory acid digestion of aliquots. Consequently, the organic phosphate incorporations supplied the soil with quantities of phosphorus far greater than those intended, and greater than those supplied by the inorganic ortho phosphate controls.

ANALYTICAL STUDIES

Preliminary observations indicated that the analysis of the liquid hexaethyl tetraphosphate called for both oxidation and ionization, prefatory to the molybdate precipitation. The dual objective was sought through a nitric acid + perchloric acid digestion of a 10-ml. aliquot of a 250-ml. solution of a 1-gm. charge of the organic phosphate. This aliquot had a P_2O_5 equivalence of approximately 20 mgm. Extended to 5 hours, the combined acid digestion gave a phosphorus recovery of only 40 per cent, which was about two-thirds of the recovery obtained through a comparable digestion with aqua regia. Further analytical studies established the fact that the assumed requirement for oxidative treatment was not essential. Comparisons demonstrated that complete transition of the phosphorus content of the organic phosphate into ionized form could be effected by prolonged digestion in relatively concentrated hydrochloric acid, in concentrated nitric acid and in aqua regia. The analytical results shown in Table 1 were obtained when the first sample of hexaethyl tetraphosphate and the two other samples of that compound were subjected to the described analytical treatments and durations.

Ionization had not occurred in the aqueous medium after the solution had stood quiescent 48 hours at room temperature, and but little ionization was induced when a 1-gm. charge of the sample S-1275 was diluted with 150 ml. of water and boiled 3 hours, down to 10 ml. After 24 hours of standing in the aqua regia system at room temperature, a mere trace of phosphorus transition to ortho form had occurred. Moreover, *only small fractions of the potential transitions were induced by conventional digestions in either HNO_3 or HCl at 120°C., even when the digestions were for periods 8 to 10 times longer than those ordinarily imposed.* The quantity of gener-

[2] *Ind. Eng. Chem.*, 29, 224 (1937).

ated PO_4 showed a progressive increase when 10-ml. aliquots of the organic phosphate were digested in aqua regia at 120°C. up to 24 hours. The transitions then registered were apparently absolute, since they were virtually identical with those registered by 20-, 24-, and 28-hour digestions. Upon basis of those determinations, sample S-1275 was a 98.75 per cent product in relation to formula.

Since the sample of hexaethyl tetraphosphate yielded a distinct odor, it

TABLE 1.—*Effect of various ionization treatments upon the conversion of the phosphorus content of three commercial hexaethyl tetraphosphates into ortho form*

TREATMENT OF ALIQUOT		P_2O_5 EQUIVALENCE OF PRODUCT[a]		
REAGENT	DIGESTION PERIOD	S-1271[b,c]	S-1275[b,c]	S-1280[d,e]
In water at room temperature	48 hrs.	None	None	—
In aqua regia at room temperature	24 hrs.	Trace	Trace	Trace
In acids		—	2.1	—
5 ml. HNO_3, 120° C.[f]	15 min.	6.2	—	—
5 ml. HNO_3, boiling	30 min.	—	9.0	—
5 ml. HNO_3, 120° C.[f]	20 hrs.	—	46.1	—
5 ml. HCl, 120° C.[f]	30 min.	4.4	—	—
10 ml. HCl, boiling	30 min.	4.7	19.0	—
10 ml. HCl, 120° C.[f]	1 hr.	6.1	—	—
5 ml. HCl, boiling	30 min.	9.2	—	—
5 ml. HCl, 120° C.[f]	2 hrs.	8.7	—	—
5 ml. HCl, 120° C.[f]	20 hrs.	8.8	55.2	26.9

[a] Determined volumetrically on a 10 ml. treated aliquot of a solution of the sample, 1-gm. charge diluted to 250 ml.
[b] Aqueous solution.
[c] Theoretical P_2O_5 content 56%, upon basis of formula $C_{12}H_{30}O_{13}P_4$ for hexaethyl tetraphosphate.
[d] Theoretical P_2O_5 content 28%, upon basis of formula $C_{12}H_{30}O_{13}P_4$ for hexaethyl tetraphosphate (product labelled as 50% solution. The inert ingredient was an organic solvent).
[e] Alcoholic solution of sample.
[f] Slightly below boiling point of the mixture of sample and acid.

seemed desirable to ascertain whether the observed volatility would entail a loss of phosphorus. Therefore, a 150-ml. aqueous solution of 1 gram of the organic phosphate S-1275 was evaporated to 10 ml. through two hours of gentle boiling in a beaker. The resultant concentrate then was dilute to volume in a 250-ml. flask and 10-ml. aliquots were subjected to digestions identical with those imposed upon corresponding aliquots of an untreated aqueous solution of the initial sample. Since the analytical finding of 55.2 per cent P_2O_5-equivalence for the boiled aliquot was identical to the value shown for the aqueous aliquot that was acid-digested, as in Table 1, it is obvious that no loss of phosphorus occurred during the prolonged unrefluxed boiling.

When a 10-ml. aliquot of the aqueous solution of hexaethyl tetraphos-

phate was evaporated to dryness, the result was a black charry mass, which contained a white substance that was insoluble in dilute nitric acid. Aliquots of the 250-ml. solution of a 1-gm. charge of that phosphate also were subjected to treatments with the peroxides of hydrogen, calcium, sodium, and barium, prefatory to the presently prescribed acid digestion, but none of these effected the desired transition of the organic phosphorus into ortho form. The period of digestion requisite for the complete transition of sample S-1275 was diminished by several hours when 10-ml. aliquots were processed by evaporation with a like volume of a saturated solution of magnesium nitrate, cautious incineration, and dissolution of the incinerate in dilute nitric acid. Although such processing served to effect complete transitions of the phosphorus content of 10-ml. aliquots of certain organic phosphates, it did not induce complete transitions in some cases. Moreover, such processing of aliquots introduces undesirable mechanical features, and is not recommended presently. The prescribed "*Analytical Technique*" is the only direct digestion procedure yet found to meet fully the requirement for the determination of the P_2O_5-equivalences of those samples of hexaethyl tetraphosphate that have been subjected to the present analytical evaluations.

ANALYTICAL TECHNIQUE FOLLOWED IN THE DETERMINATION OF THE PHOSPHORUS CONTENT OF HEXAETHYL TETRAPHOSPHATE

The following steps proved requisite for the conversion of the highly stable hexaethyl tetraphosphate to the form essential for full phosphorus recovery through precipitation as ammonium phosphomolybdate:

A 1-gm. charge of the sirupy organic phosphate was weighed into a 250-ml. flask, diluted to volume and mixed. A 10-ml. aliquot was transferred to a 200-ml. Erlenmeyer flask fitted with tube for air refluxing. Additions of 5 ml. each of concentrated hydrochloric and nitric acids were made and the solution was digested at least 16 hours at 120°C. The digestate was allowed to cool and the sides of the flask and the reflux tube were washed with a small volume of water. The diluted digestate was boiled in an open flask to expel free chlorine. The solution was allowed to cool, made slightly alkaline by means of ammonium hydroxide (1+1), and again allowed to cool. A few drops of methyl orange were added and the system was made slightly acidic through addition of nitric acid (1+1). Phosphorus content was determined as in the A.O.A.C. volumetric procedure for fertilizers.[3]

OBSERVATIONS CONCERNING OTHER ORGANIC PHOSPHATES

Since hexaethyl tetraphosphate and similar organics may be used extensively, there should be an analytical procedure adapted to the verifica-

[3] *A.O.A.C. Official and Tentative Methods of Analysis*, Sixth Edition, 1945.

tion of the phosphorus content indicated by the formula. The experience with the hexaethyl tetraphosphate was applied in the analytical evaluation of certain other organic phosphates, several of which were subjected to the conditions detailed in Table 2. The P_2O_5 values obtained by the procedure prescribed for a 24-hour digestion of the hexaethyl tetraphosphate were in accord with those computed from the formulas attributed to three of the samples, whereas only partial P_2O_5 values were registered in the case

TABLE 2.—*The progressive conversion of the phosphorus of certain organic phosphates into ionized ortho form by means of Aqua Regia digestion*[a]

DIGESTION PERIOD	DETERMINED P_2O_5 VALUES[b]—PERCENTAGES						
	S-1271[c,d]	S-1275[c,d]	S-1280[e,f]	S-1276[c,g]	S-1277[f,h]	S-1278[f,i]	S-1279[c,j]
Hrs.							
¼	7.0	3.9	—	—	—	—	[k]
½	8.6	5.0	—	—	—	—	—
1	11.4	7.4	None	None	None	None	17.10
2	15.6	11.4	—	Trace	None	None	—
3	25.4	17.1	—	2.8	None	None	—
4	27.6	22.2	None	3.2	None	None	36.0
5	35.6	30.6	—	9.4	—	—	—
6	40.1	38.6	—	—	—	—	—
7	47.6	44.6	—	—	—	—	—
16	53.7	54.8	23.7	—	3.0	11.9	57.2
20	57.0	54.8	—	37.4	—	—	—
24	57.5	55.1	28.7	37.3	4.9	18.1	57.5
28	—	55.3	—	—	—	—	—

[a] 10 ml. aliquots of 1 gm. to 250 ml. of sample solution digested with 10 ml. aqua regia in flask with air reflux at 120° C., the temperature just below boiling point.
[b] Determined volumetrically after neutralization of digested aliquot.
[c] Aqueous solution.
[d] Theoretical P_2O_5 content 56%, upon basis of formula $C_{16}H_{40}O_{13}P_4$ for hexaethyl tetraphosphate.
[e] Same as (d), but labelled as containing "50% active ingredients."
[f] Alcoholic solution.
[g] Theoretical P_2O_5 content 38.6%, upon basis of formula $(C_2H_5)_3O_4PO$ for triethyl phosphate.
[h] Theoretical P_2O_5 content 19.2%, upon basis of formula $(C_7H_7CH_3)_3O_4PO$ for tricresyl phosphate.
[i] Theoretical P_2O_5 content 21.75%, upon basis of formula $(C_6H_5)_3O_4PO$ for triphenyl phosphate.
[j] Theoretical P_2O_5 content 56.3%, upon basis of formula $C_2O_5H_7PO_4$ for monoethyl orthophosphate.
[k] An untreated aqueous aliquot gave 8.75% P_2O_5 through molybdate precipitation, as indicative of the amount in the ionized ortho form.

of samples S-1277 and S-1278 purported to be tricresyl phosphate and triphenyl phosphate, respectively.

To determine the phosphorus content of organics by means of the conventional molybdate precipitation, that content must be converted into the ionized ortho form through prefatory treatment known to be *specifically* adequate. Although the described analytical technique was found applicable for the exact determination of the phosphorus content of the particular hexaethyl tetraphosphate used in the greenhouse studies, and for a like determination upon certain other organic phosphates, that technique did not induce absolute ionization of the phosphorus content of the

tricresyl and triphenyl phosphates. Until the evolution of an analytical procedure that will induce complete ionization of the phosphorus content of all organic phosphates, it seems imperative that each new product be analyzed by a procedure of proved specific applicability and adequacy.

NOTE: Subsequent to the submittal of the foregoing findings, the following notation was carried by *Chemical and Engineering News*, p. 2926, October 6, 1947:

"The compound prepared by the reaction of triethyl phosphate and phosphorus oxychloride and sold commercially as "*hexaethyl tetraphosphate* does not, according to J. W. Hansen, California Spray Chemical Corporation, Richmond, exist in this form, but is a fortuitous mixture of organic phosphates which has an average molecular weight and combustion analysis percentages agreeing with the assigned formula. Chemical analyses of fractions obtained by distillation have shown that the active agent is a tetraethyl phosphate, probably tetraethyl peroxydiphosphate."

A PROCEDURE FOR THE DETERMINATION OF THE "AVAILABLE" MAGNESIUM ENGENDERED IN MIXTURES OF SUPERPHOSPHATE WITH OLIVINE, SERPENTINE, MAGNESITE, AND THEIR CALCINES*

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The role of magnesium in plant nutrition has been the objective of much research and the importance of this element as a fertilizer component is being accorded increasing recognition (6, 7, 12, 14, 27, 28, 37, 40). The soils of extensive areas are deficient in magnesium and this deficiency is registered by crops through visual symptoms that are well known. With recognition of the need for nutrient magnesium, the manufacture of magnesium-fortified fertilizers was a logical sequence.

PREVIOUS PRACTICES

The inclusion of dolomite as a source of nutrient magnesium in phosphatic fertilizers, and as a conditioner and neutralizer, was begun industrially in 1919, upon recommendation of the Tennessee Experiment Station. The P_2O_5 transitions that are induced by such inclusions and by admixtures of selectively calcined dolomite were dealt with in several papers (10, 17, 18, 24, 26). When the fertilizer effectiveness of dolomited phosphate had been demonstrated, and after Garner's classical experiments had established the need for magnesium in the growing of tobacco (7), the practice of the inclusion of dolomite in mixed fertilizers became

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* A study conducted in the Cooperative Chemical Research Laboratory of the Department of Chemical Engineering, Tennessee Valley Authority, at the Tennessee Agricultural Experiment Station.
† Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 20–22, 1947.

widespread in the South Atlantic Coast States. Some 15 years ago, however, the "tobacco fertilizer group" contended that an adequate supply of nutrient magnesium was not developed with sufficient rapidity from included dolomite, and that tobacco fertilizers should contain magnesium sulfates.

Substantial proportions of "available" magnesia may be engendered when adequately moistened mixtures of acidic phosphates and finely ground dolomite are aged. In the formulation of higher analysis mixtures, however, the admissible quantity of included dolomite does not provide an adequate percentage of "available" magnesium. This difficulty would be overcome by the use of a low-cost magnesic material of such form and concentration that its inclusion in "high analysis" goods will meet the requirements for nutrient magnesium.

In correspondence with one of the present authors, the late G. H. Holford revealed that, because of the scarcity of dolomite in New Zealand, the mineral magnesium silicates were tried as conditioners of superphosphate. The resultant mixtures were of good physical condition and chemical composition and proved to be effective fertilizers. According to Andrews (1), the early New Zealand practice of neutralization of the H_3PO_4 of superphosphate by 8 to 10 per cent additions of dunite ($MgSiO_4$) was superseded by the use of serpentine ($Mg, Fe)_3Si_2O_7 \cdot 2H_2O$. The attendant reactions were dealt with by Askew (2), who concluded that $Ca_3(PO_4)_2$ was the preponderant end-product in the mixtures and that the "available" P_2O_5 content was not lessened. Similar benefits had been claimed in an early patent (31).

Extensive formations of olivine and serpentine occur in Western North Carolina and Northern Georgia and have been mapped through surveys conducted by the State of North Carolina in collaboration with the Tennessee Valley Authority (9). When incorporated into soils directly in amounts beyond those requisite for saturation with magnesium, a mineral magnesium silicate was found effective (27), but in more recent pot culture studies the initial crop gave virtually no response to incorporation of olivine at conventional liming rates. Findings by the present authors (15) served to demonstrate that adequate contents of "available" magnesium were generated in moistened mixtures of concentrated superphosphate with olivine and with serpentine, and without diminution in P_2O_5 availability.

PREVIOUS ANALYTICAL PROPOSALS

When calcined Kieserite was included in mixed fertilizers to provide immediately available magnesium, there arose the demand for an analytical procedure for the determination of water-soluble magnesium content. Pursuant to a request by the tobacco fertilizer group, L. G. Willis (38) proposed an analytical procedure to register that content. It was found, however, that the proposed aqueous extraction did not effect full

recovery of the magnesium that was inmixed as magnesium sulfate. Moreover, a fertilizer fortified with MgO, or with selectively calcined dolomite, might be virtually devoid of water-soluble magnesium before analysis and yet the stipulated aqueous extraction would register a substantial percentage of magnesium as being in water-soluble form, because of the magnesium phosphates that develop during analysis (18).

Several helpful analytical procedures have been proposed for the determination of the "available" magnesium contained in mixed fertilizers (11, 30, 29, 33, 34, 35, 36). An acceptable procedure should effect full recovery of the magnesium present in the "available" forms of oxide, hydroxide, carbonate, sulfate, and phosphate, without appreciable concomitant dissolution of any incorporated magnesic or dolomitic materials, the chemical nature and particle size of which would delimit their reactivity in mixed fertilizers, even though such materials undergo disintegration slowly after incorporation into the soil and thus ultimately afford nutrient magnesium. However, the only official procedure for the determination of the magnesium carried by mixed fertilizers is the one prescribed for the acid-soluble content (3).

