FIRST DAY

MONDAY—MORNING SESSION

REPORT ON ALCOHOLIC BEVERAGES

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

At this writing, nine Associate Referee reports and one note have been submitted on alcoholic beverages in support of about 20 recommendations dealing with deletions of methods, revision and adoption of other methods, and plans for future work. These reports speak for themselves and the writer offers comment on only one of them, that is, the one dealing with the determination of methanol. As Associate Referee, Dr. Guymon was appointed to investigate both A.O.A.C. procedures **16.25** to **16.28** and **39.161** to **39.162** for methanol in distilled liquors and in drugs respectively, with a view to bringing about conformity in these procedures, so far as possible. A start has been made this year as reported by Dr. Guymon, with respect to one of the procedures, namely, **16.25**, and it is hoped that next year **39.162** can be studied and brought into harmony with **16.25**. Specifically, we do not question the part of **39.162** which provides for the removal of esters, etc., with petroleum benzene. That step should no doubt be retained in the drug method. Nor do we question the use of a series of color standards instead of measuring the color with a neutral wedge photometer or spectrophotometer. The procedure that is questioned in that method is the use of 5 milliliters of distillate containing 0.25 milliliter of total alcohols for oxidation with 2 ml of 3 per cent potassium permanganate solution. This amount of potassium permanganate is not sufficient to insure oxidation of all of the methyl alcohol in the presence of such an excess of ethyl alcohol. **16.25** corrects this defect. In this latter method, only 0.25 ml of a 22 per cent solution of total alcohols is used for oxidation, the actual amount of total alcohols being only 0.055 ml, as compared with 0.25 ml in **39.162**. However, there is a possibility that the same proportion of methanol will be oxidized in the standards as in the sample, even though the total alcohols are not completely oxidized, due to insufficiency of potassium permanganate. This is one of the important points to investigate next year.

In 1945 Beyer and Reeves published the results of an investigation of the immersion refractometer method, **16.29**, for methanol in distilled liquors (*This Journal*, 28, 800, 1945). Among other things they found that if the methanol content is less than about 2 per cent in the sample prepared for the refractometer reading, that method is not to be recommended. Also, the authors found that the scale readings for methanol did not agree with all of the corresponding specific gravities in the table

in 16.30. Method **16.29** should be revised accordingly, even though the discrepancies are very small, and the writer is making a recommendation to that effect.

For the convenience of the Associate Referees, who in the course of their duties arrange to test, revise, and recommend change of status of our tentative A. O. A. C. methods, I am listing below the section numbers of such tentative methods for alcoholic beverages, including this year's recommendations, as well as those of 1945, 1946, and 1947. They are as follows:

Beer (Chapter 14.)
.16, .21 to .25 incl., .28 to .31 incl., .33, .34, .36, .38. *This Journal*, **30**, 67 (1947).
Malt (Chapter 14.)
.42, .43, .44, .46, .47, .48, .57, .58, .59, .91, .94, .100, .101.
Yeast (Chapter 14.)
.112 to .115, incl.
Spent Grains (Chapter 14.)
.116 to .124, incl.
Wines (Chapter 15.)
.1, .25, .26, .30, .36, .37.
Spirits (Chapter 16.)
.1, .20, .21, .25 to .28 incl., .35, .36, .37. *This Journal*, **31**, 183 (1948).
Artificial Colors (Chapter 16.)
.38, .40.
Tannin (Chapter 16.)
.42, .43
Cordials and Liqueurs (Chapter 16.)
.44 to .67, incl., .70, .73 (See *This Journal*, **31**, 183 (1948) under "Distilled Liquors").

The fifth edition of the Book of Methods provided for the use of the immersion refractometer for checking the per cent alcohol in all three chapters (Malt Beverages, Wines, Distilled Liquors) and the procedure was classed as official. In the sixth edition the procedure was retained without change in the chapter on Wines, but was not included in the chapters on Malt Beverages and Distilled Liquors. The use of the immersion refractometer facilitates analysis when a number of samples are to be run and the appointment of an Associate Referee is recommended to consider the advisability of including this procedure in Chapters 14 and 16.

All of the recommendations on alcoholic beverages are appended.

RECOMMENDATIONS*

Malt Beverages, Brewing Materials, and Allied Products:
It is recommended—

* For report of Subcommittee D and action of the Association, see *This Journal*, 32, 59 (1949).

(1) That study of methods for determination of essential oil and resins in hops be continued.

(2) That the dye color method for the estimation of color in wort and beer, described in the Associate Referee's report for 1948, be adopted as official, (first action).

(3) That the tentative method for color in beer and wort, *This Journal*, **30**, 68 (1947), be dropped.

(4) That work on photometric beer color evaluations be continued.

(5) That study of beer turbidity methods be continued.

(6) That the tentative method **14.112–14.115** for total solids in yeast be adopted as official, first action, including the revision of **14.114,** Preparation of sample, for total solids as described in the report of the Associate Referee; and that the description of preparation of sample, **14.114,** for total solids in yeast, be revised as described in the report of the Associate Referee on Yeast for 1948.

(7) That the Milos test for caramel, **14.35,** be deleted, final action.

(8) That the Mathers test for caramel, *This Journal*, **31**, 76 (1948), be studied collaboratively with respect to its application to beer.

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

(10) 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 further **(a)** with a view to eliminating the use of the reducing agent, hydroxylamine hydrochloride, and **(b)** use of crystalline ferrous ammonium sulfate in place of metallic iron for standardization.

(11) That further work on copper be postponed, pending outcome of proposed work by the Referee on metals in foods.

(12) That work be continued on polarographic-spectrographic methods for tin in beer.

Wines:

It is recommended—

(1) That chromatographic studies of wines be continued.

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

(3) That the official, first action, Mathers test, *This Journal*, **31**, 76 (1948) be adopted as official, final action.

Distilled Liquors:

It is recommended—

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

(2) That the Mathers test for caramel, *This Journal*, **31**, 76 (1948), be adopted as official, final action, for distilled liquors, and that it be studied collaboratively, with respect to its application to cordials and liqueurs, before adoption as final action.

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

(4) That the study of colorimetric methods for fusel oil be continued.

(5) That the study of the method **(16.22** and **16.23)** for fusel oil be continued.

(6) That the official method, **16.29,** for methanol in distilled liquors by the immersion refractometer method be studied in the light of the findings of Beyer and Reeves, *This Journal,* 28, 800 (1945).

(7) That an Associate Referee be appointed to determine if the immersion refractometer method for alcohol in **15.4(c)** should be incorporated in Chapters 14 and 16.

(8) That the rapid method for proof of distilled spirits, as recommended in the report of the Associate Referee on obscuration test for proof in distilled spirits, be adopted as tentative.

(9) That study be continued of the official modified Denigès method for methanol, **16.25,** and the tentative method for methanol in **39.161** and **39.162,** to bring about uniformity in these procedures so far as possible.

Cordials and Liqueurs:

It is recommended—

(1) That section "**16.45** Specific Gravity, see **14.3**" be changed to read "**16.45** Specific Gravity, proceed as under **16.2.**"

(2) That a collaborative study be made of methods for caramel in cordials and liqueurs.

(3) That a collaborative study be made of the two tentative methods for total solids, *i.e.,* **16.51 (a)** From sp. gr. of dealcoholized sample and **16.51 (b)** By evaporation.

(4) That a collaborative study be made of the tentative method for total acidity, **16.62.**

REPORT ON SOLIDS IN YEAST

By Robert I. Tenney (Wahl-Henius Institute, Chicago, Ill),
Associate Referee

The criticism which has been directed to method **14.115** has been largely that agreement could not be obtained between laboratories in different cities, although it could be obtained between several analysts working with the same sample at the same time. Studies recently completed[1] by the American Society of Brewing Chemists were designed to test this method upon a carefully controlled sample and to determine whether the differences were due to the method or to fermentive changes within the sample.

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[1] R. I. Tenney, *et al.* Proceedings, Annual Meeting, A.S.B.C., 1948.

Nine laboratories participated in the work and compared results obtained upon each of three different days—all working with a split sample of a yeast which had been autoclaved to destroy all enzymatic action. They also compared the total solids content of various local yeasts over a similar period, but permitted autolysis and other fermentation changes to occur.

Changes in the samples were found to occur which could result in a difference of as much as 4.84 per cent in the total solid content within forty-eight hours and as much as 5.87 per cent in seventy-two hours. However, the standard deviation of all laboratories collaborating was only 0.4 per cent when the sample itself was known not to change.

The conclusion can be drawn, therefore, that method **14.115** is capable of giving close agreement and that the differences noted and criticized are due chiefly to changes within the sample itself.

A few studies of a non-miscible solvent distillation method were also made, but such a method involves the use of equipment not commonly available and is also only as accurate as the sampling. Since the present method does give satisfactory results, there seems no need to include an alternate procedure at this time.

RECOMMENDATIONS*

In view of the above, it is recommended—

(1) That the tentative method **14.112–14.115** for total solids in yeast be adopted as official, first action.

(2) That the present description of sample preparation, **14.114,** be revised.

Details of this revision are given in *This Journal,* **32,** 82 (1949).

REPORT ON THE OBSCURATION TEST FOR PROOF ON DISTILLED SPIRITS

By GEORGE F. BEYER (Bureau of Internal Revenue, Washington 1, D. C.), *Associate Referee*

In the past it has been the accepted practice in distilleries, rectifying plants, and by others to determine the proof of distilled spirits by the use of a hydrometer, preferably one that had been standardized by the National Bureau of Standards. It has also been known for a long time that any substance lighter or heavier than a mixture of alcohol and water will have some effect on a hydrometer.

Blended spirits (whiskey) may contain substances like blending sherry wine, glycerine, prune juice, etc., all of which are heavier than water and,

* For report of Subcommittee D and action of the Association, see *This Journal,* **32,** 59 (1949).

therefore, will affect a hydrometer by obscuring the true proof of the spirits.

It is a well-known fact that the most accurate method of determining the true proof (per cent of alcohol) in spirits is the distillation method outlined in the 6th Edition of the *Methods of Analysis, A.O.A.C.* However, for this purpose, considerable apparatus is necessary and also an expert operator. On the other hand, it has been found by hundreds of determinations in nearly all of the branch laboratories as well as in the Washington laboratories that, a procedure of determining the apparent proof of the spirits with a standard hydrometer and the solid contents of the sample according to **16.8**, and for every 100 mg. of residue (solids) found per 100 ml. of sample adding 0.4 of 1° proof to the apparent proof, produces very accurate results, with only a minimum amount of simple apparatus which requires no expert to manipulate. The procedure, however, has its limitations; for if the spirits contain 600 or more mg. per 100 ml. this method of correction becomes inaccurate. In other words, the correction factor of 0.4 of 1° proof for every 100 mg. of solids is not sufficiently accurate for use when the residue of the spirits analyzes 600 or more mg. per 100 ml., in which case the distillation method must be used.

In the light of these findings it is recommended* that a method be adopted for the determination of the proof of distilled spirits according to the following procedure.

Determine the apparent proof of the distilled spirits with an accurately standardized hydrometer, preferably one graduated in $\frac{1}{5}$ degrees in proof. Determine the extract (solids) according to Section **16.8**, and for every 100 mg extract add 0.4° proof to the apparent proof. NOTE:—If the extract amounts to more than 600 mg, this method does not apply.

REPORT ON FUSEL OIL IN DISTILLED SPIRITS

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

Since the last report was made on the shortening of the method for the determination of fusel oil in distilled spirits,[1] further work has been done which shows that still more time may be saved without interfering with the accuracy of the method.

Referring to that place in the determination directing the extraction of the saturated salt solution 4 times with varying amounts of carbon tetrachloride and washing the combined carbon tetrachloride extracts with saturated sodium chloride solution and then with saturated sodium sulfate solution, no time for the period of shaking is mentioned. However, the

* For report of Subcommittee D and action of the Association, see *This Journal*, **32**, 60 (1949).
[1] Beyer, G. F., *This Journal*, **31**, 184 (1948).

accepted practice has been to shake the solution when extracting, and the extract when washing, for 2 minutes. Experiments have shown that one minute shaking accomplishes the same purpose. Further time may be saved by reducing the time of oxidation to 2 hours instead of 4.

Work is being continued to determine how to prevent the losses that appear inherent in this method, and it is also recommended* that a colorimetric method for this determination be investigated. Some work along this line has been done with the use of a spectrophotometer, but not sufficient to make a report at this time.

REPORT ON CHROMATOGRAPHIC ADSORPTION OF WINES

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

This subject has been listed for a report for several years, but since the original and interesting paper was delivered by George K. Hamil in 1942,[1] no comprehensive report has been made. However, during this interval the original method, its improved procedures and its principles, have been in routine use in some laboratories. During the past two years Paul Simonds and Arthur Etienne have been improving its operation and broadening its scope and application up to the present moment.

At the present time we are found in the center of this work so that a paper on this subject at this time would be incomplete and premature. For this reason it is recommended[2] that the subject be continued until next meeting, at which time it is hoped that a report or a contributed paper will be ready for publication.

REPORT ON CARAMEL IN ALCOHOLIC BEVERAGES

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

At the last meeting of the A.O.A.C. the Associate Referee made a report on the above subject[3] in which was discussed the collaborative results on a method devised by Alex Mathers, and recommended its adoption.

This method has given good results both before and since its collaborative approval, but its adoption was limited to wine and spirituous liquors. Because it has also been successfully applied to beer and cordials, it has been suggested that collaborative work be done on that class of material during the ensuing year.

* For report of Subcommittee D and action of the Association, see *This Journal*, 32, 60 (1949).
[1] *This Journal*, 25, 220 (1942).
[2] For report of Subcommittee D and action of the Association, see *This Journal*, 32, 59 (1949).
[3] *This Journal*, 31, 178 (1948).

Before the next meeting in 1949, it is planned to send out samples of beer and cordials, with and without caramel, for collaborative work, to obtain a report and recommendation at the next October meeting.

No report was given on cordials and liqueurs or carbon dioxide in beer.

REPORTS ON HOPS

By D. E. Bullis (Chemist, Oregon Agricultural Experiment Station, Corvallis, Oregon), *Associate Referee*

No collaborative work has been done on the methods for hop analysis during the past 12 months. However, two points in the methods have been examined in a preliminary way in the Associate Referee's laboratory. Because of a lack of adequate analytical assistance more extensive studies could not be undertaken this year. It is hoped that some collaborative work on these items may be completed before the next annual meeting.

Under **14.86(b)**, page 173 of the 1945 edition of the *Methods of Analysis*, 18–20 ml. is specified as the proper volume in which to carry out the final precipitation of alpha resin. This laboratory has some evidence that incomplete precipitation of alpha resin may result when the above volume is employed with hops of less than average alpha resin content. During the past season we have had occasion to analyze a number of hybrid samples from the U.S.D.A. hop breeding yard at Corvallis, many of which contain less than 4 per cent alpha resin. In many of these the alpha resin-lead compound precipitates with difficulty and incompletely from a 20 ml. volume. By reducing the volume, precipitation takes place normally and apparently much more completely.

The following table illustrates the variation that has been observed.

TABLE 1.—*Variation in precipitation according to volume*

PRECIPITATING VOLUME	ALPHA RESIN-LEAD PRECIPITATE	ALPHA RESIN
ml	*gram*	*per cent*
20	.0322, .0310 } .0316	1.63, 1.57 } 1.60
15	.0399, .0418 } .0409	2.02, 2.11 } 2.07
12	.0442, .0449 } .0446	2.23, 2.27 } 2.25
10	.0451, .0469 } .0460	2.28, 2.37 } 2.33
7	.0487, .0486 } .0487	2.46, 2.46 } 2.46

In the light of these data it may be that the volume of 20 ml. now specified for the precipitation of alpha resin should be reduced, when dealing with old hops or with low alpha resin content hops such as were many lots of the 1947 crop. Further data on the influence of this factor will be obtained during the coming year.

The second point given some preliminary study concerned the precipitation of alpha resin by using a fixed quantity of lead acetate solution. Details of this modification of the usual procedure for this determination are covered in the last two paragraphs of **14.86(b)** on page 173 of the 1945 edition of the official methods.

The hops from which the following data were obtained contained 15.97 per cent soft resins, and the results are typical of the errors which are introduced in the alpha resin determination by too little or too much precipitating reagent.

TABLE 2.—*Variation in precipitation according to quantity of reagent*

LEAD ACETATE SOLUTION	ALPHA RESIN-LEAD SALT	APPARENT ALPHA RESIN
ml	*grams*	*per cent*
5.2	.0795	5.02
5.6	.0845	5.34
6.0	.0875	5.53
6.4	.0908	5.74
6.8	.0915	5.78
7.2	.0912	5.76
7.6	.0908	5.74
8.0	.0902	5.70

It will be noted that a considerable error results from 1 ml. deficiency of lead acetate, whereas 1 ml. excess does not cause the results to deviate seriously from the correct value.

It is true that 7 ml. of lead acetate is sufficient for precipitating the alpha resin of most hops of 16–19 per cent soft resin content. However, in routine hop analysis, determination of the soft resin content is not usually completed until after the alpha resin determination is finished. Consequently, the analyst has no way of knowing whether he is dealing with a hop of low, average, or high soft resin content, and so has no information on which to base the amount of lead acetate needed for the alpha resin precipitation.

If a rapid estimation of alpha resin is desired it would seem that reasonable speed combined with greater accuracy could best be obtained by omitting the preliminary titration **14.86(a)**, and carry out the determination according to the first paragraph under **14.86(b)**. Paragraphs 2 and 3 of **14.86(b)** could then be deleted.

Your Associate Referee expects to have some collaborative results on this point to report next year.

REPORT ON INORGANIC ELEMENTS IN BEER

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

IRON

INTRODUCTION

On the basis of the last report (1) and recommendations made on the determination of iron in beer the direct (non-ashing) orthophenanthroline method, based on Nissen's method, were submitted to collaborative study. This Referee has noted that several of the laboratories using a direct procedure for the routine analysis of iron in beer have worked out their own modifications of the Nissen direct orthophenanthroline procedure. The procedure used in this study is as follows:

REAGENTS

10% Hydroxylamine hydrochloride.—Dissolve 100 g of hydroxylamine hydrochloride in 1000 ml of Fe-free distilled water and store in pyrex glassware.

0.3% Orthophenanthroline.—Dissolve 1.5 g orthophenanthroline in 500 ml of Fe-free distilled water heated to 70°C. Cool and store in pyrex glassware.

Iron-free distilled water.—If necessary, water should be redistilled from pyrex glassware.

Glassware:

Pyrex glassware is used. Clean all equipment used with trisodium phosphate, rinse with hot water, rinse with conc. hydrochloric acid, rinse with tap water, and finally rinse with redistilled or Fe-free distilled water.

Standard iron solution:

Dissolve 0.5000 grams of reagent grade iron free of oxide in 5 ml of 20% HCl plus 1 ml of HNO_3. Cover with watch-glass, heat, and evaporate to dryness; add water and evaporate to dryness again. Take up with conc. HCl, cool, and rinse into 100 ml volumetric flask. This is Solution A. Take 10 ml of Solution A+2 drops of bromine water and make up to 500 ml. This is the working standard Solution B; 1 ml = 0.0001 grams of Fe.

Standardization:

Prepare a series of five standards equivalent to 0.5, 1.0, 1.5, 2.0, and 2.5 p.p.m. of iron in a twenty-five milliliter volume. Treat these samples as outlined below and read per cent transmission at 505 millimicrons, using a 4 or 5 centimeter cell in a suitable spectrophotometer. Plot results on semi-logarithmic paper in the usual manner.

PROCEDURE

Pipet 25 ml portions of test beer into two 125 ml Erlenmeyer flasks. Add 5 ml of 10% hydroxylamine hydrochloride to each, mix well, and allow to stand 30 min. Then to one aliquot add 5 ml of orthophenanthroline reagent and to the second aliquot add 5.0 ml of iron-free distilled water. Mix well and allow to stand for 30 min. Using distilled water set at 100% transmission read the two aliquot samples of beer at wave length 505 millimicrons. A blank must be run on each sample of beer analyzed. The p.p.m. iron in the beer is determined as follows:

The p.p.m. Fe read from curve for beer with phenanthroline, less p.p.m. Fe read from curve for beer blank (without phenanthroline).

TABLE 1.—*Results of collaborative study*

COLLABORATOR NO.	METHOD	IRON P.P.M.					
		SAMPLE A DUPLICATES		SAMPLE B DUPLICATES		SAMPLE C DUPLICATES	
			Ave.		*Ave.*		*Ave.*
1	Nissen	0.96		0.08		0.47	
	This report	0.99		0.08		0.47	
		—	0.98	0.09	0.08	—	0.46
2	Nissen	1.12		0.18		0.64	
	This report	1.14		0.15		0.63	
		1.13	1.13	0.15	0.16	0.63	0.63
3	Nissen	(1)	1.00	(1)	0.12	—	
	This report	(2)	0.97	(2)	0.09	—	
	Average		1.02		0.11		0.55
	Max. Deviation		0.11		0.05		0.09
	Average Deviation		0.05		0.03		0.09
4	Ashed—	1.18		—		0.83	
	Orthophenanthroline	1.26		—		0.80	
		1.12	1.19	—	0.44	0.85	0.83
1	Wet ashed	1.15		0.07		0.55	
	Orthophenanthroline	1.03	1.09	0.10	0.09	0.67	0.61
	Average		1.14		0.27		0.72
3	Direct	(1)	1.11	(1)	0.07	—	—
	Bipyridine	(2)	1.02	(2)	0.09	—	—

(1) Sample turbid and run as received.
(2) Sample filtered and then run.

SAMPLES

Three samples were to be run in duplicate by each collaborator. Sample A consisted of canned beer that had been in storage for some time; therefore, a high iron beer; Sample B was a bottled beer, or low iron beer; and Sample C was made up of equal portions of the A and B beers.

DISCUSSION OF RESULTS

The results of Table 1 indicate that the modified Nissen method used in this study is capable of producing results with an average deviation of 0.10 p.p.m. iron or less in the range of 0 to 1 p.p.m. iron in beer. Ashing procedures tend to give high results even when used with adequate blanks. The 2-2′ Bipyridine direct method of Gray and Stone compares favorably with the collaborative method outlined herein.

COLLABORATORS COMMENTS

Collaborator No. 2:

"The iron method is very similar to the one we are using, varying only in the relative volumes of beer and hydroxylamine. We have been using an automatic pipet delivering about 45 ml. and only 2 ml. of hydroxylamine. In early work on the method, it was ascertained that results obtained on freshly opened beer were identical even when hydroxylamine was omitted. Our method of standard preparation is slightly different in that we buffer the solutions with citrate-citric acid at a *p*H of 3.5. The two standard curves, however, differ only slightly."

Collaborator No. 3:

"In our tests with orthophenanthroline and 2-2′ Bipyridine, we found that the use of reducing agents, such as hydroxylamine in the direct method is unnecessary. The elimination of this unnecessary step in the method would shorten the manipulations and get rid of an unneeded reagent."

"The use of metallic iron as a standard seems unnecessarily lengthy, especially as the Nissen Method calls for complete oxidation of the iron to the ferric form. Since the ferrous ion is the one that gives the coloration with the reagent the use of crystalline ferrous ammonium sulfate for preparing the standards is suggested."

COLLABORATORS

1. Thomas Blumer, Continental Can Company, Inc., Chicago, Illinois.
2. O. R. Alexander, American Can Company, Inc., Maywood, Illinois.
3. Philip R. Gray, Wallerstein Laboratories, New York City, New York.
4. B. H. Nissen, Anheuser-Busch, Inc., St. Louis, Missouri.

COPPER

The procedure for the determination of copper in foods has been studied collaboratively by the Referee. The results of this study do not warrant a recommendation of a method for determining copper in beer at this time.

TIN

Since the last report, a spectrographic method for the determination of tin in beer was reported on by O. R. Alexander at the 1948 St. Louis meeting of the American Society of Brewing Chemists. Additional work has been done by the Referee's laboratory on a polarographic procedure, but not sufficient to warrant publication of a method.

The Associate Referee is indebted to W. C. Stammer and his associate, Thomas Blumer, for assisting in the above study and for their helpful comments.

RECOMMENDATIONS*

In view of the material reported in the foregoing study, it is recommended—

(1) That the modified Nissen method be studied further to eliminate the use of the reducing agent, if possible.

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* For report of Subcommittee D and action of the Association, see *This Journal*, 32, 59 (1949).

(2) That the standardization be made using crystalline ferrous ammonium sulfate versus metallic iron to determine the best procedure.

(3) That the potassium thiocyanate procedure previously adopted as tentative be dropped from the Seventh Edition of *Methods of Analysis*.

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

(5) That no collaborative work be done on tin in beer until the pending polarographic and reported spectrographic methods can be tried in the same study.

LITERATURE CITED

(1) BENDIX, G. H., *This Journal*, **31**, 172 (1948).
(2) NISSEN, B. H., Proceedings of the Eleventh Annual Meeting of the American Society of Brewing Chemists. p. 32 (1946).

REPORT ON COLOR OF WORT AND BEER

By B. H. NISSEN (Anheuser-Busch, Inc., St. Louis, Mo.),
Associate Referee

This report describes the final draft of the Dye Color Reference Solution method for the determination of color of wort and beer, which was originally presented to the Association (*This Journal*, **29**, 287, 1946) as an alternative for the present Lovibond procedure (official). (For details of the method, see *This Journal*, **32**, 81 (1949).)

The new Dye Color method has now become the official method of the American Society of Brewing Chemists, and accepted for publication in the Book of Methods of A.S.B.C. as an alternative method. The Dye Color method has been in practice for several years, and is now in use in some 200 brewery laboratories.

It is practically impossible to secure Lovibond apparatus and glasses, so the alternative method is really the only one available.

In the later report* on Color and Turbidity in beer and wort, comment was made on the stability of several of the Dye Color Reference Solutions. These colors showed little, if any, change when tested at yearly intervals; a number of sets have been sent out to various users, all of whom report favorable results. Again this year we can make the same report and repeat that "This Dye Color Reference method appears fully satisfactory for beer and wort color estimation, and furthermore, that their stability is very good."

As a fully proven method it is recommended again for consideration and for inclusion as official in the A.O.A.C. Book of Methods.

* Nissen, B. H., *This Journal*, 30, 217 (1947).

A photometric procedure is now being studied which it is hoped will terminate in a definition of Lovibond in terms of optical density, with reference to easily reproducible standards.

REPORT ON MALT BEVERAGES, SIRUPS, AND EXTRACTS, AND BREWING MATERIALS

By STEPHEN LAUFER (Schwarz Laboratories, Inc., New York, N. Y.), *Associate Referee*

It is well known that for the past fifteen years the A.S.B.C. has cooperated with the A.O.A.C. in developing and testing methods for the analysis of brewing materials and products. Because of the efforts of the members of both Associations, most of these methods, after careful collaborative checking, received the status of "Official, final action."

During the past year the activities of the technical subcommittees of the A.S.B.C. were concerned with a number of projects, some of which are also of interest to the A.O.A.C.

STANDARD METHODS

In place of the annual collaborative analysis of one or two samples of malt, a quarterly check sample service was instituted this year. The malt subcommittee has undertaken the distribution of samples and of the summary of results of analysis on two malts each quarter year. This summary is forwarded promptly to the participating laboratories to permit the frequent comparison of results. Over forty laboratories in the United States, Canada, and Denmark are employing this check sample service. A full report will become available at the end of the calendar year; it will also be submitted at the next annual meetings of the A.S.B.C. and A.O.A.C.

A subcommittee on sampling was organized to conduct collaborative studies in order to evaluate quantitatively the variations involved in sampling, particularly of bulk materials such as grains. A preliminary report may be available next year.

A subcommittee on statistics was also appointed, which will assist the other subcommittees, such as those on malt and sampling, in the statistical evaluation of analytical data.

The subcommittee on color of wort and beer continued its work on the Dye Color Reference solutions. Associate Referee B. H. Nissen will submit this procedure for adoption by the A.O.A.C., as an alternate method for determination of color in wort and beer.[1]

The subcommittee on yeast continued its collaborative tests on the

[1] *This Journal*, 32, 59 (1949).

determination of total solids in yeast. The results show good agreement between collaborators, and Associate Referee R. I. Tenney will present his report on the subject.

Collaborative work on determination of carbon dioxide in beer has also been concluded. A final report including the revised procedure is now under consideration by the A.S.B.C., and will be submitted next year for adoption by the A.O.A.C.

NEW METHODS

In the past few years two subcommittees were active on the development of methods for barley examination and for biological examination of yeast. In addition to the regular feed analysis on barley, the collaborative tests were concerned with the development of methods which are of main interest to maltsters and brewers, such as germination, potential extract, and potential amylase content. The biological tests on yeast dealt with microscopical examination of yeast, dead cells, counting of cells with the haemacytometer, etc. If the A.O.A.C. is interested in these methods, arrangements will be made for presentation of reports including procedures and supporting data.

NEW EDITION OF METHODS OF A.S.B.C.

A new edition of these methods is in preparation and will be published soon. In addition to new procedures on barley and biological examination of yeast, the new edition will contain minor editorial changes clarifying the old methods. These changes will also be brought to the attention of the Association of Official Agricultural Chemists.

REPORT ON METHANOL IN DISTILLED SPIRITS

By JAMES F. GUYMON (University of California, Davis, California), *Associate Referee*

The reliability of the method for determination of methanol in distilled spirits has been questioned by Morison,[1] who obtained inconsistent results both in preparation of a concentration curve using known standards and when applied to brandy. However, his procedure departed in several respects from the official A.O.A.C. method.[2] During the oxidation step using potassium permanganate, 5 ml. of 5 per cent alcohols (0.25 ml.) were present, whereas the A.O.A.C. method specifies 0.25 ml. of 22 per cent alcohols (0.055 ml.). Beyer[3] found that the intensity of the color produced is decreased as the quantity of ethyl alcohol present is increased above the

[1] *Wines and Vines*, **28**, (9) 25–27 (1947).
[2] *Methods of Analysis*, A.O.A.C. (1945), **16.25.**
[3] *This Journal*, **22**, 151–156 (1939).

quantity specified in the official method. Morison reports using a blue filter with the filter photometer employed, whereas Beyer has also shown that maximum absorption occurs in the region of 580 mμ, indicating the desirability of a yellow filter in order to obtain maximum sensitivity. Furthermore, Morison did not employ a distilling head and fractionating column but chose to distill a volume equal to 75 per cent of the volume of the sample. These departures from the official procedure, together with other uncontrolled factors such as temperature, may have caused the inconsistencies reported. Careful examination of his data would not appear to justify the conclusion made that the method fails to distinguish which aldehydes in a mixture may have been derived from methanol.