OBJECTIVE

The objective of the present study was to develop a much-needed method for the determination of the magnesium present as "available" in magnesium-fortified fertilizers. The designation "available" is deemed to include magnesium content attributable to mono-, di-, trimagnesium phosphates, to magnesium ammonium phosphate, to the oxide, hydroxide, carbonate, or sulfate. Excluded from that designation would be the component magnesium of materials whose inactivity in adequately moist phosphatic mixtures indicate that magnesium nutrition to plants would be slight and delayed.

EXPERIMENTAL

The several magnesium materials that were admixed with the concentrated superphosphate and the mixtures of Tables 1 and 2 were used in the exploratory analyses that led to the present proposal. The reactions that occurred in those mixtures are indicated by the following equations:

$$(1)\ CaH_4(PO_4)_2 \cdot H_2O + MgSiO_3 + 3H_2O \rightarrow CaHPO_4 \cdot 2H_2O + MgHPO_4 \cdot 3H_2O + SiO_2$$

$$(2)\ CaH_4(PO_4)_2 \cdot H_2O + MgCO_3 + 3H_2O \rightarrow CaHPO_4 \cdot 2H_2O + MgHPO_4 \cdot 3H_2O + CO_2 .$$

The several reagents and techniques used in the early digestions of 1-gm charges, and comments upon the results, are summarized as follows:

1. *With 1 per cent citric acid—direct digestion*

(a) 30 minutes, 100 ml @ 30°C.

This solution was found to exert little effect upon separate 1-gm.

TABLE 1.—*Comparison of P_2O_5 transitions and engendered $MgHPO_4 \cdot 3H_2O$ in mixtures[a] of Wilson Dam triple superphosphate with olivine, serpentine, and magnesite*

CODE	T.S.P. MIXTURE	P_2O_5 ANALYSIS[b]				MONOCALCIUM PHOSPHATE				MgO IN MIXTURE	MgO AS W.S.[c]	MgO AS PHOSPHATE	
		TOTAL	W.S.[c]	C.I.[d]	AVAIL.	INITIAL CONTENT[e]	FINAL CONTENT[f]	CONVERSION				COMPUTED[g]	CONVERTED[h]
								ACTUAL	OF INITIAL				
		%	%	%	%	%	%	%	%	%	%	%	%
	Olivine												
P-1265	1+1	23.00	5.20	0.85	22.15	39.38	4.74	34.64	88.0	23.58	1.42	5.54	23.5
P-1266	4+1	37.25	16.10	1.40	35.85	55.00	21.68	33.32	60.6	9.11	2.20	5.32	58.4
P-1267	9+1	42.25	25.30	1.50	40.75	63.50	39.40	24.10	38.0	4.67	1.75	3.86	82.6
	Serpentine												
P-1271	1+1	24.25	5.50	0.90	23.35	37.25	4.58	32.67	87.7	16.34	1.66	4.75	22.9
P-1272	4+1	38.00	18.00	1.40	36.60	57.10	23.78	33.24	58.4	7.09	2.67	5.33	75.1
P-1273	9+1	44.00	28.60	1.55	42.45	69.50	42.64	26.86	38.6	3.56	2.56	4.12	89.8[i]
	Magnesite												
P-1277	1+1	24.75	12.90	0.90	23.85	37.10	20.75	16.35	44.0	21.65	0.68	2.62	12.1
P-1278	4+1	39.50	26.90	1.40	37.10	55.88	33.28	22.60	40.4	8.36	1.42	3.61	43.2
P-1279	9+1	43.25	34.10	1.35	41.90	67.40	53.65	13.75	24.0	4.18	2.19	2.39	57.1

[a] Mixtures of 20-mesh T.S.P. and 100-mesh magnesic materials in the proportions indicated were moistened to 10 per cent H_2O content and cured at 30°C. After 4 week analyses (unreported) showed appreciable P_2O_5 transitions and, in some cases, the values were comparable to those shown for the 10-week period.
[b] Upon 50-mesh samples.
[c] 1 gm. leached with 250 ml. H_2O at room temperature.
[d] A.O.A.C. neutral citrate digestion.
[e] Computed from water-soluble P_2O_5 content of T.S.P.
[f] Final water-soluble P_2O_5 content corrected for $CaH_4(PO_4)_2 \cdot H_2O$ equivalence of water-soluble MgO.
[g] From equation: $CaH_4(PO_4)_2 \cdot H_2O + MgSiO_3 + 3H_2O \rightarrow MgHPO_4 \cdot 3H_2O + CaHPO_4 \cdot 2H_2O + SiO_2$.
[h] Of the total MgO in the mixture.
[i] Corrected value.

TABLE 2.—*Comparison of P_2O_5 transitions and engendered $MgHPO_4 \cdot 3H_2O$ in mixtures[a] of Wilson Dam triple superphosphate with calcines of olivine, serpentine, and magnesite*

CODE	T.S.P. MIXTURES	P_2O_5 ANALYSIS[b]				MONOCALCIUM PHOSPHATE				MgO IN MIXTURE	MgO AS W.S.[c]	MgO AS PHOSPHATE	
		TOTAL	W.S.[c]	C.I.[d]	AVAIL.	INITIAL CONTENT[e]	FINAL CONTENT[f]	CONVERSION				COMPUTED[g]	CONVERTED[h]
								ACTUAL	OF INITIAL				
		%	%	%	%	%	%	%	%	%	%	%	%
Cal. Olivine													
P-1268	1+1	23.25	5.90	0.85	22.40	35.48	4.97	30.50	85.9	23.54	1.76	4.88	20.5
P-1269	4+1	37.00	18.00	1.40	35.60	55.45	23.03	32.40	58.4	8.16	2.85	5.28	64.6
P-1270	9+1	43.00	29.90	1.60	41.40	67.40	47.00	20.40	30.2	4.57	1.94	3.26	71.3
Cal. serpentine													
P-1274	1+1	23.50	5.50	0.80	22.70	36.20	5.22	31.00	85.6	20.57	1.28	4.95	24.0
P-1275	4+1	37.25	16.40	1.35	35.90	55.90	25.00	30.90	55.3	8.41	1.31	4.94	58.7
P-1276	9+1	41.50	24.10	1.55	39.95	65.30	36.35	29.00	44.0	4.43	2.05	4.63	104.5
Cal. magnesite, 750° C.													
P-1280	1+1	23.25	2.00	0.85	22.40	34.80	0.95	35.85	97.3	22.82	0.83	5.41	23.6
P-1281	4+1	35.50	3.80	1.50	34.00	53.04	2.52	50.52	95.2	9.50	1.35	7.07	74.4
P-1282	9+1	41.75	22.80	1.55	40.20	62.40	33.53	28.87	44.3	4.85	2.21	4.60	94.9
Cal magnesite, 950° C.													
P-1283	1+1	22.25	2.10	0.90	21.35	32.62	1.97	30.65	93.9	21.60	1.13	4.90	22.7
P-1284	4+1	36.00	4.60	1.50	34.50	53.93	3.41	50.52	93.6	8.90	1.52	7.07	79.4
P-1285	9+1	41.75	22.12	1.50	40.25	65.64	34.40	31.24	47.4	4.86	2.61	4.96	102.0

[a] Mixtures of 20-mesh T.S.P. and 100-mesh calcined magnesic materials in the proportion indicated were moistened and cured at 30°C. (P-1268 to P-1270 and P-1274 to P-1276 were moistened to 10 per cent H_2O content, P-1280 to P-1285 to 20 per cent content). After 4 weeks, analyses (unreported) showed appreciable P_2O_5 transitions and, in some cases, the values were comparable to those shown for the 10-week period.

[b] Upon 50-mesh samples.

[c] 1 gm. leached with 250 ml. H_2O at room temperature.

[d] A.O.A.C. neutral citrate digestion.

[e] Computed from water-soluble P_2O_5 content of T.S.P.

[f] Final water-soluble P_2O_5 content corrected for $CaH_4(PO_4)_2 \cdot H_2O$ equivalence of water-soluble MgO.

[g] From equation: $CaH_4(PO_4)_2 \cdot H_2O + MgSiO_3 + 3H_2O \rightarrow MgHPO_4 \cdot 3H_2O + CaHPO_4 \cdot 2H_2O + SiO_2$.

[h] Of the total MgO in the mixture.

charges of the magnesic minerals, but it did not dissolve like charges of $MgHPO_4 \cdot 3H_2O$, and hence this reagent was rejected.

2. *With 1 per cent ammonium citrate, pH 4.0—direct digestion*

	Minutes	*Volume*	*Degrees C.*
(a)	30	100 ml	30
(b)	60	100 ml	30
(c)	30	100 ml	50
(d)	30	100 ml	30
(e)	60	200 ml	30

3. *With 1 per cent ammonium citrate, pH 4.0—extraction and digestion*

(a) Leached with 100 ml of reagent at room temperature and residue digested through end-over-end agitation 30 minutes with 100 ml @ 30°C.

(b) Ditto, 60-minute agitation

(c) Ditto, 30-minute agitation, at 50°C.

When used under the several conditions listed under 2 and 3 the acidulated ammonium citrate reagent effected complete dissolution of a 0.95-gm charge of $MgHPO_4 \cdot 3H_2O$, a 1-gm. charge of MgO, and a 0.90-gm charge of $MgNH_4PO_4 \cdot 3H_2O$, yet it registered only meager reactivity upon 1-gm charges of the magnesic minerals. However, when the conditions detailed under 2 and 3 were imposed upon the phosphatic mixtures of Tables 1 and 2, the acidulated 1 per cent ammonium citrate reagent did not effect complete recovery of the P_2O_5 present as "available," and therefore did not dissolve all of the $MgHPO_4 \cdot 3H_2O$ that had been engendered in those mixtures.

4. *With 2 per cent ammonium citrate, pH 4.0—direct digestion*

The more concentrated reagent also effected complete dissolution of 1-gm. charges of MgO, dimagnesium phosphate, and magnesium ammonium phosphate, and likewise was relatively inert upon the magnesium minerals. Nevertheless, this reagent did not effect complete recovery of the "available" P_2O_5 content of the phosphatic mixtures of Tables 1 and 2, and hence was rejected.

5. *With 2 per cent citric acid—direct digestion*

Charges were digested as in a, b, c, d, and e of procedure 2, and also after being leached with 100 ml of the reagent at room temperature, as in a, b, and c of procedure 3.

The 2 per cent citric acid reagent was tested for its capacity to dissolve the magnesium content of several representative magnesic materials and the magnesium phosphate content of the mixtures of Tables 1 and 2, under the conditions specified in a, b, c, d, and e of procedure 2, and in a, b, and c of procedure 3. Upon basis of the analytical results, the use of the 2 per cent citric reagent, with prefatory leaching and digestion with continuous agitation 30 minutes at 50°C., was decided upon as being the only procedure found to meet all requirements.

THE PROPOSED EXTRACTION PROCEDURE

The foregoing observations indicate the progressively severe conditions, including trials with more concentrated solvents, that were imposed throughout the studies. The severe conditions of the exploratory studies were applied until it was found that the "available" P_2O_5 values obtained through the technique of the proposed procedure were in accord with those registered by the "official" method (3). This criterion was adopted for two reasons. Obviously the determination of phosphorus is more expeditious than that of magnesium and the values for the P_2O_5 recoveries register the extent to which the engendered magnesium phosphates undergo dissolution. After the effectiveness of the procedure had been indicated through the P_2O_5 determinations, the applicability of the procedure was substantiated through direct determinations of the extracted magnesium.

Although $MgHPO_4 \cdot 3H_2O$ is appreciably dissolvable by water, that compound is not removed quantitatively by the aqueous extraction prescribed as "official" for the removal of water-soluble P_2O_5. This serves to explain the differences between the values obtained by the direct determination of the MgO content of the 250-ml leachings of 1-gm charges of Table 3 and the corresponding values that were computed from the determined phosphate transitions that occurred in the mixtures of Tables 1 and 2. Transition to di-magnesium phosphate was computed upon basis of the difference between initial and final water-soluble P_2O_5 values, with appropriate correction for the P_2O_5 associated with the magnesium that was extracted concurrently from the engendered magnesium phosphate.

As the result of the findings obtained in the several approaches, the following technique is proposed:

Leach a 1-gm charge of a 50-mesh sample with 100 ml of 2% citric acid at room temperature, by means of successive decantations thru a Shimer filter. Digest the residue and filter pad with an additional 100 ml of the reagent in a 250-ml fertilizer flask 30 min. at 50°C., by means of the end-over-end rotator in a constant-temperature apparatus (16). Filter by suction and wash the residue five times with 5-ml portions of water at 65°C. Combine this digestate with the leachate in a 250-ml fertilizer flask, dilute to volume, and mix. Transfer a 10- or 25-ml aliquot of this soln into a 400-ml beaker; separate calcium content by double precipitation as oxalate, and determine the extracted magnesium by the conventional magnesium ammonium phosphate procedure. Pertinent suggestions and precautions for that determination will be included under "discussion."

REAGENT CAPACITY FOR THE COMPLETE DISSOLUTION OF THE ENGENDERED MAGNESIUM PHOSPHATES

As noted, the primary objective of the analytical studies was to develop a procedure whereby engendered magnesium phosphates and other magnesium compounds of recognized "availability" would be extracted quantitatively from fertilizer mixtures, but with meager dissolution of any residues of the admixed magnesic materials.

TABLE 3.—*"Available" magnesia attributed to engendered phosphates in mixtures of concentrated superphosphate and various magnesic materials, as determined by the proposed procedure and as computed from phosphate transitions*

MIXTURES	MgO OCCURRENCES IN THE MIXTURES			
	AS ACID-SOLUBLE[a]	IN WATER-EXTRACTS[b]	COMPUTED AS $MgHPO_4 \cdot 3H_2O$[c]	AS 2% CITRIC ACID-SOLUBLE[d]
	per cent	*per cent*	*per cent*	*per cent*
T.S.P. with:				
Olivine				
1—1	23.58	1.42	5.54	5.69
4—1	9.11	2.20	5.32	5.56
9—1	4.67	1.75	3.86	3.78
Calc. olivine				
1+1	23.54	1.76	4.88	5.83
4—1	8.16	2.85	5.28	5.29
9+1	4.57	1.94	3.26	2.92
Serpentine				
1+1	16.34	1.66	4.75	5.45
4+1	7.09	2.67	5.33	5.13
9+1	3.56	2.56	4.27	3.42
Calc. serpentine				
1+1	20.57	1.28	4.95	9.88
4+1	8.41	1.31	4.94	6.41
9+1	4.43	2.05	4.63	4.25
Magnesite				
1+1	21.65	0.68	2.62	3.87
4+1	8.36	1.42	3.61	2.83
9+1	4.18	2.19	2.39	2.06
Calc. magnesite[e]				
1+1	22.82	0.83	5.41	22.73
4+1	9.50	1.35	7.07	9.52
9+1	4.85	2.21	4.60	4.65
Calc. magnesite[f]				
1+1	21.60	1.13	4.90	20.99
4+1	8.90	1.52	7.07	8.96
9+1	4.86	2.61	4.96	4.99

[a] By A.O.A.C. procedure.
[b] Charge of 1 gm leached with 250 ml. H_2O (approximately 5 times the amount necessary to dissolve the maxima: $MgHPO_4 \cdot 3H_2O$ content of the samples).
[c] Computed from the P_2O_5 transitions.
[d] Charge of 1 gm was leached with 100 ml 2 per cent citric acid and the residue was digested in additional 100 ml 2 per cent citric acid, with end-over-end agitation 30 minutes at 50°C.
[e] At 750°C. Virtually all of the magnesium content was as MgO.
[f] At 900°C. Virtually all of the magnesium content was as MgO.

A sample of each of the 21 experimental mixtures of Tables 1 and 2 was extracted by the proposed procedure to establish its capacity to effect complete dissolution of engendered magnesium phosphates. The resultant values for "available" P_2O_5 content were in accord with those registered by the "official" neutral citrate digestion procedure. There were twelve minus variations in range between 0.02 per cent and 0.70 per cent and eight plus deviations in range between 0.05 per cent and 0.37 per cent. The

mean of the the minus deviations was 0.34 per cent and 0.37 per cent for the plus values, with an average deviation of minus 0.06 per cent.

Since the proposed 2 per cent citric acid dissolved all of the contained P_2O_5, other than that in the residues of raw rock, it follows that it also effected complete dissolution of the engendered magnesium phosphates. This was substantiated by the parallels of direct determination of the magnesium content of the extracts.

THE ANALYTICAL DETERMINATION OF THE MAGNESIUM OF THE 2 PER CENT CITRIC ACID EXTRACTS

The following precautionary measures were taken in the determination of magnesium carried by the 2 per cent citric acid extracts:

1. The aliquot should be such that its contained calcium will be as little as possible, but its magnesium content should be such as to assure a suitable precipitate of magnesium ammonium phosphate, regardless of the proportion of calcium. Aliquots of 10 or 25 ml from a 250-ml extract of a 1-gm charge are recommended, since the magnesium contained therein was found to produce a calcine of from 20 to 50 mgm of pyrophosphate.

2. The initial precipitation of calcium was made by the oxalic acid-ammonium oxalate procedure. It is important that the precipitation be made in a solution of near 200 ml that contains the NH_4Cl formed by the neutralization of at least 10 ml of concentrated HCl. The second calcium precipitation was made in similar manner from a solution of 150–175 ml.

3. After filtration of the second percipitate of calcium-oxalate, the ammonium salts contained in the combined filtrates were metathesized with HNO_3 and the resultant residue was brought into dilute HCl solution. Necessity for removal of iron and aluminum is obviated through the addition of 2 to 3 grams of citric acid to that solution prior to the magnesium ammonium phosphate precipitation. In case high degree of accuracy is essential, any appreciable manganese content should be removed. To assure that the precipitating reagents effect a complete throw-down of magnesium ammonium phosphate in the initial precipitation, 25 ml of concentrated NH_4OH should be added and a quiescent period of 30 minutes should be allowed, after which the solution should be stirred vigorously and then allowed to stand 4 hours before filtration.

4. The incomplete precipitation of calcium in the oxalate procedure would induce an inadmissible contamination in the subsequent magnesium ammonium phosphate precipitate. Such a contamination would be serious, especially when the solute magnesium is relatively low. The $Mg_2P_2O_7$ calcines from 14 representative determinations by the prescribed procedure were examined for this source of error. In every instance, absence of calcium was indicated by the ammonium oxalate test and also by Wolf's photometric method (39).

5. Other sources of error in the determination of magnesium is through loss as a co-precipitate in the calcium oxalate precipitation and its occlu-

sion by the calcium oxalate precipitate. Therefore, twelve of the second calcium oxalate precipitates were analyzed by the modified Stolberg procedure (8) and were found devoid of magnesium.

6. The removal of solvated silicia from the citric acid extracts necessitates a long and tedious procedure. Hence, to ascertain whether such removal is essential for analytical accuracy, magnesium determinations were made upon the extracts of 12 samples, with and without prior removal of silica. The results demonstrated that the presence of solvated silica did not induce higher values for magnesium. Hence, the tedious step of silica removal is not included in the directions for the analysis of the 2 per cent citric acid extracts of ordinary fertilizer mixtures.

Oxidation of the citric acid of the extracts, and the dehydration of the silica content, was effected by means of digestions with HNO_3 and $HClO_4$. Although oxidation of the organic matter of the filtrates with $HClO_4$ alone is precarious, the operation can be effectuated safely by starting it with a mixture of HNO_3 and $HClO_4$ in the cold, gradually increasing the temperature, and then boiling until the oxidation is completed.

7. To prevent carbonization of the citrate during the metathesis of the filtrates prefatory to the precipitation of $MgNH_4PO_4$, the solution should not be allowed to go to dryness. In case of carbonization, however, reclarification of the solution can be effected by another addition of HNO_3 and further digestion.

Utility of the Proposed Procedure:

In the development of the proposed procedure, the primary objective was to determine the quantity of magnesium generated as "available" by concentrated superphosphate in its mixtures with the relatively insoluble magnesium minerals—olivine, serpentine, and the crystalline type of magnesite.

The citric acid extractions and digestions of olivine, serpentine, or magnesite served to establish the fact that the described procedure effected only meager dissolution of either of those three minerals, as such, or when they were components of the phosphatic mixtures. It should be noted, however, that the prescribed volume of the 2 per cent citric acid is capable of effecting complete dissolution of the magnesium that may be present as a residue of MgO in a 1-gm charge along with any magnesium phosphate content. The MgO values determined directly by the 2 per cent citric acid digestion procedure were usually in close agreement with the MgO values that were computed from corresponding phosphate-transition values. In general, the directly determined "available" MgO values were higher than those computed from the P_2O_5 transitions, but in most cases the differences were within tolerance. The occasional lack of concordance may have been caused by a slight dissolution of the residues of the minerals in the charges. It may be, however, that the differences between the MgO values obtained by the two approaches were due as

much to cumulative errors in the computations based upon the P_2O_5 transitions as to any inadequacy inherent to the proposed procedure.

Upon basis of the agreement between the values that were computed for magnesium from the P_2O_5 transitions of Table 3 and the values obtained by the direct determination of the magnesium content of the extracts, the proposed extraction-digestion procedure appears well adapted to the determination of "available" magnesium content of such phosphatic mixtures as those of Tables 1 and 2.

Effect of the Proposed Procedure upon Undecomposed Dolomite Residues:

After it had been found suitable for the extraction of "available" magnesium compounds derived from the several magnesic materials, the ques-

TABLE 4.—*The applicability of the proposed leaching-digestion procedure[a] for the determination of the "available" magnesium content of several magnesic materials, dolomite, and their phosphatic mixtures*

MIXTURES	DETERMINED MgO VALUES		
	TOTAL	AVAILABLE BY THE PROPOSED PROCEDURE[a]	
		ACTUAL	OF THE TOTAL
	per cent	*per cent*	*per cent*
Mineral Material Separates			
Olivine, −100	49.30	1.34	2.7
Serpentine, −100	38.90	1.67	4.3
Magnesite, −100	45.80	2.55	5.5
Calc. olivine, −100	50.00	1.66	3.3
Calc. serpentine, −100	43.50	3.42	7.8
Calc. magnesite, −100	89.80	89.80	100.0
Dolomite (W. Va.), −100	21.25	12.55	59.0
−60+70	21.25	9.81	40.4
−50+60	21.25	8.97	42.2
−30+50	21.25	6.95	32.2
−20+30	21.25	4.90	23.0
Prepared Materials			
$MgHPO_4 \cdot 3H_2O$, C.P.	23.12	23.12	100.0
$MgNH_4PO_4$, Powd.	20.74	20.74	100.0
MgO, Powd.	99.00	99.00	100.0
Calc.-Serp.-Phos.[b]	21.27	21.02	98.8
Calc.-Olivine-Phos.[c]	16.38	16.25	99.2
$MgSO_4 \cdot 7H_2O$, Cryst.	16.36	16.36	100.0
Kieserite	23.50	23.50	100.0
Mixtures			
0.25 Dolomite, −100 0.25 T.S.P. 0.25 $CaHPO_4 \cdot 2H_2O$ 0.25 $MgHPO_4 \cdot 3H_2O$	10.59 (5.62)[d]	10.47	98.8

TABLE 4.—*Continued*

MIXTURES	DETERMINED MgO VALUES		
	TOTAL	AVAILABLE BY THE PROPOSED PROCEDURE[a]	
		ACTUAL	OF THE TOTAL
	per cent	*per cent*	*per cent*
0.25 Dolomite, −100 0.25 Quartz 0.25 $CaHPO_4 \cdot 2H_2O$ 0.25 $MgHPO_4 \cdot 3H_2O$	10.59 (5.62)[d]	10.01	94.5
0.15 Dolomite, −100 0.3 $MgHPO_4 \cdot 3H_2O$ 0.3 $CaHPO_4 \cdot 2H_2O$ 0.25 T.S.P.	10.73 (6.94)[d]	9.65	89.9
0.25 Dolomite, −60+70 0.25 T.S.P. 0.25 $CaHPO_4 \cdot 2H_2O$ 0.25 $MgHPO_4 \cdot 3H_2O$	10.59 (5.62)[d]	10.01	94.5
0.25 Dolomite, −60+70 0.25 Quartz 0.25 $CaHPO_4 \cdot 2H_2O$ 0.25 $MgHPO_4 \cdot 3H_2O$	10.59 (5.62)[d]	8.52	80.4
0.15 Dolomite, −60+70 0.3 $MgHPO_4 \cdot 3H_2O$ 0.3 $CaHPO_4 \cdot 2H_2O$ 0.25 T.S.P.	10.73 (6.94)[d]	8.62	80.3
0.3 $MgSO_4 \cdot 7H_2O$ 0.3 $MgHPO_4 \cdot 3H_2O$ 0.3 $CaHPO_4 \cdot 2H_2O$ 0.1 T.S.P.	11.85	12.12	102.3
0.75 g. P-1280[e] 0.25 g. MgO	41.79	41.22	98.6
Com. Fert., dolomited[f]	3.98	3.50	90.0

[a] Charges of 1-gm. were leached with 100 ml. of 2% citric acid and the residues digested with 100 ml additional for 30 min. at 50°C.
[b] Commercial product.
[c] Wilson Dam product.
[d] The amount from sources other than dolomite.
[e] Three parts of the 1+1 mixtures of MgO and superphosphate, P-1280 of Table 1, were supplemented by 1 part of MgO, reagent grade.
[f] A "4-8-4" product.

tion was raised as to whether the procedure would register "availability" for the magnesium content of mixed fertilizers attributable to residues of dolomite, the coarse particles of which show limited dissolvability. Although the answer to that question was not a primary objective in the study of a procedure for the quantitative recovery of "available" magnesium engendered in mixtures of superphosphate and magnesium silicate minerals, certain exploratory and integrated studies afforded the findings that are presented in Table 4.

The data show that the prescribed reagent and procedure exerted (a) complete dissolution of MgO, of $MgSO_4 \cdot 7H_2O$, of Kieserite, of $MgHPO_4 \cdot 3H_2O$, of $MgNH_4PO_4$, alone and as inclusions, and near-complete extraction of the magnesium contained in fused Ca-Mg phosphates, (b) only slight dissolvent action upon the separate charges of olivine, serpentine, and crystalline magnesite. Moreover, the proposed procedure did effect differential dissolution of a representative dolomite, in relation to its particle size. This is shown by the limited attack upon charges of the $-20+30$-mesh separate alone (and as an inclusion in phosphate mixtures), and by the near-complete dissolution of the -100-mesh separate inclusion in the experimental phosphatic mixtures. Because of the foregoing findings and in view of the demonstrated dissolubility and rapidity with which 100-mesh dolomite undergoes disintegration in acidic soils, in contrast with the persistence of its larger separates (4, 5, 13, 19, 20, 21, 22, 23, 25, 32), it seems logical and equitable to accord "availability" to magnesium that fertilizers may contain as residues of dolomite fines that are extractable by the proposed procedure.

CONCLUSION

Through interpretation of the present data, and upon basis of related findings as to rate of reactivity of dolomite separates in fertilizers, and after incorporation into soils (9, 18, 4, 5), it is concluded that the proposed procedure provides the means for the direct and accurate determination of any contained magnesium that is entitled to classification as "available," in the chemical control evaluation of commercial fertilizers.

In presenting their findings, the authors hope that the procedure proposed for the determination of available magnesium content of fertilizers will be found applicable for that objective and that their findings and conclusions will be substantiated through collaborative studies.

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THE DETERMINATION OF FLUORINE ATTRIBUTABLE TO SOIL INCORPORATIONS OF CALCIUM FLUORIDE*

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The 1945 Edition of the Book of Methods contains the first prescribed procedure for the determination of the florine content of soils (1). Until 1933, there had been no analytical technique for the titrative determination of the fluorine in concentrations so meager as those obtained in soil

* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 20–22, 1947.

distillates. After the thorium-nitrate titration procedure had been developed by Willard and Winter (11), however, determination of the fluorine content of soil became mainly the problem of a complete transposition of that content into a distillate that could be titrated.

The prefatory incineration and fixative treatment of soil charges for perchloric acid distillations was evolved mainly from studies conducted in the laboratory of the Association's Referee on Soils and Liming Materials (6, 8), and after proof of the inadequacy of fusions with $Na_2CO_3-K_2CO_3$ and incinerations with magnesic materials—peroxide, carbonate, nitrate. Passage of a current of steam throughout the perchloric acid digestion, as advocated by MacIntire, Shaw, and Hardin for purge of liberated NH_3 (9), also was adopted. That technique served to eliminate bumping during distillation and diminished by half the time requisite for the complete expulsion of the fluorine from the $HClO_4$ digestate. In a contribution based upon the findings in the Referee's laboratory, it was stated: "Prerequisites for the distillation of entire fluorine content of soils are (a) preparatory fixation of the element and its conversion to compounds that undergo dissolution during digestion and yield the element to the distillates, (b) elimination of 'volatiles' that would pass into the distillates and vitiate their titration, (c) proper temperature control, and absence of colloidal silica and the prevention of bumping during distillation"(**7**, p. 107).

In the original directions (1), the prescribed ratio of soil charge to admixed CaO_2 was 1 to 3. Fluorine-free peroxide was not always obtainable, however, and Clifford substituted $Ca(OH)_2$ in his directions for the fixation of fluorine in the prefatory incineration of organic materials (2). In a subsequent presentation, the present senior author noted the alternative adopted by Clifford and prescribed, "Mix the charge intimately with either the peroxide or the hydroxide of calcium in a nickel or a platinum crucible. Incinerate thoroughly in an electric furnace below 500°C., and then ignite 30 minutes at 900C°." (**7**, p. 108).

It was assumed that the hydroxide would be as efficacious as the peroxide in the prevention of fluorine escape during incineration of the soil charges and in the fixation of component fluorine in forms from which complete evolution would be effected by the $HClO_4$ distillation. The present contribution stems from studies as to the admissibility of $Ca(OH)_2$ as a substitute for the prescribed CaO_2, and as to appropriateness of calcination temperature above 500°C.

PRESENT STUDIES

When soils of known content of additive fluorine were subjected to the prefatory incineration with $Ca(OH)_2$, in lieu of CaO_2, then to 30-minute calcination at 900°C., and then to three successive 100-ml perchloric acid distillations, the recoveries of fluorine were only fractional and as little as 6 per cent of the quantity that had been incorporated as CaF_2 in pre-

TABLE 1.—*Comparison of three distillation procedures for recovery of fluorine from experimentally phosphated soils that contained added fluorides, with and without limestone and dolomite*

SOIL NO.	OVERALL INCORPORATIONS	ADDED FLUORINE	FLUORINE RECOVERIES FROM 1-GM. CHARGES		
			METHOD I[a]	METHOD II[b]	METHOD III[c]
			SINGLE DISTILLATION WITH $HClO_4$ AT 135°C.	DOUBLE DISTILLATION FROM $HClO_4$ AT 165° & 135°C.	DIRECT DISTILLATION FROM H_2SO_4 AT 165° AND $HClO_4$ AT 135°C.
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*
337	None	None	60	68	88
	Rock phosphate[d]				
338	Alone	64	62	133	163
339	plus limestone	64	81	109	163
423	plus dolomite	64	124	101	150
424	plus $MgCO_3$	64	120	109	150
	TriCa phosphate[e]				
425	Alone	None	58	85	94
426	plus CaF_2	1200	79	135	1250
427	plus CaF_2+L.S.	1200	129	170	1265
428	plus CaF_2+Dol.	1200	88	168	1288
	DiCa phosphate				
429	Alone	None	77	60	110
430	plus CaF_2	1200	82	119	1287
431	plus CaF_2+L.S.	1200	77	118	1265
432	plus CaF_2+Dol.	1200	71	129	1288
	$Ca(PO_3)_2$				
433	Alone	None	73	33	103
434	plus CaF_2	1200	113	124	1250
435	plus CaF_2+L.S.	1200	116	101	1254
436	plus CaF_2+Dol.	1200	116	97	1288
437	plus L.S.	None	55	33	103
438	plus L.S.	None	59	34	100
439	plus Dol.	None	81	38	100

[a] Aggregate of three successive 100-ml. distillates by $HClO_4$ from 900°C. 30 minute calcine of soil +$Ca(OH)_2$.
[b] Double distillation with $HClO_4$ soil at 165°C. +aliquot at 135°C. from calcine as in (a).
[c] From sulfuric acid distillation from unignited soil, without additive $Ca(OH)_2$ and with H_2SO_4 and $HClO_4$ distillation of an aliquot.
[d] The 2-ton per acre incorporations of rock phosphate were made 10 years previously and they enriched the soil by 64 p.p.m. of fluorine.
[e] The other phsophated soils had been enriched with eight incorporations of CaF_2 that gave a 1200 p.p.m. enrichment of fluorine in 10 years.

vious years. The dependability of $Ca(OH)_2$ as a fixative agent for fluorine in the ashing of organics had been questioned by Cox, *et al.* (3), by Dahle (4), and by Clifford (2), who believed that "the danger of loss of fluorine . . . through 'wetting' by a fixative solution" could be eliminated through distillation with H_2SO_4 at 165°C. and a $HClO_4$ distillation of the evaporated distillate at 135°C., as proposed by Wichmann and Dahle (10).