Prior to more collaborative study of the method for methanol, it was decided to further investigate the influence of certain factors such as temperature, time of standing and light conditions, the spectral characteristics of the color formed by the modified Schiff reagent, and the efficiency of recovery in the distillation step.

The wave length corresponding to maximum light absorption was found to be 570 to 580 mμ when the spectral transmittance curves were prepared from readings made upon two concentrations of methanol employing a Beckman spectrophotometer. Subsequent measurements were made with a Model 11, Coleman Universal spectrophometer at 575 mμ.

The time of standing necessary for maximum color development is dependent upon methanol concentration as well as temperature. Extinction readings (−log T) for both 0.05 and 0.5 per cent methanol in 22 per cent total alcohols at different temperatures after varying periods of standing are shown in Table 1. As the temperature is increased, the color not only forms more rapidly but also develops a greater depth. The higher concentration of methanol resulted not only in a more rapid development of color than the lesser amount when compared at the same temperature, but also led to greater depth of color based upon apparent extinction coefficients. The dependence of both rate of development and depth of

TABLE 1.—*Effect of temperature upon color development*

METHANOL	HOURS OF STANDING	EXTINCTION		
		15°C.	20°C.	25°C.
per cent				
0.05	0.5	0.002	0.017	0.031
	1.0	0.023	0.041	0.068
	2.0	0.048	0.100	0.102
	5.5	0.094	0.131	0.135
0.50	0.5	0.234	0.453	0.886
	1.0	0.580	1.000	1.569
	2.0	1.125	1.658	1.959
	5.5	1.699	1.721	1.745

color upon temperature and concentration shows that Beer's law is not followed, as already reported.[3]

It is recommended* that further study be made of the influence of temperature, methanol concentration, and other physical factors, with a view toward establishing conditions suitable for employment of photoelectric measurements. It is also recommended that further study be made of the efficiency of recovery of methanol by distillation in laboratory columns.

REPORT ON CACAO PRODUCTS

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

In the report of the Referee last year, the work outlined for this year included five different ingredients of cacao products, viz., lecithin, lactose, maltose, pectic acid, and cacao ingredient. Work has been reported on three of these subjects this year.

Lecithin.—A collaborative study of the method reported in 1946 was made by the Associate Referee. Results obtained show great improvement over those of the previous year. An average recovery of 92 per cent of the added lecithin was obtained, as compared with 78 per cent in 1946. Considering the nature of the material and the quantity of constituent present, the recoveries this year are approaching sufficient accuracy. The Referee concurs in the recommendation of the Associate Referee that the work be continued for another year.

Lactose in the presence of other reducing sugars—As indicated in the Associate Referee's report on this subject, a fermentation procedure for lactose was developed by chemists at the Walter Baker Chocolate and Cocoa Division of General Foods, with some suggestions by the Referee. Results indicate the methods to be reproducible and quite close to the theoretical. In general, the results are a little high on the samples reported, but as almost all of these samples contained "Frodex" (corn syrup solids), the difference is no doubt due to the small amount of higher sugars in the corn syrup which were unfermented by the yeasts used. In the opinion of the Referee, a correction for these could be made by obtaining the rotation of the solution as well as the copper reducing power, and estimating the corn syrup solids by the difference in rotation as found and that calculated from the apparent lactose found. The Referee's one criticism of the method is the time required for an analysis. Since the sample is incubated for about 5 days with the *Sacchromyces fragilis* fermentation, it is entirely too long as a control method. It is recommended that a study be made of ways of shortening the method by the use of larger quantities of the ferment, or by other suitable means, and that the work be continued.

* For report of Subcommittee D and action of the Association, see *This Journal*, **32**, 60 (1949).

Maltose and cacao.—No report will be made on these materials this year and it is recommended that the work be continued.

Pectic acid.—Collaborative work was done on milk chocolate. A sample of milk chocolate was prepared, containing 15.6 per cent of chocolate liquor, representing 6.97 per cent of fat-free cacao in milk chocolate. To 1986 grams of this material was added 14 grams of cacao shell. The original liquor used in the milk chocolate contained 0.5 per cent of cacao shell, making in all 10.2 per cent of shell in the fat-free cacao present in the final sample. Portions of the sample were sent to several collaborators, with the request that they determine the per cent pectic by both the present tentative method and an alternative method, and to report also the weight of cacao residue found by the two methods.

The alternative method differs from the tentative method in that it involves the use of sodium acetate in place of ammonium oxalate to extract the milk protein from the cacao portion, and the separation of any pectin from the extracted milk protein by precipitation with barium hydroxide. The method also makes use of a continuous glass stirrer during the extraction of pectin from the cacao with ammonium oxalate.

Results received from three collaborators, and those of the Referee, are given in Table 1.

TABLE 1.—*Extraction of pectin*

COLLABORATOR	PECTIC ACID				DRY FAT-FREE RESIDUE FOUND×2			
	TENTATIVE METHOD		ALTERNATIVE METHOD		TENTATIVE METHOD		ALTERNATIVE METHOD	
	per cent		*per cent*		*g*		*g*	
1	0.43	0.42	1.11*	0.376	6.38	6.47	10.77	10.91
2	0.28	0.28	0.33	0.38	6.229	6.465	11.239	11.306
3	0.44		0.245	0.35	5.923	5.842	10.146	10.996
4	0.24		0.69		6.392		10.475	

* Probably contaminated with some milk protein.

A study of the results indicates that the milk protein was not as thoroughly extracted with the sodium acetate as was anticipated, and that some of the protein contaminated the cacao residue and the pectic acid precipitate. The residue showed a great increase in weight and developed into a horny mass on drying. Results by the tentative method were more satisfactory, and the cacao residue found was fairly close to the theoretical.

It is the Referee's opinion that the best features of both methods could be combined for a more rapid and accurate method. A feature which could be incorporated in the tentative method to advantage is the use of a continuous stirring device during the extraction of the pectin with

ammonium oxalate. This would no doubt give a better and more uniform extract.

The following change is proposed in the tentative method:

Neutralize to litmus with NH_4OH (1+1) (ca 1 ml), then make slightly acid with acetic acid and add 50 ml of 2% NH_4 oxalate soln. Place power driven glass rod stirrer with a vertical loop at the end in the flask with the shaft thru tube inserted in a No. 10 rubber stopper. Place flask in water bath held at 90–92°C. and stir contents gently and continuously for three hours.

RECOMMENDATIONS*

It is recommended—

(1) That the method for lecithin in cacao products be further studied.

(2) That the method for lactose in cacao products, reported this year, be studied to increase the rapidity of the method and to correct for the effect of the presence of higher sugars in corn syrup solids.

(3) That the study of methods for maltose and cacao ingredient be continued.

(4) That the tentative method for pectic acid, **19.16**, be revised as recommended by the Associate Referee.

(5) That the method for pectic acid in milk chocolate be further studied.

(6) That the method of separation of fat, **19.25**, when used on milk chocolate, be studied and compared with the method for refractory sample proposed by Ferris. *This Journal*, **31**, 728 (1948).

REPORT ON LECITHIN IN CACAO PRODUCTS

By JOHN H. BORNMANN (Food & Drug Administration, Federal Security Agency, Chicago, Ill.), *Associate Referee*

The collaborative results reported by the Associate Referee in 1946, (*This Journal*, **30**, 281), showed fairly good agreement among analysts, but the recovery of added lecithin (78%) was considered to be too low. No samples were sent out for collaboration in 1947 because the Associate Referee was not able to improve materially on the per cent recovery, although a fresh sample of lecithin was used and samples were carefully prepared.

Two samples were sent to collaborators this year for the determination of lecithin by the method outlined in the 1946 report referred to above. One sample was a sweet chocolate (A) and the other (B)—a portion of the same with 0.25% pure lecithin added.

Results reported by collaborators are given in the following table.

* For report of Subcommitteee D and action of the Association, see *This Journal*, **32**, 60 (1949).

SAMPLE A		SAMPLE B		
COLLABORATOR	LECITHIN	LECITHIN	ADDED LECITHIN FOUND	RECOVERY
	per cent	*per cent*	*(B—A)*	*per cent*
1	0.186	0.407	0.221	88.4
	0.186	0.402	0.216	86.4
2	0.200	0.443	0.243	97.2
	0.182	0.443	0.261	104.4
3	0.152	0.439	0.287	114.8
	0.205	0.439	0.234	93.6
4	0.239	0.432	0.193	77.2
	0.230	0.425	0.195	78.0
5	0.151*	0.371*	0.220	88.0
	0.160*	0.382*	0.222	88.8
6	0.202	0.425	0.223	89.2
	0.200	0.421	0.221	88.4
7	0.205	0.455	0.250	100.0

* Corrected for reagent blank.

The Associate Referee believes that, with sufficient experience, an analyst can check his results to within 2 per cent of the value. His results (Collaborator No. 6) represent the maximum and minimum of 5 determinations on A and 4 on B, obtained on three different dates. The average recovery of all collaborators was 92 per cent. No reason for the low recovery has been found.

Lecithin was determined on an aliquot of a chocolate extract and on an aliquot of a lecithin solution. Equal aliquots of the two solutions were mixed and the lecithin determined on the mixture equalled the sum of the determinations on the separate aliquots, within experimental error (0.221 to 0.224 mg). Thus there is no loss under these conditions; however, when lecithin is incorporated in chocolate it does not appear to be completely recoverable by extraction.

In order to determine whether phosphoric acid is lost in the digestion with larger amounts of fat present, an aliquot of the lecithin solution was digested with 1.13 g. olive oil, free from phosphoric acid, added. No loss was detected.

The advantage of the proposed method is due to the fact that the extraction involves less work than a method whereby a sufficiently large sample of chocolate is extracted to yield enough phosphoric acid for a determination by the phosphomolybdate method. The main difficulty

lies in the application of the molybdenum blue method, which, however, is necessary for the determination of the small amounts of phosphoric acid involved. The fact that recovery is about 90 per cent is not a great disadvantage when it is considered that accurate estimation of the added lecithin depends on a knowledge of the amount naturally present, which Winkler & Sale (*This Journal*, **14**, 543(1931)) found to vary from 0.25 to 0.46 per cent in different varieties of cacao.

SUMMARY

Collaborative results reported in 1946 showed an average recovery of only 78 per cent of the lecithin which was added. No samples were sent out in 1947 because the Associate Referee was unable to improve recovery materially. This year the average recovery of 7 collaborators was 92 per cent. No adverse comments were reported.

The proposed method is considered advantageous because of the ease with which an extract is obtained for determination of phosphoric acid.

Digestion of lecithin in presence of large amount of fat does not result in loss of phosphoric acid. Results obtained on a mixture of a lecithin solution with a chocolate extract are the same as the sum of equivalent aliquots run separately. When lecithin is incorporated in chocolate there is an unexplained loss of lecithin. Because of the variation in lecithin naturally present in cacao, a loss of 10 per cent in recovery of added lecithin is not considered of great importance.

It is recommended* that work on lecithin be continued.

ACKNOWLEDGMENT

The writer is indebted to the following collaborators:

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G. McClellan, New Orleans District.

REPORT ON LACTOSE IN CHOCOLATE

By MARY E. HANLON, F. V. KENNEY, JR.† and D. G. MITCHELL (*Associate Referee*), (Walter Baker Chocolate and Cocoa Division, General Foods Corporation, Dorchester, Mass.)

SUMMARY

A method suggested by W. O. Winkler of the Food and Drug Administration for the determination of lactose in milk chocolate in the presence of other reducing sugars has been developed. The method is considered

* For report of Subcommittee D and action of the Association, see *This Journal*, 32, 60 (1949).
† Mr. Kenney's present address is White Rock Beverage Corporation, Brooklyn, New York.

of value in view of the reproducibility of results and the moderate error from the theoretical quantity added to the milk chocolate.

INTRODUCTION

The determination of lactose in milk chocolate containing other reducing sugars by the regular methods of lactose analysis has been impossible because of the interference of other materials. Such reducing sugars as dextrose and maltose when present with lactose give similar reactions in such methods as the Munson and Walker, Lane-Eynon, Soxhlet, Defren, and others. (1) The determination of lactose in the presence of sucrose has presented no problem and several methods have been established.

Magraw, Copeland, and Sievert (2) developed an improvement on the method of Magraw and Sievert (3) for the determination of lactose in the presence of other carbohydrates. This method is based on an enzymatic treatment using animal diastase, invertase-melibase scales, and bakers' yeast for destruction of interfering materials. The lactose is then determined by the Munson and Walker method. This procedure has been tried unsuccessfully on milk chocolate. When tried on milk chocolate containing corn syrup solids, quantities of lactose in excess of the known amount were found. It is possible that the enzymes used are not effective in the presence of chocolate as they were intended for complex mixtures of mixed feeds.

A similar method for the determination of lactose in milk chocolate has been described by F. Th. Van Voorst (4). This method was found unsuitable when other reducing sugars are present.

The method presented below is that suggested by W. O. Winkler of the Food and Drug Administration. The use of *Saccharomyces fragilis*[1] was not originally suggested but was later recommended by Mr. Winkler when the yeasts were not found to be sufficient.

PROCEDURE

Place 12 grams of chocolate sample (accurately weighed) in a 250 ml centrifuge bottle; extract twice by shaking with 100 ml portions of petroleum ether, centrifuging and decanting supernatant liquid each time. Allow ether to evaporate by placing the bottle in a warm place (about 40–45°C.)

Add 60–70 ml of water at 60°C. to the residue in the bottle, stopper, and shake well for 3 min. to dissolve sugars and disperse residue.

Add one g of dry brewers' yeast. Add 6–7 g of Hyflo supercel and a suspension of 6–7 g of washed bakers' yeast in water to centrifuge bottle and mix. Allow to stand at temp. of ca 35°C. for 4 hours, rotating flask occasionally. Conduct a blank using 4 g of sucrose and 1 g of dextrose. Filter the mixture on an 11 cm Büchner funnel with suction, using an 11 cm CS & S blue ribbon filter paper and a suction flask. Wash centrifuge bottle and filter several times with about 15 ml portions of water. Remove the paper and residue from the funnel, strip off paper and place residue in a 600 ml beaker. Add about 40 ml of water and triturate to thoroly disperse the material. Return paper to funnel and decant mixture in the beaker again to the

[1] The *Saccharomyces fragilis* was obtained from American Type Culture Collection, Georgetown University, School of Medicine, Washington, D. C.

funnel, rinse beaker, and suck dry. Transfer combined filtrate to 250 ml volumetric flask. Add 1 ml of 10% tannic acid, and wash down with a little water, and add 2 or 3 ml of basic lead acetate slowly with rotation to clarify the soln. Allow to settle and test with 1 drop of the clarifying soln for complete precipitation. Make to the 250 ml mark at 20°C., mix, and filter. Discard about the first 15 ml of the filtrate. Add a slight excess of dry $Na_2C_2O_4$ to delead the filtrate by adding salt in small amounts with stirring and, after settling, test for complete precipitation with a few crystals of oxalate. Filter off precipitate, discard the first 15 ml.

Inoculate the filtrate with a loopful of *Saccharomyces fragilis*, plug with sterile cotton, and incubate for five days.

Filter and determine the reducing sugars by the Munson and Walker method of copper reduction (*Methods of Analysis, A.O.A.C.* 1945) on 50 ml of the soln. Obtain the lactose from Table **44.11**, 6th Ed. Calculate the total lactose in the sample taken and divide by the weight of the sample to obtain the per cent of lactose.

The *Saccharomyces fragilis* was cultured in an agar medium made up as follows:

Water	—1000 ml
Bacto-peptone—	15 g
Glycerol	— 5 g
Dextrose	— 20 g
Agar	— 20 g

Incubate at 30–35°C.

RESULTS

The samples of milk chocolate used for analysis in these tests were of a normal formulation but were especially prepared for accuracy of composition. Sample A of composition shown below was prepared using whole milk solids of known lactose content. "Frodex," or commercial corn syrup solids, was added as a source of glucose and maltose.

Sample A

	Per cent
Sucrose	50.5
Lactose	4.42
"Frodex"	4.76

Analyses were made of successive samples of this chocolate over a period of a week according to the procedure described above. Blanks were run in all cases as prescribed in the method.

Analysis of Sample A

Anal. No.	*% Lactose*
1	4.74
2	4.73
3	4.70
4	4.70
5	4.70
6	4.70
Average	4.71
Theoretical	4.42

Analysis was made of another lot of milk chocolate, Sample B, whose composition was slightly different. The composition of this chocolate was as shown below:

Sample B

	Added *Per cent*	*By Analysis* *Per cent*
Sucrose	40.0	40.16
Glucose	4.55	—
"Frodex"	4.55	—
Lactose	5.60	5.45

In order to produce an accurate composition and without an interference from other substances, a special sugar feed, Sample C, was compounded from pure substances. The materials were added in approximately the same ratio as would be found in a milk chocolate. Below is shown its composition and the analysis obtained.

Sample C

Sucrose	4.4 gm.
"Frodex"	0.6 gm.
Lactose	0.6 gm. (theoretical)
Lactose	0.57 gm. (by analysis)

Another test was made, where additional quantities of dextrose and maltose over and above that present in the corn syrup solids were added. The composition of this sample is shown below:

Sample D

	Per cent
Sucrose	46.00
Dextrose	4.35
"Frodex"	4.35
Maltose	4.35
Lactose	4.03 (theoretical)
Lactose	2.96 (by analysis)

Only about 75 per cent of the lactose was recovered on this sample. It is possible that the higher percentage of reducing sugars was greater than could be reacted with the quantities of reagents used. A recheck of this sample was made using a longer fermentation to determine if a better recovery of lactose could be made. After ten days fermentation, lactose was found to be 4.70 per cent. Further fermentation was impossible because of the interference of bacteria at this time.

This proportion of reducing sugars might be further investigated to determine if the quantity of maltose is a limitation of the method or if the quantity of reagents is insufficient. However, it is felt that this proportion of maltose is greater than would normally be encountered in a sample of chocolate, therefore the results may be more or less of academic interest.

Since the above analyses were all performed by the same analyst, prepared samples, Sample E, were sent to Central Laboratories of General Foods Corporation, Hoboken, N. J. for analysis. The method was prescribed and a sample of *Saccharomyces fragilis* supplied. They used the method previously described with one modification. The sugars were determined by the Lane and Eynon titration rather than the Munson and Walker method, because of their greater familiarity with it.

Sample E

	Per cent
Sucrose	52.00
"Frodex"	5.00
Lactose	5.20

The chocolate sample was run in triplicate. Two blanks were used consisting of one gram of dextrose and four grams of sucrose in each, and two controls were also used consisting of one and two grams of lactose with one gram of dextrose and four grams of sucrose. The results are presented below:

SAMPLE	LACTOSE	ADDED DEXTROSE	SUCROSE	LACTOSE FOUND
				per cent
E	—	—	—	5.75
E	—	—	—	5.76
E	—	—	—	5.82
Blank	—	1	4	—
Blank	—	1	4	—
Control	1	1	4	99.3 (recovery)
Control	2	1	4	98.9 (recovery)

As will be noted, the results are slightly higher than the amount present and the difference is a little greater than that obtained in the Munson-Walker method. Although it is believed there is no difference in the results by the two methods for determining the sugars it is possible that this is the cause of the discrepancy. This analysis has been run and the lactose content determined as 5.53 per cent. In view of the Central Laboratory's result, it is felt that there is a reasonably good check between two analysts using the same method independently.

From the work outlined above it is considered that the method is successful. The analyses indicate that the results are reproducible with a moderate difference from the theoretical value. Samples containing higher sugars were not prepared as it was felt that they would not be found normally and would be only of academic interest.

It is recommended* that the method for lactose be studied collaboratively.

* For report of Subcommittee D and action of the Association, see *This Journal*, 32, 61 (1949).

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(3) *Ind. Eng. Chem., Anal. Ed.*, 7, 106 (1935).
(4) *Chemisch Weekblad*, 34, 803 (1937).

No reports were given on malt solids, pectic acid, cacao ingredients, or fat.

The contributed paper entitled "Milk Fat in Milk Chocolate," by L. W. Ferris, was published in *This Journal*, **31**, 728 (1948).

REPORT ON FRUITS AND FRUIT PRODUCTS

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

PRELIMINARY REPORT ON METHODS FOR THE DETERMINATION OF FILL OF CONTAINER OF FROZEN FRUITS

Work is now in progress on methods for the determination of fill of container for the smaller (1 lb.) packages of frozen fruit. It appears that the per cent fill can be obtained by a displacement procedure which is a modification of the tentative procedure of this Association for Ice Cream and Frozen Desserts—weight per unit volume of packaged ice cream (*Methods of Analysis, A.O.A.C.*, 6th ed., **22.143**). An overflow can filled with deodorized kerosene[1] is placed in a deep freeze cabinet at or near 0°F. and the frozen food, removed from its wrapping, is immersed in the liquid. The overflow of liquid is the measure of the capacity of the frozen food. In order to provide for interstices within the frozen food, use is made of a synthetic latex bag.[2] The frozen food is placed in the bag which is evacuated and tied before immersion. The work on this project is still in progress and a more complete report may be expected later.

A rapid method for water-insoluble solids and also one for determining the weight of seeds in berry fruit are recommended for adoption.

NOTE ON THE SODIUM COBALTINITRITE REAGENT FOR THE DETERMINATION OF POTASSIUM IN FRUITS AND FRUIT PRODUCTS

Under **26.19**, sodium cobaltinitrite solution is used in the determination of potassium. The analyst is directed to prepare and use a control sample of potassium chloride containing 2 mg. of potash per ml. as a check on the suitability of the reagent. It is stated "If recoveries are low, reagent should

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* W. B. White, Chief.
[1] Bayol D, manufactured by Standard Oil Co. of New Jersey.
[2] Cry O Vac bag, manufactured by Dewey and Almy Chemical Co., Cambridge, Mass.

be rejected; if slightly high, blank corrections may be made on the K estimations."

Those who are familiar with the reagent have doubtless observed that different lots may vary in physical properties, particularly in color. The writer has been advised by those engaged in the manufacture of the reagent that it is not a definite chemical compound. Since the analyst is

TABLE 1.—*Comparisons of lots of sodium cobaltinitrite reagent*
(Recoveries using 20 mg K_2O in KCl solution)

FIRM	LOT	K_2O RECOVERED	RECOVERY
		Mg	*per cent*
A	1	20.16	100.8
A	1	20.49	102.4
A	1	20.50	102.5
A	1	20.53	102.6
A	1	20.59	102.9
B	2	20.22	101.1
B	2	20.41	102.0
B	3	19.89	99.5
B	3	19.93	99.7
C	4	19.95	99.8
C	4	19.99	99.9
C	5	21.11	105.5
C	5	21.17	105.8
C	6	19.43	97.2
C	6	19.85	99.2
C	7	20.74	103.7
C	7	20.84	104.2
C	7	20.95	104.7
C	7	21.01	105.0
D	8	19.58	97.9
D	8	19.72	98.6

primarily concerned with its suitability as a reagent in the quantitative determination of potassium, the fact that the reagent does not have a definite chemical composition is immaterial if reasonable recoveries of potash are obtained by its use. The writer has tested samples of the cobaltinitrite reagent manufactured by each of four firms located in the United States. In carrying out the comparisons 10 ml. of a potassium chloride solution was taken, each ml. of which contained an equivalent of 2 mg. of potash.

It will be observed from Table 1 that the tendency is for slightly high rather than low recoveries. In the interpretation of the results of a fruit

analysis, the greater than 100 per cent recovery of potash would lead to a slightly higher estimation of fruit content. Two of the lots examined gave potash recoveries in the neighborhood of 105 per cent. It would be preferable to reject such lots and use a lot which will give recoveries more nearly 100 per cent. It is recommended that the reagent be described merely as sodium cobaltinitrite rather than by a formula, that each lot of the reagent be tested before use, and that lots be rejected which give recoveries of less than 98 per cent or more than 102 per cent with 20 mg amounts of K_2O.

RECOMMENDATIONS*

It is recommended—

(1) That study of methods for determining fruit and sugar content of frozen fruits be continued.

(2) That further collaborative study be made of the method for the electrometric titration of acidity.

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

(4) That procedures as given by the Associate Referee in his 1948 report, for the rapid determination of water-insoluble solids and for the determination of seeds of berry fruits be adopted as tentative and that the procedures be subjected to collaborative study.

(5) That 26.18(a) be changed to read as follows:

> (a) *Sodium cobaltinitrite soln.*—Prepare an aqueous soln containing 2.0 g of sodium cobaltinitrite in each 10 ml, from a previously tested lot of the reagent giving recovery of not less than 98 per cent and not more than 102 per cent with 20 mg amounts of K_2O. Filter soln before use and prepare fresh soln before each set of determinations.

REPORT ON FRUIT AND SUGAR CONTENT OF FROZEN FRUITS

By R. A. Osborn (Division of Food,† Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

In an article by J. Walter Sale entitled "Interpretation of Chemical Analyses of Preserves and Jams," *This Journal*, **21**, 502 (1938), much authentic information is given regarding the chemical composition of fruits grown in the United States, and the procedure for determining the ratio of fruit to sugar in preserves is described. The amounts of fruit and sugar (or sugars) in frozen fruit mixtures can be calculated using the procedure

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* For report of Subcommittee D and action of the Association, see *This Journal*, 32, 63 (1949).
† W. B. White, Chief.

described by Sale. The problem is somewhat less complicated since the mixtures are not concentrated by the application of heat as is the case with jams, jellies, and fruit butters. It is necessary to obtain a representative sample of frozen fruit "A" and subject it to chemical analysis by the well-known procedures of this Association (Methods of Analysis, *A.O.A.C.* 6th ed., Chapter 26 (1945)) for such constituents as water-insoluble solids, ash, potash, phosphate, and total sugars after inversion; and then by comparison with authentic data (averages of known samples of the fruits analyzed for their content of sugars, water-insoluble solids, ash, potash, etc.) calculate the fruit and sugar contents of the sample "A." This procedure involves much analytical work. If possible, simple and accurate alternate procedures should be developed. In contested court cases it has been suggested that the comparison of the analytical values obtained should be made with the minimum values of the authentic samples rather than the averages. Sale (*loc. cit.*) points out the fallacy of this proposal.

A considerable amount of preliminary work has been carried out in the Food and Drug Administration with frozen fruits, to determine whether the drained weight under carefully controlled conditions, together with the determination of the soluble solids (as sucrose) by refractometer can be employed as a rapid procedure for determining their fruit and sugar content. The results so far obtained are encouraging. However, the problem is not a simple one, since ripeness (softness) of the fruit is a factor which has an effect on the amount of drained fruit left on the screen.

The work on this problem is still in progress and a report at this time would be premature. Accordingly, it is recommended* that the work be continued.

REPORT ON WATER-INSOLUBLE SOLIDS OF FRUITS AND FRUIT PRODUCTS

By R. A. Osborn (Division of Food,† Food and Drug Administration, Federal Security Agency, Washington 25, D. C.), *Associate Referee*

PART I. RAPID METHOD

A former report on this subject[1] deals chiefly with a study of the existing procedure for the determination of water-insoluble solids, as published in *Methods of Analysis, A.O.A.C.*, 6th ed., **26.7** (1945).

A rapid procedure for the determination of water-insoluble solids of fruits and fruit products has now been developed. Speed is obtained by rapid preparation of the sample through (1) the use of a Waring Blendor,

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* For report of Subcommittee D and action of the Association, see *This Journal*, 32, 63 (1949).
† W. B. White, Chief.
[1] Osborn, R. A., *This Journal*, **30**, 260 (1947).

which in two minutes comminutes and intimately mixes samples weighing up to 2 lbs.; (2) by shortening the period of boiling to a few minutes, since the sample is finely divided; (3) by rapid filtration on a loose textured filter paper of greater area (15 cm diameter circle in a 12½ cm diameter Büchner funnel); and (4) by rapid drying of the filter and water insoluble solids (by the use of an instrument which works on the principle of an electric hair dryer in that hot air is forced on the sample and exhausted through a 500-mesh Monel metal filter cloth bottom). Drying can ordinarily be completed in 10–15 minutes, depending on the size of sample taken for analysis and the amount of material to dry. The procedure does not differ materially from the existing longer procedure and results that have been obtained are comparable with it. The rapid procedure should appeal to the ordinary analyst, and it should find application in factories that process fruit products into preserves, fruit butters, fountain fruits, etc., where it is desirable to control the insoluble solids content of the finished products.

WATER-INSOLUBLE SOLIDS (RAPID METHOD)

APPARATUS

Waring Blendor (or other suitable comminuting device).

Balance (sensitive to ±1 milligram) and weights.

1-*liter suction flask* (provision for vacuum).

Büchner funnel (Coors 4 5¼″ diam.).

Filter paper.—15.0 cm fast; open texture (Whatman #4, or equivalent).

Weighing dishes.—Aluminum or tinned iron 5¼″ diam. × ¾″ high, with close-fitting cover (16 mm film holders obtainable from camera stores). (Al. dishes weigh approximately 40 g, tinned iron ca 85–90 g.)

Rapid drying device:

(*a*) Moisture Teller Model 271 T, manufactured by Harry W. Dietert Co., 9330 Roselawn Ave., Detroit 4, Mich., or

(*b*) Forced Draft Drying Oven operating at 100°C.