Therefore, the 2-acid distillation was tested as to the recovery of fluorine from 10-gm charges of unignited soil and 0.0120-gm and 0.0240-gm additions of F as CaF_2. The distillations with H_2SO_4 were at 165°C. and the succeeding perchloric acid distillations of aliquots were at 135°C. This

TABLE 2.—*The effectiveness of the direct double distillation of fluorine-fortified[a] soils of known history in lysimeter experiments*

SOIL NO.	OVERALL INCORPORATIONS	FLUORINE ADDITIONS AND ANALYTICAL RECOVERIES					
		ADDED	LEACHED	RESIDUAL	FOUND	RECOVERY OF RESIDUAL FLUORINE[b]	
		lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*per cent*
337	None	—	—	—	176	—	—
	Rock phosphate						
338	Alone	128	5	123	306	130	106
339	plus limestone	128	5	123	306	130	106
423	plus dolomite	128	6	122	300	124	102
424	plus $MgCO_3$	128	7	121	300	124	102
	TriCa phosphate						
425	Alone	None	—	—	188	—	—
426	plus CaF_2	2400	56	2344	2500	2312	99
427	plus CaF_2+L.S.	2400	63	2337	2530	2342	100
428	plus CaF_2+Dol.	2400	64	2336	2576	2388	102
	DiCa phosphate						
429	Alone	None	—	—	220	—	—
430	plus CaF_2	2400	40	2360	2574	2354	100
431	plus CaF_2+L.S.	2400	38	2362	2530	2310	98
432	plus CaF_2+Dol.	2400	41	2359	2576	2356	100
	$Ca(PO_3)_2$						
433	Alone	None	—	—	206	—	—
434	plus CaF_2	2400	51	2350	2500	2294	98
435	plus CaF_2+L.S.	2400	35	2365	2508	2302	98
436	plus CaF_2+Dol.	2400	40	2360	2576	2370	100
437	plus L.S.	None	—	—	206	—	—
438	plus L.S.	None	—	—	200	—	—
439	plus Dol.	None	—	—	200	—	—

[a] Through 8 incorporations of CaF_2 that supplied 2400 pounds of fluorine per 2,000,000 pounds of soil.

[b] From the quantities computed as differences between the amounts of fluorine incorporated and those found in the rain-water leachings.

procedure effected 100 per cent recoveries of the two fluorine additions.

The comparisons were extended through the use of the four soils that had received rock phosphate at the 2-ton rate 10 years previously, and 12 other soils that had been enriched in fluorine through 8 incorporations of CaF_2 during the 10 years. The data of Table 1 register low fluorine recoveries from the 900°C. calcines of the mixtures of fluorine-enriched soils and $Ca(OH)_2$ by means of the single and double perchloric distilla-

tions, in contrast to the higher recoveries obtained by H_2SO_4 and $HClO_4$ distillations of the unignited soil.

The recoveries by the $HClO_4$ distillations from the soils that contained rock phosphate were erratic, but all recoveries were much larger percentagely than any recovery from the common input of CaF_2 that supplied more than 18 times the 64 p.p.m. enrichment that was supplied by the apatite of the single incorporation of rock phosphate.

The procedure used in the direct distillation of fluorine from unignited soil was as follows:

A charge of either 0.5 gm or 1 gm of 325-mesh soil was distilled from 20 ml of fluorine-free H_2SO_4 (1+1) in a current of steam at 165°C., until a distillate of 250 ml was obtained. A 25-ml aliquot of that distillate then was likewise distilled at 135°C. with 15 ml of 60 per cent $HClO_4$ that contained 0.2 gm of Ag_2SO_4, to obtain a 100-ml distillate. The acidity of 20 ml of this distillate was determined by titration with 0.05 *N* NaOH and the acidity of the remaining 80 ml was adjusted to contain the equivalent of 4 ml of 0.05 *N* $HClO_4$. This sodium-free 80-ml aliquot then was titrated with $Th(NO_3)_4$ [0.20 gm $Th(NO_3)_4 \cdot 4H_2O$ per liter] that had been standardized against the double distillates obtained from known amounts of NaF, as prescribed.

TABLE 3.—*The effect of variance in proportion of $Ca(OH)_2$ in soil charge upon recovery of fluorine from calcines[a] of a slagged soil[b]*

SOIL	RATIO OF SOIL TO $Ca(OH)_2$ IN CHARGE	CALCINATION OF CHARGE		FLUORINE RECOVERY[c]
		TEMP.	DURATION	
		°C.	*Minutes*	*p.p.m.*
Alone	None	—	None	1108
With $Ca(OH)_2$	1:0.5	900	30	63
With $Ca(OH)_2$	1:2.0	900	30	150
With $Ca(OH)_2$	1:7.0	900	30	875
With $Ca(OH)_2$	1:9.3	900	30	925

[a] Of 0.5 gm. of soil.
[b] One that had received incorporations of quenched calcium silicate slag 6 years previously.
[c] By double (H_2SO_4 and $HClO_4$) distillations.

The 2-acid distillation procedure then was checked further for accuracy and for reproducibility of results through the comparative analyses of a series of fluorine-enriched soils, records of which had been kept in a 10-year lysimeter experiment. Respective incidences of fluorine were computed as the differences of the amounts incorporated and the quantities found in the leachings per annum. In terms of pounds of fluorine per 2,000,000 pounds of soil, and as percentage of the quantities computed to be residual from the incorporations of CaF_2, the analytical values of Table 2 demonstrate that fluorine content of soils is registered accurately by the direct distillation of unignited charges by H_2SO_4 and $HClO_4$ in succession, at respective temperatures of 165°C. and 135°C.

THE REASON FOR THE FRACTIONAL RECOVERIES BY THE $HClO_4$ DISTILLATES FROM THE CHARGES OF SOIL+$Ca(OH)_2$

An explanation then was sought as to why the recoveries of fluorine obtained by the $HClO_4$ distillations from calcined charges of lime-plus-soil were far from complete, as shown in Table 1, although full recoveries of additive fluorine had been registered by the $HClO_4$ distillations of soils

TABLE 4.—*The effect of variance in the temperature, and in the duration, of calcination of soil+$Ca(OH)_2$ upon recovery of fluorine through double distillation*[a]

CALCINATION		FLUORINE RECOVERY[b]
TEMP.	DURATION	
°C.	minutes	p.p.m.
—	None	1250
500	5	1265
500	15	1288
500	30	1275
500	60	1275
500 and 900	60 and 5	150
500 and 900	60 and 15	25
500 and 900	60 and 30	25
500 and 900	60 and 60	0

[a] From H_2SO_4 at 165°C., 1-gm. charges of soil and $Ca(OH)_2$ [1 to 1], and from aliquot by $HClO_4$ at 135°C.

[b] Basis of moisture-free soil, No. 426 (Tables 1 and 2), which had received 8 inputs of CaF_2 to the aggregate of 1200 p.p.m. in 10 years.

incinerated with CaO_2 and ignited at 900°C., as reported by MacIntire and Hammond (8). It was assumed that the deficiency in recovery might be a question of (a) adequacy in the proportion of the fixative $Ca(OH)_2$ in the analytical charge and (b) the temperature and duration of the prefatory calcination.

To test its adequacy, the hydroxide fixative was admixed with a soil of high fluorine content in the four proportions listed in Table 3, and the respective mixtures were calcined 30 minutes at 900°C. It is obvious that all of the resultant recoveries of fluorine were fractional and that disparity

between content and recovery became less as the proportion of $Ca(OH)_2$ per soil-charge was increased. But, even with a 1 to 9 ratio of soil to $Ca(OH)_2$—which is thrice the ratio of 1:3 that had proved adequate for additive CaO_2—the maximal recovery of fluorine from the 900°C. calcines did not equal the one obtained by the direct H_2SO_4 and $HClO_4$ distillation of the unignited soil. This disparity might be attributable to two factors, singly or jointly. The hydroxide may not have prevented the loss of fluorine during the prefatory incineration and calcination, or it may not have prevented the formation of those alumino combinations of fluorine that do not undergo dissolution and yield fluorine to the perchloric acid distillations.

The volatility of CaF_2 at 900°C., alone and in mixtures with quartz, was determined. When charges of 0.2 gm were heated alone 60 minutes at 900°C. and then distilled with $HClO_4$ at 135°C., fluorine recovery was complete. But when identical charges of CaF_2 were mixed with pulverized quartz and the mixture heated 60 minutes at only 500°C., the recoveries were only 39 per cent, as shown in Table 4. When the heating of companion charges at 500°C. was followed by 15-minute and 30-minute calcinations at 900°C., there were further losses of fluorine, up to 50 per cent of content. Obviously, an adequate proportion of a protective additive, such as CaO_2. or the hydroxide of calcium in much larger proportion, is essential to prevent volatilization of fluorine when an analytical charge of CaF_2 is heated to 900°C. in contact with quartz.

The near-recovery from the 900°C. calcine of soil with 9 parts $Ca(OH)_2$ serves to explain the disparities between full recoveries from the 500°C. calcines of the $Ca(OH)_2$-fortified charges and the fractional recoveries from corresponding 900°C. calcines.

An experimental soil of known history (No. 426 of Table 2) and a determined fluorine content of 1250 p.p.m. then was used in the four 500°C. heatings and those extended to 900°C., as detailed in Table 4. Each 1-gm charge of soil was impregnated with a slurry of calcium hydroxide that provided 0.3 gm of $Ca(OH)_2$. Four of the slurried charges were dried and calcined at 500°C. for periods of 5, 15, 30, and 60 minutes, and then subjected to the H_2SO_4 and $HClO_4$ distillation. Four identical charges were heated 60 minutes at 500°C. with further respective heating periods of 5, 15, 30, and 60 minutes at 900°C. The values registered by direct distillation of the unheated charge and by the distillations of the 500°C. calcines can be rated as identical. In contrast, the additional heatings at 900°C. caused progressive diminution in fluorine recoveries with increased duration, and even no recovery from the calcine that was subjected to the 60-minute experimental heating. Hence, when the 1 to 1 mixtures of soil and $Ca(OH)_2$ were calcined at 500°C. for periods between 5 and 60 minutes, the hydroxide proved adequate as a fixative that allowed full release of component fluorine in the double distillations.

SUMMARY AND CONCLUSIONS

A correlation of the earlier Referee studies with those now reported serves to establish the following conclusions.

Fluorine present as apatite in the soil is recoverable far more readily than fluorine supplied by inputs of CaF_2, when calcined charges of soil are subjected to distillation with either H_2SO_4 or $HClO_4$.

The prefatory incineration and calcination of soil charge with CaO_2, as now prescribed in the *Book of Methods*, has been found adequate to prevent loss of fluorine from the analytical charge and to assure full release of fluorine content by the prescribed perchloric acid distillation in a current of steam. Also, the further calcination at 900°C. was proved unnecessary, and even unadmissible.

Used in the proportion of at least 10 parts to one part of soil, $Ca(OH)_2$ can be substituted for CaO_2 in calcination at 900°C., but such large proportion and high temperature are not advocated.

The findings establish the adequacy of $Ca(OH)_2$ as a fixative for the fluorine content of an equal weight of soil, when the calcination of the mixture is at 500°C. for a period so brief as 5 minutes.

It is established also that full recovery of fluorine content of rock-derived soils is effected by direct H_2SO_4 distillation of the charge of *unignited* soil at 165°C., with sequential $HClO_4$ distillation and with titration against a thorium nitrate solution, *standardized through identical double distillation*, as prescribed in the foregoing procedure.

Since it assures (a) full recovery of fluorine, (b) obviates the use of a fixative, (c) eliminates incineration and calcination, and (d) is more expeditious than the peroxide procedure, the direct distillation technique is deemed as meritorious and even preferable to the present accepted procedure, which prescribes that a mixture of soil and CaO_2 be calcined at 500°C., and further at 900°C., as prefatory to the distillation with $HClO_4$.

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THE DETERMINATION OF TOTAL BORON IN PLANT MATERIAL WITH "CHROMOTROPE-B"*,†

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The role of boron in living plants is being investigated in this laboratory. To facilitate this study a rapid method for the determination of boron was required. A search of the literature revealed only three types of methods that might be applicable. These were examined critically.

The electrometric titration of boric acid used by Wilcox (8), McCormick (3) and others which requires 5–25 gm. samples lacks the sensitivity required. The Naftel procedure (6) was eliminated on the basis of previous work at this laboratory (4) and elsewhere (1). The quinalizarin method described in *Methods of Analysis, A.O.A.C.* (6th Edition) is subject to several disadvantages. Particularly objectionable are the hazardous use of fuming sulfuric acid in the process and the difficulty experienced in maintaining the critical sulfuric acid concentration. The above methods all require long ignitions at 450°C. or less. This is a serious disadvantage when a considerable number of samples are to be analyzed.

The addition of a base is reported unnecessary by the above workers. Piper (7), however, describes two different ignition procedures; in one sodium hydroxide is added to the sample and in the other calcium hydroxide. Both procedures entail complicated manipulative detail and are time consuming and inconvenient.

These disadvantages are reduced to a minimum in the method described. The ignition lasts for two hours and is made at 600° C. with barium hydroxide added to the sample. The reagent, p-nitrobenzeneazo-1, 8-dihydroxynapthalene-3, 6-disulfonic acid ("Chromotrope-B") is used as a 0.005 per cent solution in concentrated (95.5 per cent) sulfuric acid. Feigl (2) has recorded the use of the reagent for the detection of boron, but its use for the determination has not been previously reported.

METHOD

APPARATUS

Coleman Model 11 Universal spectrophotometer with ½ inch square cuvettes. In this determination no filter is used.

10 ml automatic burette protected from atmospheric moisture with calcium chloride tubes.

15 ml centrifuge tubes—pyrex glass tubes are satisfactory.

125 ml flat bottom boiling flasks of "Corning" Brand Alkali Resistant (boron free) glass.

Platinum dishes.

REAGENTS

Acetic acid—70 per cent.—Made by dilution of glacial acetic acid (Reagent grade).

* The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

† Presented at the Annual Meeting of the Association of Official Agricultural Chemists held at Washington, D. C., October 20–22, 1947.

Concentrated sulfuric acid (Reagent grade, 95.5 per cent H_2SO_4).—"Baker's Analyzed" acid has been used satisfactorily without analysis.

Saturated solution of recrystalized barium hydroxide.

A 0.005 per cent solution by weight of p-nitro-benzenazo-1, 8-dihydroxynaphthalene-3, 6-dusilfonic acid (Eastman Organic Chemicals No. 4411), "Chromotrope-B" in concentrated sulfuric acid.

PREPARATION OF STANDARD CURVE

Dissolve 0.0930 g anhydrous $Na_2B_4O_7$ in several hundred ml of 70% acetic acid. Add 100 g of recrystallized $Ba(OH)_2 \cdot 8H_2O$ and allow to dissolve. Dilute to one liter with 70% acetic acid. This solution contains 20 mmg of boron per ml. Dilute portions of this stock solution so that a series of standards containing 0 to 15 mmg of boron per ml is obtained. Treat one ml of each solution as described below and determine per cent transmission at a convenient but reproducible temperature between 20° and 30°C. Draw a concentration vs. transmission curve from these data.

PROCEDURE

Place a 0.2–0.5 g sample of plant tissue (air-dried and 60-mesh) in a platinum dish. Determine the moisture content on a separate sample by drying for four hours at 100°C. Add 5 ml of a saturated soln of barium hydroxide and place the dish in a cool muffle furnace. Raise the temperature to 600°C. and ignite for two hours. Remove the dish from the furnace, cool, and add 5.0 ml of 70% acetic acid (10.0 ml may be necessary when large amounts of boron are present). Triturate with a rubber policeman and pour the suspension into a 15 ml centrifuge tube. After centrifuging transfer 1.0 ml of the clear supernatant liquid to a 125 ml flask (boron-free glass) and add 10.0 ml of the 0.005% solution of "Chromotrope-B" in concentrated sulfuric acid. Mix and allow the soln at least 30 min. for color development and for cooling to the same temperature used in preparing the standard curve. Using ½ inch square cuvettes, determine the per cent transmission at 620 mμ against a reference soln prepared similarly but with 1 ml of 70% acetic acid instead of the boron soln. (Contrary to the usual practice no filter is used while making transmission readings.) Determine micrograms of boron in the aliquot from the standard curve and calculate total boron in the sample by the equation:

$$\text{p.p.m. boron (dry basis)} = \frac{\text{ml acetic acid added to ash} \times \text{micrograms boron found/ml}}{\text{oven dry weight of sample}}$$

Duplicate analyses can be easily completed in about three hours.

EXPERIMENTAL

Interfering Elements and Ions:

Tests were made on 28 cations and 21 anions for interference with the reaction. A small quantity (five to 10 milligrams) of a compound of the ion to be tested was added to the boron solution on the spot plate and the reagent added. Silver, lead, and strontium interfered somewhat with color development. With smaller amounts of these ions no interference could be detected. Several organic anions interfered by modification of the boron "Chromotrope-B" color, but as such ions are absent after ignition this is not significant. The inorganic anions NO_3, NO_2, Cr_2O_7, and SeO_4 interfere with the test by reacting with the reagent to form a red compound.

Fluorides prevent color development by forming the stable fluoboric acid or boron fluoride. No ion tested gave a false positive test.

Effect of Drying Procedure on Boron Recovery:

A sample of wild cherry leaves was collected, finely shredded, and well mixed. Random samples were taken from the main sample and treated as shown in Table 1. Analytical results are based on total dry matter.

TABLE 1.—*Boron recovery as influenced by pre-ignition drying*

TREATMENT	BORON FOUND (DRY BASIS)	RECOVERY
	p.p.m.	*per cent*
Air dried, 48 hours	22.9	100.0
Air dried, 48 hours then dried 3 hrs. at 100°C.	21.2	92.6
Dried 70° for 24 hours	19.9	86.9
Dried 4 hours (to constant weight) at 100°C.	19.8	86.5

Effect of Variations in Temperature of Ignition and in Base Added:

The procedure was the same as that described previously except that the ignition temperature and the base added were varied. Results obtained are shown in Table 2. The time of ignition was two hours.

TABLE 2.—*Effect of varying ignition temperature and base added on boron recovered from the synthetic sample (97.2 p.p.m. of boron) as anhydrous* $Na_2B_4O_7$

IGNITION TEMPERATURE	BASE ADDED	BORON FOUND
		p.p.m.
500°C. (placed in cool furnace)	None	11
500°C. (placed in cool furnace)	$Ba(OH)_2$ (satd. soln)	92.0
500°C. (placed in cool furnace)	K_2CO_3 (solid)	89.0
500°C. (placed in cool furnace)	K_2CO_3 (20% soln)	49.0
500°C. (placed in cool furnace)	$CaCO_3$ (solid)	69.0
600°C. (placed in cool furnace)	$Ba(OH)_2$ (satd. soln)	92.7
750°C. (placed in cool furnace)	$Ba(OH)_2$ (satd. soln)	89.5
900°C. (placed in cool furnace)	$Ba(OH)_2$ (satd. soln)	82.7
400°C. (placed in cool furnace)	$Ba(OH)_2$ (satd. soln)	87.8
600°C. (placed in cool furnace)	$Ba(OH)_2$ (solid)	89.8
600°C. (placed in hot furnace)	$Ba(OH)_2$ (satd. soln)	84.1

Similar results were obtained with kelp and bean seed samples. The highest result obtained was considered the most accurate, and in each case this was obtained with the 600° ignition.

There is some loss of boron on ignition of the sample. This is minimized in the recommended procedure. For example, the sample of kelp had been

previously analyzed by the quinalizarin method and the boron content reported as 270 p.p.m. (5). This result is 14 per cent lower than the 314.5 p.p.m. of boron found by the method herein described.

Small variations in temperature of the solutions when reading per cent transmission are inconsequential. Variations of as much as 1 per cent in the concentration of the sulfuric acid do not affect results.

RESULTS

The results of anlyases of several plant samples and a synthetic sample containing 97.2 p.p.m. of boron as anhydrous $Na_2B_4O_7$ are shown in Table 3. A number of analyses were run on each sample. The average deviation of a single observation indicates the precision of the method.

TABLE 3.—*Results of Analyses*

SAMPLE	BORON ADDED	NUMBER OF DETERMINATIONS	MEAN BORON FOUND	AVERAGE DEVIATION OF SINGLE OBSERVATION	RECOVERY
	p.p.m.		*p.p.m.*	*p.p.m.*	*per cent*
Synthetic	97.2	9	92.7	1.1	95.4
Bean Seed		6	21.8	.4	
White Pine Needles (N)		5	38.0	1.1	
White Pine Needles (D)		5	42.6	1.1	
Wild Cherry Leaves (1)		4	17.1	.2	
Wild Cherry Leaves (2)		3	22.9	.4	
Kelp		6	314.5	2.8	

The color does not strictly obey Beer's law. It is, however, reproducible with different batches of the reagent solution. The standard curve is shown in Figure 1. The concentration versus per cent transmission curve for the quinalizarin method is also shown for comparative purposes.

SUMMARY

A method for the determination of boron in plant materials is proposed based on the colored complex or ester of boric acid and "Chromotrope-B" (p-nitrobenzeneazo-1, 8-dihydroxynapthalene-3, 6-disulfonic acid).

Oxidizing agents, nitrates, nitrites, and fluorides interfere with the reaction.

The method of drying the sample has been shown to have a significant effect on the amount of boron recovered.

The ignition of the sample is carried out at 600°C. after the addition of barium hydroxide. It is essential that the sample be placed in a cool furnace and the temperature then raised to 600°C.

Several plant samples as well as a synthetic sample have been analyzed by the method. In the synthetic sample, recovery was 95.4 per cent.

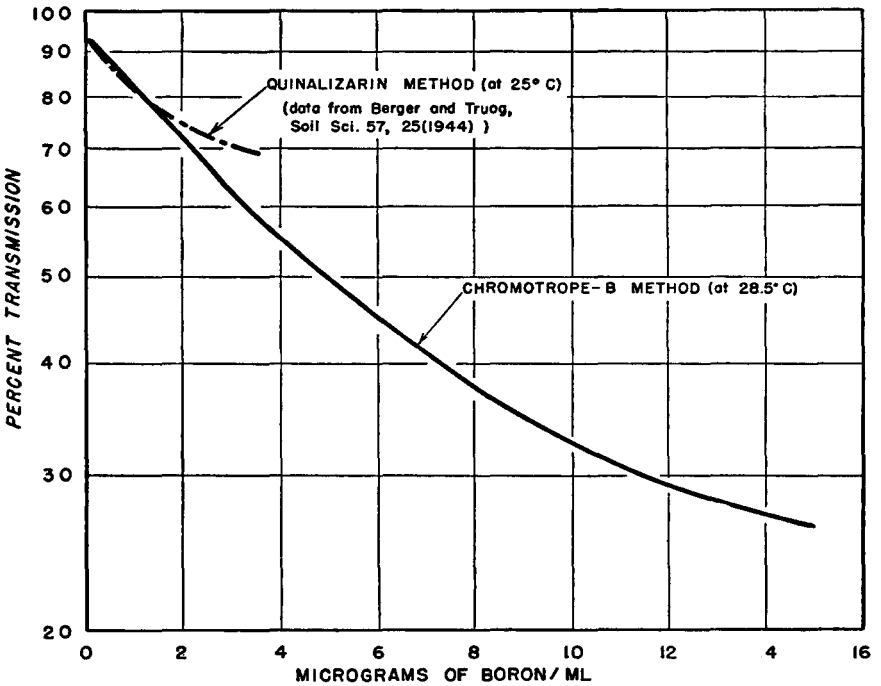

FIG. 1.—Concentration vs. per cent transmission for chromotrope-B and quinalizarin

The authors wish to express their appreciation to Dr. L. K. Wood of this Department for his helpful suggestions and criticisms in the preparation of this manuscript.

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NOTES ON THE DISTILLATION OF AMMONIA IN THE KJELDAHL DETERMINATION. A NEW CONNECTING BULB*

By C. O. Willits, H. J. John, and L. R. Ross (Eastern Regional Research Laboratory,† Philadelphia 18, Pennsylvania)

In a study to modify the Kjeldahl method for nitrogen so that it would be suitable for all types of nitrogenous organic compounds, inconsistent "blank" values were obtained even when the same reagents were used. Since the nitrogen "blank" values should be constant for any set of reagents, this inconsistency could be accounted for only by assuming that droplets of the alkaline reaction mixture were entrained in the vapors and carried through the Kjeldahl connecting bulb. The same difficulty was experienced with several commonly used connecting bulbs which have either been described in the literature (1, 3, 4,) or listed as catalogue items. This "distillation" of nonvolatile alkali had been recognized previously by Davisson (1) and by Lovecy (4). The variation of the "blank" depends upon the efficiency of the bulb in removing droplets of entrained alkali from the vapor stream and upon the concentration of the droplets in the vapor caused by the reagent or by the rate of distillation. The blank, therefore, becomes a possibly serious source of error in the Kjeldahl method for the determination of nitrogen. Obviously, with a varying blank, the selection of the correct blank becomes impossible. It does not necessarily follow that reproducible blank values are correct, since the conditions of the distillation of the samples and blank may be different. To eliminate this nonvolatile alkali error and still permit complete recovery of ammonia, a new connecting bulb has been designed, and has been used satisfactorily during the past year as part of a 24-unit Kjeldahl apparatus in which a large number of nitrogen determinations were made.

Description of bulb.—The new connecting bulb (Fig. 1) is unique in that it has two wire screens, which serve as condensers to form water films at the beginning of the distillation, as plates to support the condensed water, and as diffusors of the distilled vapors.

The application of the countercurrent extraction principle to Kjeldahl connecting bulbs is not new. Davisson (1) made use of this principle, but his bulb failed in that it did not start to scrub the vapors until sufficient distillate had collected in the bulb to cover the holes in the small inner bulb. During this time any droplets that occurred in the vapor were free to be carried through the bulb by the vapor stream. Lovecy (4) also made use of this principle, but unless the distillation rate was kept slow the scrubbing action was not effective and the condensate was carried over into the receiver.

* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 20–22, 1947.

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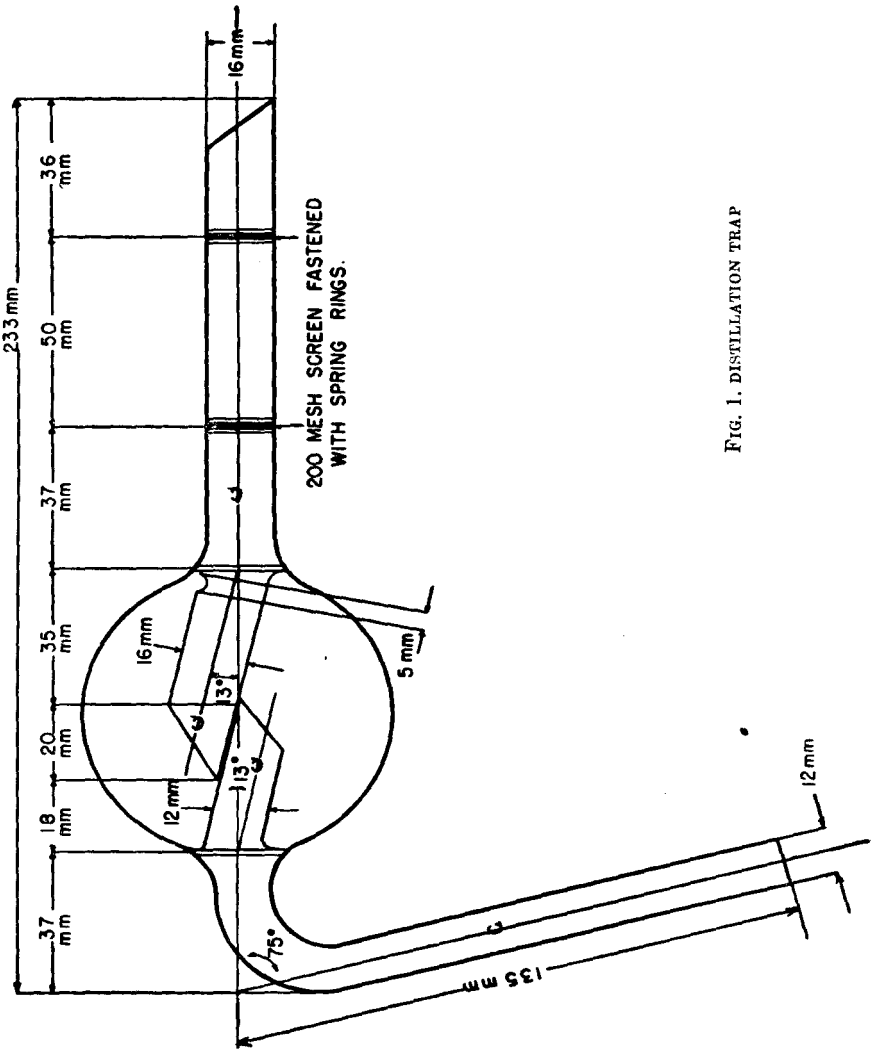

FIG. 1. DISTILLATION TRAP

The experimental connecting bulb scrubs all the vapors passing through it, since a water film forms on the wire screen plates the instant water vapor reaches them, and these films are maintained throughout the distillation. In passing through the layer of water condensate built up on the upper screen plate, the vapors carry water into the bulb vapor trap. The amount of condensate carried into the bulb seldom exceeds 35 ml., even with long distillation times.

The condensate in the bulb is maintained above *p*H 7 by the nonvolatile alkali scrubbed from the vapor, and it is kept at or near the boiling point by the passing steam. Under these conditions, no ammonia should be retained in the condensate in the connecting bulb. That no ammonia was retained was confirmed by collecting the bulb condensate at the end of a nitrogen determination and testing it with Nessler's reagent.

A comparison was made of four of the commonly used Kjeldahl connecting bulbs with the experimental bulb. The Lovecy bulb was not considered in these tests because of its fragile structure and because of the care that must be exercised in controlling the necessarily slow distillation rate.

The distillation mixture consisted of 100 ml of 50 per cent sodium hydroxide, which had been boiled to remove any ammonia, 250 ml of water and 10 grams of mossy zinc. Two hundred milliliters of distillate were collected in receivers containing 50 ml of 0.0100 *N* hydrochloric acid at the rate of 100 ml in 30 minutes for the slow distillation and 200 ml in 30 minutes for the rapid distillation. This test mixture, which is similar to that used by Davisson, produces a heavy spray rich in nonvolatile alkali. The results are given in Table 1.

The zinc was responsible for the large amounts of sodium hydroxide in the vapor, since when no zinc was used in the distillation mixture, the amount of sodium hydroxide carried over was small and nearly independent of the type of bulb used. The form of the zinc was also an important factor, since mossy zinc caused almost twice as much sodium hydroxide to be carried over as did an equal weight of 20-mesh zinc.