DETERMINATION

Fit a 15 cm circle of filter paper into a 12½ cm Büchner funnel, add ½ of a 7 cm circle of filter paper (to be used to wipe any insoluble solids from Büchner after filtration and washing the sample), wash with boiling water, apply suction, and dry, using drying device (a) or (b). Transfer to weighing dish, cool and weigh in balance using a tare consisting of weighing dish and paper. (Approximate time of drying, 5 min. at 215°F. ±5°F.

Weigh 25 or 50 g of well mixed sample (Waring Blendor) to nearest .01 g, transfer the sample with hot water to a 400 ml beaker, adjust to approximately 200 ml with hot water, stir, and boil gently for a few minutes. Place prepared filter in Büchner, attach to suction flask but do not attach flask to suction line. Pour 50 to 100 ml of boiling water on filter and when a steady flow of water passes thru filter, transfer the sample to the filter, portionwise if necessary. Wash insoluble solids with boiling water and collect approximately 850 to 900 ml of filtrate. (In the washing operation keep the solids from forming a tight mat on the surface by portionwise additions of the boiling water.) Apply suction after concluding the washing operation and aspirate thoroly. Transfer paper and water-insoluble solids and dry as above, us-

ing extra piece of weighed filter paper to complete the transfer, and dry at 215°F. ±5°F. (approximately 15 min., depending on amount of water-insoluble solids). After drying, transfer sample to weighing dish, cool in desiccator, and weigh. Weight of water-insoluble solids ÷ wt. of sample × 100 = % water-insoluble solids.

Tabulated data (Table 1) contain comparative results for per cent water-insoluble solids for four commercial preserves analyzed by the rapid procedure as given herein and by the regular published procedure. It will be observed that the results are not materially different.

TABLE 1.—*Comparison of results for water-insoluble solids, regular A.O.A.C. procedure*[1] *vs. rapid procedure*
All samples 25 grams

SAMPLE NO.	TYPE OF COMMERCIAL PRESERVE	WATER-INSOLUBLE SOLIDS (PER CENT)			
		RAPID PROCEDURE		REGULAR PROCEDURE	
			Average		*Average*
51591 F	Raspberry	2.48		2.47	
		2.51		2.58	
		2.57			
		2.61	2.54		2.53
62770 F	Peach	0.48		0.49	
		0.50	0.49	0.50	0.49
51494 F	Strawberry	1.20		1.13	
		1.20	1.20	1.14	1.14
52791 F	Cherry	0.56		0.57	
		0.62	0.59	0.59	0.58

[1] *Methods of Analysis, A.O.A.C.*, 6th ed., 26.7 (1945).

PART II. SEED AND NON-SEED WATER-INSOLUBLE COMPONENTS OF BERRY FRUITS

During a study of methods for the determination of water-insoluble solids of fruits and fruit products,[1] a number of samples of commercial berry preserves and a few samples of authentic berry fruits were examined for their content of seeds free from adhering fruit tissue, in addition to the determination of water-insoluble solids. C. L. Hinton and T. Macara in a manuscript entitled "The Composition of Some Jam Fruits and the Determination of the Fruit Content of Jams"[2] have reported their findings of insoluble solids and seed contents of several berry fruits using analytical procedures which differ from those described here. The procedure for seeds which we employ is simple and rapid.

[1] Osborn, R. A., *This Journal*, **31**, 185 (1948).
[2] The Analyst, **65**, 540 (1940).

SEEDS IN BERRY FRUITS

Prepare the sample by thoro mixing, using a Waring Blendor. Take 50 g ±.01 g of the sample, transfer with ca 500 ml of hot water to the mixing chamber of Waring Blendor and mix for 1–2 min. Transfer mixture to a 20-mesh screen and use additional hot water to transfer and wash the bare seeds. (Hot water from the tap is suitable for use in this procedure.) Transfer the seeds on the screen to a 70 mm aluminum dish, previously weighed, with close-fitting cover. (This is readily accomplished by transfer to a 7 cm Whatman #4 circle of filter paper in a Coors 2A Büchner funnel with suction. The paper is previously dried and weighed with the aluminum dish.) Dry at 100°C. in a forced draft oven for 30 min. and weigh. To determine average weight of one seed, count out and weigh separately several 100-unit lots.

TABLE 1.—*Commercial preserves with seeds*

SAMPLE NO.	WATER INSOLUBLE SOLIDS		BARE SEEDS		INSOLUBLE SOLIDS NOT SEEDS BY DIFFERENCE	NON-SEED OF TOTAL	BARE SEEDS OF TOTAL	AVERAGE WEIGHT OF 1 SEED
	per cent		*per cent*		*per cent*	*per cent*	*per cent*	*Mg*
				Blackberry				
54164 F	1.38		0.90					
	1.40	1.39	0.94	0.92	0.47	33.8	66.2	3.49
79220 F	2.44		1.81					
	2.64	2.54	1.83	1.82	0.72	28.2	71.8	2.93
55028 F	2.49		1.75					
	2.65	2.57	1.79	1.77	0.80	31.1	68.9	3.24
60470 E	2.93		2.37					
	2.95	2.94	2.38	2.37	0.57	19.4	80.6	2.82
19412 E	3.52		2.60					
			2.81	2.71	0.81	23.0	77.0	2.04
62772 F	3.52		2.81					
	3.53	3.52	2.88	2.84	0.68	17.9	82.1	1.76
54166 F	3.51		2.83					
	3.80	3.66	2.94	2.89	0.77	21.0	79.0	2.98
Average						24.9	75.1	2.75
				Logan-(Black) berry				
55128 E	2.70		2.20					
	2.70	2.70	2.30	2.25	0.45	16.7	83.3	1.84
63101 E	3.16		2.47					
	3.34	3.25	2.55	2.51	0.74	22.8	77.2	2.20

TABLE 1.—*Continued*

SAMPLE NO.	WATER INSOLUBLE SOLIDS		BARE SEEDS		INSOLUBLE SOLIDS NOT SEEDS BY DIFFERENCE	NON-SEED OF TOTAL	BARE SEEDS OF TOTAL	AVERAGE WEIGHT OF 1 SEED
	per cent		*per cent*		*per cent*	*per cent*	*per cent*	*Mg*
				Red Raspberry				
54158 F	2.02		1.55					
	2.12	2.07	1.58	1.56	0.51	24.6	75.4	1.33
62774 F	2.23		1.90					
	2.38	2.30	2.02	1.96	0.34	14.8	85.2	1.22
54154 F	2.37		1.74					
	2.39	2.38	1.86	1.80	0.58	24.4	75.6	1.14
51589 F	2.42							
	2.49		2.06					
	2.63	2.51	2.13	2.09	0.42	16.7	83.3	1.12
51493 F	2.50							
	2.51		2.29					
	2.56	2.52	2.37	2.33	0.19	7.5	92.5	1.30
55045 F	2.67		2.18					
	2.80	2.74	2.35	2.27	0.47	18.9	81.1	1.16
55026 F	2.75		2.46					
	2.84		2.48					
	2.89	2.83	2.61	2.52	0.31	11.0	89.0	1.14
26054 E	3.80		3.52					
	3.88	3.84	3.58	3.55	0.29	7.6	92.4	1.37
Average						15.6	84.3	1.22
				Black Raspberry				
8286 F	2.77		2.10					
	3.08	2.92	2.16	2.13	0.79	27.1	72.9	1.39
33945 F	5.09							
	5.20		4.72					
	5.38	5.26	4.93					
	5.39		4.99	4.88	0.38	7.2	92.8	1.62
				Strawberry				
62775 F	1.14		0.74					
	1.15	1.14	0.76	0.75	0.39	34.2	65.8	0.53

TABLE 2.—*Water-insoluble solids and seed content of some authentic berry fruits*

SAMPLE NO.	WATER-INSOLUBLE SOLIDS		SEEDS		WATER-INSOLUBLE SOLIDS—NOT SEEDS	TOTAL NON-SEED	WATER-INSOLUBLE SOLIDS DUE TO SEEDS	AVG WT. 1 SEED	NO. SEEDS 100 G FRUIT	SOLUBLE SOLIDS REFRACTOMETER
Subs	*per cent*		*per cent*		*per cent*	*per cent*	*per cent*	*Mg*		*per cent*
A—Pacific Blackberry	5.00		3.64							
	5.38	5.19	3.69	3.66	1.53	29.5	70.5	1.73	2119	13.2
B—Black Raspberry	9.60		8.05							
	9.64	9.62	8.24	8.15	1.47	15.3	84.7	1.31	6221	16.3
C—New Washington	5.00		3.97							
Red Raspberry	5.26		4.09	4.03	1.22	23.2	76.8	1.12	3598	14.5
	5.48	5.25								
D—Youngberry	4.59		3.46							
	4.84	4.71	3.66	3.56	1.15	24.4	75.6	3.08	1156	11.7
E—Cuthbert Red	6.09		4.64							
Raspberry	6.14	6.11	4.78	4.71	1.40	22.9	77.1	1.02	4618	13.1
F—Boysenberry	4.09		3.13							
	4.45	4.27	3.32	3.22	1.05	24.6	75.4	3.35	961	12.0
G—Loganberry	6.35		4.61							
	6.57	6.46	4.71	4.66	1.80	27.9	72.1	1.73	2694	13.3

Report average weight of one seed in milligrams, number of seeds per 100 g of sample and after determination of the water-insoluble solids content of the sample, calculate and report the per cent of the total that is due to bare seeds and the per cent that is due to non-seed water-insoluble solids.

The procedure for seeds is more rapid than that for water-insoluble solids. A 50-gram weight of sample can be analyzed for seeds more readily than a 25-gram weight of the same sample can be analyzed for water insoluble solids since difficulties of filtration and washing are eliminated. It may develop that data which include the percentage of seeds in the sample, average weight of the seed, and number of seeds per 100 grams of sample will be more informative and useful in the interpretation of the analyses of berry fruit products than the mere determination of their water-insoluble solids content. Such a conclusion must await the accumulation of authentic data on which comparisons can be made.

Table 1 contains results obtained from the analysis of a number of samples of commercial preserves, including blackberry, loganberry, red raspberry, black raspberry, and strawberry. No conclusions are to be drawn here in regard to the exact proportions of fruit and sugar employed in the preparation of these samples. Some idea can be obtained as to the agreement between duplicates, the relative amounts of seeds to total water-insoluble solids, and the unit weight of seeds.

Table 2 contains a limited amount of information on the water-insoluble solids and seed content of some authentic berry fruits which were analyzed by these procedures.

No report was given on titration of acids, or on fruit acids.

REPORT ON SUGARS AND SUGAR PRODUCTS

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

RECOMMENDATIONS*

It is recommended—

(1) That the method of the Java Sugar Experiment Station, adopted as a tentative method last year, be made official, with the following changes: "Reagents **(f)**" should read "dilute sulfuric acid. Three volumes of water plus 1 volume of conc. H_2SO_4." Under "Fermentation" the sentence beginning at the end of the fifth line should read: "Place the flask in a water bath kept at 30°C. and allow to ferment for 4 hours or more, shaking the flask from time to time; if desired, an incubator may be used and the flask may be left overnight."

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* For report of Subcommittee D and action of the Association, see *This Journal*, 32, 63 (1949).

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

(3) That the study be continued on tables of density of solutions of sugar at various temperatures.

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

(5) That the study of methods for the detection of adulteration of honey be continued.

(6) That the method for determination of shellac on confectionery, *This Journal*, **31,** 196 (1948), modified as described in this year's report of the Associate Referee, be adopted as official, first action, and that study be continued of methods applicable to confectionery.

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

(8) That tentative methods, **34.133–34.155,** inclusive, be subjected to further study.

(9) That the procedures described in NBS Circular C440, pp. 324–334, for measurement of transmittancy of solutions of commercial sugar products be subjected to collaborative study with a view to their future adoption.

(10) That micro methods for reducing sugars be studied.

(11) That method **34.8** be amended by the following addition: "In liquid products containing invert sugar, correct the per cent solids obtained from 44.7 by adding 0.022 for each per cent of invert sugar present in the sample."

REPORT ON UNFERMENTED REDUCING SUBSTANCES IN MOLASSES

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

At the 1947 meeting of the Association the Java method was adopted as a tentative method for the determination of unfermented reducing substances in molasses.[1] In accordance with the recommendation made, the collaborative work has been repeated this year, by the method as published.[2] Samples of a raw sugar blackstrap and a refiners' blackstrap were sent to nine collaborators who had expressed willingness to carry out the analyses. Reports have been received from all of these. The results are shown in Table 1.

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

[1] *This Journal*, 31, 61 (1948).
[2] *This Journal*, 31, 192 (1948).

TABLE 1.—*Percent unfermented reducing substances, expressed as invert sugar*
In raw sugar blackstrap and refiners' blackstrap

ANALYST	RAW SUGAR BLACKSTRAP	REFINERS' BLACKSTRAP
1. Sam Byall, New Orleans, La.	4.42	3.75
	4.31	3.64
	4.25	3.87
	4.42	3.70
2. J. K. Dale, Terre Haute, Ind.	4.20	3.64
	4.25	3.64
	4.09	3.64
	4.14	3.61
3. Carl Erb (New York yeast), New York, N. Y.	4.40	3.82
	4.46	3.82
4. Same, (New Orleans yeast)	4.32	3.71
	4.26	3.71
5. W. J. Hughes, New York, N. Y.	4.35	3.74
	4.40	3.80
6. W. L. Porter, Philadelphia, Pa.	4.30	3.80
	4.35	3.81
	4.32	3.92
7. F. E. Randall, Buffalo, N. Y.	4.64	4.20
	4.64	4.20
	4.70	4.25
	4.76	4.14
8. D. J. Smith, Boston, Mass.	4.24	3.75
	4.28	3.80
	4.31	3.81
9. W. O. Winkler, Washington, D. C.	4.48	3.92
	4.48	3.93
10. R. T. Wisthoff, Baltimore, Md.	4.47	3.77
	4.47	3.77
	4.42	3.71
	4.36	3.82
	4.42	3.87
	4.36	3.82
Averages		
1	4.35	3.74
2	4.17	3.63
3	4.43	3.82
4	4.29	3.71
5	4.38	3.77
6	4.32	3.84
7	4.68	4.20
8	4.28	3.79
9	4.48	3.93
10	4.42	3.79
Grand Averages	4.38	3.82
Grand Averages, omitting No. 7	4.35	3.78

TABLE 2.—*Deviations of individual averages from grand averages*
Per cent of invert sugar

ANALYST	RAW SUGAR BLACKSTRAP	REFINERS' BLACKSTRAP	NUMERICAL AVERAGE
1	−0.03	−0.08	0.055
2	−0.21	−0.19	0.200
3	+0.05	0.00	0.025
4	−0.09	−0.11	0.100
5	0.00	−0.05	0.025
6	−0.06	+0.02	0.040
7	+0.30	+0.38	0.340
8	−0.10	−0.03	0.065
9	+0.10	+0.11	0.105
10	+0.04	−0.03	0.035
Average deviations	+0.049	+0.051	
	−0.049	−0.049	
	±0.098	±0.100	

The maximum spread between the results of repeated determinations by any of the individual analysts is 0.17 for the raw sugar blackstrap, and 0.23 for the refiners' blackstrap; the maximum differences for the ten sets of analyses average 0.085 and 0.081, respectively.

The results of the collaborators check satisfactorily, except for Analyst No. 7, who had no previous experience with the method. If his results are omitted and the other nine are averaged, the maximum deviation from the grand averages is only 0.18 for the raw sugar blackstrap, and 0.15 for the refiners' blackstrap. The average deviation is only ±0.065 and ±0.054, respectively. This is very good agreement for analyses of this nature, requiring many manipulations.

The results also show that the particular lot of Fleischmann's baker's yeast, produced in different localities, has no noticeable effect. Sam Byall (New Orleans), who in previous years had obtained unusually high results, and D. J. Smith (Boston), whose results had been unusually low, checked well this time with each other and with the averages, on both samples. Carl Erb (New York) ran analyses with yeast secured in New York, and also with yeast sent by Mr. Byall in a well insulated package via air mail from New Orleans. The results were in good agreement with the averages.

W. L. Porter and R. T. Wisthoff have called attention to the indistinctness of the starch end point in the iodine titration, and J. K. Dale had mentioned this point in earlier work. This difficulty, according to Mr. Wisthoff, can be overcome by acidifying with sulfuric acid, diluted with 3 instead of 5 volumes of water. This observation has been confirmed in this laboratory.

W. L. Porter has reported that four hours was insufficient for complete fermentation. The directions adopted last year read: "for at least 4 hours, shaking the flask from time to time. When fermentation is complete, etc." To avoid misunderstanding, this should be changed to read as stated below.

RECOMMENDATIONS*

It is recommended that the method of the Java Sugar Experiment Station, adopted as a tentative method last year, be made official, first action, with the following changes:

REAGENTS, **(f)** should read: "*Dilute sulfuric acid.*—3 volumes of water plus 1 volume of conc. H_2SO_4."

Under FERMENTATION, the sentence beginning at the end of the fifth line should read: "Place the flask in a water bath kept at 30°C. and allow to ferment for 4 hours or more, shaking the flask from time to time; if desired, an incubator may be used, and the flask may be left overnight. When fermentation is complete, etc."

REPORT ON CONFECTIONERY

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

According to the recommendation of last year,[1] some further investigation was made of the lac method with reference to the recovery of relatively large amounts of added shellac. On checking the solubility of the lac in the isoamyl alcohol-benzol mixture, at times on cooling and standing during the water wash, some of the lac settled out. To obviate this difficulty, the method[2] was changed by inserting the words "hot (about 60°)" before "water" in line 18 of the method, changing "reject the wash water" to "filter wash water if necessary" in line 19, and changing "filter" to "filters" in line 22. In line 15 following isoamyl alcohol, "B.P. 129–132" was inserted.

Three samples were prepared for collaborative testing using the same shellac and sugar candy as employed last year.

Sample A—	Sugar	candy	with	0.90%	lac	added.
Sample B—	"	"	"	0.67%	"	"
Sample C—	"	"	"	0.45%	"	"

Results are shown in Table 1.

The results on the higher lac samples now seem fairly good. There will always be some tendency to low results, since drying the shellac for an

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* For report of Subcommittee D and action of the Association, see *This Journal*, 32, 64 (1949).

[1] *This Journal*, 31, 61, 1948.

[2] *This Journal*, 31, 196, 1948.

TABLE 1.—*Collaborative results*

COLLABORATOR	LAC FOUND		
	A	B	C
	per cent	*per cent*	*per cent*
J. L. Hogan	0.89	0.58	0.42
	0.90	0.60	0.44
C. A. Wood	0.90	0.66	0.44
	0.90	0.65	0.44
Average	0.90	0.62	0.435

appreciable time gradually changes it to a benzol alcohol insoluble form.

It is recommended*—

(1) That the method as published in *This Journal*, **31**, 196 (1948) with modifications as indicated above be adopted as tentative.

(2) That further collaborative work be done.

REPORT ON REDUCING SUGARS

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

A collaborative study has been made of Ofner's Method for the determination of small quantities of invert sugar in the presence of sucrose. This method is now a tentative method of the Association of Official Agricultural Chemists.

Four analysts were provided with three different sucrose samples and with levulose and dextrose. Analyses were made in duplicate on the three sucrose samples and determinations were also made on 10, 5, and 1 mg. of invert sugar. The duplicate results obtained by the different collaborators were in good agreement, differing by approximately 0.1 mg. Bearing in mind this consistency within the results of each analyst, the overall results of the collaboration can be considered. The pure invert analyses agreed with the invert present within an average of 0.2 mg. with a maximum error of 0.5 mg. Sucrose samples I and II contained an average of 4.7 and 2.2 mg. of invert in 10 grams of sugar, or .047 and .022 per cent of invert, respectively. The results of the various analysts differed by .005 per cent and .003 per cent invert, respectively, in the two samples. Sucrose sample III was reported to contain 0.1 and 0.2 mg. of invert per 10 g. of

* For report of Subcommittee D and action of the Association see *This Journal*, 32 63 (1949).

sugar by two investigators and 0.8 and 1.0 mg. by the remaining two. There is some question as to the stability of the sample since the first two analysts obtained their results 5 months prior to those reporting the higher percentages of invert. A summary of the collaborative work is given in the following table.

TABLE 1.—*Collaborative results*

	SUCROSE I	SUCROSE II	SUCROSE III	10 MG INVERT	5 MG INVERT	1 MG INVERT
Analyst A	4.85	1.98	0.11	9.96	5.06	0.91
	4.85	2.04	0.18	9.88	5.12	0.88
Analyst B	3.70	2.39	0.23	10.07	5.04	0.97
	3.74	2.12	0.18	10.07	5.14	1.03
Analyst C	5.66	2.65	0.93	10.20	5.52	1.42
	5.49	2.77	1.03	10.34	5.14	1.39
Analyst D	4.76	1.85	.84	9.63	4.99	.96
	4.80	1.89	.84	9.60	4.94	.98
		SUCROSE I		SUCROSE II		SUCROSE III
Average Invert		4.73 mg		2.21 mg		.42 mg
Average Deviation		±.51 mg		±.29 mg		±.32 mg
		Invert				
Average Error		10 mg		5 mg		1 mg
		±.20		±.14		±.14

Ofner's method uses a carbonate copper solution. The reduced copper is not filtered but is determined in the reaction mixture by the addition of iodine and back titration with thiosulfate. The mild action of the carbonate solution compared with that of alkali reagents on sucrose, the fact that the reduced copper can be determined in the reaction mixture, and finally, and of greatest importance, the consistency of results obtained by different analysts are the basis on which it is recommended* that Ofner's method be made an official method.

No reports were given on drying methods, densimetric and refractive methods, honey (free acid and commercial syrup adulterants), corn syrup and corn sugar, color and turbidity in sugar products, or micro sugar methods.

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* For report of Subcommittee D and action of the Association, see *This Journal*, 32, 64 (1949).

REPORT ON FLAVORS AND NON-ALCOHOLIC BEVERAGES

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

No collaborative work has been reported on any of the several projects under this refereeship for this meeting. However, plans have been drawn up which should result in definite progress during the coming year.

Mr. R. D. Stanley, Associate Referee on Organic Solvents in Flavors, has made an informal report to the Referee concerning a considerable amount of work on procedures designed to remove acetone from products containing both acetone and isopropyl alcohol, to make it possible to apply the method given in *Methods of Analysis*, **25.26**, which is applicable only when acetone is absent. So far the work is encouraging but not conclusive and a definite answer to the problem is expected before the next meeting.

No report has as yet been received on Maple Flavor Concentrates.

The Referee is recommending the continuation of all previously recommended projects with the addition of a new item on collaborative study of the tentative procedures for vanilla resins.

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 on 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 Secker-Kirby Method for determination of esters in lemon and orange oils (Dept. of Agri. Bull. 241) be continued.

(6) That collaborative studies of methods for extract 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.

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* For report of Subcommittee D and action of the Association, see *This Journal*, 32, 62 (1949).

(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 the Referee study collaboratively the modification of **25.23** as given in last year's report (*This Journal*, **31**, 202 (1948)).

(13) That the Referee study collaboratively the modification of **25.54** as given in last year's report (*This Journal*, **31**, 203 (1948)).

(14) That the methods for vanilla resins in vanilla extract, **25.15** and **25.16**, be studied collaboratively.

No report was given on beta-ionone, lemon oils and extracts, organic solvents, vanillin, emulsion flavors, maple flavor concentrates, or diacetyl.

REPORT ON FERTILIZERS

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

During the year, one Associate Referee was appointed to study methods of analysis for inert materials in fertilizers. Most of the Associate Referees previously appointed have done some work on problems related to their respective assignments. However, not all of them have reported.

Considerable interest has been shown during the year on problems of sampling and segregation of fertilizer materials. Research has been initiated at the Indiana and Maryland laboratories to determine the correct number of cores to be drawn from a given lot of fertilizer in order that a statistically sound sample can be obtained. It is hoped that the results of these studies will stimulate other States to carry out similar experiments, and that by the end of another year we will have established without question the correct number of bags which an inspector should sample under a given set of circumstances.

RECOMMENDATIONS*

Sampling.—The recommendations of the Associate Referee, which are as follows, are approved:

(1) That study of sampling equipment and method of sampling be continued.

(2) That preparation of sample for analysis be studied.

Phosphoric Acid.—The recommendations of the Associate Referee are approved.

Moisture.—The recommendations of the Associate Referee are approved.

Nitrogen.—The recommendations of the Associate Referee, which are as follows, are approved:

* For report of Subcommittee A and action of the Association, see *This Journal*, **32**, 43 (1949).

(1) That the formaldehyde titration method be adopted as official, first action, for determining nitrogen in ammonium nitrate.

(2) That the study of nitrogen in high nitrate-chloride mixtures be continued.

Potash and Platinum Recovery Methods.—The recommendations of the Associate Referee, which are as follows, are approved:

(1) That a survey be made of the different types of mills being used for preparation of the sample.

(2) That collaborative potash work on samples prepared by the different mills be conducted on a greater variety of samples.

No reports were received on seven subjects:

Magnesium and manganese
Acid- and base-forming quality
Potash and platinum recovery methods
Sulfur
Copper and zinc
Boron
Inert materials

It is recommended* that work of all Associate Referees be continued.

REPORT ON SAMPLING FERTILIZERS

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

This report consists of results of (1) a questionnaire on sampling fertilizers and preparation of samples for analysis; (2) a collaborative study of the effectiveness of 3 kinds of sample containers in preventing change in moisture; (3) a comparison of the slotted single-tube and the slotted double-tube sampler in taking samples; (4) a comparison of preparation of samples for analysis with and without screening the sample through 10-mesh.

QUESTIONNAIRE ON SAMPLING FERTILIZERS

It was felt that work on this subject would be aided by a knowledge of the present methods used in all States of collecting samples and preparing them for analysis. This questionnaire was sent to the fertilizer control officials in each State and in the Dominion of Canada. Replies with questionnaire filled out were received from 41 officials. Five State officials replied but did not fill out the questionnaire, chiefly because only a few samples were taken. Answers were not complete in a few instances.

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* For report of Subcommittee A and action of the Association, see *This Journal*, **32**, 43 (1949).

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

Results of the questionnaire are given in Table 1. They show considerable variation in procedures used in the different States. One of the greatest variations is in the number of bags sampled. A number of States sample a smaller number of bags than is prescribed by the A.O.A.C. procedure.

TABLE 1.—*Questionnaire on sampling fertilizers*

(Numbers in column at right refer to number of States)

I. METHOD OF COLLECTING SAMPLES

1. Type of sampler used;	
A. Use single-tube sampler	20
B. Use double-tube sampler	17
C. Use tube-and-rod sampler	4
2. Number of bags sampled:	
A. Less than 10 bags present:	
All bags sampled	30
6 bags sampled	5
3 or 4 bags sampled	1
1 bag sampled	2
No bags sampled	2
B. From 10 to 100 bags present:	
10 bags sampled	27
10 per cent of bags sampled	3
5 bags sampled	3
4 to 20 bags sampled	2
20 per cent sampled	1
15 bags sampled	1
10 to 20 bags sampled	1
C. More than 100 bags present:	
10 per cent of bags sampled	15
A.O.A.C. procedure* followed	10
10 bags sampled	6
20 bags sampled	3
5 bags sampled	2
20 to 40 bags sampled	1
5 bags plus 1 bag for each ton over 5	1
3. Method used in taking sample:	
A. 1 core from each bag parallel to sides	25
B. 2 or more cores from each bag parallel to sides	4
C. Cores taken diagonally	17
4. Samples sent to laboratory:	
A. Entire sample	17
B. A portion of sample	24
5. Weight of sample sent to laboratory:	
A. Less than 1 pound	4
B. 1 to 2 pounds	27
C. 2 pounds or more	10

* Before changed at last meeting.

6. Container for samples:

A. Type of container in which samples are sent to laboratory:

Glass containers	21
Paper containers†	11
Paste-board or ice-cream containers	7
Cotton bag (paraffined)	2
Metal cans (2 States use more than one type)	2

B. Type of container in which reserve portion is stored:

Glass containers	27
Waxed ice-cream containers	3
Metal can	1
Paper bag with liner	1
Do not save reserve	2
No answer	4

7. Are liquid fertilizers sampled?

Yes	26
No	11

A. Type of sampler used:

Sample small packages and purchase 1 package	18
Sample large containers, using one of following: steel bomb, rubber tube, stainless steel tube, syphon, modified milk sampler, stainless steel dipper	8

II. METHOD OF PREPARING SAMPLES FOR ANALYSIS

1. Mesh of screen through which inspector's sample is passed before it is subdivided for laboratory sample:

A. 2 mm. or 10 mesh	13
B. 20 mesh	2
C. Samples not screened	17

Note—Whole sample ground in 2 States.

2. Weight of sample prepared for laboratory:

A. Less than $\frac{1}{4}$ pound	10
B. $\frac{1}{4}$ to $\frac{1}{2}$ pound	20
C. $\frac{1}{2}$ to 1 pound	8

3. Size of screen through which ground sample is passed:

A. 1 mm. circular opening	14
B. 20 mesh	7
C. 0.5 mm. circular opening or finer than 20-mesh	13

4. Method of grinding samples:

A. Mortar and pestle used	15
B. Mill used	23

NOTE 1. 8 using mill also use mortar and pestle when necessary.
NOTE 2. 6 use mortar and pestle who would use mill if a satisfactory one is developed.
NOTE 3. 10 Mikro-samplmills are in use.

† Some States use inner liner.

A number of States send the whole sample collected by the inspector to the laboratory. When a large stock of fertilizer is sampled, 30 or more sample cores are required and a larger container is needed if the whole sample is sent in. It seems essential to develop a suitable container for such samples. The container should be one which would keep the moisture content of the sample unchanged. A container which is easily transported to place of sampling is desirable. A number of States now use ordinary paper bags, some of which have an inner liner.

The questionnaire results show that about 8 States sample liquid fertilizers in larger than package quantity, using a variety of sampler types. A number of States do not screen the inspector's sample through 10-mesh as prescribed by the A.O.A.C., before grinding the portion for the laboratory. Three or more States grind the whole sample, in which case the above provision is unnecessary.