In these tests, those bulbs which did not provide for scrubbing the distilled vapors (the Hopkins, Iowa State and the Kjeldahl) passed the largest amount of sodium hydroxide. The poorest duplicate check results were obtained with the bulbs which passed the most sodium hydroxide. Of the nonscrubbing-type connecting bulbs the Hopkins bulb allowed the greatest amount of sodium hydroxide to pass and the Kjeldahl bulb the least. Much less nonvolatile alkali passed through the two scrubbing-type bulbs. The experimental bulb was more than three times as effective as the Davisson and more than thirteen times as effective as the best nonscrubbing type. These data indicate that bulbs having only baffles are ineffective, and when used, the conditions of the distillation must be controlled to give a minimum amount of droplets in the vapor stream. Only

those bulbs which scrub the vapors will perform satisfactorily independent of the distillation conditions.

Using the Davisson scrubber, the best of the common connecting bulbs, as shown by the preceding experiment, the blank values obtained under ordinary Kjeldahl conditions ranged from a low of 0.2 ml to a high of 0.6 ml of 0.1 *N* hydrochloric acid. The experimental connecting bulb under

TABLE 1.—*Comparison of various types of Kjeldahl connecting bulbs for removal of entrained nonvolatile alkali*

BULB	DISTILLATION OF 200 ML. PER 30 MIN.						DISTILLATION OF 100 ML PER 30 MIN.	
	WITH MOSSY ZINC		WITH 20-MESH ZINC		WITHOUT ZINC		WITH MOSSY ZINC	
Scrubber Type								
Experimental bulb	1.82*		1.30		1.33		1.40*	
	1.89		1.12		1.06		1.68	
	Ave.	1.9	Ave.	1.2	Ave.	1.2	Ave.	1.5
Davisson bulb	5.69		3.00*		1.57*		2.79	
	6.45		2.60		1.72		3.67	
	Ave.	6.1	Ave.	2.8	Ave.	1.7	Ave.	3.2
Nonscrubber Type								
Kjeldahl bulb	23.02		2.16				19.4	
	26.92		1.36				24.0	
	Ave.	24.9	Ave.	1.8			Ave.	21.7
Iowa State bulb	56.96		91.14		1.67		77.0	
	69.93		36.89		1.83		61.0	
	Ave.	63.5	Ave.	64	Ave.	1.8	Ave.	69.0
Hopkins bulb	152.0		164.0				64.0	
	161.0		187.0				210.0	
	Ave.	156	Ave.	176			Ave.	137

* Amount of nonvolatile alkali carried to the receiver expressed as milliliters of 0.01 *N* HCl.

the same conditions gave a blank value of 0.0 to 0.04 ml of 0.1 *N* hydrochloric acid.

An attempt was made to evaluate the two parts of the new bulb, the screen plates and the bulb trap, but it was found that the values obtained by independent measurements of each part were of little significance and that the bulb could be considered only as a whole. The bulb was especially useful as an antifoam trap. In this respect it was far superior to the other bulbs tested.

The rate at which ammonia was distilled into the receiver was determined by preparing several Kjeldahl flasks, all containing the same amounts of reagents and sample. After digestion, alkali was added, and

the ammonia distilled into boric acid. The rate of distillation, 200 ml per 30 minutes, was the same for all flasks. The receiving flasks were then withdrawn at different time intervals, beginning at 7½ minutes from the start of boiling. The results are given in Table 2. At the rate of 200 ml per 30 minutes, essentially all the nitrogen as ammonia was distilled during the first 22.5 minutes, and a prolonged distillation did not yield appreciably higher values.

TABLE 2.—*Rate of distillation of ammonia from Kjeldahl digestion mixtures*

DISTILLATION TIME (MIN.)	VOLUME DISTILLED ML. (APPROX.)	NITROGEN FOUND, PER CENT	PER CENT OF TOTAL (THEORY) NITROGEN DISTILLED
7.5	50	7.96 7.93 Ave. 7.94	95.9
15.0	100	8.19 8.12 Ave. 8.16	98.6
22.5	150	8.25 8.26 Ave. 8.26	99.8
30.0	200	8.26 8.31 Ave. 8.29	100.1
37.5	250	8.25 8.23 Ave. 8.24	99.5

Tests were also made to determine whether any ammonia was lost during the initial heating period by being carried through the trapping liquid in the large bubbles of the displaced air. Using distilled water and indicator, we demonstrated that no ammonia was carried by the displaced air, since the indicator did not change color until condensed water vapor had reached the receiver. Loss of ammonia by this cause is therefore improbable.

Although the new bulb made it possible to measure accurately the nitrogen blank of the reagents, it did not account for the slightly low nitrogen values obtained on analysis of pure nitrogenous compounds. This loss could be accounted for only by assuming that all the ammonia was not being caught by the trapping liquid. In an attempt to eliminate this source of error, a Goessman trap (2) was used on the receiver. Results of analyses of S-benzyl thiuronium chloride with the new connecting bulb, with and without the Goessman trap, are given in Table 3. The Goessman

trap caused an increase of 0.5 per cent of the total nitrogen found when boric acid was the trapping liquid and 0.7 per cent when 0.1 *N* hydrochloric acid was used. Results most closely approximating the theoretical were obtained when boric acid was used.

Of the many mixed indicators proposed in the past few years, a mixture of methyl red and methylene blue is often used in Kjeldahl nitrogen titrations. This indicator has been used in many proportions of methyl red to methylene blue, but 4 parts of methyl red to 1 part of methylene blue, with a final concentration of methyl red of 0.1 per cent wt./vol. in 95% alcohol was the most satisfactory with boric acid. Under these conditions

TABLE 3.—*Nitrogen analysis of S-benzyl thiuronium chloride* by the Kjeldahl method with a new connecting bulb*

LIQUID IN RECEIVER	WITH GOESSMAN TRAP		WITHOUT TRAP	
	NITROGEN, %	RECOVERY, %	NITROGEN, %	RECOVERY, %
Boric acid	13.80		13.82	
	13.78		13.81	
	13.84		13.70	
	13.81		13.78	
	13.81		13.76	
	13.80		13.70	
	Ave. 13.81	99.9	Ave. 13.76	99.6
HCl 0.1 *N*	13.73		13.63	
	13.72		13.66	
	13.70		13.56	
	13.76		13.60	
	13.75		13.64	
	13.67		13.57	
	Ave. 13.72	99.3	Ave. 13.61	98.5

* % Nitrogen found by the Dumas method equals 13.82.

ammonium hydroxide is titrated with the mineral acid, usually hydrochloric. The grey, neutral color of this mixed indicator coincides exactly with the stoichiometric end point (*p*H 4.7). A 4% boric acid solution, however, is acid to the above mixed indicator, and a correction must be made for this in the determination. By the use of the proposed connecting bulb, or by observing the necessary precautions with reagents suitable for Kjeldahl nitrogen analysis, the alkali distilled in the blank is not sufficient to neutralize the boric acid. The acidity of the boric acid of the blank may be neutralized by back titration with a standard base, but a standard base tends to nullify most of the advantages of boric acid as the trapping liquid. The standard base can be omitted if the color of the indicator in the partially neutralized boric acid of the blank is taken as the end-point color. It will be found that this slightly acid shade of the indicator

(*p*H 4.2) is easily matched, and in the analysis of a sample the titration will be carried through indicator color changes from green to grey to light purple (end point shade). The grey shade serves as a warning of the approach to the end point. By taking the sample titration to the same indicator color as that developed in the blank, no blank correction need be used in the calculation, since this procedure automatically corrects for the reagent nitrogen and the boric acid acidity.

SUMMARY

Some of the sources of errors which occur during distillation in the Kjeldahl method for determination of nitrogen have been pointed out. Chief among these is the carry-over of nonvolatile alkali from the alkaline distilling mixture to the trapping liquid in the receiver. By the use of a new connecting bulb, the carry-over of non-volatile alkali is greatly reduced, practically eliminating this source of error. A titration technique is proposed with the mixed indicator, methyl red-methylene blue, which eliminates the standard base and simultaneously corrects for any base from the reagents as well as for the acidity of the boric acid.

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ADDITION OF SELENIUM TO WET-ASH PROCEDURE FOR THE DETERMINATION OF MERCURY IN APPLE PEEL*

By FRIEDA M. KUNZE (Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.)

The method reported by Laug and Nelson[1] for the determination of mercury in biological material was investigated for its adaptability to the mercury analyses of apples sprayed with phenyl mercuric ammonium lactate. It was found that the recovery of mercury added to animal diet, tissues, or excreta in an order of 0.2 p.p.m. could be effected with ease and accuracy, but that the recovery of much larger quantities of mercury added to apple peel or pulp was uniformly poor. Complete recovery of mercury could be obtained, however, if the mercury was added to the

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* Presented at the Annual Meeting of The Association of Official Agricultural Chemists, held at Washington, D. C., October 20–22, 1947.
[1] Laug, E. P., and Nelson, K. W., *This Journal*, **XXV**, 400 (1942).

acid apple digest subsequent to the wet-ashing procedure, indicating that in the instances of low recoveries the mercury was either complexed during the oxidation procedure or that it was lost through volatilization. Analysis of the acid contained in a trap fitted to a Friedrichs extraction condenser showed that no loss of mercury occurred by refluxing if the oxidizing mixture was added cautiously during the violent frothing period. To minimize the diluting effect, wet apple peel was dried overnight on a flat plate in an oven under forced draft at 50°C. This step increased the efficacy of the oxidizing mixture, but appreciable loss of mercury occurred by volatilization during the drying.

An adaptation of Hubbard's method[2,3] essentially a sulfuric-nitric digestion followed by $KMnO_4$, was rejected because of a variable mercury blank due, no doubt, to impure $KMnO_4$. Cognizance of the use of selenium as a catalyst in nitrogen determinations and of the fixative properties of mercuric oxide in selenium determinations[4] prompted an investigation of its use here. Selenium was added either as the metallic powder or as an aqueous solution of sodium selenite. Both effected quantitative recovery of mercury from apple peel.

PREPARATION OF SAMPLE

The modifications in the ashing method of Laug and Nelson are detailed below. In every other respect the procedure is unchanged.

REAGENTS

(1) *Metallic selenium or sodium selenite, reagent grade.*

(2) *NaOH, 50% W/V, a.c.s.*—Prepare a stock supply and store in a paraffin-lined bottle.

(3) *Superoxol.*—Hydrogen Peroxide, 30%, c.p.

(4) All other reagents listed by Laug and Nelson.

Transfer 50 grams wet apple peel to the distilling flasks; add *ca.* 0.1 gram metallic selenium or an equivalent amount of sodium selenite and shake until the selenium is well equalized throughout the sample. Over an interval of 5–10 min., add cautiously, through the drip funnel, 15 ml of concentrated sulfuric acid and 5 ml of concentrated nitric acid. (An initial 3:1 sulfuric nitric ratio reduces frothing and decreases the reflux time. Considerable variation will be found in the amount of acid necessary to complete digestion. Usually 50 grams of wet apple peel require 20 ml of sulfuric and 30–40 ml of nitric acid.) Whenever charring occurs add small amount of nitric acid. As the frothing abates, increase the heat and reflux at full flame for about 2½ hours or until the soln is clear; cool; add 10 ml H_2O_2; reflux for another half hour. Since relatively large amounts of acid and small amounts of mercury are involved and in order to prevent oxidation of dithizone during the extraction procedure, neutralize the acid digest with 50% NaOH to a *p*H of 1 to 1.5, or use an aliquot containing no more than 2.5 ml of H_2SO_4. (Over-neutralization will result in mercury loss.) Proceed with the determination of Hg as outlined by Laug and Nelson.[1]

[2] Cholak, J., and Hubbard, D., *Ind. Eng. Chem., Anal. Ed.*, **12**, 768–771 (1940); Hubbard, D., *Ind. Eng. Chem., Anal. Ed.*, **18**, 149 (1946).

[3] Unpublished experiments. Food Division, Food and Drug Administration, Federal Security Agency.

[4] Klein, A. K., *This Journal*, **XXVI**, 347 (1943).

TABLE 1.—*Mercury recovered from apple peel*

1:1 SULFURIC NITRIC			1:1 SULFURIC NITRIC+Se			SULFURIC NITRIC+$KMnO_4$			
Hg ADDED	Hg RECOVERED	PER CENT RECOVERED	Hg ADDED	Hg RECOVERED	PER CENT RECOVERED	Hg ADDED	Hg RECOVERED	BLANK	PER CENT RECOVERED
mmg	*mmg*	*mmg*	*mmg*	*mmg*	*mmg*	*mmg*	*mmg*	*mmg*	*mmg*
				Dry peel—8 grams					
69.0	21.7	36	20	20.3[a]	101				
55.0	30.8	56	60	60.3[a]	100				
75	30.0	40	60	62.4[a]	104				
43	22.5	52							
				Wet peel—50 grams					
			20.0	20.4[a]	102	8.0	8.0	1.41	83
			70.0	62.6[b]	89	8.0	8.76	1.58	90
			30.0	30.4[b]	101	9.0	9.75	1.75	89
			2.0	1.76[b]	88				
			5.0	4.86[b]	97				
			4.0	4.20[b]	105				

[a] Unneutralized aliquot used.
[b] Partially neutralized aliquot.

Acid digestion will not destroy completely the wax present in the sample. Filter through glass wool and make up to suitable volume (100–200 ml). The sample or aliquot to be analyzed must be cooled before extraction to prevent precipitation of Se by $NH_2OH \cdot HCl$. A rust-red color which sometimes appears in the stem of the funnel, however, does not interfere with mercury determination.

DISCUSSION

The neutralization procedure introduces a small blank. After the insoluble matter in the NaOH solution had settled, however, this blank became constant. For instance, 50 ml of 50% sodium hydroxide contributed 0.85 microgram of mercury.

There is evidence that selenium exerts a fixative action on mercury; therefore, a study was undertaken to determine the feasibility of a Kjeldahl digestion procedure. Under carefully controlled conditions fairly good recoveries were obtained from apple peel, but the same procedure applied to rat diet and beef liver yielded low results. The time and care required and the questionable reproducibility invalidates the open Kjeldahl digestion.

RESULTS

Table 1 shows the recoveries obtained when the apple peel was ashed by three different methods. The addition of selenium to sulfuric-nitric oxidizing mixtures effected quantitative recovery without contributing to the blank. Neutralization of the ash with 50% NaOH adds an analytical blank.

The author wishes to thank Edwin P. Laug, Alfred K. Klein, and Hugo J. Wichmann, Food and Drug Administration, for their suggestions and comments.

SEPARATION OF THE SATURATED STRAIGHT-CHAIN FATTY ACIDS C_{11} TO C_{19}*

By L. L. RAMSEY and W. I. PATTERSON (Food Division,† Food and Drug Administration, Federal Security Agency, Washington 25, D. C.)

For analysis of fats, the fatty acids are usually separated by the fractional distillation of their methyl or ethyl esters, and apparatus is available for separating less than a gram of the mixed esters (1). Low temperature crystallization is also being used more frequently for fatty acid purification and separation, but this technique is not readily adapted to micro quantities. Both distillation and crystallization are either time consuming or else require special apparatus. In a search for a more reliable and simpler procedure for small quantities of fatty acids the technique termed "partition chromatography" (2) was investigated. The relatively easy

* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 20–22, 1947.

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quantitative separation of the fatty acids with less than 11 carbon atoms by partition chromatography (3) suggested that the same principle might apply to the higher fatty acids. Earlier use of adsorption chromatography for complete separation of these fatty acids was not too promising, although the separation of the higher acids, including palmitic from myristic, was achieved (4).

Whereas water was used as the immobile solvent for the lower acids (5), the low solubility of the higher acids in water precluded its use in a partition system for their separation. Nitromethane had already been used as the immobile solvent, with n-hexane as the mobile solvent, for the separation of some isomers of hexachlorocyclohexane (6), but these solvents were not suitable for separating the higher fatty acids. Methanol and 2,2,4-trimethylpentane were an acceptable combination for the acids through capric (3), but again were unsuitable for the higher acids.

In the further search for a suitable pair of immiscible solvents for separating the higher fatty acids, preliminary experiments indicated that furfural, furfuryl alcohol, tetrahydrofurfuryl alcohol, or β-hydroxyethyl pyridine as the immobile solvent, with 2,2,4-trimethylpentane or n-hexane as the mobile solvent, would separate lauric from palmitic acid, but would not separate palmitic from stearic acid completely. Finally, a mixture of furfuryl alcohol and 2-aminopyridine as the immobile solvent, with n-hexane as the other solvent, gave a satisfactory separation of palmitic and stearic acids, and a mixture of the even-numbered acids C_{12}–C_{18}.

The synthetic acids containing an odd number of carbon atoms from C_{11} through C_{19} were also tested on this column and found to separate in a manner similar to that of the even-numbered acids. When the C_{18} acid (stearic) was mixed with the C_{19} acid (nonadecanoic) in equal amount, separation was only partial; however, the first portion of the percolate containing ca $\frac{1}{4}$ of the eluted acids was pure C_{19} within the limits of detection. Suitable refractionation of the remaining eluate results in recovery of more of the pure C_{19} acid, and also of part of the C_{18} acid. Thus a single passage through the column is capable of completely separating from each other, within the limits of detection, only the even-numbered members of the series C_{12}–C_{18}, and only the odd-numbered members of the series C_{11}–C_{19}. The purity of the separated acids is rather difficult to establish, since by the usual criteria of purity even 1 per cent of a homolog, present as an impurity in one of these acids, is difficult to detect.

The identification of each acid may be made tentatively by measuring its threshold volume as was described in an earlier paper, for the C_5 to C_{10} acids (3). This tentative identification may be confirmed by a melting point determination or by ascertaining the chromatographic homogeneity of a mixture of the unknown with an approximately equal amount of an authentic sample of the suspected acid.

With the weakly basic 2-aminopyridine as an essential part of this solvent system, an equilibrium of the free fatty acids with their 2-amino-

pyridine salts is probable, and this may contribute to the success of the method. However, the solvent properties of the 2-aminopyridine also seem to be an important factor, since experiments with a series of aqueous buffers as the immobile phase, and n-hexane as the mobile one, gave no separation at all. Attempts to determine whether it is the free acids or their 2-aminopyridine salts that are actually separated led to inconclusive results.

Whereas bromocresol green was used as an indicator for the C_5 to C_{10} acids, in the presence of the basic 2-aminopyridine an indicator will not change color with the relatively minor amounts of acid going through. Even in the separation of lauric from palmitic acid with no 2-aminopyridine present, the indicators previously used (3, 5) showed no appreciable change in color in the presence of small quantities of the acids above lauric. Thus the separation of these higher acids, as now carried out, is followed by the collection of a series of percolate fractions and the measured titration of each.

The purity of the solvents, 2-aminopyridine and furfuryl alcohol, does not seem to affect the separation of the fatty acids, as technical and purified material gave the same threshold volume, recovery, and other behavior.

With columns of the size employed, the optimum working range of the method is from about 5 mg to 20 mg of each acid, the lower limit being defined by the sensitivity of the titration rather than by the sensitivity of the separation.

The saturated straight-chain fatty acids below hendecanoic (C_{11}) do not interfere with the separation of the C_{11}–C_{19} straight-chain members of the series. The naturally-occurring unsaturated fatty acids, such as oleic, do interfere and must be removed by suitable means before the method can be applied to the acids of a hydrolyzed fat.

METHOD

APPARATUS

(1) Chromatographic tubes.

(a) Tube 27 mm O. D. ×200 mm long prepared from Pyrex tubing, or from Pyrex test tubes by sealing into the bottom of each a piece of glass tubing 6 mm O. D. ×25 mm long.

(b) Any other suitable container.

(2) Suitable pressure source, such as compressed air or a cylinder of nitrogen; and a means of keeping the pressure constant, such as a column of mercury or a diaphragm type pressure regulator.

REAGENTS

(1) *Silicic acid.*[1]

(2) *Furfuryl alcohol*[2]*—2-aminopyridine solvent:*—Dissolve sufficient sodium hydroxide in furfuryl alcohol to make it strongly alkaline, and distil. Dissolve 50 g of 2-aminopyridine in 50 g of purified furfuryl alcohol.

[1] Mallinckrodt's S.L. grade precipitated powder was used in this work.

[2] Purification of these solvents is included in order to remove any acidic materials which might be present.

(3) *n-Hexane*[2] *solvent:*—If not free of acid, wash with alkali, dry, and distil. To ca. one l of n-hexane add 25 ml of the furfuryl alcohol-aminopyridine solvent, shake vigorously, allow to separate, draw off the furfuryl alcohol-aminopyridine layer, and reserve it for saturating a future batch of n-hexane.

(4) *Phenolphthalein soln:*—Dissolve 1 g of phenolphthalein in 100 ml of absolute alcohol and neutralize with sodium ethylate.

(5) *Sodium ethylate, standard 0.02 N soln.*—Prepare this soln by the usual procedure using aldehyde-free absolute alcohol.

(6) *Sodium hydroxide, standard 0.02 N soln.*

(7) *Ethyl alcohol,* 70% v/v:—Dilute 700 ml of 95% alcohol to 950 ml. Neutralize just before use with the 0.02 *N* NaOH.

(8) *Ether, A.C.S.*

(9) *Sulfuric acid,* ca *N.*

(10) *Hendecanoic, lauric, tridecanoic, myristic, pentadecanoic, palmitic, margaric, stearic, and nonadecanoic acids.*

PROCEDURE

(1) *Preparation of the chromatographic column and determination of the optimum ratio of furfuryl alcohol-aminopyridine solvent to the silicic acid:*

To 20 g of silicic acid in a mortar, add 10 ml of the furfuryl alcohol-aminopyridine solvent and mix well with a pestle. Add ca 65 ml of the n-hexane solvent and make a slurry. Place a very small cotton plug firmly in the neck of the constriction at the bottom of the chromatographic tube and clamp the tube in an upright position. Pour the slurry into the tube, preferably by transferring from the mortar to a 100 ml beaker and pouring from the beaker into the tube; connect the top of the tube to a suitable pressure source thru the gas pressure regulator; and apply 2–5 pounds of pressure. During the packing down process, tap the tube from time to time to aid in obtaining a smooth, level surface. Observe whether the gel packs down quite firmly without cracking or drying out, and whether the flow rate is convenient—in the range 3–5 ml per minute. If the flow rate greatly exceeds 5 ml per minute and/or the gel fails to pack down firmly without cracking or drying out, it is possible that too much furfuryl alcohol-aminopyridine (immobile) solvent has been used. On the other hand if the flow rate is extremely slow (less than 2 ml per minute), the silicic acid probably has a greater capacity for the immobile solvent. Accordingly, increase or decrease by ca 2 ml the amount of the immobile solvent to be added to another 20 g portion of silicic acid, and prepare another column as above. Observe its behavior. Proceed in this manner until the optimum ratio of furfuryl alcohol-aminopyridine solvent to silicic acid (*i.e.*, the ratio which produces a column that packs down firmly without cracking or drying out, and with a convenient flow rate) has been found. If a column prepared with the optimum ratio of immobile solvent to silicic acid begins to crack or dry out because the pressure was left on too long in the packing down process, remove the gel from the tube, slurry again with the hexane, retransfer to the tube, and repack.

(2) *Testing the silicic acid for its suitability and standardization of the chromatographic column:*

Pipet onto a column, prepared as in (1) above, 2 ml of a standard solution of lauric, myristic, palmitic, and stearic acids[3] in hexane containing about 10 mg of each

[3] Since the odd- and even-numbered carbon acids are ordinarily not found together, and since a single passage through the column will not completely separate a mixture of contiguous fatty acids, the standardization is made with a mixture of the acids with either an even or an odd number of carbon atoms. For brevity the procedure is given only for the even-numbered acids.

acid. Place a graduated cylinder under the column to collect the percolate, and allow the solvent to sink into the gel under pressure. At the instant all the solution has sunk into the gel, release the pressure, and add 1 ml of hexane. Renew the pressure until this wash solvent has sunk into the gel, and repeat the washing again using 1 ml of hexane. Fill up the tube with solvent and renew the pressure. (A separatory funnel or other suitable container may be fitted into the chromatographic tube to serve as a reservoir for the solvent.)

After 30 ml of solvent has percolated through the column, begin to collect 2 ml fractions in a 5 ml graduated cylinder. When the first 2 ml fraction has been collected, transfer it immediately to an Erlenmeyer flask and titrate with the 0.02 *N* sodium ethylate using phenolphthalein as the indicator. Add the second fraction to the first in the Erlenmeyer flask and again titrate; continue adding successive fractions to this flask, titrating each time, until a titration is obtained greater than the preceding one by several hundredths of a ml. The cumulative volume just before this fraction is the threshold volume for stearic acid. Continue titrating and recording both the cumulative volumes and titrations, until the volume of alkali decreases to ca 0.1 ml for a 2 ml fraction. Now transfer each 2 ml fraction to flask 2 until only 0.05 ml or less of alkali is required. (Another flask is used in order to obtain sensitivity of titration for these small amounts of acid.) Combine flasks 1 and 2 and label "stearic acid."

At this stage begin collecting, in flask 3, 5 ml fractions and titrate each as before; continue to titrate each fraction until only ca 0.05 ml is required. Transfer the next fraction to flask 4 and continue as before until only ca 0.02 ml of alkali is required. Combine the percolates in flask 3 and 4 and label "palmitic acid."

Continue collecting and titrating 5 ml portions of percolate as for palmitic acid, first in flask 5 and then in flask 6. Combine the material in flasks 5 and 6 and label "myristic acid." At this point collect, in flask 7, 10 ml portions until the titration becomes 0.02 ml or less. Label flask 7 "lauric acid."

From the record of volumes collected ascertain the threshold volume for each of the other three fatty acids. These threshold volumes constitute the standardization of the column.

Using the above procedure, run through the process, omitting the addition of the acids to the column, in order to obtain the blank. Correct the titrations of the 4 acids for the blank and calculate the approximate amount of each acid recovered based on the titration.[4]

For more accurate results, distil off the solvent from the sodium salts of the four acids, dissolve each of the salts in ca 20 ml of water, and transfer to individual separatory funnels with a small amount of water. Acidify each of the solutions with 2 ml of the ca *N* H_2SO_4 and extract successively with 25 and 10 ml portions of ether, drawing off and combining the ether extracts for each acid in another separatory funnel. Wash each of the combined ether extracts once with ca 5 ml of water and pour the ether solution through a small folded filter into a 125 ml Erlenmeyer flask. Wash the funnel and filter paper with three 10 ml portions of ether. Evaporate the ether on the steam bath, dissolve the acid in neutral 70% alcohol, using heat if necessary to effect solution, and titrate with the standard 0.02 *N* NaOH, using phenolphthalein as indicator. From the titration in each case, calculate the amount of acid present.

Consider the silicic acid suitable if, upon following the above procedure, the recoveries of the added acids are in the range 90–100%.

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[4] The titration of a fatty acid in hexane in the presence of 2-aminopyridine, using phenolphthalein as the indicator, is almost quantitative, the results being only a trifle high; but the total titration of a series of cumulative fractions as required by the method leads to high and somewhat non-reproducible results even when the titrations are corrected for a blank.

(3) *Determination and identification:*

Determine the acids in an unknown exactly as described above in the standardization and suitability procedure.

Compare the threshold volumes of the unknown acids with those of the known acids and make a tentative identification of the acids.

To confirm the tentative identification proceed as follows: (a) Where at least 10 mg of the acid is available, crystallize the acid from hexane in a 15 ml centrifuge tube (or small test tube) by cooling to ca minus 10°C. Isolate the acid either by centrifuging at that temperature, washing, and drying in the centrifuge tube; or by filtering with suction at ca minus 10°C. on a small filter. Determine the melting point. (b) With the smaller amounts proceed as follows: Pipet 1 ml of a hexane solution containing 5 to 10 mg of the unknown acid onto a freshly prepared column. Pipet an approximately equal amount of the authentic acid dissolved in ca 1 ml of hexane onto the column; gently swirl to mix; and proceed to develop the chromatogram. If only one band is obtained, *i.e.*, if all the acid elutes in the same volume of solvent required in the standardization process to elute the authentic acid, the tentative identification is confirmed.

EXPERIMENTAL RESULTS AND DISCUSSION

Table 1 shows the recoveries of the fatty acids with an even number of

TABLE 1.—*Recoveries and threshold volumes of lauric, myristic, palmitic, and stearic acids when present in admixture*

ACID	ADDED	FOUND	RECOVERY	THRESHOLD VOLUME
name	*mg*	*mg*	*per cent*	*ml*
		Mixture A		
Stearic	10	10.0	100	68
		9.3	93	66
Palmitic	10	9.7	97	106
		9.8	98	102
Myristic	10	9.7	97	170
		9.7	97	165
Lauric	10	9.4	94	275
		10.0	100	280
		Mixture B		
Stearic	10	9.3	93	68
Palmitic	20	18.8	94	102
Myristic	5	5.5	110	175
Lauric	3	3.1	103	295

carbon atoms when present in admixture in the range 3 to 20 mg. each. Two different mixtures were analyzed, one of them in duplicate. As can be seen, recoveries are generally within 6 or 7 per cent. No corrections

have been made for homologs found (as later described) as impurities in the acids used, but in no case would the results in this table be appreciably affected by such impurities. Table 1 also shows the threshold volumes obtained in each experiment for each acid. The threshold volume of an acid is not appreciably affected by the quantity of acid, within the working range of the method.

Table 2 shows the recoveries of the fatty acids with an odd number of carbon atoms when such acids are present in admixture. As can be readily seen from the data the recoveries are of about the same degree of precision as those of the even-numbered acids.

TABLE 2.—*Recoveries and threshold volumes of hendecanoic, tridecanoic, pentadecanoic, margaric, and nonadecanoic acids when present in admixture*

ACID	ADDED	FOUND	RECOVERY	THRESHOLD VOLUME
name	*mg*	*mg*	*per cent*	*ml*
		Mixture A		
Nonadecanoic	10	9.4	94	54
Margaric	10	9.4	94	80
Pentadecanoic	10	10.0	100	121
Tridecanoic	10	9.5	95	220
Hendecanoic	10	9.3	93	370
		Mixture B		
Nonadecanoic	20	18.8	94	54
Margaric	20	19.7	98	82
Pentadecanoic	20	19.4	97	121
Tridecanoic	20	19.1	95	215
Hendecanoic	20	18.9	94	360

The melting points of the separated acids (Table 3) were determined on a Fisher-Johns melting point apparatus. A more precise melting point was unnecessary to distinguish between adjacent acids with an even number of carbon atoms, or between adjacent acids with an odd number of carbon atoms, after each had been separated from its homologs by passage through the column and recrystallized once.

An experiment was performed to determine the probable degree of contamination of the even-numbered acids with their even-numbered homologs after separation by the proposed method. Refractionation of the separated acids was not feasible because of the difficulty of measuring the small amounts of acid involved. For each of the four even-numbered acids, the threshold volume and the volume of eluate required to elute 98–100 per cent of each acid (10 mg. quantity) were determined on the same

TABLE 3.—*Melting points of the acids after chromatographic separation of admixtures of 20 mg each followed by recrystallization from hexane, compared with melting points from literature (7)*

ACID	M.P., ° C. (7)	M.P., ° C. FOUND
Admixture of acids containing an even number of carbon atoms		
Stearic	69.6	68–69
Palmitic	63.1	61–63
Myristic	53.9	52–53
Lauric	44.2	41–42
Admixture of acids containing an odd number of carbon atoms		
Nonadecanoic	68.6	67–68
Margaric	61.3	59–61
Pentadecyclic	52.3	51–52
Tridecylic	41.5	40–41
Hendecanoic	29.3	23–26*

* Satisfactory recrystallization of this acid (20 mg) was difficult.

column, a single acid being placed on the column and then completely eluted before the next was added. After all four acids had been passed through the column, the threshold volume for stearic acid was checked to determine whether the column had changed; this threshold volume was found to remain the same. The results are tabulated in Table 4. Based on the data in Table 4 it appears that the separations are at least 98 per cent complete in all cases, and probably better than that in the case of lauric and myristic acid and in the case of myristic and palmitic acid.

TABLE 4.—*Fractions containing the even-numbered fatty acids*

ACID	FRACTION CONTAINING 98–100% OF THE ACID*
Name	*ml*
Stearic	68–98
Palmitic	100–145
Myristic	160–225
Lauric	275–390

* First figure of a fraction is the threshold volume.