While a number of States still use mortar and pestle for grinding samples the use of mills seems to be increasing. A number now using mortar and pestle stated that they would use a mill when a satisfactory one is developed. Ten or more States now use the new "Mikro-samplmill."

Comments of Officials.—Twenty officials were satisfied with the present procedures for taking samples and preparing same for analysis, and nine were not satisfied. Many offered suggestions for study. Among them, six thought study should be made to determine the least number of bags necessary to give a representative sample, three listed need of suitable container in which to ship sample to the laboratory, three listed sampling and preparation of sample in general, one thought a suitable sample divider for use in the field would be desirable, and one suggested a study of mixing and quartering sample in the field versus sending in the whole sample.

COLLABORATIVE STUDY OF SAMPLE CONTAINERS

Through the assistance of Dr. S. F. Thornton, the Bemis Brothers Bag Company developed a double-wall asphalt-impregnated sample bag made of heavy paper. This bag measures 4 by 6 inches on the bottom and it is about $12\frac{1}{2}$ inches tall. It will hold 30 to 35 average sample cores.

Collaborative work was conducted on moisture change in samples stored in a quart fruit jar, in the Bemis bag, and in an ordinary nail sack in a moist and in a dry atmosphere, under partially controlled humidity conditions.

The collaborators were:

(1) Leo Fanuef and Kenneth Helrich, Agricultural Experiment Station, Rutgers, N. J.
(2) J. W. MacKay, North American Cyanamid, Ltd., Niagara Falls, Canada.
(3) Philip McG. Shuey, Shuey & Co., Savannah, Ga.
(4) C. T. McCloud, F. S. Royster Guano Co., Norfolk, Va.
(5) H. R. Allen, Agricultural Experiment Station, Lexington, Ky.
(6) H. K. White, Purdue Agricultural Experiment Station, Lafayette, Indiana.

DIRECTIONS FOR COLLABORATIVE STUDY

Procure at least 3 quarts of a mixed fertilizer, preferably one containing 5 or 6 per cent nitrogen. The mixture may be sampled in the regular way or it may be composed of a composite of your reserve samples. Grind whole amount (not too fine, preferably to pass a 1 mm sieve). Mix well, spread out, and divide whole amount into 3 portions. Take a representative sample for moisture determination from each portion and place each in a separate airtight sample bottle. It is best to mix each portion again before taking this sample.

Place 1 portion (about 1 quart) in a Mason jar with zinc top and rubber gasket, and screw lid tight. Place equal portions in the Bemis bag (No. 2) and in the nail sack (No. 3). Fold the top of each bag twice and use paper clips (5 or 6) to keep tight. The side edges may be sealed with adhesive tape.

Place the 3 containers under a bell jar on a glass plate bottom. Put a 250 ml beaker of water at room temp. and a relative-humidity indicator and thermometer under the bell jar. Let sample stay under the bell jar for 4 days. The humidity indicator can be dispensed with if not available. If used, take humidity and temperature readings at beginning and end of each test.

Take out samples, mix quickly on oilcloth, and take a portion of each for moisture determination as before. Put samples back into their respective containers, seal as before, and place in a warm, dry atmosphere for 3 days (about 2 feet from the window getting direct sunlight for part of the day is satisfactory). A source of heat such as a small hot-plate may be used near the samples for short periods to dry the air if it is too moist. Place the humidity-indicator near the samples and take readings. If samples are warm at end of test, remove to a cooler place for an hour, then mix and take portions for moisture determinations as before.

Make all moisture determinations for each group as soon as possible after placing portions in sample bottle. Use a 2-gram sample and place it in an air oven for 3 hours at 98° to 100°C. Use dishes equipped with covers while cooling in desiccator, preferably the aluminum dish (Fisher-E. & A. catalog No. 8-722). Report as per cent moisture.

Please report any necessary variations from these directions. If bell jar is not available, use some substitute, such as a cardboard box.

If time permits, report moisture tests on a sample of commercial ammonium nitrate, except do not grind whole portion. Grind small amounts quickly for moisture determinations.

Results of collaborative study are given in Table 2. Since each collaborator used his own samples, the results of each must be studied as a unit. Some of the divergent results are probably due to changes in moisture during mixing to obtain portion for moisture determination. To reduce such change, it was specified that all samples be ground previous to the tests. Collaborators 2, 5, and 6 reported very little change in moisture of the samples stored in glass or in the Bemis bag, either in the moist or dry atmosphere. The same collaborators found that the sample stored in the nail sack gained from 0.68 to 4.50 per cent moisture in the moist atmosphere and lost 1.25 to 4.88 per cent moisture in the dry atmosphere.

Collaborators 1 and 3 found the sample in the nail sack lost appreciable moisture in the dry atmosphere but did not gain it in the moist atmosphere. Collaborator 4 found the sample in the nail sack gained 2.25 per cent moisture in the moist atmosphere but that the moisture remained the same in the dry atmosphere.

TABLE 2.—*Collaborators results in percent moisture on moisture tests with different containers*

COLLABORATOR NUMBER	CONTAINER	BEFORE TEST	MOIST ATMOSPHERE	CHANGE	DRY ATMOSPHERE	CHANGE
			Sample 1, 7-7-7 Grade			
1	Quart jar	5.72	5.62	−0.10	5.56	−0.06
	Bemis bag	5.65	5.64	−0.01	5.45	−0.19
	Nail sack	5.38	5.39	+0.01	5.07	−0.32
			Sample 2, Same			
	Quart jar	5.55	5.76	+0.21	5.54	−0.22
	Bemis bag	5.64	5.69	+0.05	5.54	−0.15
	Nail sack	5.52	5.43	−0.09	4.76	−0.67
			Sample 1, Mixed Fertilizer			
	Quart jar	4.98	4.98	0.00	4.97	−0.01
	Bemis bag	4.98	4.99	+0.01	4.97	−0.02
	Nail sack	4.99	5.67	+0.68	2.64	−3.13
2			*Sample 2, Aeroprills*			
	Quart jar	0.20	0.20	0.00	0.19	−0.01
	Bemis bag	0.20	0.22	+0.02	0.21	−0.01
	Nail sack	0.19	1.49	+1.30	0.24	−1.25
			Sample 1, Mixed Fertilizer			
3	Quart jar	7.94	7.50	−0.44	7.27	+0.23
	Bemis bag	7.94	7.61	−0.33	7.59	−0.02
	Nail sack	7.94	7.97	+0.03	6.89	−1.08
			Sample 1, 5-10-5 Grade			
4	Quart jar	5.95	5.90	−0.05	5.85	−0.05
	Bemis bag	5.90	6.20	+0.30	6.15	−0.05
	Nail sack	5.85	8.10	+2.25	8.05	−0.05
			Sample 1, 6-8-6 Grade			
5	Quart jar	3.75	3.67	−0.08	3.55	−0.12
	Bemis bag	3.75	3.78	+0.03	3.52	−0.26
	Nail sack	3.82	4.80	+0.98	2.54	−2.26

COLLABORATOR NUMBER*	CONTAINER	SAMPLE NUMBER	MOIST ATMOSPHERE PER CENT CHANGE	DRY ATMOSPHERE PER CENT CHANGE
	Quart jar	1	0.00	0.00
	Quart jar	2	0.00	0.00
	Quart jar	3	0.00	0.00
	Bemis bag	1	+0.37	−0.21
6	Bemis bag	2	0.00	−0.16
	Bemis bag	3	+0.29	−0.48
	Nail sack	1	+4.50	−3.86
	Nail sack	2	+4.07	−4.88
	Nail sack	3	+1.60	−4.70

* Collaborator 6 weighed the whole sample and container in each case and compared per cent change from the gain or loss in weight. Sample 1 was 8-8-8 grade, Sample 2 was 4-12-4, and Sample 3 was 5-10-5.

Collaborator 6 could not obtain uniform results by following the directions. He attributed this to loss or gain while taking sample for moisture determination. He modified the procedure as follows: Samples were placed in the containers as directed, and the whole sample and container weighed in each instance. Then containers and samples were placed in desiccators, connected to a pump forcing air of 100 per cent relative humidity through the desiccators in the moist atmosphere test, and air of 0 to 8 per cent humidity in the dry atmosphere test. Gain or loss of weight of each container plus sample was recorded. Reported weights were corrected for gain or loss of moisture due to the containers.

Humidity readings were taken by only 2 collaborators, so they have been omitted. The results show that the Bemis bag is almost as effective in moisture resistance as the glass fruit jar sealed with the rubber gasket, and that it is greatly superior to the nail sack for this purpose. It is believed that folding the top of the bag twice and use of ordinary letter clips or a stapling machine makes a satisfactory closure.

COMPARISON OF SINGLE-TUBE AND DOUBLE-TUBE SAMPLERS

Previous study[1] showed little difference in samples obtained with these two types of samplers when the fertilizers were ordinary mixed goods. It was suggested that the samplers be compared under more extreme conditions.

A wood box, open at the top, was built. The inside dimensions were: 23 inches long, 5 inches wide, and 5 inches deep. Two holes side by side were bored in each end and in a partition placed $1\frac{1}{2}$ inches from the front end. The holes were lined up so the inserted samplers were parallel to sides of the box. Holes in the inner partition served as a guide for the samplers. Paper was glued on the holes before placing sample in the box to prevent loss of sample. In sampling, samplers were pushed through the rear holes so that only the slot of each sampler was within the box.

Materials used were ammonium sulfate, superphosphate, and muriate of potash. Materials were placed in the box in 8 blocks of 895 grams each. Four of the blocks were superphosphate, 2 were ammonium sulfate, and 2 were muriate of potash. The order of the blocks from front to rear were superphosphate, ammonium sulfate, superphosphate, muriate of potash, ammonium sulfate, superphosphate, muriate of potash, superphosphate.

The 2 samplers were the ones described in a previous paper.[1] The samplers were inserted at the same time by 2 operators, the single-tube sampler with the slot down and the double-tube sampler with the slot closed. The single-tube sampler was turned over, the double-tube sampler was opened and the sample was taken. The slot was closed and both samples were removed. Cores of samples from this sampling were placed

[1] Allen, H. R., *This Journal*, 31, 205–209 (1948).

in sample bottles and the operation repeated to obtain a second and third set of cores.

The sample materials in the same proportion were thoroughly mixed, the mixture was placed in the box and 3 sets of cores taken as before. Samples were ground and analyzed for nitrogen, total phosphoric acid, and potash. Results are given in Table 3.

TABLE 3.—*Comparison of results on samples obtained with slotted single-tube and slotted double-tube samplers. Materials sampled in blocks (unmixed) and in mixed form*

Results in percent

TYPE OF SAMPLE	SAMPLER	CORE NUMBER	NITROGEN	TOTAL PHOSPHORIC ACID	POTASH
Block*	single-tube	1	2.09	10.50	24.58
Block	double-tube	1	2.40	10.65	22.94
Block	single-tube	2	1.86	11.90	20.78
Block	double-tube	2	1.55	11.50	22.78
Block	single-tube	3	3.96	12.45	14.86
Block	double-tube	3	3.63	11.10	18.22
Mixture	single-tube	1	5.40	9.75	16.88
Mixture	double-tube	1	5.38	9.90	16.86
Mixture	single-tube	2	5.36	9.95	16.90
Mixture	double-tube	2	5.39	9.90	16.90
Mixture	single-tube	3	5.43	9.80	16.92
Mixture	double-tube	3	5.39	9.80	16.88

* Blocks were composed of 895 g each in this order from front to rear: superphosphate, ammonium sulfate, superphosphate, muriate of potash, ammonium sulfate, superphosphate, muriate of potash, superphosphate.

Results on materials in blocks would not be the theoretical percentages because the same weights were used in each block and, since the materials differed in density, they did not occupy equal spaces. Equal volumes of the materials in the order ammonium sulfate, superphosphate, and muriate of potash were in the ratio 4.5:5.3:7.5. This does not seem sufficient to cause the large difference in results obtained from this sampling. Results for the mixed samples were markedly uniform. The experiment did not indicate that either sampler was more accurate than the other. It did indicate in uniform mixtures there is practically no difference in analyses of samples taken with the 2 types of samplers and that insertion of the samplers several times in the same space does not change concentration of nutrients in that space. It is believed experiments of this type might be continued to advantage.

COMPARISON OF PREPARATION OF SAMPLES FOR ANALYSIS, WITH AND WITHOUT SCREENING WHOLE SAMPLE THROUGH 10-MESH

Replies to the questionnaire show many laboratories do not screen the whole sample through 10-mesh before grinding the portion for analysis. In this laboratory previous practice has been to grind the whole sample (1 quart), in which case screening is not necessary. Recently one of the new Mikro-samplmills was installed and, while it will grind a quart or more of sample, this is not very practical, especially for samples with excessive moisture. The following procedure was tried in order to check accuracy of results obtained when the whole sample was not screened through 10-mesh.

All samples were well mixed and large lumps were broken up with spatula. One-half pint of each sample (one-fourth of whole sample) was ground in the Mikro-samplmill and usual analyses made. On each sample showing a deficiency of 0.50 per cent in any guarantee, all the reserve portion was screened through 10-mesh, and the coarse portion was ground as before. Comparison of results from the two procedures is given in Table 4.

TABLE 4.—*Comparison of analyses of samples with and without screening through 10-mesh**

DIFFERENCE IN PER CENT IN CHECK ANALYSES	NUMBER OF CHECK ANALYSES ON—		
	NITROGEN	AVAILABLE PHOSPHORIC ACID	POTASH
0–0.05	12	9	17
0.60–0.10	5	3	5
0.11–0.15	3	4	6
0.16–0.20†	0	6	6
>0.20	0	4	1
Totals	20	26	35

* Original analyses: One-fourth of quart sample ground in Mikro-samplmill without screening. Check analyses: Remainder of quart sample screened through 10-mesh, coarse part ground in mill, and one-fourth quart of mixture ground.

† 94 per cent of checks agree within 0.20 per cent.

The results show that 63 per cent of the checks agree within 0.10 and 94 per cent agree within 0.20. In most cases only one analysis was made on each portion of the sample and some of the differences could be due to slight analytical errors. Duplicate analyses on each portion might reduce the above differences.

Sampling is time-consuming and elimination of the screenings process reduces sampling time by one-half or more. It is assumed that even better checks would be obtained if one-half the sample is ground. This laboratory

has found the Mikro-samplmill very satisfactory. A pint sample can be ground in about 1 minute (exclusive of cleaning mill). The mill is easier to clean than any others used. All mixed fertilizers and superphosphates for the 1948 season to date have been ground in this mill.

ACKNOWLEDGMENT

The phosphoric acid analyses shown in Table 3 were made by Lelah Gault and the potash analyses in Table 4 were made by members of the laboratory staff of this Station.

SUMMARY

Results of a questionnaire on sampling and preparation of sample for analysis are tabulated.

Number of bags sampled and preparation of sample for analysis varied most in the different States. Replies indicate much interest in investigation of various parts of the procedures.

Collaborative study on sample containers in which samples are sent to the laboratory shows the experimental Bemis bag is about as effective as glass in keeping moisture of the sample unchanged.

Comparison of the single-tube and double-tube sampler, using a specially built sample box, did not show that either type sampler was more accurate than the other.

Comparison of preparation of samples for analysis with and without screening whole sample through 10-mesh showed that 80 per cent of analyses using the two procedures agree within 0.15 and that 94 per cent agree within 0.20.

Much time is saved when the screening process is omitted.

RECOMMENDATIONS*

It is recommended—

(1) That study of sampling equipment and method of sampling be continued.

(2) That preparation of sample for analysis be studied.

* For the report of Subcommittee A and action by the Association, see *This Journal*, 32, 43 (1949).

REPORT ON PHOSPHORIC ACID IN FERTILIZERS: COMPARISON OF NEUTRAL AMMONIUM CITRATE AND TWO PER CENT CITRIC ACID SOLUTIONS AS SOLVENTS FOR BASIC SLAG

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In 1886 Paul Wagner (40) proposed the use of an acid ammonium citrate solution for the determination of available P_2O_5 in superphosphate and precipitated phosphate. For the evaluation of the P_2O_5 content of basic slag he (41) recommended in 1894 the use of a solution containing 60 grams of crystallized citric acid and 11.17 grams of ammonia per liter, the composition of which corresponds to 14 grams of free citric acid per liter. In 1896 Gerlach and Passon (9) reported that in tests on 84 samples of basic slag the same results for soluble P_2O_5 were usually obtained by the use of a solution containing no ammonia and only 14 grams of citric acid as by the use of Wagner's acid ammonium citrate. They further reported that a solution containing 46 grams of citric acid and 11.17 grams of ammonia per liter (corresponding to Wagner's solution without the free citric acid) gave very much lower results than did either Wagner's acid ammonium citrate or a 1.4 per cent solution of free citric acid. Gerlach and Passon concluded that free citric acid and not ammonium citrate is the active solvent for slag P_2O_5, a conclusion which is not supported by the work of Jacob, Rader, and Tremearne (24) 40 years later. On the basis of a large number of plant-growth tests, Wagner (42) recommended, in 1899, the use of a 2 per cent citric acid solution for the evaluation of basic slag, and a detailed discussion of the method was given in a later publication (43).

Beginning in 1896 with Huston's and Jones' (21) paper on the action of ammonium citrate and citric acid on basic slag, this Association's work on slag prior to 1911 has been summarized by Haskins and Patten (16). On the basis of further extensive laboratory, pot, and field experiments (15, 16, 32, 45), Wagner's 2 per cent citric acid method for the evaluation of this material was officially adopted in 1922 (12, 14, 34). The plant-growth experiments were made under the direction of the Committee on Vegetation Tests on the Availability of Phosphoric Acid in Basic Slag. The concluding paragraph of the final report (15) of the Committee is as follows:

"The results obtained by the experiment have established the fact that all four slags contained their phosphoric acid in forms freely available to the crops grown, comparing favorably, both in yield of crop and in phosphoric acid recovered, with results obtained with acid phosphate. Moreover, the availability figures established by the

vegetation pot work compare favorably with the available phosphoric acid as measured by the Wagner method for Thomas slag phosphate and the official neutral citrate of ammonia method for acid phosphate or superphosphate."

Although the Association's work that finally led to official adoption of the Wagner method was done on Thomas-Bessemer slag the procedure has subsequently been used also for the evaluation of open-hearth and other types of slag.

For some 35 years after the publication of the paper by Gerlach and Passon (9) it seems to have been the general opinion that, as compared with 2 per cent citric acid, ammonium citrate solutions are not satisfactory solvents for the P_2O_5 of basic slags (11, 39, 44). More recently, however, it has been shown that the solubility of the P_2O_5 in neutral ammonium citrate solution depends to a marked extent on the ratio of sample weight to solvent volume (23, 24, 37). Thus, the citric acid solubility is usually much higher than the neutral citrate solubility when the latter is determined on the basis of 2 grams of sample per 100 ml. of solvent in accordance with the former official method for citrate-insoluble P_2O_5 (*Methods of Analysis*, 1930, pp. 17–18), whereas there is evidence (17, 18, 23, 24, 25, 36, 37) that the difference is small when the weight of sample per 100 ml. of citrate solution is reduced to 1 gram, as directed in the present procedure (*Methods of Analysis*, 1945, pp. 23–24).

In view of the indicated close agreement between the solubility values obtained by the 2 per cent citric acid method and the present official citrate procedure the Referee on Fertilizers in his report to the 1946 meeting of the Association (8) recommended "that a study be made of the applicability of the ammonium citrate method to basic slag, with the object of adopting it in place of the citric acid method if such change is found to be desirable." 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 1, which also shows the total P_2O_5 and the fluorine content of the materials. Table 2 gives the mechanical composition of 11 of the 14 samples as received from the manufacturers and distributors. For use in the investigation all the samples were ground to pass a 100-mesh sieve. With one exception (Collaborator 19) complete sets of the slags were not issued to the collaborators because of the large number of samples and the short supply of some of them.

Open-Hearth Slag, with Fluorspar.—Samples 1 and 2 are from materials made at two plants in the United Kingdom in late 1947 or early 1948. Sample No. 3 is a tapping slag produced at Sydney, Nova Scotia, in or about 1930.

TABLE 1.—*Samples for collaborative study of solubility of basic slag phosphorus in neutral ammonium citrate and 2 per cent citric acid solutions*

SAMPLE	TYPE AND SOURCE OF SLAG	TOTAL P_2O_5		FLUORINE[b]
		RANGE[a]	AVERAGE	
		per cent	*per cent*	*per cent*
	Open hearth, with fluorspar:			
1	United Kingdom	8.78–10.37	9.40	1.00
2	United Kingdom	11.00–11.82	11.33	0.58
3	Canada	10.67–11.30	11.00	1.50
	Open hearth, without fluorspar:			
4	United Kingdom	7.15– 7.96	7.62	0.08
5	United Kingdom	10.64–11.12	10.91	0.27
6	United Kingdom	10.78–11.88	11.44	0.11
7	United Kingdom	12.97–14.71	14.11	0.11
8	United Kingdom	14.60–15.72	15.11	0.09
	Open hearth:			
9	Alabama	8.09– 8.73	8.45	0.37
10	Alabama	8.20– 8.98	8.64	0.33
11	Alabama	11.48–12.09	11.86	0.14
	Bessemer:			
12	Europe	15.52–16.67	16.17	0.05
13	United Kingdom	16.18–17.07	16.60	0.09
14	Europe	17.67–18.25	17.98	0.10

[a] Averages of replicate determinations on each sample by 11 collaborators.
[b] The analyses were made by E. J. Fox of this Bureau.

TABLE 2.—*Mechanical composition of slag samples as received from the manufacturers and distributors*

SAMPLE	COMPOSITION, MESH[a]						
	+35	−35, +60	−60, +80	−80, +100	−100, +150	−150, +200	−200
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	4.6	9.8	8.6	8.4	9.8	13.6	45.2
2	5.2	2.6	6.0	6.8	10.8	14.8	53.8
4	6.0	3.6	4.0	4.6	9.0	17.0	55.8
5	1.2	3.8	4.4	4.4	7.6	14.6	64.0
6	1.2	3.8	5.4	5.4	9.2	14.6	60.8
7	2.8	6.2	6.6	6.6	9.6	13.8	54.4
8	2.4	6.4	6.4	6.2	8.6	13.6	56.4
10	0.2	1.4	1.4	3.0	7.0	14.0	73.0
11	13.8	7.2	4.4	4.6	6.6	10.6	52.8
13	0.6	3.8	4.4	4.4	6.8	11.0	69.0
14	3.0	1.2	1.4	2.5	5.2	11.6	75.1

[a] Screen openings in sieve series were 420, 250, 177, 149, 104, and 74 microns, respectively.

Open-Hearth Slag, without Fluorspar.—These samples (Nos. 4–8), from three plants in the United Kingdom, are from materials produced in late 1947 or early 1948.

Open-Hearth Slag from Alabama.—Commercial production of basic slag in the United States is confined to the Birmingham, Alabama, area. Samples 9 and 10 are from materials made prior to 1942, while Sample 11 is from slag produced in early 1948.

Bessemer Slag.—Samples 12 and 14 are materials manufactured in Europe prior to 1940. Sample 13 was produced in the United Kingdom in late 1947 or early 1948.

COLLABORATORS' DIRECTIONS FOR ANALYSIS

1. Determine total P_2O_5 by the volumetric method as directed in *Methods of Analysis, A.O.A.C.*, 1945, p. 23, sec. **2.12(a)** or **(b)**. Prepare the solution as directed on pp. 21–22, sec. **2.8(b)**.

2. Determine citrate-insoluble P_2O_5 as directed on p. 24, sec. **2.16(b)**. Dissolve the citrate-insoluble residue as directed in sec. **2.8(b)** and determine P_2O_5 as directed in sec. **2.12(a)** or **(b)**.

3. Repeat the determinations of citrate-insoluble P_2O_5 as follows: Proceed as directed in sec. **2.16(b)** through the point where the flask is first shaken vigorously to reduce the filter paper to a pulp. Next place the tightly stoppered flask in a continuous agitation apparatus provided with means for maintaining the contents of the flask at 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. Only those collaborators having access to continuous agitation, constant temperature devices are requested to determine citrate-insoluble P_2O_5 by the procedure outlined in this paragraph.

4. Prepare citric acid extracts of the samples as directed on p. 25, sec. **2.18**, using one of the following modifications depending on the type of agitation apparatus available, and determine P_2O_5 as directed in sec. **2.12(a)** or **(b)**. It is important that the initial temperature of the citric acid solution be adjusted to 17.5°C.; that caking of the sample during addition of the citric acid solution be avoided; that the citric acid extract be filtered on a dry paper immediately after the digestion is completed; and that the clear extract be analyzed for P_2O_5 at once.

Modification I. Make the citric acid extraction with the aid of an end-over-end agitation apparatus (20–50 r.p.m.) and a wide-mouth, 250 ml. volumetric "fertilizer" flask, using a 2.5-gram sample, 2.5 ml. of alcohol, and sufficient citric acid solution to give a total flask content of 250 ml. As the 500-ml. Wagner flask specified in the official method is not commonly available in the fertilizer laboratories of the United States, this deviation from the official procedure is permissible because it has been shown (30) that with a constant ratio of weight of sample to volume of citric acid solution the results for citric acid-soluble P_2O_5 are not affected by variations in the sample weight. It has also been shown (28) that with end-over-end agitation in the range of 21–52 r.p.m. the results are not dependent on the speed of rotation of the flask.

Modification II. If an end-over-end agitation apparatus is not available, make the citric acid digestions (a) with the aid of continuous stirring or (b) with the use of a shaking apparatus such as the Ross-Kershaw machine or the Fisher "Gyrosolver." In such cases, add 1 ml. of alcohol and 99 ml. of citric acid solution to 1 gram of the sample in a 250-ml. beaker for (a) or a 250-ml. "fertilizer" flask for (b).

5. Make all the determinations in triplicate, each on a separate portion of the sample, and report the individual results on the form enclosed with these Directions. If for any reason it is necessary to repeat a determination the repetition should be made in triplicate and the three results reported should be those obtained in simultaneous replications.

6. Your comments and observations concerning this investigation are requested.

COLLABORATORS

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PROCEDURES

For comparison with results by manual agitation at 5-minute intervals, eight of the collaborators determined citrate-insoluble P_2O_5 with the aid of continuous agitation, constant-temperature devices. For this purpose Collaborators 1, 10, 13, 15, 18, and 19 used the end-over-end rotation (20–22 r.p.m.) apparatus described by MacIntire, Marshall, and Meyer (29). Collaborator 3 used continuous stirring at 1,000 r.p.m., while Collaborator 11 employed an apparatus that moved the flask in a straight line in the horizontal plane at 70 oscillations per minute.

With the exception of Collaborator 18 who omitted this analysis, the determinations of citric acid-soluble P_2O_5 were made with the aid of

several types of continuous agitation devices as listed in Table 3, which also shows the weight of sample used in the extractions.

RESULTS OF ANALYSES

The results for citrate-insoluble P_2O_5 as determined respectively with the aid of continuous agitation and of manual shaking at 5-minute inter-

TABLE 3.—*Type of continuous agitation apparatus and weight of sample used in determining citric acid-soluble P_2O_5*

COLLABORATOR	APPARATUS		WEIGHT OF SAMPLE[a]
	TYPE	REVOLUTIONS OF OSCILLATIONS PER MINUTE	
			grams
1	End-over-end[b]	22	2.5
10	End-over-end[b]	21	2.5
13	End-over-end[b]	21	1.0
15	End-over-end[b]	22	2.5
19	End-over-end[b]	22	2.5
2	End-over-end	32	5.0
7	End-over-end	30	2.0
11	End-over-end	18	1.0
14	End-over-end	20	2.5
21	End-over-end	30	5.0
3	Stirring[c]	300	1.0
4	Stirring[d]	670	1.0
6	Stirring	650	1.0
8	Stirring[e]	250	1.0
17	Stirring	275	1.0
20	Stirring	360	1.0
9	Ross-Kershaw shaker[f,g]	120	1.0
12	Ross-Kershaw shaker[f]	180	1.0
16	Fisher "Gyrosolver"[h]	200	1.0
5	Kahn-type shaker[i]	165	2.5

[a] In all cases the ratio of sample weight to solvent volume was 1 gram per 100 ml.
[b] MacIntire-Marshall-Meyer type (29); Catalog No. 5960, Precision Scientific Co., Chicago, Ill.
[c] To prevent caking, the contents of the beaker were stirred by hand preliminary to the continuous agitation.
[d] Stirred with rod, 6 mm diameter with a right-angle bend 22–23 mm long, reaching close to bottom of beaker. The contents of the beaker were stirred by hand while adding the citric acid solution to the sample, and the beaker was turned in a 90° arc at 5-minute intervals during the continuous agitation period.
[e] Stirred with straight rod revolving in a circle of 13 mm radius.
[f] Eccentric rotary movement in the horizontal plane (38); Catalog No. 30873, Eimer & Amend, New York, N. Y., 1936.
[g] Flask was shaken by hand at 10-minute intervals during the continuous agitation period.
[h] Movement in 3 planes; Catalog No. 14-258, Fisher Scientific Co., Pittsburgh, Pa., 1942.
[i] Straight-line oscillation in the horizontal plane; Catalog No. 8926, Arthur H. Thomas Co., Philadelphia, Pa., 1931. Flask was shaken by hand at 5-minute intervals during the continuous agitation period.

vals during the citrate digestion are shown in Table 4. Table 5 summarizes the results for citric acid-insoluble P_2O_5 and citrate-insoluble P_2O_5, the latter determined with manual shaking at 5-minute intervals

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

COLLABORATOR	CITRATE-INSOLUBLE P_2O_5 BY—				DIFFERENCE IN AVERAGE RESULTS[c]
	INTERMITTENT AGITATION[a]		CONTINUOUS AGITATION[b]		
	RANGE	AVERAGE	RANGE	AVERAGE	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
			Sample 1		
1	6.80–6.85	6.82	6.55–6.65	6.60	0.22
15	5.73–6.28	6.04	6.03–6.05	6.04	0.00
19	6.92–6.99	6.96	6.48–6.61	6.55	0.41
Group	5.73–6.99	6.61	6.03–6.65	6.40	0.21**
			Sample 2		
3	8.12–8.16	8.15	8.00–8.16	8.09	0.06
10	7.92–7.98	7.95	7.86–7.98	7.92	0.03
11	7.60–7.68	7.63	7.68–7.82	7.77	−0.14
13	7.70–7.80	7.77	7.60–7.75	7.68	0.09
18	7.86–7.96	7.91	7.88–8.05	7.94	−0.03
19	8.11–8.31	8.20	8.07–8.10	8.09	0.11
Group	7.60–8.31	7.94	7.60–8.16	7.92	0.02
			Sample 3		
15	8.55–8.70	8.62	9.10–9.10	9.10	−0.48
19	9.11–9.39	9.23	8.71–9.00	8.86	0.37
Group	8.55–9.39	8.92	8.71–9.10	8.98	−0.06
			Sample 4		
3	0.68–0.72	0.71	0.68–0.72	0.71	0.00
10	0.67–0.79	0.75	0.83–0.94	0.90	−0.15
11	0.88–0.92	0.89	0.58–0.78	0.71	0.18
13	0.90–0.95	0.93	0.85–0.90	0.88	0.05
18	0.68–0.73	0.70	0.73–0.77	0.75	−0.05
19	0.81–0.81	0.81	0.78–0.82	0.80	0.01
Group	0.67–0.95	0.80	0.58–0.94	0.79	0.01
			Sample 5		
15	0.58–0.67	0.63	0.78–0.82	0.80	−0.17
19	1.31–1.40	1.35	1.19–1.23	1.21	0.14
Group	0.58–1.40	0.99	0.78–1.23	1.00	−0.01
			Sample 6		
1	1.30–1.30	1.30	1.15–1.20	1.17	0.13
15	0.72–0.73	0.73	0.57–0.62	0.59	0.14
19	0.97–1.16	1.05	1.12–1.16	1.13	−0.08
Group	0.72–1.30	1.03	0.57–1.20	0.96	0.07*

TABLE 4—(*continued*)

COLLABORATOR	CITRATE-INSOLUBLE P_2O_5 BY—				DIFFERENCE IN AVERAGE RESULTS[c]
	INTERMITTENT AGITATION[a]		CONTINUOUS AGITATION[b]		
	RANGE	AVERAGE	RANGE	AVERAGE	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
			Sample 7		
1	1.45–1.50	1.48	1.25–1.25	1.25	0.23
15	1.15–1.16	1.16	0.68–0.75	0.71	0.45
19	1.44–1.48	1.46	1.12–1.27	1.17	0.29
Group	1.15–1.50	1.36	0.68–1.27	1.04	0.32***
			Sample 8		
3	1.16–1.20	1.19	1.04–1.12	1.07	0.12
10	1.28–1.36	1.33	1.09–1.24	1.17	0.16
11	1.24–1.28	1.26	1.18–1.28	1.24	0.02
13	1.30–1.65	1.48	1.20–1.25	1.22	0.26
18	1.20–1.28	1.24	1.07–1.15	1.11	0.13
19	1.32–1.38	1.35	0.96–1.07	1.01	0.34
Group	1.16–1.65	1.31	0.96–1.28	1.14	0.17***
			Sample 9		
3	3.08–3.12	3.09	3.12–3.16	3.13	−0.04
10	3.22–3.27	3.25	3.30–3.40	3.35	−0.10
11	3.16–3.20	3.17	3.06–3.06	3.06	0.11
13	3.05–3.30	3.20	3.10–3.20	3.17	0.03
18	3.22–3.24	3.23	3.20–3.23	3.21	0.02
19	2.99–3.11	3.05	3.01–3.12	3.06	0.01
Group	2.99–3.30	3.16	3.01–3.40	3.16	0.00
			Sample 10		
1	2.25–2.30	2.28	2.20–2.20	2.20	0.08
15	1.84–1.89	1.87	1.89–1.97	1.92	−0.05
19	2.18–2.23	2.21	2.01–2.18	2.10	0.11
Group	1.84–2.30	2.12	1.89–2.20	2.07	0.05*
			Sample 11		
3	1.48–1.52	1.49	1.44–1.48	1.45	0.04
10	1.58–1.68	1.61	1.50–1.71	1.63	−0.02
11	1.60–1.64	1.63	1.32–1.36	1.34	0.29
13	1.40–1.60	1.50	1.50–1.55	1.52	−0.02
18	1.55–1.60	1.58	1.46–1.50	1.48	0.10
19	1.55–1.57	1.56	1.49–1.73	1.61	−0.05
Group	1.40–1.68	1.56	1.32–1.73	1.50	0.06*
			Sample 12		
1	1.70–1.75	1.73	1.50–1.50	1.50	0.23
15	1.40–1.46	1.42	1.36–1.37	1.37	0.05
19	1.67–1.69	1.68	1.55–1.67	1.60	0.08
Group	1.40–1.75	1.61	1.36–1.67	1.49	0.12***

TABLE 4—(*continued*)

COLLABORATOR	CITRATE-INSOLUBLE P_2O_5 BY—				DIFFERENCE IN AVERAGE RESULTS[c]
	INTERMITTENT AGITATION[a]		CONTINUOUS AGITATION[b]		
	RANGE	AVERAGE	RANGE	AVERAGE	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
			Sample 13		
3	1.80–1.80	1.80	1.80–1.84	1.81	−0.01
10	1.95–1.98	1.97	1.80–1.98	1.91	0.06
11	1.92–2.16	2.05	1.84–1.88	1.86	0.19
13	1.70–1.85	1.78	1.75–1.85	1.80	−0.02
18	1.84–1.87	1.86	1.74–1.80	1.78	0.08
19	1.66–1.85	1.75	1.82–1.83	1.83	−0.08
Group	1.66–2.16	1.87	1.74–1.98	1.83	0.04
			Sample 14		
3	1.60–1.64	1.63	1.56–1.60	1.57	0.06
10	1.62–1.68	1.64	1.65–1.83	1.76	−0.12
11	1.48–1.60	1.53	1.48–1.56	1.53	0.00
13	1.55–1.70	1.63	1.60–1.70	1.65	−0.02
18	1.69–1.74	1.71	1.55–1.60	1.57	0.14
19	1.55–1.78	1.67	1.52–1.76	1.61	0.06
Group	1.48–1.78	1.63	1.48–1.83	1.61	0.02
			Group Averages		
d	—	2.92	—	2.85	0.07[e]
f	—	3.23	—	3.13	0.10[e]
g,h	—	2.61	—	2.56	0.05[e]
g,i	—	2.62	—	2.59	0.03[e]
g,j	—	2.58	—	2.55	0.03[e]
g,k	—	2.59	—	2.50	0.09[e]

[a] Manual shaking at 5-minute intervals during the citrate digestion.
[b] Collaborators 1, 10, 13, 15, 18, and 19 used end-over-end agitation by the MacIntire-Marshall-Meyer apparatus; Collaborator 3 used continuous stirring at 1,000 r.p.m.; and Collaborator 11 used continuous shaking in a straight horizontal plane at 70 oscillations per minute.
[c] The minus sign denotes that the result by continuous agitation is higher than that by intermittent agitation. Tests for significant differences were made only on the group results. One, two, and three asterisks denote that the differences are significant at the 5%, 1%, and 0.1% levels, respectively.
[d] All results.
[e] Not analyzed statistically.
[f] Samples 1, 3, 5, 6, 7, 10, 12.
[g] Samples 2, 4, 8, 9, 11, 13, 14.
[h] All collaborators.
[i] Collaborators 1, 10, 13, 15, 18, 19; continuous agitation by end-over-end rotation.
[j] Collaborator 3; continuous agitation by stirring.
[k] Collaborator 11; continuous agitation by straight-line oscillation in the horizontal plane.

during the citrate digestion. For each collaborator and each sample the citric acid-insoluble P_2O_5 values were computed individually from the reported replicate results for citric acid-soluble P_2O_5 and the collaborator's average result for total P_2O_5 in the sample.

TABLE 5.—*Citrate-insoluble and citric acid-insoluble P_2O_5 in basic slags*

COLLABORATOR	CITRATE-INSOLUBLE P_2O_5[a]		CITRIC ACID-INSOLUBLE P_2O_5[b]		DIFFERENCE IN AVERAGE RESULTS[c]
	RANGE	AVERAGE	RANGE	AVERAGE	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
			Sample 1		
1	6.80–6.85	6.82	6.45– 6.50	6.48	0.34***
4	6.55–6.61	6.58	6.27– 6.32	6.29	0.29***
5	6.55–6.55	6.55	7.35– 7.39	7.36	−0.81[d]
6	6.17–6.18	6.18	5.71– 5.86	5.79	0.39***
7	6.77–6.85	6.81	5.88– 5.93	5.90	0.91***
8	6.60–6.70	6.67	6.42– 6.92	6.63	0.04
12	6.52–6.61	6.56	6.34– 6.36	6.35	0.21**
14	6.97–7.10	7.02	5.74– 5.75	5.75	1.27***
15	5.73–6.28	6.04	7.07– 7.12	7.10	−1.06[d]
17	6.40–6.66	6.54	6.55– 6.62	6.59	−0.05
19	6.92–6.99	6.96	5.87– 6.13	5.99	0.97***
Group[e]	6.17–7.10	6.68	5.71– 6.92	6.20	0.48***
Group[f]	5.73–7.10	6.61	5.71– 7.39	6.38	0.23[g]
			Sample 2		
2	7.95–8.02	8.00	6.82– 6.97	6.87	1.13***
3	8.12–8.16	8.15	7.13– 7.17	7.16	0.99***
9	7.68–8.10	7.90	7.56– 7.71	7.61	0.29***
10	7.92–7.98	7.95	7.08– 7.18	7.13	0.82***
11	7.60–7.68	7.63	7.29– 7.35	7.32	0.31***
13	7.70–7.80	7.77	7.35– 7.40	7.38	0.39***
16	7.95–8.08	8.03	7.38– 7.51	7.45	0.58***
19	8.11–8.31	8.20	6.97– 7.13	7.04	1.16***
20	8.21–8.25	8.22	7.72– 7.77	7.75	0.47***
21	8.17–8.20	8.18	7.77– 7.85	7.82	0.36***
Group[f]	7.60–8.31	8.00	6.82– 7.85	7.35	0.65***
			Sample 3		
4	9.23–9.27	9.25	8.88– 8.97	8.92	0.33***
5	9.28–9.30	9.29	10.36–10.38	10.37	−1.08[d]
6	9.75–9.85	9.78	9.27– 9.47	9.35	0.43***
7	9.55–9.75	9.63	9.14– 9.21	9.18	0.45***
8	9.30–9.60	9.47	8.87– 8.95	8.91	0.56***
12	8.14–8.54	8.31	9.50– 9.60	9.55	−1.24[d]
14	9.75–9.78	9.77	8.88– 8.91	8.89	0.88***
15	8.55–8.70	8.62	8.96– 8.96	8.96	−0.34***
17	9.45–9.64	9.55	9.46– 9.52	9.49	0.06
19	9.11–9.39	9.23	9.21– 9.52	9.34	−0.11
Group[h]	8.55–9.85	9.41	8.87– 9.52	9.13	0.28***
Group[f]	8.14–9.85	9.29	8.87–10.38	9.30	−0.01[g]
			Sample 4		
2	0.75–0.80	0.77	−0.03– 0.07	0.04	0.73***
3	0.68–0.72	0.71	0.25– 0.29	0.28	0.43***
9	0.68–0.74	0.70	0.88– 0.98	0.91	−0.21***

TABLE 5—(*continued*)

COLLABORATOR	CITRATE-INSOLUBLE P_2O_5[a]		CITRIC ACID-INSOLUBLE P_2O_5[b]		DIFFERENCE IN AVERAGE RESULTS[c]
	RANGE	AVERAGE	RANGE	AVERAGE	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
10	0.67–0.79	0.75	0.51– 0.58	0.56	0.19***
11	0.88–0.92	0.89	0.53– 0.77	0.67	0.22***
13	0.90–0.95	0.93	1.03– 1.08	1.05	−0.12**
16	0.58–0.70	0.65	0.20– 0.35	0.26	0.39***
19	0.81–0.81	0.81	0.51– 0.66	0.57	0.24***
20	0.79–0.80	0.79	0.67– 0.73	0.70	0.09*
21	0.78–0.86	0.81	0.69– 0.86	0.75	0.06
Group[f]	0.58–0.95	0.78	−0.03– 1.08	0.58	0.20***
			Sample 5		
4	1.13–1.22	1.18	0.74– 0.78	0.75	0.43***
5	0.98–1.02	1.00	2.62– 2.67	2.65	−1.65[d]
6	1.22–1.26	1.24	0.75– 1.05	0.89	0.35***
7	1.05–1.12	1.10	0.61– 0.69	0.65	0.45***
8	2.07–2.10	2.08	1.94– 2.52	2.16	−0.08
12	1.01–1.06	1.03	0.52– 0.57	0.54	0.49***
14	1.28–1.33	1.31	0.17– 0.19	0.18	1.13***
15	0.58–0.67	0.63	0.44– 0.49	0.46	0.17*
17	1.14–1.16	1.15	0.80– 0.92	0.84	0.31***
19	1.31–1.40	1.35	0.54– 0.61	0.58	0.77***
Group[i]	0.58–2.10	1.23	0.17– 2.52	0.78	0.45***
Group[f]	0.58–2.10	1.21	0.17– 2.67	0.97	0.24[g]
			Sample 6		
1	1.30–1.30	1.30	0.67– 0.72	0.70	0.60***
4	1.19–1.27	1.23	0.61– 0.65	0.62	0.61***
5	0.86–0.91	0.89	3.12– 3.22	3.17	−2.28[d]
6	1.12–1.18	1.15	1.05– 1.27	1.15	0.00
7	1.10–1.12	1.11	0.67– 0.71	0.69	0.42***
8	1.20–1.40	1.30	1.77– 2.57	2.10	−0.80[d]
12	0.88–0.96	0.91	0.38– 0.41	0.39	0.52***
14	1.28–1.36	1.32	0.50– 0.69	0.59	0.73***
15	0.72–0.73	0.73	−0.02– 0.03	−0.02	0.75***
17	1.11–1.16	1.13	0.82– 0.95	0.88	0.25***
19	0.97–1.16	1.05	0.93– 1.03	0.97	0.08
Group[i]	0.72–1.36	1.10	−0.02– 1.27	0.66	0.44***
Group[f]	0.72–1.40	1.10	−0.02– 3.22	1.02	0.08[g]
			Sample 7		
1	1.45–1.50	1.48	1.80– 1.85	1.82	−0.34
4	1.16–1.20	1.18	0.51– 0.64	0.55	0.63***
5	1.60–1.62	1.61	3.50– 3.58	3.54	−1.93[d]
6	1.38–1.44	1.41	1.25– 1.46	1.37	0.04
7	1.27–1.32	1.30	0.77– 0.81	0.80	0.50**
8	1.40–2.00	1.73	−0.33– 1.12	0.15	1.58***

TABLE 5—(*continued*)

COLLABORATOR	CITRATE-INSOLUBLE P_2O_5[a]		CITRIC ACID-INSOLUBLE P_2O_5[b]		DIFFERENCE IN AVERAGE RESULTS[c]
	RANGE	AVERAGE	RANGE	AVERAGE	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
12	1.29–1.43	1.37	0.43– 0.53	0.50	0.87***
14	1.59–1.66	1.63	0.49– 0.54	0.52	1.11***
15	1.15–1.16	1.16	0.29– 0.29	0.29	0.87***
17	1.14–1.28	1.21	1.52– 1.76	1.64	−0.43*
19	1.44–1.48	1.46	0.84– 0.90	0.87	0.59**
Group[i]	1.14–1.66	1.39	−0.33– 1.85	0.85	0.54***
Group[f]	1.14–1.66	1.41	−0.33– 3.58	1.09	0.32[g]
			Sample 8		
2	1.30–1.35	1.33	0.50– 0.65	0.55	0.78***
3	1.16–1.20	1.19	0.76– 0.80	0.79	0.40***
9	0.90–1.00	0.93	1.89– 2.15	2.04	−1.11[d]
10	1.28–1.36	1.33	0.63– 0.68	0.65	0.68***
11	1.24–1.28	1.26	0.50– 0.65	0.58	0.68***
13	1.30–1.65	1.48	1.92– 2.07	2.00	−0.52[d]
16	1.17–1.27	1.20	0.48– 0.57	0.52	0.68***
19	1.32–1.38	1.35	0.86– 0.93	0.89	0.46***
20	1.40–1.45	1.42	0.76– 0.77	0.76	0.66***
21	1.30–1.60	1.47	1.03– 1.08	1.05	0.42***
Group[k]	1.16–1.60	1.32	0.48– 1.08	0.72	0.60***
Group[f]	0.90–1.65	1.29	0.48– 2.15	0.98	0.31[g]
			Sample 9		
2	3.15–3.20	3.18	2.92– 2.92	2.92	0.26***
3	3.08–3.12	3.09	2.94– 3.02	2.97	0.12**
9	3.36–3.40	3.37	3.19– 3.26	3.21	0.16***
10	3.22–3.27	3.25	2.94– 3.09	2.99	0.26***
11	3.16–3.20	3.17	2.81– 2.87	2.84	0.33***
13	3.05–3.30	3.20	3.12– 3.22	3.17	0.03
16	3.02–3.10	3.06	2.79– 2.96	2.90	0.16***
19	2.99–3.11	3.05	2.85– 2.89	2.88	0.17*
20	3.06–3.08	3.07	3.03– 3.04	3.03	0.04
21	3.06–3.20	3.12	3.16– 3.18	3.17	−0.05
Group[f]	2.99–3.40	3.16	2.79– 3.26	3.01	0.15***
			Sample 10		
1	2.25–2.30	2.28	2.13– 2.18	2.15	0.13**
4	2.04–2.04	2.04	1.81– 1.82	1.82	0.22***
5	2.10–2.15	2.13	2.88– 2.93	2.90	−0.77[d]
6	2.23–2.26	2.25	2.13– 2.23	2.19	0.06
7	2.10–2.12	2.11	2.04– 2.08	2.05	0.06
8	1.20–1.30	1.25	1.69– 1.82	1.74	−0.49***
12	2.11–2.13	2.12	1.67– 1.89	1.81	0.31***
14	2.14–2.16	2.15	1.79– 1.86	1.82	0.33***
15	1.84–1.89	1.87	1.45– 1.60	1.52	0.35***

TABLE 5—(*continued*)

COLLABORATOR	CITRATE-INSOLUBLE P_2O_5[a]		CITRIC ACID-INSOLUBLE P_2O_5[b]		DIFFERENCE IN AVERAGE RESULTS[c]
	RANGE	AVERAGE	RANGE	AVERAGE	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
17	2.00–2.03	2.02	2.00– 2.09	2.04	−0.02
19	2.18–2.23	2.21	1.76– 1.87	1.81	0.40***
Group[i]	1.20–2.30	2.03	1.45– 2.23	1.90	0.13***
Group[f]	1.20–2.30	2.04	1.45– 2.93	1.99	0.05[g]
			Sample 11		
2	1.57–1.62	1.60	1.00– 1.10	1.05	0.55***
3	1.48–1.52	1.49	1.23– 1.35	1.28	0.21**
9	1.60–1.68	1.64	2.72– 3.02	2.87	−1.23[d]
10	1.58–1.68	1.61	1.33– 1.38	1.36	0.25***
11	1.60–1.64	1.63	1.13– 1.18	1.15	0.48***
13	1.40–1.60	1.50	2.17– 2.32	2.25	−0.75[d]
16	1.55–1.65	1.60	0.91– 0.96	0.93	0.67***
19	1.55–1.57	1.56	1.11– 1.65	1.42	0.14*
20	1.66–1.66	1.66	1.37– 1.38	1.38	0.28***
21	1.56–1.66	1.59	1.58– 1.65	1.62	−0.03
Group[k]	1.48–1.68	1.59	0.91– 1.65	1.27	0.32***
Group[f]	1.40–1.68	1.59	0.91– 3.02	1.53	0.06[g]
			Sample 12		
1	1.70–1.75	1.73	2.83– 2.88	2.86	−1.13[d]
4	1.56–1.57	1.56	1.34– 1.42	1.38	0.18***
5	1.88–1.94	1.91	5.92– 5.97	5.93	−4.02[d]
6	1.48–1.52	1.49	0.66– 0.70	0.68	0.81***
7	1.95–1.95	1.95	1.39– 1.43	1.41	0.54***
8	1.70–2.00	1.83	1.38– 1.43	1.41	0.42***
12	1.69–1.69	1.69	1.48– 1.68	1.55	0.14**
14	1.75–1.82	1.79	1.21– 1.23	1.22	0.57***
15	1.40–1.46	1.42	0.70– 0.75	0.73	0.69***
17	1.48–1.48	1.48	1.67– 1.88	1.77	−0.29***
19	1.67–1.69	1.68	1.43– 1.48	1.45	0.23***
Group[l]	1.40–2.00	1.65	0.66– 1.88	1.29	0.36***
Group[f]	1.40–2.00	1.68	0.66– 5.97	1.85	−0.17[g]
			Sample 13		
2	1.87–1.90	1.89	1.08– 1.18	1.13	0.76***
3	1.80–1.80	1.80	1.51– 1.67	1.62	0.18***
9	2.00–2.08	2.05	3.12– 3.85	3.51	−1.46[d]
10	1.96–1.98	1.97	1.33– 1.48	1.43	0.54***
11	1.92–2.16	2.05	1.53– 1.68	1.61	0.44***
13	1.70–1.85	1.78	2.32– 2.72	2.47	−0.69[d]
16	1.72–1.87	1.80	1.47– 1.55	1.50	0.30***
19	1.66–1.85	1.75	1.50– 1.52	1.51	0.24***
20	1.75–1.79	1.77	1.53– 1.55	1.54	0.23***

TABLE 5—(*continued*)

COLLABORATOR	CITRATE-INSOLUBLE P_2O_5[a]		CITRIC ACID-INSOLUBLE P_2O_5[b]		DIFFERENCE IN AVERAGE RESULTS[c]
	RANGE	AVERAGE	RANGE	AVERAGE	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
21	1.94–2.06	1.99	1.86– 1.89	1.88	0.11*
Group[k]	1.66–2.16	1.88	1.08– 1.89	1.53	0.35***
Group[f]	1.66–2.16	1.89	1.08– 3.85	1.82	0.07[g]
			Sample 14		
2	1.65–1.70	1.67	0.90– 0.95	0.92	0.75***
3	1.60–1.64	1.63	1.21– 1.29	1.24	0.39***
9	1.84–1.96	1.89	3.18– 5.31	4.29	−2.40[d]
10	1.62–1.68	1.64	1.26– 1.34	1.30	0.34***
11	1.48–1.60	1.53	1.37– 1.37	1.37	0.16***
13	1.55–1.70	1.63	2.15– 2.35	2.27	−0.64[d]
16	1.60–1.62	1.61	1.12– 1.17	1.15	0.46***
19	1.55–1.78	1.67	1.22– 1.34	1.27	0.40***
20	1.53–1.55	1.54	1.33– 1.36	1.35	0.19***
21	1.88–2.04	1.96	1.44– 1.57	1.50	0.46***
Group[k]	1.48–2.04	1.66	0.90– 1.57	1.26	0.40***
Group[f]	1.48–2.04	1.68	0.90– 5.31	1.67	0.01[g]
			Group Averages		
[m]	—	2.99	—	2.61	0.38[g]
[n]	—	2.98	—	2.82	0.16[g]

[a] Manual shaking at 5-minute intervals during citrate digestion.
[b] Computed from the collaborator's results for total P_2O_5 and citric acid-soluble P_2O_5.
[c] The minus sign denotes that the value for citric acid-soluble P_2O_5 is higher than that for citrate-insoluble P_2O_5. One, two, and three asterisks denote that the differences are significant at the 5%, 1%, and 0.1% levels, respectively.
[d] Collaborator's results are not included in the statistical analysis.
[e] Excluding Collaborators 5 and 15.
[f] Including all collaborators.
[g] Not analyzed statistically.
[h] Excluding Collaborators 5 and 12.
[i] Excluding Collaborator 5.
[j] Excluding Collaborators 5 and 8.
[k] Excluding Collaborators 9 and 13.
[l] Excluding Collaborators 1 and 5.
[m] Results included in the statistical analyses.
[n] All results.

COMMENTS OF COLLABORATORS

Collaborator 5.—With the room temperature and the initial temperature of the citric acid solution at 29°C. and 17.5°C., respectively, the temperature of the citric acid extract was 24°C. at the end of the 30-minute digestion period. No difficulty was experienced with caking of the samples during the citric acid digestion.

Collaborator 6.—More difficulty was experienced with Sample 1 than with the other samples, as regards dehydration of the silica in the hydrochloric-nitric acid digestion of the citrate-insoluble residues and of the original materials.

Collaborator 7.—Before adding the molybdate solution it was necessary to acidify the citric acid extract more strongly with nitric acid than the official method calls for. Without the additional acid the solution developed a green color and no pre-

cipitate was formed. This condition appeared to be unaffected by the interval between the citrate digestion and addition of the molybdate reagent. All the reported values were determined on extracts that were filtered and analyzed immediately after the 30-minute citric acid digestion. With an initial citric acid temperature of 17.5°C. the temperature of the extract was 30°C. at the end of the digestion period.

Collaborator 8.—The results are not as good as desired, as we have had very little previous experience with basic slags, but shortness of time did not permit additional determinations. After most of the work was finished we suspected the following possible errors.

1. In digesting the sample for determination of total P_2O_5, separation of gelatinous silica interferes with solution of the sample and may account for some of the discrepancies.

2. In the continuous stirring for extraction of citric acid-soluble P_2O_5, we conclude that a straight stirring rod does not properly disperse the sample throughout the liquid. A mass of coarse particles collects in the center of the beaker, where it remains almost stationary.

Collaborator 9.—For the citric acid digestion the Ross-Kershaw machine at 120 oscillations per minute does not provide sufficient agitation. Without additional manual shaking a firm cake of the material forms on the bottom of the flask. The citrate extracts of Samples 2 and 8 were difficult to filter and wash, requiring 1 hour and 3 hours, respectively.

Collaborator 10.—For determination of total P_2O_5 the samples were digested with 10 ml. of perchloric acid plus 5 ml. of nitric acid. It is the opinion of this collaborator that only one value for total P_2O_5 in a sample should be used. This would make the values for available P_2O_5 in the sample independent of variations in the results for total P_2O_5 obtained thereon by the different collaborators.

Collaborator 13.—In the determination of total P_2O_5 the samples were dissolved by means of hydrochloric and nitric acids, as directed. Higher values were obtained on solutions prepared with sulfuric and nitric acids and analyzed by the volumetric method with precipitation of the phosphomolybdate at 25–30°C. and subsequent continuous agitation. The results are as follows:

SAMPLE	TOTAL P_2O_5 BY DIGESTION WITH—	
	HCl-HNO_3	H_2SO_4-HNO_3
	per cent	*per cent*
2	11.00	11.82
4	7.53	7.81
8	15.12	15.48
9	8.37	8.66
11	11.82	12.07
12	16.47	16.90
14	17.90	18.16

Collaborator 15.—Considerable difficulty was experienced with the hydrochloric-nitric acid method of dissolving the sample for total P_2O_5 determination owing to the presence of much gelatinous silica which made filtration very tedious. In addition to the reported P_2O_5 values which were obtained with this method of solution, determinations were also made on solutions prepared with the aid of hydrofluoric and perchloric acids, whereby the silica was volatilized and the hydrofluoric acid was expelled by fuming the mixture in a platinum dish. The results are as follows:

SAMPLE	TOTAL P_2O_5 BY DIGESTION WITH—	
	HCl-HNO_3	HF-$HClO_4$
	per cent	*per cent*
	10.40	8.55
1	10.35	8.70
	10.35	8.65
	10.98	9.95
3	10.55	10.00
	11.05	10.40
	10.65	9.85
5	10.45	9.90
	10.83	10.00
	10.15	10.90
6	10.78	11.00
	11.40	11.10
	13.59	14.00
7	13.84	13.99
	14.10	14.05
	8.10	8.65
10	8.06	8.55
	8.45	8.55
	15.27	15.75
12	15.75	15.90
	15.93	15.80
56a[a]	—	32.77

[a] National Bureau of Standards sample of Tennessee phosphate rock. Certificate value for total P_2O_5 is 33.01%.

Collaborator 19.—Thorough washing of the citrate-insoluble residues was tedious, requiring 3 to 4 hours in some cases.

INTERPRETATION OF RESULTS

Intermittent Shaking vs. Continuous Agitation During Citrate Digestion.—In last year's report on P_2O_5 (25) it was concluded that, in comparison with manual shaking at 5-minute intervals, continuous agitation during the citrate digestion tends to give somewhat lower values for citrate-insoluble P_2O_5 in basic slag and other furnace-made phosphates. Although the results of the present investigation generally confirm this conclusion, the differences are statistically significant for only 7 of the 14 samples submitted to the collaborators (Table 4). Even so, the significant differences between the group results for citrate-insoluble P_2O_5 by intermittent and continuous agitation—all in the direction of lower values

by the latter procedure—are quite small; they range from 0.05 to 0.32 per cent of the sample or 0.6 to 2.2 per cent of the group-average total P_2O_5. In these comparisons, significance was determined by the *F* test using the variance within replicates as the error term. When collaborator variance was included in the error term, the differences between group averages for intermittent and continuous agitation did not attain significance in a single case.

In agreement with previous results (24, 25, 29) the differences in the average values for citrate-insoluble P_2O_5 (Samples 2, 4, 8, 9, 11, 13, and 14) by intermittent and continuous agitation, respectively, are the same or nearly so (Table 4), whether the latter was by end-over-end rotation (Collaborators 1, 10, 13, 15, 18, and 19), stirring (Collaborator 3), or straight-line oscillation in the horizontal plane (Collaborator 11).