An experiment was conducted to determine the sensitivity of the method in detecting the even-numbered homologs of an even-numbered acid added to such acid as impurities. Using a 40 mg. sample and a single fractionation, 5 per cent of added homolog could be detected; with a larger sample and suitable refractionation, about 0.5 per cent could be detected.

The even-numbered fatty acids used in this work were examined by the proposed method to determine their purity. The results are tabulated in Table 5. A weighed quantity of the fatty acid in the range 200 to 300 mg. was dissolved in 5 to 10 ml of hexane and transferred to the standard 20 g column. The fraction beginning after about 50 ml of solvent had passed through the column and including a small part of the main band (5 to 10%) was collected and designated as fraction 1. (This fraction was not collected for stearic acid.) The middle portion of the main band was collected as fraction 2 and discarded. Beginning 5 to 10 ml before the next lower homolog should begin to elute, fraction 3 was collected and collection was continued until no more acid would elute. Fraction 1 containing a small amount of the main band and the higher homologs, if any, was refractionated on a fresh column. The higher homologs, if any, were

TABLE 5.—*Method applied to the even-numbered acids used in this work to determine their purity*

ACID	GRADE	HOMOLOGS FOUND AS IMPURITIES
Stearic	Pure	2% palmitic acid
Palmitic	Pure	0.5% stearic acid
Myristic	Pure	0.5% palmitic acid and 0.8% lauric acid
Lauric	Pure	1.5% capric acid

identified by threshold volume and determined by titration. A third or fourth refractionation was made when necessary. Fraction 3 containing the tailings of the acid in the main band plus its lower homologs, if any, was refractionated on a fresh column and the fractions identified and measured in a manner similar to that for fraction 1.

The purity of the odd-numbered acids (obtained from commercial sources) used in this work was also investigated by the same general procedure used above for the even-numbered acids. The margaric acid was found to contain about 30 per cent of non-acidic material and 17 per cent of nonadecanoic acid. Tridecanoic acid was found to contain 12 per cent of lauric acid and 7 per cent of hendecanoic acid. Each of these acids was purified chromatographically for the recovery experiments reported above, approximately 200 mg of each being prepared. For this purification a standard 20 g column was used. In the case of margaric acid, about 150 mg of the acid dissolved in ca 10 ml of hexane was fractionated on the column. The fraction 88–110 ml containing 35 to 40 mg of acid was collected, the forerun and tailings being discarded. The same column was usually used in succession for three 150 mg batches of the acid. A total of six fractions from six 150 mg batches of the acid were combined; the hexane solution was washed once with approximately 0.1 *N* sulfuric acid and 3 or 4 times with water to remove the 2-aminopyridine. The solution was then dried over anhydrous sodium sulfate, filtered, and evaporated

on the steam bath until the concentration was about 5 mg per ml, the concentration being accurately determined on an aliquot by titration. A refractionation of 50 mg of this purified acid (solution concentrated to about 3 ml before addition to the column) showed that no nonadecanoic acid was present. Since the nonadecanoic acid is almost completely eluted before the margaric acid begins to come through near the 80 ml point, beginning the collection of the "pure" fraction at 88 ml in the purification process above gives about an 8 ml fraction as a margin of safety.

In the case of tridecanoic acid ca 100 mg of the acid dissolved in about 5 ml of hexane was placed on a standard 20 g column and the fraction 205–275 ml (containing ca 60 mg acid) collected, the forerun and tailings being discarded. The fractionation of each 100 mg batch of acid was made on a fresh column, a total of 4 columns being used to produce about 200 mg of tridecanoic acid free of lauric acid. The hexane solution of the tridecanoic acid was washed, dried, filtered, and concentrated as above for

TABLE 6.—*Threshold volumes of the even-numbered fatty acids with different mobile solvents and with 2-aminopyridine-furfuryl alcohol as the immobile solvent*

ACID	MOBILE SOLVENT, ML		
	HEXANE	CYCLOHEXANE	2,2,4-TRIMETHYLPENTANE
Stearic	68	52	102
Palmitic	106	80	170
Myristic	170	128	295
Lauric	275	220	530

margaric acid. A refractionation of 50 mg of the purified acid indicated that not more than a trace of lauric acid was present; 98 per cent of the acid was eluted in the fraction 215–280 ml.

The nonadecanoic acid contained no homologous acids, but did have ca 40 per cent of non-acidic impurities. This acid was also purified chromatographically before use in a procedure similar to the above.

Certain other solvent combinations in which the threshold volume of stearic acid is higher may be useful for separating still higher fatty acids, assuming that their threshold volumes will be less than those of the acids with fewer carbon atoms. As the threshold volume increases, the width of the band containing a given acid also increases; thus the completeness of separation of two acids may be just as good with relatively low threshold volumes as it is with higher ones. Table 6 affords a comparison of the threshold volumes of the even-numbered acids C_{12}–C_{18} using three different mobile solvents with the same immobile solvent. It was also found that the separations with cyclohexane were of about the same order of completeness as with hexane or 2,2,4-trimethylpentane.

It was found that the threshold volumes for palmitic acid shown in

Table 6 are in line with its distribution coefficients in the various solvent systems, one member of which was always a mixture of 2-aminopyridine and furfuryl alcohol. The distribution coefficients were determined as follows:

The 2-aminopyridine-furfuryl alcohol solvent was prepared by dissolving sufficient 2-aminopyridine in furfuryl alcohol to double the volume of the furfuryl alcohol taken. The cyclohexane and 2,2,4-trimethylpentane were pure grade reagents and were used directly without redistilling. The solvents were saturated with each other and 25 ml of each solvent were pipetted into a separatory funnel. One hundred mg of palmitic acid were added and the funnel shaken vigorously for several minutes. After the phases separated the lower layer was drained off and discarded. A 20 ml aliquot of the upper, or hydrocarbon, layer was pipetted into a beaker and the hydrocarbon boiled off on a steam bath. The residue was dissolved in neutral 70% alcohol and titrated with 0.02 *N* NaOH using phenolphthalein as the indicator. From the titration the amount of palmitic acid present in the hydrocarbon layer was calculated, and the amount of acid present in the 2-aminopyridine-furfuryl alcohol layer obtained by difference. The distribution coefficient in the results below is defined as the ratio of the quantity of palmitic acid in the 2-aminopyridine-furfuryl alcohol to the quantity of the acid in the hydrocarbon. For the solvent system with hexane the distribution coefficient of palmitic acid was found to be 5.6; with cyclohexane, 3.7; and with 2,2,4-trimethylpentane, 8.3.

The threshold volume of a fatty acid is also affected by the particular silicic acid used, probably by its adsorptive capacity, which varies somewhat from one batch of silicic acid to another. For example, with one batch of silicic prepared in the laboratory according to a recommended procedure, (8) the threshold volume of stearic acid on a standard column was 96 ml and that of palmitic acid was 150 ml. (See Table 1 above for the threshold volumes of these acids using a commercial silicic acid as the column medium.)

2-AMINOPYRIDINE STEARATE

When stoichiometric amounts of 2-aminopyridine and stearic acid were dissolved in each of three solvents, alcohol, ether, and furfuryl alcohol, and then set aside to crystallize, the crystals obtained in each case were found to be stearic acid. When stearic acid was mixed with a large excess of 2-aminopyridine in alcohol (2 parts of aminopyridine to 1 part of alcohol, by weight), somewhat impure 2-aminopyridine stearate precipitated. The crude product was purified by recrystallization from n-hexane. However, 2-aminopyridine stearate is best prepared by dissolving stoichiometric amounts of the acid and base in hot hexane and allowing the salt to crystallize. The detailed preparation follows:

Stearic acid, 2.85 g, and 2-aminopyridine, 0.94 g, were dissolved in ca 90 ml of hexane by heating on the steam bath. A few mg of decolorizing carbon were added and the solution gently boiled ca 5 minutes. The solution was filtered, reheated on the steam bath, and set aside to crystallize at room temperature. The fine needle-like crystals were filtered on a Büchner funnel, washed with two small portions of hexane, and dried. The neutral equivalent of the salt was determined by dissolv-

ing 378.6 mg in 70% alcohol and titrating against 0.1 *N* NaOH using phenolphthalein as the indicator. The neutral equivalent was found to be 377; theory is 378.6. The product melted at 62–64°C.

The salt, 2-aminopyridine stearate, can be separated into its component parts by dissolving it in hexane and washing the hexane solution 4 or 5 times with water. The stearic acid remains in the hexane while the 2-aminopyridine goes into the aqueous phase. One millimol of the 2-aminopyridine stearate (378.6 mg) was dissolved in 50 ml of hexane and the solution washed with four 25 ml portions of water. The wash water portions were combined and titrated against 0.1 *N* HCl using bromocresol green as the indicator; 9.5 ml of 0.1 *N* HCl were required, whereas 10 ml is the calculated amount.

SUMMARY

A method, based on partition chromatography, is presented for the separation of the straight-chain saturated fatty acids C_{11}–C_{19}. The separation of the even-numbered acids from each other and of the odd-numbered members from each other is fairly complete in a single fractionation and recoveries of added acids are essentially quantitative.

The fatty acids are separated on a column of silicic acid using a mixture of furfuryl alcohol and 2-aminopyridine as the immobile solvent, and n-hexane as the mobile solvent. The separation is followed by titration of percolate fractions of suitable volume with standard sodium ethylate, and the separated acids are determined by titration in 70 per cent alcohol with standard sodium hydroxide. Each acid is tentatively identified by its threshold volume, and the identification is confirmed either by a melting point determination or by adding an approximately equal amount of an authentic sample of the suspected acid to the unknown and testing the chromatographic homogeneity of the mixture on a fresh column.

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BOOK REVIEWS

The Chemistry of Organic Compounds—A Year's Course in Organic Chemistry. By JAMES BRYANT CONANT and ALBERT HAROLD BLATT. Third edition. x+665 pages. The Macmillan Co., 60 Fifth Ave., New York, N. Y., 1947. Price $5.00.

The regulatory chemist's need for a general up-to-date knowledge of the basic principles of organic chemistry is becoming almost a necessity, with the multitude of new synthetic drugs and food adjuncts. Without this elementary knowledge of the recent developments in organic chemistry, it is more difficult for the control chemist to develop or apply analytical procedures suitable for effective enforcement of food and drug laws. On the other hand, the average control chemist does not have time for the details and more involved theories of organic chemistry. An elementary treatment of the subject such as this third edition of "The Chemistry of Organic Compounds" is perhaps a desirable answer. Even though the book is planned as an elementary text, Conant and Blatt have succeeded in describing and illustrating organic chemical principles in a simple, easily readable style which tends to hold the reader's interest.

With examples, accompanied by some discussion, the authors illustrate the significance of certain physico-chemical principles in organic reactions. This recognition of the role which certain thermodynamic principles play in organic reactions is a noteworthy step in the treatment of elementary organic chemistry, as it tends to put previous statement of fact upon a logical and, to some extent, predictable basis.

Industrial products from petroleum, polymerization mechanisms, and the chapter on "Certain Biochemical Processes" are subjects in the book deserving specific mention. Some of the newer antimalarials and other drugs are also described. The few errors which were noted are of the obvious typographical kind. This book is to be recommended to those who desire an up-to-date presentation of organic chemistry.

W. I. PATTERSON

Cottonseed and Cottonseed Products, edited by ALTON E. BAILEY. Interscience Publishers, Inc., New York, 905 pp. Price $17.50.

This book on the chemistry and technology of the cottonseed industry contains chapters written by the editor and twenty-four collaborating authors. While the various chapters are grouped into sections, mention will be made only of the specific chapters. The authors have presented their subject matter in such a well organized manner that there is a minimum of duplication and over-lapping in the various chapters.

The first chapter of 50 pages on the "History of Cotton and the U. S. Cottonseed Industry," by Maurice R. Cooper, gives a historical background and outlines the development of the various phases of the cottonseed industry. Cottonseed processing, production, and consumption in the important cotton-producing nations are discussed in 50 pages by Charles E. Lund. The chapter of 11 pages by John Leahy on the "Structure of the Cottonseed" is enhanced by numerous photographs of the structural characteristics and of the microscopic sections of the cottonseed. In 39 pages, W. H. Thorp covers "Cottonseed Composition—Relation to Variety, Maturity, and Environment of the Plant," which includes numerous tables of data on composition of cottonseed and its different parts. "Biological Processes of the Cottonseed," 55 pages, by Aaron M. Altschul, will be of special interest and benefit in

connection with cottonseed storage, which is more specifically discussed under "Handling and Storage of Cottonseed," 20 pages, by O. H. Alderks. The chapter of 150 pages on "Pigments of Cottonseed" by Charlotte H. Boatner, seems rather lengthy, although it will be of immense value to the scientist or student specializing in this field.

The material under "Cottonseed Oil," 44 pages, by the editor, Alton E. Bailey, is impressive for its completeness and relative conciseness. Thomas D. Fontaine's chapter, 56 pages, on "Cottonseed Proteins" deals with chemical and physical properties; and those proteins constituting the primary commercial value of cottonseed meal and cake are treated from the standpoint of their nutritional significance under "Cottonseed as a Source of Animal Feedstuffs," 42 pages, by Fred Hale and Carl M. Lyman. Under "Miscellaneous Constituents," by F. G. Dollear and K. S. Markley, there are 27 pages on chemical properties and physical characteristics of linters, hulls, and kernels. The portion on sampling under "Grading and Evaluation of Cottonseed," 30 pages, by Guy S. Meloy, should be of special interest to the chemist. The material on sampling, methods of analysis, and collaborative check work is also of unusual interest under "Grading and Evaluation of Cottonseed Oil, Cake, and Meal," 23 pages, by E. R. Barrow. Another chapter of 12 pages by Guy S. Meloy, covers "Grading of Cotton Linters." The details of the technology of cottonseed processing are well presented in the following chapters: "Mechanical Pretreatment of the Seed," 21 pages, by A. Cecil Wamble; "Cooking of Meats and Recovery of the Oil," 30 pages, by O. H. Alderks; and "Economics of Cottonseed Crushing," 37 pages, by John F. Moloney.

A chapter on "Processing of Cottonseed Oil," 40 pages, by Edward M. James, is followed by one on "Edible Cottonseed Oil Products," 29 pages, by Howard C. Black, which covers particularly well the various types of shortening for specific bakery uses. Another chapter of nutritional importance deals with the role of fat in human nutrition under "Nutrition Aspects of Cottonseed Oil Utilization," 48 pages, by Harry J. Deuel, Jr. Information is furnished on the relatively small proportion of cottonseed oil entering the non-edible field, under "Non-edible Cottonseed Oil Products," 13 pages, by O. H. Wurster, W. J. Govan, Jr., and G. J. Stockmann. A few pages are devoted to "Miscellaneous Products from Seed and Meal," by A. E. Bailey, which include some information on cottonseed flour and its use. The two remaining chapters are on "Cottonseed Hulls," 20 pages, by John W. Dunning, and "Cotton Linters," 11 pages, by Peter Van Wyck.

"Cottonseed and Cottonseed Products" encompasses a wide variety of subjects pertaining to the cottonseed industry. It is very readable and well illustrated, and the typography is good. Although some sections will be more useful than others, the reviewer recommends this book to anyone engaged in the cottonseed industry.

V. E. MUNSEY

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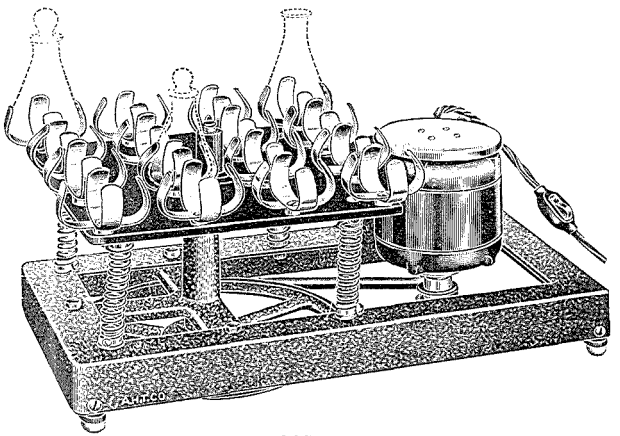

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