Citrate-Insoluble P_2O_5 vs. Citric Acid-Insoluble P_2O_5.—As previously pointed out, the values for citric acid-insoluble P_2O_5 (Table 5) are computed from the reported results for total P_2O_5 and citric acid-soluble P_2O_5. Consequently these values, unlike those for citrate-insoluble P_2O_5, also reflect such discrepancies as may occur in the results for total P_2O_5. Careful inspection of the detailed data, partly omitted from this report, indicates that this is not an important factor in the differences between the tabulated values for citrate-insoluble and citric acid-insoluble P_2O_5. In most cases these differences reflect principally the disparity, usually small, in the ultimate solvent action of the two reagents on the slag P_2O_5 under the specific conditions of the determinations.

Previous work (17, 18, 23, 24, 25, 36, 37) has indicated that with the present official methods the percentage of citrate-insoluble P_2O_5 in a slag can be expected, in general, to exceed that of the citric acid-insoluble P_2O_5. This is true of 113 (78 per cent) of the 145 individual comparisons listed in Table 5. For the other 32 comparisons, widely distributed among 13 samples, close study of the data leads to the conclusion that the reversal in the sign of the differences is due mostly to too high values for citric acid-insoluble P_2O_5 relative to citrate-insoluble P_2O_5, rather than the contrary. A possible major cause of such discrepancy is agglomeration and caking of the slag particles during the early stages of the digestion—a condition that is apt to occur, especially with citric acid—and subsequent failure to effect thorough dispersion of the sample. On this basis and in view of the large preponderance of positive differences (citrate-insoluble values higher than citric acid-insoluble values) omission from the statistical analyses of the comparisons that show negative differences greater than 0.5 per cent is considered justifiable.

Statistical analyses were made of the data for each sample. Significance was determined by the *F* test using the variance within replicates as the error term. Collaborator variance was excluded. The statistically significant differences at the 5, 1, and 0.1 per cent levels are indicated by ap-

propriate asterisks in the last column of Table 5. In 19 of the 145 comparisons the values for citric acid-insoluble P_2O_5 exceeded those for citrate-insoluble P_2O_5 by more than 0.5 per cent; these comparisons were excluded from the statistical analysis.

Among the 126 individual comparisons that were tested statistically, 109 show significant differences between the values for citrate-insoluble and citric acid-insoluble P_2O_5. Only 13 of the comparisons show higher values for citric acid-insoluble P_2O_5 than for citrate-insoluble P_2O_5, and in 7 of the 13 the differences are not statistically significant. The distribution of the individual comparisons as regards statistical significance is as follows:

COMPARISONS	NUMBER	FRACTION OF TOTAL
		per cent
Tested statistically	126	100
Statistically significant	109	86.5
5.0% level	6	4.8
1.0% level	8	6.3
0.1% level	95	75.4

All the average results on the 14 samples show statistically significant differences at the 0.1 per cent level, and these differences are all in the direction of higher values for citrate-insoluble P_2O_5. For 8 samples (Nos. 3, 4, 5, 6, 7, 8, 11, and 12) the collaborator averages for citric acid-insoluble P_2O_5 are significantly more variable than those for citrate-insoluble P_2O_5. Of the other 6 samples all but No. 10 show this trend though not attaining significance.

For the 145 individual comparisons the differences between the respective values for citrate-insoluble and citric acid-insoluble P_2O_5 range from -4.02 to $+1.58$ per cent (Table 5). Positive differences (citrate-insoluble P_2O_5 greater than citric acid-insoluble P_2O_5), averaging 0.45 per cent of P_2O_5, are shown by 112 of the comparisons. For all of the 126 comparisons that were analyzed statistically, including 13 that show negative differences (range -0.02 to -0.49 per cent, average -0.20 per cent), the differences average $+0.38$ per cent of P_2O_5. Of the statistically tested comparisons, 88 (69.8 per cent) show differences of not more than 0.5 per cent of P_2O_5, while only 6 (4.4 per cent) show differences of more than 1 per cent. The distribution of the differences is shown in the table on the next page.

The data of Table 4 indicate that use of continuous agitation during the citrate digestion may narrow the gap between the citrate-insoluble and the citric acid-insoluble P_2O_5 values.

For the individual samples the statistically analyzed comparisons show average P_2O_5 differences—all positive—ranging from 0.13 per cent (Sample

CITRATE-INSOLUBLE P_2O_5 MINUS CITRIC ACID-INSOLUBLE P_2O_5	COMPARISONS	
	NUMBER	FRACTION OF TOTAL
per cent		*per cent*
−4.02 to −0.50[a]	19	13.1
−0.49 to −0.25	5	3.5
−0.24 to 0.00	9	6.2
0.01 − 0.10	10	6.9
0.11 − 0.20	14	9.7
0.21 − 0.30	16	11.0
0.31 − 0.40	18	12.4
0.41 − 0.50	16	11.0
0.51 − 0.60	9	6.2
0.61 − 0.80	15	10.4
0.81 − 1.00	8	5.5
1.01 − 1.58	6	4.1
−4.02 to +1.58	145	100.0

[a] Not analyzed statistically.

10) to 0.65 per cent (Sample 2) and averaging 0.38 per cent. On the basis of total P_2O_5 the differences range from 1.5 per cent (Sample 10) to 5.7 per cent (Sample 2) and average 3.1 per cent; these differences are of the same order as those previously found with finely ground alpha phosphates (17, 27).

With the possible exception of the Kahn-type shaker the values for citric acid-insoluble P_2O_5 show no clearly defined effects of variations in the type of continuous agitation device or the speed of agitation (Tables 3 and 5). This agrees with the findings of MacIntire, Hardin, and Meyer (28).

There appears to be no definite relation between the difference in the citrate-insoluble and the citric acid-insoluble P_2O_5 of a slag and either its type, source, total P_2O_5, or fluorine (Tables 1 and 5). As would be expected however, the high-fluorine slags (Samples 1, 2, and 3) show low solubilities in both neutral ammonium citrate and 2 per cent citric acid; this is because the P_2O_5 in these samples is present principally in the form of fluorapatite (2, 27).

Although this report is concerned chiefly with the differences in the respective values for citrate-insoluble and citric acid-insoluble P_2O_5, rather than with the actual values themselves, it should be noted that for a given sample the actual values for these determinations, as well as those for total P_2O_5, are generally less consistent than is desirable in studies pertaining to official methods of analysis.

EFFECT OF PARTICLE SIZE

Hoffmeister (19) reported that when samples of ground basic slag were separated into different particle sizes the chemical composition of the

fractions and the solubility of the P_2O_5 in Wagner's acid ammonium citrate solution varied with the particle size, the solubility increasing with increase in fineness. Popp (33) also showed that the chemical composition of the mechanical fractions of ground basic slag varies with the particle

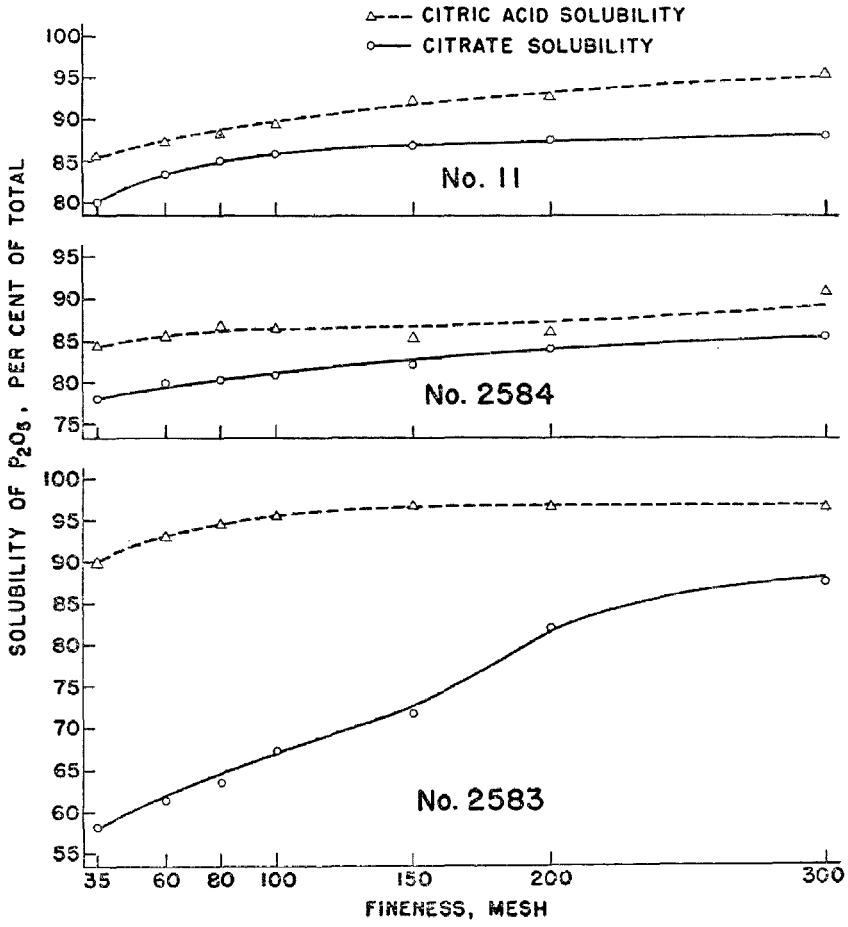

FIG. 1.—Effect of fineness on solubility of basic slag.

size; he reported, however, that the solubility of the P_2O_5 in 2 per cent citric acid did not vary greatly with the different fractions. Previous work in the writers' laboratory showed that the citrate and citric acid solubilities of a basic slag were not greatly affected by the fineness of the sample in the range −40 to −300 mesh (17, 18).

Solubility-fineness results obtained by the writers on three samples of slag are presented in Figure 1. Sample 11, an Alabama open-hearth slag, is

from one of the collaborative materials. Samples 2583 and 2584, containing 18.73 and 20.70 per cent of total P_2O_5, respectively, are Bessemer slags recently manufactured in France. The fineness series of Samples 11 and 2584 were prepared from the commercially ground materials in such a way that all members had the same chemical composition as the original sample. Sample 2583 was received in the unground condition, and its fineness series was prepared in a manner similar to that for the other two samples.

As shown in Table 6 the fineness series of Samples 11 and 2584 have

TABLE 6.—*Mechanical composition of slags ground to different finenesses*

SAMPLE	FINENESS[a]	COMPOSITION, MESH						
		−35, +60	−60, +80	−80, +100	−100, +150	−150, +200	−200, +300	−300
	Mesh	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
	− 35	10.2	7.6	4.8	7.6	7.9	9.7	55.2
	− 60	—	10.2	6.3	8.2	9.2	10.6	55.5
11	− 80	—	—	8.9	11.3	10.2	11.3	58.3
	−100	—	—	—	13.7	11.8	12.3	62.2
	−150	—	—	—	—	14.2	16.5	69.3
	−200	—	—	—	—	—	16.5	83.5
	− 35	25.0	20.4	10.8	13.7	9.4	7.3	13.4
	− 60	—	25.6	14.9	18.8	12.4	9.7	18.6
2583	− 80	—	—	21.4	25.0	16.0	12.2	25.4
	−100	—	—	—	32.0	20.1	15.5	32.4
	−150	—	—	—	—	28.7	23.1	48.2
	−200	—	—	—	—	—	48.4	51.6
	− 35	7.8	6.8	4.4	8.0	9.1	12.4	51.5
	− 60	—	7.7	5.3	8.9	10.1	13.2	54.8
2584	− 80	—	—	5.9	10.3	11.1	14.0	58.7
	−100	—	—	—	11.0	12.0	15.4	61.6
	−150	—	—	—	—	13.6	17.7	68.7
	−200	—	—	—	—	—	10.4	89.6

[a] Screen openings in sieve series were 420, 250, 177, 149, 104, 74, and 46 microns, respectively.

about the same distribution of particle size in the corresponding members, while Sample 2583 has considerably higher percentages of material in the coarser fractions. The determinations were made by sieving 10-gram samples, previously dried at 100°C., for 30 minutes in a Ro-Tap machine on U. S. standard screens that had been warmed to hinder condensation of moisture during the analysis. Because of extensive agglomeration of the finer particles in the presence of small amounts of moisture, the use of undried samples and cold screens during summer periods of high atmospheric humidity, as was the case at the time this solubility-fineness

study was made, may easily lead to erroneous particle-size distribution values in the screen series finer than 150 mesh. This is illustrated by the following results which were obtained by sieving 10-gram portions of 150-mesh material (Sample 11) in a Ro-Tap machine for 30 minutes:

CONDITIONS	COMPOSITION, MESH		
	−150, +200	−200, +300	−300
	per cent	*per cent*	*per cent*
Undried sample, cold screens	15.3	55.9	28.8
Dry sample, warmed screens	14.2	16.5	69.3

The solubility-fineness data for Samples 11 and 2584 (Fig. 1) indicate that with slags commercially ground to a fineness of 85 per cent or more through a 35-mesh screen the gap between the citrate- and the citric acid-solubility values is narrowed but little, if any, by regrinding the analytical samples completely to pass screens finer than 35 mesh. For such samples, a rigid interpretation of the results points, however, to a fineness of −200 to −300 mesh in order to reduce the particle-size effect on the solubility to negligible values. Nevertheless, a fineness of −100 mesh sacrifices only 2 to 5.5 per cent of the P_2O_5 to the insoluble fraction.

That variation of the particle-size distribution in the fineness series has a much smaller effect on the citric acid solubility of slag P_2O_5 than on its citrate solubility is indicated by the data for Sample 2583. Especially as regards citrate solubility, this situation raises the question of particle-size distribution and its reproducibility in the preparation of slag samples for analysis, which apparently has an important bearing on analytical variability when the fineness of the sample does not lie in the range of a nearly horizontal portion of the solubility-fineness curve. The limited evidence (Fig. 1 and Table 6) indicates that for citrate solubility the slag fineness corresponding to the nearly horizontal portion of the solubility-fineness curve is 100 per cent through a 100-mesh screen and at least 95, 75, and 60 per cent through 150-, 200-, and 300-mesh screens, respectively.

SUMMARY AND CONCLUSIONS

In last year's report (25) it was shown for basic slag and a wide variety of other phosphatic materials (a) that the results for citrate-insoluble P_2O_5 by continuous agitation during the citrate digestion differ only slightly from those by shaking at 5-minute intervals and (b) that variation in the method of continuous agitation is not an important factor. The current work with basic slag supports these findings.

Triplicate determinations of citrate-insoluble and citric acid-insoluble P_2O_5 in 14 samples of different types and sources of basic slags were made by 10–11 collaborators using the present official methods with manual

shaking at 5-minute intervals during the citrate digestion. The slags, all received in the commercially ground condition, were reground to pass a 100-mesh screen for analysis.

The values reported by the individual collaborators for citrate-insoluble P_2O_5 are usually higher than those for citric acid-soluble P_2O_5 in the same sample. However, for the individual samples the average differences are small though statistically significant. Excluding certain results that are obviously considerably out of line, they range from 0.13 to 0.65 per cent and average 0.38 per cent of P_2O_5 on the sample, or 1.5 to 5.7 per cent (average 3.1 per cent) of the total P_2O_5, all in the direction of higher values for citrate-insoluble P_2O_5.

The results of this study, together with those of previous investigations, point to the present official neutral ammonium citrate method as a suitable procedure for the evaluation of the P_2O_5 in basic slags.

CITRATE-SOLUBLE AND AVAILABLE PHOSPHORIC ACID

In the *Methods of Analysis*, 1940, p. 24, sec. **17,** the following directions are given for obtaining citrate-soluble and available P_2O_5:

"Subtract sum of water-soluble and citrate-insoluble P_2O_5 from total to obtain the citrate-soluble P_2O_5. Subtract citrate-insoluble P_2O_5 from total to obtain available P_2O_5."

At the 1944 meeting of this Association it was recommended by the Referee on Fertilizers (7) and by Subcommittee A on Recommendations of Referees (10) that these directions be revised to change the last sentence to read, "Subtract citrate-insoluble P_2O_5 from the total to obtain chemically available P_2O_5 in dicalcium phosphate, precipitated bone phosphate, and precipitated bone" (official, final action). As printed in the *Methods of Analysis*, 1945, p. 25, sec. **2.17,** this sentence is further changed to include "acidulated samples."

Thus, aside from the citric acid procedure for basic slag, there is at present no official method for the determination of available P_2O_5 in materials other than acidulated samples and the three so-called "non-acidulated" products—dicalcium phosphate, precipitated bone phosphate, and precipitated bone. Though the official citrate procedures make no mention of mixed fertilizers, the available P_2O_5 in such materials is commonly determined by the procedure for acidulated samples, regardless of the type of phosphate (acidulated or non-acidulated) present in the mixture. This situation leaves the manufacturer and the State fertilizer control authorities without an official method for available P_2O_5 in any other non-acidulated phosphatic material until such time as the Association may designate a specific method for that particular material. However, the material loses its identity when it is included in a mixed fertilizer, and its P_2O_5 content is then subject to evaluation by the procedure for acidulated samples. Furthermore there is uncertainty as to the specific

kinds of phosphatic materials and compounds, aside from mixed fertilizers, that are covered by the respective terms, "acidulated samples" and "non-acidulated samples."

In view of the fact that a number of investigations (1, 3, 4, 5, 6, 13, 18, 20, 22, 26, 31, 35, 36, 37), including several (26, 35, 36, 37) conducted under the auspices of this Association, have shown a close relation between the citrate solubility of the P_2O_5 of a wide variety of phosphatic materials and their effectiveness in promoting plant growth, when the citrate digestion is made with a ratio of 1 gram of sample per 100 ml. of solvent, it seems appropriate that the Association reconsider the present directions for obtaining citrate-soluble and available P_2O_5, with the object of specifying the use of the neutral ammonium citrate procedure for the evaluation of all types of phosphatic materials until such time as it may be demonstrated to the satisfaction of the Association that other procedures are better adapted for specific materials. The results of the present study of basic slag lend further support to this suggestion.

ACKNOWLEDGMENT

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

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RECOMMENDATIONS*

It is recommended—

(1) That in the *Methods of Analysis, A.O.A.C.*, 1945, sec. **2.10(b)** be changed by substituting "324.03" for "323.81" in line 1 and "32.40" for "32.38" in line 2 (final action).

(2) That sec. **2.11** be changed by deleting "and dilute to 200 ml with H_2O," designating the paragraph as "**(a),**" and adding a second paragraph as follows:

> "**(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**" (final action).

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* For report of Subcommittee A and action by the Association, see *This Journal*, **32**, 43 (1949).

(3) That sec. **2.12** be changed by:

(1) Adding to sec. **2.12(a)** the following as the first sentence: "Prepare soln of sample as directed under **2.11(a).**"

(2) Deleting the phrase, in sec. **2.12(b)** first line, "Not applicable to superphosphate and other fertilizers that contain sulfates (5)," and adding the following as the first sentence: "Prepare soln of sample as directed under **2.11(b).**"

(3) Adding to sec. **2.12** a new paragraph, as follows:
"**(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)**" (final action).

(4) That the first six sentences of sec. **2.16(a),** lines 1–11, be changed (final action) as follows:

"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 thru Whatman filter paper No. 5 or other paper of equal speed and retentiveness."

(5) That sec. **2.16** be altered by:

(1) Changing the phrase, in sec. **2.16(a)** first line, "*Acidulated samples*" to "*Acidulated samples and mixed fertilizers.*"

(2) Deleting the words, in sec. **2.16(b)** first line, "*other than basic slag*" (first action).

(6) That sec. **2.17** be changed by deleting the words, in second sentence, "in acidulated samples, dicalcium phosphate, precipitated bone phosphate, and precipitated bone" (first action).

(7) That the methods for citric acid-soluble phosphoric acid in basic slag, sec. **2.18, 2.19,** and **2.20,** be deleted (first action).

(8) That work on methods for phosphoric acid be continued, with emphasis on:

(1) Evaluation of sintered, fused, and calcined alpha phosphates as fertilizers.

(2) Aging of the molybdate solution used in the volumetric method to determine if a time limit should be put on its use or an addition made to preserve it.

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REPORT ON MOISTURE IN FERTILIZERS

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The work on moisture this year covers the results of the collaborative study of methods ordered last year, and also a discussion of the distillation method for moisture in fertilizers, as well as requested suggestions as to needed editorial changes in the Book of Methods. These three topics and the recommendations comprise the subjects, respectively, of the four sections of this report.

SECTION A. COLLABORATIVE STUDY OF DETERMINATION OF MOISTURE IN FERTILIZERS BY OVEN-DRYING, VACUUM-DESICCATION, AND AIR-FLOW METHODS

This study was recommended and approved last year[1] for the second time. It marks the beginning of another organized effort to do something about the long-recognized need for a method that is more satisfactory than the present official procedure for determining moisture in fertilizers. A rather full discussion of the problems of moisture determination in fertilizers was presented before the Association a few years ago.[2]

Two points of view regarding moisture determination in fertilizers have been prominent during the past few years. The fertilizer analyst tends to view water in fertilizers merely as a diluent He is interested mainly in a moisture determination that will enable him to reduce his analytical results to a common basis as regards water content. The determination of water for this purpose need not involve considerations as to the actual state of the water in the sample so long as the result includes all or a definite part of the total water. The fertilizer technologist or manufacturer, on the other hand, has a vital interest in the free water since this is

[1] *This Journal*, **31**, 234 (1948); **31**, 42 (1948).
[2] *This Journal*, **25**, 132 (1942).

the part of the total water that so largely affects the physical condition of the fertilizer. But since the free water value can often be used to reduce the results of analysis to a standard base, a very strong case can be made for setting up a procedure for the rapid determination of free water and requiring its use as a general method for all fertilizer purposes. The principal obstacle to this approach is the condition that free water does not always stay put—under some circumstances free water today may in part be bound as water of crystallization tomorrow, in which cases the analyst needs to know the sum of the two interchangeable forms, or else condition the sample at a definite temperature. Thus, in order to meet the needs of all concerned, at least two general methods will be required—one for free water, and another for the sum of the free water and that bound as water of crystallization. Although methods known to fulfill the necessary requirements are not at hand, the realization that any search for a single method for determining water in fertilizers for all purposes holds little promise, is a first step toward the solution of the problem.

With this consideration as a background the recommendation has been pursued by having determinations by the three methods—oven drying at 100°C. (official), vacuum desiccation at room temperature, and air flow at 60°C—performed on six typical fertilizers and fertilizer materials in a dozen fertilizer laboratories and comparing the variabilities of the data with respect to collaborators, materials, and methods. The results thus obtained are presented in this section of the report.

MATERIALS AND METHODS

Descriptions of procedures, special apparatus and other details, except the samples, are given below in the instructions to collaborators. The samples were ground to pass a U. S. No. 30 standard sieve. Sample I,

TABLE 1.—*Formulation of mixed fertilizer samples*

INGREDIENT	AMOUNT OF INGREDIENT IN—		
	SAMPLE IV 10-6-4 MIXTURE[1]	SAMPLE V 10-10-10 MIXTURE[2]	SAMPLE VI 5-10-5 MIXTURE[2]
	Lb. per ton	*Lb. per ton*	*Lb. per ton*
Nitrogen solution IIA	—	200	140
Ammonium sulfate	330	278	96
Ammonium nitrate	300	200	67
Superphosphate	600	782	1012
Double superphosphate	—	100	—
Potassium chloride	140	330	160
Magnesian limestone	—	110	325
Cocoa shell meal	—	—	200
Soybean meal	630	—	—

[1] Laboratory preparation.
[2] Commercial fertilizer.

the reference sample, was potassium sulfate, reagent quality (A.C.S. Standard) that had stood for some time in a closed container after the addition of about 4 per cent of water by spraying. Sample II was run-of-pile superphosphate, without added conditioner of any kind, that had been stored in an air-tight container for 12 months. Sample III, cocoa shell meal, was received from a fertilizer concern in December 1947. Samples IV, V, and VI were mixed fertilizers; their formulations are shown in Table 1.

GENERAL INFORMATION AND INSTRUCTIONS FOR COLLABORATORS

1. The official samples will be shipped about January 14, 1948. Six samples are to be analyzed in triplicate for moisture by twenty collaborators with the use of three methods.

2. The methods are: (A) oven drying (official), (B) vacuum desiccation, and (C) air flow at 60 °C. The procedures to be followed are given below. The higher temperature prescribed in the Book of Official and Tentative Methods for potassium salts is to be disregarded in this study.

3. A complete set of samples (18 bottles) consists of the following materials: I. Potassium sulfate, II. Ordinary superphosphate, III. Organic material, IV. Mixed fertilizer, 10-6-4 grade, V. Mixed fertilizer, 10-10-10 grade, and VI. Mixed fertilizer, 5-10-5 grade. There are three identical bottles of each material, which will enable the collaborator to segregate the bottles into *three identical sets of the six samples*. A single determination by each of the three methods is to be made on the contents of each bottle. DO NOT BREAK THE SEAL ON A BOTTLE UNTIL READY TO WEIGH SAMPLES FROM ITS CONTENTS.

4. Practice determinations shall be made on a suitable material obtainable in the analyst's laboratory. Then single determinations shall be made on one set of the official samples by the three methods with due regard for the order of weighing prescribed below. Because of the necessity for making the three weighings from a bottle in rapid succession, a set of single determinations on the contents of a bottle will need to be run simultaneously. It is not absolutely necessary that all materials be run simultaneously. Each of the other two sets of official samples shall be used in a like manner to obtain replicate results.

5. The result in percentage to two decimals for each single determination on the official samples shall be reported on the attached form. Only spills, mechanical failure, or other *known* sources of gross error afford sufficient justification for not reporting a result. If such loss of data should occur, the partial set of results shall be reported, and in addition, another set of determinations by the three methods shall be made on the same bottle and the results shown on the margin of the report.

6. The order of weighing from a bottle for determinations by the different methods has been randomized among the collaborators. You are requested always to allocate your weighings in the following order:

1st weighing to Method _____
2nd weighing to Method _____
3rd weighing to Method _____

The three weighings should follow one another as rapidly as the skill of the operator will permit. In case the analyst should desire for his own information to run duplicate determinations a second set of three weighings shall be made and allocated to the methods in the foregoing order. However, except in the case of accidental loss

of a determination, only results on the first set of weighings are to be included in the formal report.

7. Collaborators are respectfully urged to follow the instructions with great care in order to avoid the introduction of spurious variability into the assembled results and consequent impairment of the validity of the tests this study has been designed to provide. Comments on, and studied criticism of, both method of study and procedures will be appreciated.

PROCEDURES

Note on weighing technique:

It is requested that the analyst catch the first weight on adding the sample to the weighing dish without any attempt to adjust the weight of the sample to an exact predetermined value.

Method A. Official oven drying:

Heat 2 grams of sample for 5 hours in water oven at temperature of boiling water (98°–100°C.). Report percentage loss in weight as moisture.

Method B. Vacuum desiccation:

Place 2 grams of sample in a tared low-form weighing dish (4 grams of sample may be used with large weighing dishes, 1.5–2 inches in diameter) and place it in a vacuum desiccator at 25–30°C. over anhydrous magnesium perchlorate (or equivalent desiccant) for 16–18 hours under not less than 20 inches of vacuum. Report percentage loss in weight as moisture.

Method C. Air flow at 60°C.:

Place 2 grams of sample in a tared fritted glass crucible and seat the crucible on a manifold in the oven at 60°C. Aspirate for 2 hours under 15 inches of vacuum. Cool in a desiccator and report the percentage loss in weight as moisture.

The air-flow method is described in a recent article entitled "The Air-flow Method for the Determination of Moisture in Fertilizers."[3] The necessary equipment consists of a constant-temperature oven, provided with a horizontally-disposed U-shaped manifold that is connected through a gage to a source of vacuum, and a supply of fritted glass crucibles. Any standard laboratory oven will suffice, but a type with vents so placed that the incoming air passes directly over the heating coils is to be preferred. The vacuum gage is a standard instrument.

A 6-crucible manifold can be readily assembled (Fig. 1) in the laboratory with the use of the following pipe fittings:

5 tees, $\frac{1}{2}'' \times \frac{1}{2}'' \times \frac{1}{4}''$
2 ells, $\frac{1}{2}'' \times \frac{1}{4}''$
2 ells, $\frac{1}{2}''$
8 close nipples, $\frac{1}{2}''$
7 nipples, $\frac{1}{4}'' \times 1\frac{1}{2}''$ long

Crucible seats are provided by placing No. 6, 1-hole rubber stoppers over the vertical $1\frac{1}{2}''$ nipples. The nipple should extend about $\frac{1}{8}''$ above the

[3] *This Journal*, 30, 640 (1947).

stopper to facilitate centering of the crucible over the opening. All connections must be air-tight when the vacuum is on. To this end the lower edge of the crucible and the upper surface of the stopper must be ground smooth to insure a proper seal at this point.

The fritted glass crucibles are the Gooch type, Pyrex glass, 15-ml. capacity, with straight sides, 45 mm. high, 30 mm. in diameter at the top and 20 mm. in diameter at the bottom, with a fine-porosity disc 20 mm. in diameter. All crucibles in a set should have about the same porosity.

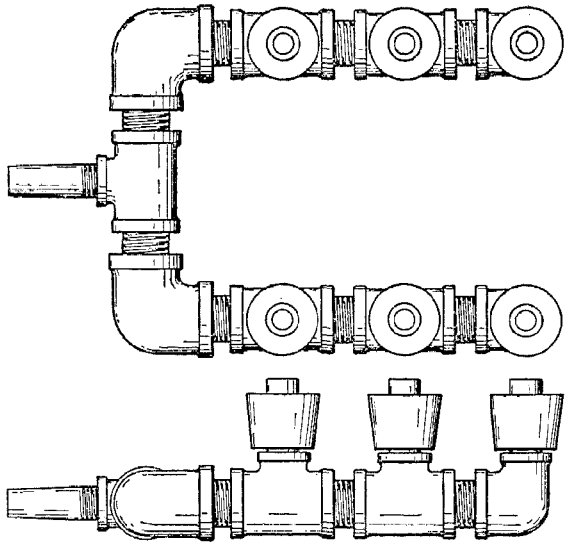

FIG. 1.—Views of manifold assembled with the use of pipe fittings.

A matched set is very desirable and can be rather simply obtained by selecting from the supply those that pass a given volume of air under constant pressure in approximately the same length of time.

COMMENTS OF COLLABORATORS

Samples.—"Bottles were too full to permit mixing before weighing out the sample."—C. A. Butt. "No difficulty was experienced in weighing samples with the exception of Sample No. 1, which was badly caked. Bottles were not too well sealed, and I would suggest that they be sealed with wax in future work."—C. T. McCloud.

Air-Flow Method.—"Our lot of 24 crucibles, when filled with water, varied in emptying times from 10 to 120 seconds."—J. B. Smith. "In setting up the air-flow apparatus we fitted a small piece of rubber tubing over the nipples, which provides a better seat than stoppers for the crucibles and also eliminates the possibility of the crucible toppling over when the vacuum is broken."—C. T. McCloud.

RESULTS AND THEIR VARIABILITY

Only twelve of the twenty-one laboratories that received sets of samples were able to perform the necessary analytical work. Two reports, re-

ceived in July, are excluded from the discussion, because, as was noted by another collaborator, the bottles were not too well sealed and certainly the closure was not adequate to carry them through the summer. The results from the ten remaining reports were obtained between January 30 and May 14—a period of 3.5 months, whereas it had been hoped that the work would all be done within a month.

TABLE 2.—*Collaborator and method means of results for percentage moisture in collaborative samples*

SAMPLE NO.	METHOD[a]	RANGE OF COLLABORATOR MEANS	METHOD MEAN		
			AVERAGE OF COLLABORATOR MEANS	STANDARD DEVIATION	COEF. OF VARIATION
I	A	2.60–4.02	3.57	0.473	13.25
(Potassium sulfate)	B	2.55–4.05	3.56	0.480	13.48
	C	2.76–4.02	3.56	0.467	13.12
II	A	5.33–6.92	6.34	0.482	7.60
(Superphosphate)	B	2.82–3.55	3.31	0.253	7.65
	C	3.66–4.70	4.20	0.328	7.80
III	A	3.63–4.47	4.21	0.229	5.44
(Cocoa shell meal)	B	0.22–2.51	1.52	0.710	46.7
	C	1.07–2.84	2.25	0.480	21.3
IV	A	6.48–8.54	7.28	0.531	7.29
(10-6-4 fertilizer)	B	3.98–4.74	4.54	0.233	5.14
	C	4.33–4.92	4.79	0.190	3.97
V	A	1.43–2.37	1.75	0.276	15.77
(10-10-10 fertilizer)	B	0.55–1.08	0.86	0.176	20.49
	C	0.70–1.19	0.99	0.148	14.94
VI	A	4.78–6.13	5.29	0.365	6.90
(5-10-5 fertilizer)	B	2.92–4.04	3.55	0.310	8.74
	C	3.45–4.21	3.90	0.229	5.88

[a] The letters A, B, and C designate the oven-drying (official), vacuum-desiccation, and air-flow methods, respectively.

Ranges of collaborator means (averages of replicates) and method means (averages of collaborator means) are given in Table 2. The individual analyses, being unnecessary for the immediate discussion, are omitted from this report. The variability of the results by the three methods is rather high (Table 2), varies from sample to sample, and amounts to 0.176 to 0.710 in terms of standard deviation. The next highest standard deviation is 0.531, which compares favorably with that of a recent set of moisture analyses on a commercial check sample.[4] The rela-

[4] E. W. Magruder's check sample No. 1, January 1947, for which the average moisture was 4.05 per cent, standard deviation (omitting analysis 76) 0.51 and average P_2O_5 was 31.42 per cent, standard deviation 0.34.

tive variability of the results by the three methods is readily appreciated with the aid of coefficients of variation (standard deviation expressed in percentage of the method mean). Thus, considering numerical values of this coefficient without regard for significance, the variability of the results by the three methods were aproximately the same on samples I and II. In the case of the cocoa shell meal (sample III) oven drying gave the most consistent results, despite the fact that this material is known to decompose in part at 100°C. In the remaining samples, all mixed fertilizers, the air-flow method gave the most consistent results.

SIGNIFICANCE OF RESULTS

In order to obtain a view of the structure of the variability and to test different effects for significance, the mean square variance of the results has been separated into four source factors— collaborators, methods, interaction of collaborators with methods, and replicates (Table 3). The statistical significance of the mean squares for the first three sources was determined by comparing them samplewise with those of the replicates. With the exception of the reference sample (I) the results are all very highly significant (odds 999:1).

TABLE 3.—*Variances contributed by certain sources of variability in moisture data*

VARIATION		MEAN SQUARES FOR SAMPLE NO.—					
SOURCE	DEGREES[a] OF FREEDOM	I	II	III	IV	V	VI
Collaborators[b]	9	1.98***	0.83***	1.60***	0.44***	0.28***	0.45***
Methods	2	0.0009	73.03***	58.00***	69.00***	6.91***	25.47***
C×M	18	0.018	0.19***	0.38***	0.34***	0.054***	0.20***
Within replicates	60	0.0552	0.0264	0.0263	0.0195	0.0175	0.0218

[a] Total of 89.
[b] Includes also sample variability, for example, change in moisture content with time either by reaction or by evaporation.
*** Significant, odds 999:1.

Collaborators.—The variance ascribed to collaborators covers, among other things, variability of sample, which admittedly was appreciable in some instances, and variability arising from the effect of atmospheric conditions on the sample during weighing and drying, which may differ widely between localities and vary markedly at the same location. The latter effect proved to be significant in four of the five instances in which this factor could be isolated. The collaborator variance (Table 3) is relatively large in the cases of samples II and III, which could be taken as an indication that these materials were susceptible to more rapid changes on exposure, especially the dried samples, than the other samples of the set. Measurements of weight gains on exposure of oven-dried samples (Fig. 2) show that absorption of atmospheric moisture could hardly ac-

count for the large collaborator variability in the case of sample II. At any rate, one could reasonably expect that rigid adherence by the analyst to the minute and tedious details of good weighing technique, both in taking the sample and in manipulating the dried sample, would markedly

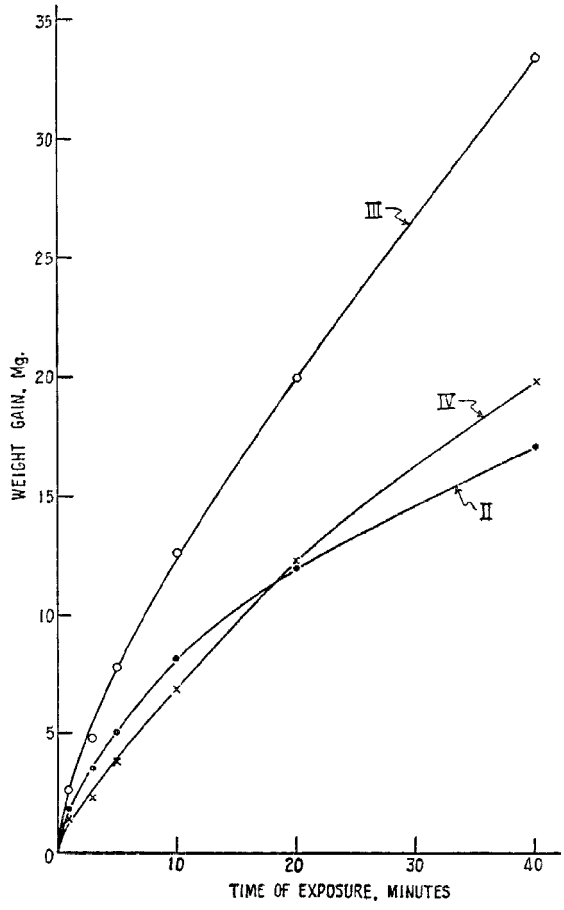

FIG. 2.—Increase in weight of oven-dried (105°) samples of superphosphate (II), cocoa shell meal (III) and a mixed fertilizer (IV) upon exposure to the atmosphere (25°C. and about 40% relative humidity).

reduce collaborator variability in the moisture determination by any method.

Methods.—The interaction of collaborator with methods (C×M, Table 3) indicates merely that the collaborators were not equally skilled in the use of all three methods, provided they all had equally satisfactory equipment. The latter condition certainly was not realized in this study.

For example, some collaborators had difficulty with the maintenance of the specified vacuum in the air-flow procedure.

The relatively large methods variance for all samples except the reference sample (sample I) in Table 3 arises mainly from the condition that with these materials oven drying yielded results 0.76 to 3.03 per cent higher than either of the other methods (Table 4). These differences were to be expected and need no further discussion at this juncture, for it is known that oven drying expels more or less water of crystallization from common hydrated fertilizer salts, whereas the other methods are supposed to show free water only. Considerable interest lies in the comparison of vacuum desiccation with the air-flow method (Table 4, last column). With the exception of the reference sample, in which case the results are practi-

TABLE 4.—*Comparison of method performances on individual samples*

SAMPLE	DIFFERENCE BETWEEN METHOD MEANS		
	A–B	A–C	B–C
I. Potassium sulfate	0.01	0.01	0.00
II. Superphosphate	3.03***	2.14***	−0.89***
III. Cocoa shell meal	2.69***	1.96***	−0.73***
IV. 10-6-4 fertilizer	2.74***	2.49***	−0.25
V. 10-10-10 fertilizer	0.89***	0.76***	−0.13
VI. 5-10-5 fertilizer	1.74***	1.39***	−0.35**

** Significant, odds 99:1.
*** Significant, odds 999:1.
Significance was tested against the combined variances of within replicates and the interaction of collaborators with methods.

cally identical, vacuum drying gave lower results than the air-flow method and the differences attained statistical significance in three of the five samples.

The differences between the results by vacuum-desiccation and air-flow methods merit further discussion, since both methods are thought to give a measure of the free water in the sample. One suggestion would be that 5 hours is insufficient time for vacuum drying. That it sometimes is, whereas other times it is not, is illustrated by the drying-rate curves for superphosphate and cocoa shell meal shown in Figure 3. These materials were similar to, but not identical with, the materials used in the moisture study. In the case of the cocoa shell meal the weight loss was still increasing at a moderate rate at the end of 5 hours—an observation that affords a plausible explanation for the poor showing of vacuum drying on this type of material (Table 2). Cocoa shell meal is hardly a fair test of the method, but similar rate curves have been encountered with mixed fertilizer samples. Thus, a longer desiccation period, preferably overnight (16–18 hours), is indicated for a general procedure. If overnight desiccation had been used in the collaborative study, the differences between the re-

sults by the two methods would no doubt be lower, but hardly below the level of significance in all instances—in the case of cocoa shell meal, for example.

Finally, a few remarks should be made relative to the reproducibility of the results recorded in Table 2. If one of the collaborators, a random selection, were to repeat the air-flow determination on sample VI, for example, the probability (with the usual assumptions as to sampling and

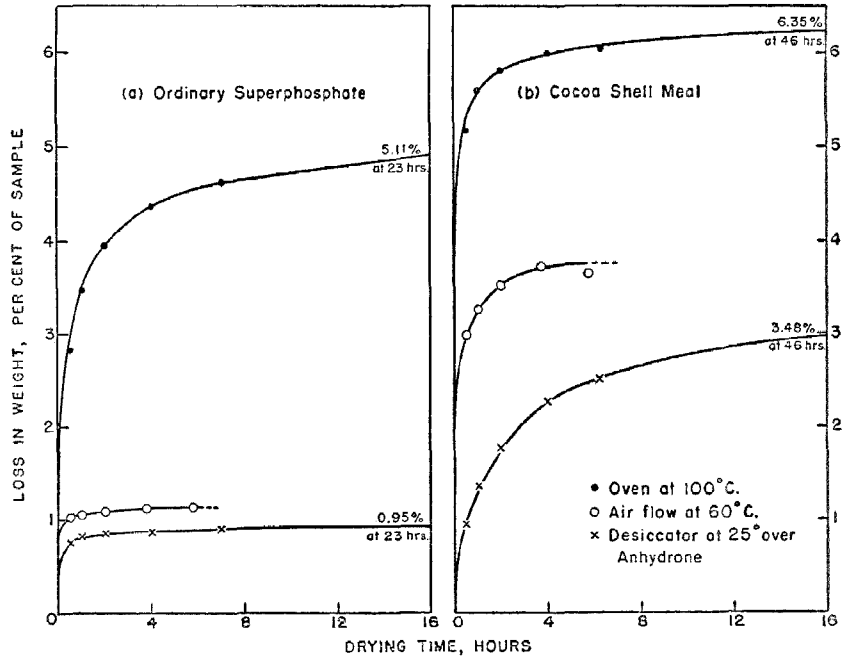

FIG. 3.—Weight loss of superphosphate and cocoa shell meal dried by different methods.

distribution) that the new result would lie within the range, recorded mean $\pm$ standard deviation, that is to say, within 3.90 ± 0.23, is about 2 to 1. Similarly, the odds in favor of the new result falling within 3.90 ± 0.46 are about 19 to 1. On the other hand, if all ten of the collaborators were to repeat the work on this sample, the odds in favor of the new mean differing from the recorded mean by less than ± 0.16 are 19 to 1. An interesting comparison can be drawn without recourse to probability. Thus, the difference between the extreme collaborator means for the air-flow procedure on all samples except I and III is of the order of 1 per cent or less, which would give rise to a difference of about 0.2 per cent, or less, between "moisture-free" results for P_2O_5 in a sample that contains 20 per cent of P_2O_5.

GENERAL CONCLUSIONS

The performance of the air-flow method in the hands of the collaborators, particularly with respect to speed and to consistency of results on mixed fertilizers, would seem to justify its recommendation for use as an official method for determining free water in fertilizers. It is realized that the method, which was designed for rapid routine analysis, will hardly separate all free water from water of crystallization in all mixtures that fertilizer manufacturers may prepare, but the same could be said of any other rapid method that might be proposed.

The simplicity of equipment necessary for overnight drying in a vacuum desiccator commends this procedure with overnight drying as an alternate method for determining free water in fertilizers. The lower temperature here used would seem to give this method preference for certain types of mixtures.

The official procedure for oven drying can perhaps be modified by a judicious choice of a higher temperature so as to yield useful approximate results for the sum of the free water and the water of crystallization on samples to which it can be properly applied.

SECTION B. STATUS OF DISTILLATION METHOD FOR FERTILIZERS

The procedure for determining moisture by distillation with toluene was adopted[5] as a tentative method in 1931 upon the recommendation of the Associate Referee on High-Analysis Fertilizers, Mr. John B. Smith, who had made a fairly extensive study of it in his search[6] for a method that could be applied to certain troublesome materials. Further work was reported[7] in 1932, at which time extensive work was contemplated for the next year. However, the contemplated work was not carried out,[8] and apparently no further work has been done on the method, although in 1939 it was recommended[9] for further study and there referred to the Associate Referee on Nitrogen.

In order to obtain some idea of the place the distillation method holds currently in fertilizer analysis, a questionnaire was sent to the collaborators last summer. The questions were: (1) Have you used this method?, (2) Do you now use it?, (3) What is your opinion as to its applicability?, and (4) Give names and addresses of those whom you know are using it. None of the twelve reporting collaborators knew anyone who now uses the method on fertilizers; one of them uses it (with benzene in place of toluene) now occasionally, but only on fish scrap—so stand the replies to questions 2 and 4. The replies to the other questions are given in Table 5. The information elicited by the questionnaire, therefore, confirms the judgment

[5] *This Journal*, **15**, 66 (1932).
[6] *This Journal*, **14**, 206 (1931); **15**, 272 (1932).
[7] *This Journal*, **16**, 220 (1933).
[8] *This Journal*, **17**, 251 (1934).
[9] *This Journal*, **23**, 51 (1940).

of one of the collaborators,[10] whose letter accompanying his report states, among other things, "the distillation method does not include all the water of hydration and (it may) include water of constitution under some circumstances." "A stronger criticism is that it is too long and requires special equipment." "So far as I know it has not been used, and I recommend that it be taken out of the Book of Methods." Accordingly, in line with the movement[11] to eliminate as far as possible tentative methods from the next edition of *Methods of Analysis*, the deletion of the distillation method is recommended solely on the basis of non-use.

TABLE 5.—*Collaborators report on use and applicability of distillation method*

COLLABORATOR NO.	HAVE YOU USED THIS METHOD?		WHAT IS YOUR OPINION AS TO ITS APPLICABILITY
	YES	NO	
1	x		Do not like it, though it yields good results
3		x	Not in position to comment
4		x	Believe it should be studied further
7	x		Gives irregular results when salt hydrates are present
9	x		Believe it would be useful in special cases
11		x	Seems impractical for many determinations per day
12		x	Requires too much undivided attention
14		x	Consumes too much time
16	x		Too cumbersome for routine work
18	x		Too complicated and requires too much apparatus
20	x		Could easily have possibilities
21		x	Not in position to comment

SECTION C. PHRASEOLOGY OF PRESENT OFFICIAL PROCEDURE

Pursuant to a request by the Committee on Classification of Methods contained in a letter from the Chairman, dated June 29, 1948, certain changes in the wording of the present official procedure for oven drying were recommended in a preliminary report furnished the Secretary August 28, 1948. These proposed changes are repeated below as items A, B, and C of Recommendation 6; item D has been added since the preliminary report was written, mainly because *water*, being the more general term, involves fewer embarrassing commitments than does *moisture*.

ACKNOWLEDGMENT

In the moisture work this year aid was promised and as far as possible contributed by the following collaborators:

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[10] John B. Smith.
[11] *This Journal*, 31, 63 (1948).

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C. A. Butt and W. H. Banks, International Minerals and Chemical Corp., East Point, Ga.
E. W. Constable, State Chemist, North Carolina Dept. of Agr., Raleigh, N. C.
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Howard Hammond, Chemist, and J. S. Overholzer, State Laboratories Dept., Bismark, N. D.
R. C. Koch, Chemist, Plant Food Div., Swift and Company, Hammond, Indiana
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C. T. McCloud, Chemist, F. S. Royster Guano Company, Norfolk, Va.
P. McG. Shuey, President, Shuey and Company, Savannah, Ga.
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John B. Smith, Chemist, and Roland Gilbert, Rhode Island Agr. Expt. Sta., Kingston, R. I.
Marvin Van Wormer, Fertilizer Div., The Farm Bureau Cooperative Assoc., Inc., Columbus 16, Ohio
R. D. Wallace, Technical Supt., Spencer Chemical Company, Pittsburg, Kans.

SECTION D. RECOMMENDATIONS*

It is recommended—

(1) That the air-flow method be made official for determining free water in fertilizers (first action).

(2) That the vacuum-desiccation method with a drying period of 16 to 18 hours be made official for determining free water in fertilizers (first action).

(3) That the official procedure for oven drying be modified to state only one drying temperature, the selection of the temperature to be determined by study, not necessarily collaborative, during the coming year.

(4) That further study be made on the applicability of the aforementioned three methods.

(5) That the tentative method for determining moisture with the use of distillation with toluene be deleted from the "Methods of Analysis."

(6) That the phraseology of the present official procedure for oven drying be modified as follows:

(A) Change parenthetical remark now worded "Not applicable to samples containing compounds other than H_2O that are volatilized at the temp. of drying." to read "Not applicable to samples that yield volatile substances other than H_2O at the temp. of drying."

* For report of Subcommittee A and action of the Association, see *This Journal*, 32, 43 (1949).

(B) Change first sentence of text now worded "Heat 2 g of prepared sample, *2.2*, for 5 hours in water oven at temp. of boiling water (98–100°)." to read "Heat 2 g of prepared sample, *2.2*, for 5 hours in oven at temp. of 99–101°."

(C) Change second sentence of text now worded "In case of potash salts, $NaNO_3$, and $(NH_4)_2SO_4$, heat at ca 130° to constant weight." to read "In case of $NaNO_3$, $(NH_4)_2SO_4$ and potash salts heat to constant weight in oven at temp. of 129–131°."

(D) Change section heading to read "Water" instead of "Moisture."

No report was given on boron in fertilizers.

The contributed paper "Determination of Borax in Mixed Fertilizers," by Donald S. Taylor, is published in *This Journal*, p. 422.

REPORT ON NITROGEN IN FERTILIZERS

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

The report to the Association of last year (*This Journal*, **31**, 236, 1948) indicated the lack of agreement on a method for total nitrogen in ammonium nitrate; therefore, it was agreed to continue studies on the analysis of this product. Owing to the possibility of the rapid formaldehyde titration procedure, it was decided to concentrate on this method and compare it to the Devarda Method.

Even more discouraging last year were the results on a high nitrate-chloride mixture. Here, again, was an opportunity for continued study, with the hope for some improvement. It was later decided to also include one sample of a low nitrate-chloride mixture.

There was a feeling among several chemists of the Association that we should revive the comparison of the Devarda Method with method **2.27** when applied to nitrate of soda. Method **2.27** is the Kjeldahl Method Modified to Include Nitrogen of Nitrates, and has the option of using either zinc or thiosulfate for reduction.

Some will remember when this comparison was made several years ago. The initial study was made by I. K. Phelps as Associate Referee, and the study was continued after A. L. Prince became Associate Referee. References to these studies are as follows: *This Journal*, **5**, 450 (1922); *ibid.*, **6**, 391 (1923); *ibid.*, **7**, 381 (1924); *ibid.*, **8**, 410 (1925). It has been said that these results were inconclusive; therefore, it was thought well to send out one sample to revive this argument.

SAMPLES

With the foregoing in mind, five samples were sent out during March to thirty-three collaborators. The descriptions of the samples follow:

(1) *Pure Ammonium Nitrate.*—This was prepared by Ralph D. Miller of Spencer Chemical Company. Owing to small impurities that yet remain, it contains approximately 34.91% Nitrogen.

(2) *Commercial Ammonium Nitrate.*—This is a regular fertilizer grade that is sold by Spencer Chemical Company. It contains approximately 34.28% Nitrogen.

(3) *High Grade Nitrate-Chloride Mixture.*—This sample was carefully prepared in the Mississippi State Chemical Laboratory from 70 per cent of reagent grade nitrate of soda, and 30 per cent superphosphate-muriate mixture. The analysis should be approximately 11.55–3.05–9.70.

(4) *Low Grade Nitrate-Chloride Mixture.*—This mixture was prepared by taking 50 per cent of last year's sample No. 3 and the other half is superphosphate with a bit of limestone added. The analysis is approximately 5.50–11.30–5.40.

(5) *C. P. Sodium Nitrate.*—This is a reagent grade product (J. T. Baker) and the nitrogen content should approach the theoretical, 16.47%.

DIRECTIONS FOR COLLABORATORS

A. Samples No. 1 and 2 are to be run by the Devarda Method **(2.31)**. Also, they are to be run by formaldehyde titration procedure as follows:

NITROGEN IN AMMONIUM NITRATE

Formaldehyde Titration Method

(May also be adapted to Ammonium Sulfate)

Weigh out 7.004 or 14.008 g of sample and make up to 250 or 500 ml. Pull off 25 or 50 ml and put into a 300–500 ml Erlenmeyer (ca. 1.5 g may be rapidly weighed and washed directly into flask). Add ca. 1 ml of reagent formaldehyde for each 0.1 g of sample in aliquot. Make total volume 150–200 ml and allow 5 min. before titrating with 0.25–0.50 *N* sodium hydroxide, using 5 drops of phenolphthalein as indicator. Titrate until there is no perceptible color change at the point of contact, or until the proper color of pink persists. Run a blank on the formaldehyde.

$$\% \text{ Nitrogen} = \frac{\text{Net ml of NaOH} \times \text{Normality} \times 2.8016}{\text{Wt. of Sample}}$$

B. Samples No. 3 and 4 are to be run by **2.27**, part 2, as amended by Mr. Ford.

We would also like these samples to be run by **2.27**, part 1, using 2 gm of salicylic in 30 ml of approximately 15% fuming sulfuric (use 0.7004 gm sample in No. 3).

Mr. Shuey would also like for his procedure to be tried again, using a Millican Spiral or Filter Disc gas washing bottle instead of the Drechsel bottle.

C. Run the nitrate of soda by Mr. Ford's procedure and by the Devarda Method.

We believe that all nitrates should be dried and reported as if moisture free. Please give a moisture on samples No. 3 and 4.

We are particularly interested in adopting the formaldehyde procedure; therefore, please give us your version of the best detail of the method. We can then set up an overall compromise procedure.

RESULTS

A reasonably good response from collaborators was obtained, and the Associate Referee feels deeply grateful to the following persons, who have made this report possible.

Ralph D. Miller, Spencer Chemical Co., Pittsburg, Kansas

W. A. Morgan, Ammonia Dept., Du Pont, Wilmington

R. L. Willis & A. C. Wark, New Jersey Exp. Sta., New Brunswick
W. S. Thompson, Dept. of Agr., Columbus, Ohio
C. A. Butt & W. H. Banks, Int. Min. & Chem. Co., East Point, Ga.
C. Reynolds Clark, State Chemist, Atlanta, Ga.
R. C. Koch, Swift & Co., Hammond, Ind.
A. N. Lineweaver, F. S. Royster Guano Co., Norfolk, Va.
Geo. E. Grattan & R. Payfer, Dept. of Agr., Ottawa
G. C. Bollinger & C. M. Fleming, Am. Agr. Chem. Co., Baltimore
Howard Hammond, Chemist, State Laboratories, Bismarck, N. D.
Frances L. Bonner, Feed & Fert. Lab., Baton Rouge, La.
O. W. Ford, Indiana Exp. Sta., Lafayette
W. C. Geagley & Percy O'Meara, Bureau Chem. Labs., Lansing, Mich.
Gordon Hart, Asst. State Chem., Tallahassee, Fla.
P. R. Bidez, Senior Chemist, Auburn, Ala.
A. F. Spelman, Senior Chemist, Mass. Exp. Sta., Amherst
V. G. Hiatt, Asst. Chem., Dept. Agr., Salem, Oregon
Guy Mitchell, Chief Chemist, Lion Oil Co., Eldorado, Ark.
H. R. Allen, Chemist, Ky. Agr. Exp. Sta., Lexington
Philip McG. Shuey, Savannah, Ga.
C. O. Willits, in charge Analyt. Section, Eastern Reg. Lab., Philadelphia
Roland Gilbert, Rhode Island Exp. Sta., Kingston
C. O. Hurst, Mississippi State Chem. Lab., State College

The results are shown in Tables 1, 2, and 3. Both ammonium nitrates are reported in Table 1 for ease of comparison of the Devarda and formaldehyde procedures. The high nitrate-chloride mixture is the only sample reported in Table 2. Finally, the low nitrate-chloride mixture is reported in Table 3 with the nitrate of soda.

DISCUSSION OF RESULTS

Ammonium Nitrate.—If the results on the ammonium nitrates are examined, it is seen that the agreement in the majority of instances is very good. Some have always claimed difficulty with the Devarda Method, and it is not too easy to decide on the end point in the formaldehyde titration.

It might be well to give a detailed answer to the questionnaire regarding the formaldehyde procedure; however, a summary will probably suffice.

Seven collaborators prefer to titrate until there is no perceptible color change at the point of contact while four prefer a definite color that persists. Three suggested potentiometric ranges from *p*H 8 to 8.4. Analyst No. 15 probably has the best suggestion when he says allow each analyst to adjust the titration to his own wishes when compared to a reagent grade sample.

Seven collaborators prefer quick weighings of approximately 1.5 gram of sample and eight prefer making an exact amount up to volume and taking an aliquot.

There are seven collaborators who think the titration procedure is preferred while an equal number think that it should be regarded only as an

alternative procedure to the Devarda Method. Two definitely prefer the latter.

Nitrate-Chloride Mixtures.—In the case of the high nitrate-chloride mixture, few obtained the theoretical amount by the fuming sulfuric pro-

TABLE 1.—*Total nitrogen in ammonium nitrate*

ANALYST	SAMPLE NO. 1		SAMPLE NO. 2	
	DEVARDA METHOD	FORMALDEHYDE TITRATION	DEVARDA METHOD	FORMALDEHYDE TITRATION
	per cent	*per cent*	*per cent*	*per cent*
1	34.54	34.83	34.23	34.36
2	34.99	34.93	34.18	34.28
3	34.92	34.70	34.14	33.90
4	34.98	34.10	34.21	33.70
5	34.99	34.96	34.25	34.31
6	34.89	34.86	33.99	34.08
7	34.96	34.94	34.25	34.26
8	34.87	34.76	34.40	34.24
9	34.70	34.50	34.20	33.80
10	35.05	34.90	34.40	34.29
11	34.78	34.90	34.14	34.26
12	34.52	34.33	34.33	34.20
13	35.02	34.97	34.32	34.28
14	35.05	34.69	34.44	34.02
15	34.73	34.86	34.21	34.24
16	34.97	35.04	34.30	34.36
17	34.84	34.73	34.42	34.08
18	34.65	34.59	34.05	34.07
19	—	34.89	—	34.26
20	34.96	35.15	34.28	34.38
21	34.97	34.97	34.45	34.48
22	35.01	34.87	34.40	34.14
23	34.56	35.02	33.91	34.32
24	34.72	34.90	34.10	34.10

cedure. None approached the theory by the Shuey Method, although the results as a whole were not extremely low.

The Ford version of the Kjeldahl Method to Include Nitrates (**2.27**, part 2) seems promising. Over a third of the analysts obtained or approached the theoretical amount. On the other hand, there were some very low results.

The results by the Ford version on the low nitrate-chloride mixture are even better than on the high mixture. This, of course, would be expected. There are also a few good results by the other two procedures. A few high results are indicative that this sample may not have been properly mixed.

Nitrate of Soda.—There were three analysts who obtained as high or

slightly higher results on the nitrate of soda by the Ford procedure. However, most analysts obtained slightly higher results by the Devarda

TABLE 2.—*Sample No. 3. Total nitrogen in high grade nitrate-chloride mixture*

ANALYST	FORD METHOD	SHUEY METHOD	FUMING SULFURIC	MOISTURE
	per cent	*per cent*	*per cent*	*per cent*
1	10.88	10.35	7.92	0.40
2	11.57	11.26	5.56	0.34
3	11.53	11.05	11.45	0.68
4	10.76	—	—	0.44
5	11.47	11.29	4.56	0.56
6	11.58	—	11.61	0.67
7	11.23	11.23	11.00	1.53
8	9.85	—	9.68	0.60
9	11.40	10.40	9.80	1.01
10	11.39	11.20	9.49	0.60
11	8.18	10.25	—	0.46
12*	11.24	11.12	11.42	—
13	11.51	—	—	0.52
14	11.46	—	11.30	0.61
15	11.20	11.16	11.34	0.64
16	11.30	—	7.18	1.22
17	11.07	—	—	0.69
18	10.98	—	—	—
19	—	—	—	—
20	11.37	10.32	10.76	0.67
21	11.13	11.29	—	—
22	11.52	—	11.68	0.40
23	11.30	—	—	0.71
24	11.48	—	—	0.49

* Dry basis.

Method. If one will refer back to the previous references on this subject he will find that this was somewhat the case twenty-five years ago.

CONCLUSIONS

(1) It is obvious that the Devarda Method and the formaldehyde titration procedure give about equal results on the analysis of ammonium nitrate for total nitrogen. Most of the analysts prefer the latter's adoption as either a preferential or alternative method. The end point is not ideal; however, it can be adapted to any laboratory. Most laboratory analysts who have only a few ammonium nitrates would probably prefer to use the Devarda Method. Most analysts will agree that the formaldehyde procedure can be used to good advantage where there are large numbers of samples.

(2) The high nitrate-chloride mixtures will not be easy to solve. It may

TABLE 3.—*Total nitrogen*

ANALYST	SAMPLE NO. 4 (LOW NITRATE-CHLORIDE)			SAMPLE NO. 5 (NITRATE OF SODA)	
	FORD METHOD	SHUEY METHOD	FUMING SULFURIC	DEVARDA	FORD METHOD
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
1	5.21	4.99	4.69	16.34	16.27
2	5.73	5.69	3.90	16.43	16.21
3	5.55	5.28	5.62	16.41	16.31
4	5.25	—	—	16.44	—
5	5.76	5.63	3.67	16.42	16.36
6	5.48	—	5.65	16.46	16.01
7	5.37	—	5.57	16.22	16.23
8	5.24	—	5.30	16.25	15.90
9	5.60	5.00	4.80	16.40	16.00
10	5.70	5.65	5.65	16.46	16.45
11	5.50	5.68	—	16.36	16.17
12	5.67	5.48	5.68	16.40	16.38
13	5.60	—	—	16.45	16.47
14	5.62	—	5.66	16.19	16.16
15	5.52	5.50	5.63	16.34	16.31
16	5.51	—	4.45	16.40	16.35
17	5.56	—	—	—	16.38
18	5.55	—	—	16.37	16.42
19	—	—	—	—	—
20	5.73	5.14	5.46	16.40	—
21	5.48	—	—	16.48	16.39
22	5.84	—	5.89	16.44	15.96
23	5.59	—	—	16.20	16.29
24	5.62	—	—	16.36	16.33

be that some variation of 2.27 as suggested by O. W. Ford will be the most practical method. Perhaps there is a limit beyond which no practical method will give good results.

(3) There is no occasion for extreme arguments in the case of nitrate of soda. A few samples can be run with a reasonable degree of accuracy by 2.27. However, analysts who have had the nitrate of sodas by the hundreds will prefer the Devarda Method. The checks may be wider by the latter; however, results will more nearly approach the theoretical amount of nitrogen.

RECOMMENDATIONS*

It is recommended—

(1) That the formaldehyde titration method be adopted as official, first action, for determining nitrogen in ammonium nitrate.

(2) That the study of nitrogen in high nitrate-chloride mixtures be continued.

* For report of Subcommittee A and action of the Association, see *This Journal*, 32, 43 (1949).

REPORT ON POTASH

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

In accordance with the recommendations of the Association "that work on the details of the method for the determination of potash in fertilizers be continued" (*This Journal*, **31**, 42, 1948) referee work was conducted in 1948 by collaboration.

A copy of the proposed work was sent to each chemist who had expressed a willingness to collaborate. This report summarizes the results of the twelve chemists who found time to do the work and report to the Associate Referee.

Collaborative work was directed to a comparison of potash and insoluble phosphoric results obtained on fertilizers when like portions of the same sample were ground in the old type Burr mill and the new type Micro-samplmill. The new type Micro-samplmill is a hammer mill in which the hammers are fixed on a rotor. This mill was one of two mills exhibited and demonstrated at the 1947 A.O.A.C. Meeting.

This work was undertaken because early trials in the Indiana Laboratory on some late 1947 fall shipments of fertilizer indicated that slightly lower insoluble phosphoric acid and higher potash values might be obtained by its use.

The lower insoluble phosphoric acid values agreed with work previously reported by Ross and Jacobs, etc., when samples were more finely ground.

1948 COLLABORATIVE WORK ON POTASH IN FERTILIZERS

With the recent use of the Micro-samplmill an increase in both potash and available phosphoric acid values have been obtained in the Indiana Laboratory over that obtained with portions of the same sample ground in a Burr mill. If this is universally true, it would be worthy of some collaborative work.

Six samples have been ground for analysis in each of the two types of mills.

Type 1 *Burr Mill*		Type 2 *Micro-samplmill*	
3841	8-10-10	3841	8-10-10
3720	4-12- 8	3720	4-12- 8
3683	3- 9-18	3683	3- 9-18
3684	3-12-12	3684	3-12-12
4069	0- 9-27	4069	0- 9-27
3999	3-12-12	3999	3-12-12

It is recommended:

1. Burr Mill Samples—
   Make three individual potash determinations on each sample.

Make three individual insoluble phosphoric acid determinations on each sample.

2. Micro-samplmill Samples—
Make three individual potash determinations on each sample.
Make three individual insoluble phosphoric acid determinations on each sample.

It should be understood that the present official method for potash will be used, and that all collaborators will use 95% ethyl alcohol or 95% formula 30 alcohol and the corresponding acid-alcohol for the potash determinations.

Dr. K. D. Jacob, Associate Referee on phosphoric acid, has sanctioned the work on the insoluble phosphoric acid.

COMMENTS ON RESULTS

The results of the twelve chemists who collaborated on the potash and insoluble phosphoric acid are reported in Tables 1 and 2.

Taken as a whole, the potash results are slightly more disappointing than the Associate Referee had expected. Only two of the six samples showed a slightly higher average of potash values when ground by the Micro-samplmill over that of the Burr mill.

This, however, is in agreement with one of the collaborators, who reported that considerably less potash was obtained in his laboratory when samples were ground too fine. It seems difficult to believe that fine grinding would produce less potash. This is also a little contradictory to past work on potash, where slightly more potash and more concordant results were obtained. One collaborator offered the suggestion that perhaps some segregation has taken place. If this is the case, there is a definite need for a thorough study of the effect of fineness of grinding and an investigation of the proper procedure of drawing a sample for analysis when a sample has been prepared for analysis. This should be taken into consideration since there is no definite instruction regarding this at present. Reports to the Associate Referee indicate that all but one of the manufacturing chemists remixed on paper all samples sent to them for analysis while the control chemists followed a procedure of drawing the sample directly from the bottle. It is the observation of the Associate Referee that many samples that have been thoroughly mixed can be segregated by remixing on paper. A survey of the results obtained indicate that the greatest variations from the average occurred when the collaborator removed the sample from the bottle and remixed it. Many but not all collaborative chemists concur in this procedure. The Associate Referee regrets the fact that definite instruction was not given to the collaborators to draw all samples directly from the bottle. In nearly all cases all collaborators obtained slightly less insoluble phosphoric acid on the Micro-samplmill samples. These differ-

TABLE 1.—*Effect of fineness of grinding on the determination of potash in fertilizers*

ANALYST NUMBER	SAMPLING PROCEDURE	BURR MILL AVERAGE OF THREE ANALYSES PER CENT POTASH						MICRO-SAMPLMILL AVERAGE OF THREE ANALYSES PER CENT POTASH					
		SAMPLES						SAMPLES					
		3683	3684	3720	3841	3999	4069	3683	3684	3720	3841	3999	4069
1	*	16.72	12.20	7.89	10.13	11.19	26.58	16.24	12.03	7.85	9.95	11.29	26.68
1	†	16.72	12.12	7.99	10.10	11.18	26.97	16.21	12.03	7.82	9.92	11.32	26.79
2	*	16.63	12.37	7.83	10.25	11.08	26.67	16.10	11.94	7.63	9.84	11.10	26.58
3	*	17.23	12.65	8.39	10.46	11.32	27.20	16.91	12.30	8.26	10.18	11.63	27.31
4	*	16.64	11.92	7.85	10.16	11.24	26.73	16.38	11.72	7.65	9.99	11.27	26.78
5	†	17.07	12.64	7.94	10.62	11.32	28.21	17.13	12.18	7.90	10.25	11.27	28.66
6	†	16.64	12.26	7.82	10.21	11.02	26.65	16.24	11.89	7.72	9.90	11.15	26.54
8	*	16.92	12.45	7.88	10.20	11.34	27.47	16.41	12.01	7.72	10.04	11.40	27.22
9	†	17.09	12.48	7.96	10.46	11.20	27.08	16.79	12.02	7.81	10.04	11.37	27.19
10	*	17.00	12.75	8.02	10.48	11.58	27.48	16.60	12.45	8.02	10.33	11.88	27.41
12	*	16.80	12.34	8.00	10.09	11.07	27.21	16.39	12.22	7.83	10.13	11.33	26.96
13	*	16.25	12.08	7.56	9.98	11.28	25.80	16.10	12.05	7.60	9.95	11.32	25.86
15	†	16.98	12.45	8.20	10.49	11.72	27.57	16.60	12.52	8.20	10.23	11.82	27.65
High		17.23	12.75	8.39	10.62	11.72	28.21	17.13	12.52	8.26	10.33	11.88	28.66
Low		16.25	11.92	7.56	9.98	11.02	25.80	16.10	11.72	7.60	9.84	11.10	25.86
Average		16.83	12.40	7.93	10.31	11.30	27.03	16.50	12.11	7.85	10.06	11.42	27.09
Maximum variation		0.98	0.67	0.83	0.64	0.70	2.41	1.03	0.63	0.66	0.49	0.78	2.80

* Sample weighed from bottle, mixed on paper and several dips taken for each sample.
† Sample weighed directly from bottle.

TABLE 2.—*Effect of fineness of grinding on the determination of insoluble phosphoric acid in fertilizers*

ANALYST NUMBER	SAMPLING PROCEDURE	BURR MILL AVERAGE OF THREE ANALYSES PER CENT INSOLUBLE P_2O_5						MICRO-SAMPLMILL AVERAGE OF THREE ANALYSES PER CENT INSOLUBLE P_2O_5					
		SAMPLES						SAMPLES					
		3683	3684	3720	3841	3999	4069	3683	3684	3720	3841	3999	4069
1	*	0.35	0.41	0.70	0.47	1.22	0.39	0.34	0.35	0.63	0.48	1.15	0.30
1	†	0.32	0.41	0.68	0.44	1.18	0.38	0.37	0.38	0.62	0.50	1.17	0.37
2	*	0.35	0.30	0.71	0.41	1.34	0.26	0.29	0.28	0.67	0.44	1.22	0.25
3	*	0.45	0.55	0.94	0.60	1.42	0.52	0.55	0.43	0.95	0.77	1.34	0.53
4	*	0.40	0.43	0.80	0.51	1.42	0.32	0.37	0.33	0.72	0.45	1.38	0.32
5	†	0.35	0.34	0.76	0.46	1.21	0.29	0.32	0.36	0.72	0.54	1.31	0.31
6	†	0.39	0.40	0.88	0.44	1.29	0.33	0.34	0.29	0.73	0.50	1.22	0.30
8	*	0.34	0.42	0.83	0.47	1.34	0.34	0.33	0.36	0.77	0.48	1.30	0.32
9	†	0.38	0.33	0.88	0.53	1.12	0.40	0.37	0.30	0.77	0.45	1.08	0.42
10	*	0.35	0.35	0.92	0.42	1.28	0.25	0.36	0.35	0.72	0.43	1.33	0.27
12	*	0.32	0.38	0.78	0.44	1.16	0.30	0.36	0.36	0.76	0.46	1.22	0.32
13	*	0.38	0.40	0.68	0.53	1.18	0.36	0.36	0.35	0.66	0.50	1.13	0.26
15	*	0.34	0.38	0.84	0.44	1.06	0.25	0.27	0.24	0.75	0.44	0.92	0.22
High		0.45	0.55	0.94	0.60	1.34	0.52	0.55	0.43	0.95	0.77	1.33	0.53
Low		0.34	0.30	0.68	0.41	1.06	0.25	0.27	0.24	0.63	0.43	0.92	0.22
Average		0.37	0.39	0.81	0.48	1.27	0.34	0.35	0.33	0.74	0.50	1.21	0.32
Maximum variation		0.13	0.25	0.26	0.19	0.28	0.27	0.28	0.19	0.32	0.34	0.41	0.31

* Sample weighed from bottle, mixed on paper and several dips taken for each sample.
† Sample weighed directly from bottle.

ences were not as great as might have been obtained had samples been selected with larger insoluble phosphoric acid values.

In general, a quicker job of grinding is done by the Micro-samplmill. This will remove the danger of moisture changes during the process of preparation of the sample. Since the grinding requires less than one minute to grind a one pound sample, the tendency, due to heating, to change the composition of the sample is reduced to a minimum. With the use of the one-eighth inch screen, no samples were encountered in the Indiana Laboratory in the spring of 1948 that could not be ground in less than a minute. This is less than one-tenth of the time formerly required to grind the same amount of sample. The cleaning of the mill between samples is no greater per sample than with the Burr type mill. For this reason, the Associate Referee will recommend that these studies be continued to take in other types of mills and a greater variety of fertilizer mixtures.

LIST OF COLLABORATORS

1. W. R. Austin and Madalane Buford, Armour Fertilizer Works, Nashville, Tenn.
2. H. C. Batton, Swift & Company, 219 Wainwright Building, Norfolk, Va.
3. R. D. Caldwell, Armour Fertilizer Works, 350 Hurt Building, P. O. Box 1685, Atlanta 1, Ga.
4. William Chapman, Consolidated Rendering Company, 178 Atlantic Avenue, Boston 10, Mass.
5. T. L. Ogier and J. F. Fudge, Texas Agricultural Experiment Station, College Station, Tex.
6. R. C. Koch, Swift & Company, 150 Marble St., Hammond, Ind.
7. John W. Kuzmeski, Massachusetts State College, Amherst, Mass.
8. H. L. Moxon, Virginia-Carolina Chemical Corporation, Richmond, Va.
9. Richard M. Smith, Agricultural Department, Chemical Division, Tallahassee, Fla.
10. A. N. Lineweaver and S. F. Thornton, F. S. Royster Guano Company, Norfolk, Va.
11. L. S. Walker, University of Vermont, Burlington, Vt.
12. A. T. Blackwell, The Davison Chemical Corporation, Baltimore, Md.
13. C. W. Byers, Armour Fertilizer Works, Carteret, N. J.
14. Wm. McAllister, Director of Laboratories, Southern States Laboratories, 2101 East Fort Ave., Baltimore 30, Md.
15. R. F. Serro and O. W. Ford, Purdue University, Department of Agricultural Chemistry.

COMMENTS OF COLLABORATORS

(1) "First set of results obtained by mixing sample on paper and dipping. This is common practice on all our work at Nashville. The second set of results were obtained by weighing directly from the bottle as you do in Lafayette. Some of the samples were set up hard when received. The matter of size of sampling rod in use in the field for certain types of materials in the fertilizer mixtures needs to be investigated."

(2) "The samples, particularly the micro-mill grindings, were so tightly packed in the bottles that we thought it best to withdraw the entire portion from each bottle, break up the solidified portions, mix and re-bottle before using."

(4) "In most all cases the insoluble phosphoric acid was lower on the finer

ground sample but in only one case was the potash higher. Checking back I found in four cases there was enough total phosphoric acid to make the potash difference. In the other case a nitrogen overage will account for some of the difference. I think that in cutting down to such small samples segregation has reared its ugly head and caused the results to come out the way they did. The results on the lower insoluble phosphoric figures check out what we have found here in working on high analysis goods."

"I weighed the samples for analysis after I had removed them from the small bottle and thoroughly mixed them. Then I placed the sample in a larger bottle so that in withdrawing the sample for analysis I could keep the sample well mixed at all times. This was borne out by several checks that I made weighing out a new portion each time."

"The micro-samplmill would not operate successfully under our conditions. Samples high in water gummed up around the rotor. Suggested modification by the manufacturer was not feasible as it would have altered the sample."

(6) "Since our potash figures did not agree with work done in your laboratory, we repeated the potash determinations with substantially the same results."

(8) "As to sample grinding, this laboratory has been collaborating with a company manufacturing a machine similar to the type of machine which you have. Exhaustive tests have been made on various fertilizers and fertilizer materials. The grinding for laboratory weighings is excellent, but we have found that the solubility, especially on nitrogen, will change. This change is not unifrom; some samples will increase materially while others stay fairly constant and for this reason we are unable to grind very many samples in our regular work."

"I hope the A.O.A.C. will make an investigation of machines of this type and if found satisfactory will adopt methods for their use."

(9) "Our results would appear to indicate that the Micro-samplmill grinds samples too fine for full leaching of potash in routine work by the A.O.A.C. method."

"These samples were run under routine conditions in which there is not time to stop and remix on paper every bottle of ground sample sent to the chemist. The worth of any mill to the commercial laboratories depends a lot on small items such as this."

(10) "A study such as the one proposed by you is very necessary now that it seems we are going into the use of sample grinding mills."

(12) "Samples were spread and mixed before portions were taken for analysis."

(13) "Samples spread on paper and mixed before portions were taken for analysis."

(15) "Samples drawn directly from bottles as all laboratory samples are so prepared for analysis. Results on insoluble phosphoric acid were in accord with original work, but in only three of the six cases was the potash higher on the Micro-samplmill samples. It would appear that more care in mixing the sample for analysis is necessary when samples are ground in the Micro-samplmill."

RECOMMENDATIONS*

It is recommended—

(1) That a survey be made of the different types of mills being used for preparation of the sample.

(2) That collaborative potash work on samples prepared by the different mills be conducted on a greater variety of samples.

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* For report of Subcommittee A and action of the Association, see *This Journal*, **32**, 43 (1949).

ACKNOWLEDGMENT

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

No reports were given on magnesium and manganese, acid- and base-forming quality, sulfur, copper and zinc, boron, or inert materials.

A paper entitled "Limitations of the Modified Kjeldahl Method for Determining the Nitrate Nitrogen in Nitrate-Chloride Mixtures," by H. K. White and O. W. Ford, is published in *This Journal*, p. 397.

REPORT ON CEREAL FOODS

By V. E. MUNSEY, (Food and Drug Administration, Federal Security Agency, Washington 25, D. C.), *Associate Referee*

In anticipation of the publication of the Seventh Edition of the "Methods of Analysis" in 1950, the Referee has reviewed the Cereal Food Chapter No. 20, looking toward either final adoption or deletion of methods. There are 7 methods for various proteins (**20.32–.38**, incl.) which were developed many years ago and have appeared as tentative methods in various editions of the "Book of Methods." These methods do not determine a specific component of flour. They are antiquated and little used except in some research work. It is recommended that these methods be dropped. There have been considerable advances in the past ten years in technical methods for the study of protein systems, which should be applied to research on cereal proteins.

The tentative method for the detection of rye flour in wheat flour, **20.60** is of doubtful value, and it is recommended it be dropped. Further consideration will be given other methods that are not official during the coming year.

RECOMMENDATIONS*

The following recommendations are based on the work of the Associate Referees:

It is recommended—

(1) That both procedures proposed by the Associate Referee last year (*This Journal*, **31**, 79) for the determination of phosphorus in cereals and cereal products be adopted as official (final action) and the study be discontinued.

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

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* For report of Subcommittee D and action of the Association, see *This Journal*, 32, 61 (1949).

(3) That the tentative method for the determination of fat acidity in grain and flour **(20.18–20.21,** incl. and **20.76)** be adopted as official (first action) and the study continued.

(4) That a study of the application of the method for reducing and non-reducing sugar in flour, **20.28–.30,** incl., be made to the determination of sugars in bread and other bakery products, with special consideration given to the article on this subject published by R. M. Sandstedt and G. C. Fleming (*This Journal,* **30,** 550–2).

(5) That the tentative method for benzoic acid in flour **(20.53)** be replaced by the method proposed by the Associate Referee for wheat flour and be adopted as official (first action) as a qualitative test, and the study continued.

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

(7) That the methods for the determination of lactose in bread be further studied.

(8) That the method for determination of fat and fat number in bread as proposed by the Associate Referee in this year's report, replace **20.86** and be adopted as official (first action).

(9) That the method proposed by the Associate Referee in this year's report for the determination of proteolytic activity of flour and malted wheat flour be adopted as official (first action), and that the work be continued.

(10) That the methods for soybean flour—**(a)** moisture, **20.77 (20.4)** with the exception that a 5 g sample be dried 130° for 2 hours; **(b)** ash, **20.78 (27.9);** (c) nitrogen, **20.79,** proceed as directed under **2.26,** using 10 g K_2SO_4 or Na_2SO_4, and 0.7 g HgO or its equivalent in Hg, with the additional option of using sodium alizarin sulfonate; **(d)** oil, **20.82,** except that ca 2 g full fat flour or 5 g low or defatted soy flour be extracted for 5 hours—be adopted as official, first action, and that the study be continued.

(11) That the study on the detection and determination of soybean flour in cereal products be discontinued.

(12) That the method proposed by the Associate Referee in this year's report for the determination of added inorganic material in phosphated flour be adopted as official, first action, and the study continued with elimination of self-rising flour.

(13) 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 bakery products containing eggs.

(14) That the study of methods for the determination of albumen in noodles and macaroni products be conducted.

(15) That the study on the determination of moisture, fat, crude fiber, ash, and protein in bakery products be continued.

(16) That the study on the determination of moisture in flour products containing sodium bicarbonate as one of its constituents be continued.

(17) That the study on the determination of bromates in flour be continued.

(18) That the tentative methods **20.32–20.38**, inclusive, and **20.60**, be dropped.

REPORT ON THE DETERMINATION OF BENZOIC ACID IN FLOUR

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

The method for the determination of benzoic acid submitted to collaborative study last year was modified on the basis of recommendations of the collaborators. The changes are largely a clarification of the directions.*

Ten collaborators were asked to analyze three samples of flour by the following procedure. The three samples consisted of an untreated flour, flour treated with usual amount of benzoyl peroxide, and a flour with about ½ normal application of benzoyl peroxide. In the preparation of the treated flours, 825 mg of the commercial preparation containing benzoyl peroxide was mixed with 300 g of flour by sifting through a flour sifter 12 times and then mixing and rolling on paper. 100 g of this master mix was mixed in a McCellan mixer with 2500 g flour, then mixed in ball mill and rolled and mixed on paper. This final mixture represented sample No. 3, containing 21.4 p.p.m. of benzoic acid based on Associate Referee's analysis of the commercial benzoyl peroxide product.

Sample No. 2 consisted of mixing equal parts of sample No. 3 and untreated flour. It should contain 10.7 p.p.m. benzoic acid. Although it is difficult to uniformly distribute these small amounts of benzoyl peroxide in flour, the above procedure seemed to accomplish a uniform mixture.

Results received from nine chemists are given in Table 1. The results, with the exception of collaborators 6, 7, and 9, indicate a fairly close agreement in found and added amounts. One collaborator pointed out the need for absolute dryness in the test tube before nitration. This essential point may explain the low results of two collaborators. One collaborator recommended the use of 30×200 mm test tube in place of 25×150 mm as an improvement. Another collaborator suggested the use of 200 ml centrifuge bottle and decantation of the ether in preference to filtration through a Büchner funnel.

Acknowledgment is made of the splendid cooperation of the following collaborators: C. G. Harrel, Pillsbury Mills, Inc.; R. C. Koehn, General Mills, Inc.; E. Stegemeyer, Kroger Food Foundation; W. L. Rainey, Commander Larabee Milling Co.; R. L. Gray, Novadel-Agene Corp.; and F. J. McNall, M. A. Braun, and E. F. Steagall, of the Food and Drug Administration.

* Details of the method are published in *This Journal*, **32**, 84 (1949).

TABLE 1.—*Benzoic acid in flour samples containing no benzoic acid, 10.7 p.p.m. and 21.4 p.p.m.*

COLLABORATOR	NO BENZOIC ACID	10.7 p.p.m.	21.4 p.p.m.
	SAMPLE 1	SAMPLE 2	SAMPLE 3
	p.p.m.	*p.p.m.*	*p.p.m.*
1	3.0	11.0	14.2
	3.2	11.0	14.6
	3.0	12.0	19.6
	av. 3.0	11.3	16.1
2	none	8.8	15.4
3	1.1	10.8	18.1
	0.8	11.9	18.8
	av. 0.9	11.3	18.5
4	1.0	9.8	19.8
	0.8	10.0	—
	av. 0.9	9.9	19.8
5	0	8.8	16.8
	—	—	14.4
			av. 15.6
6	0	4.0	6.5
7	1.5	4.0	6.0
	1.7	4.3	6.3
	av. 1.6	4.2	6.2
8	0.8	10.0	18.8
	0.9	10.6	19.8
		9.8	18.4
			20.0
	av. 0.9	10.1	19.2
9	2.3	6.3	12.1
	1.9	4.5	12.9
	2.4	7.0	12.4
	av. 2.2	5.9	12.4

It is recommended† that the method be adopted as official, first action, as a qualitative test for benzoic acid, and that study be continued on the method for improvement of the quantitative measurement.

† For report of Subcommittee D, and action of the Association, see *This Journal*, 32, 61 (1949).

REPORT ON ADDED INORGANIC MATERIAL IN PHOSPHATED FLOUR

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

In accordance with the recommendations of the Association (*This Journal*, **31**, 59, 1948) "That the method proposed by the Associate Referee for determination of the amount of added inorganic material in phosphated flour be further studied," work has been continued this year by collaboration.

In this Associate Referee's former report (*This Journal*, **31**, 259, 1948) a few collaborative results were given on flour containing added monocalcium phosphate. A modification of the carbon tetrachloride separation method of Gustafson (*This Journal*, **19**, 82, 1932) was used, collecting the sediment from the separator on weighed filter paper. This material was air dried at room temperature and weighed.

In the present instance the work has been expedited by the use of a Gooch crucible. It has been found that the carbon tetrachloride is completely removed and the weight of the Gooch becomes constant by aspirating 2 or 3 minutes. The results thus obtained by direct weighing were checked, for possible error due to any flour in the sediment, by heating the crucible to 700 degrees C. and weighing as calcium metaphosphate. (Hill et al., *Ind. Eng. Chem.*, **39**, 1667, 1947).

Commercial monocalcium phosphate monohydrate as used in the commercial phosphating of flour was used in the preparation of the samples. In the following tables Procedure No. I refers to the direct weighing of the sediment, while Procedure No. II refers to results obtained by heating the monocalcium phosphate monohydrate to convert it to anhydrous calcium metaphosphate. The details of the method are given in *This Journal*, **32**, 88 (1949).

DISCUSSION

The sediment can generally be drawn from a separator, with a minimum of solution, by flicking or quickly turning the stop-cock from side to side. Occasionally, however, a separator will be found to have a ledge just above the stop-cock from which it is difficult to dislodge the sediment. A wire has been utilized to dislodge the deposit in such cases. The low results included in the above tables may be due to some unnoticed sediment on such a ledge.

Nearly all of the inorganic material will be in the first draw-off from the 250 ml separator. It has been found that if this is drawn off into a second separator containing carbon tetrachloride, a clean separation is made as the inorganic material settles down through the solution. It is advantageous to draw off this sediment into a weighed Gooch crucible before the

TABLE 1.—*Recovery of monocalcium phosphate added to soft red winter wheat, long patent flour*

ANALYST	PROCEDURE	SAMPLE NO. 1 CONTAINING 0.21% MONOCALCIUM PHOSPHATE	SAMPLE NO. 2 CONTAINING 0.72% MONOCALCIUM PHOSPHATE
		per cent	*per cent*
1	I	.21, .21, .21	.72, .73, .72
	II	.20, .20, .19	.71, .71, .71
2	I	.21, .21, .21	.72, .72, .73
	II	.20, .19, .20	.71, .71, .71
3	I	.21, .21, .21	.72, .72, .73
	II	.19, .20, .20	.71, .71, .72
4	I	.20, .20, .20	.66, .66, .66
	II	.19, .20, .19	.62, .63, .62
5	I	.20, .21, .21	.70, .47, .62
	II	.18, .19, .20	.65, .39, .60
6	I	.23, .23	.66, .68
	II	.21, .22	.63, .68
7	I	.22, .22	.70, .60
	II	.20, .20	.70, .64
8	I	.23, .23	.73, .74

TABLE 2.—*Recovery of monocalcium phosphate added to hard wheat flour*

ANALYST	PROCEDURE	SAMPLE A HARD SPRING WHEAT FLOUR CONTAINING 0.50% MONOCALCIUM PHOSPHATE	SAMPLE B HARD WINTER WHEAT FLOUR CONTAINING 0.50% MONOCALCIUM PHOSPHATE
		per cent	*per cent*
1	I	.50, .51, .50	.51, .50, .51
	II	.49, .50, .49	.49, .48, .49
9	I	.52, .52, .53	.50, .53, .48
	II	.49, .49, .50	.50, .50, .43

second draw-off from the 250 ml separator. Any sediment adhering to the sides of the separator may be dislodged by gently swirling the funnel and its contents. The results obtained by ignition were found to be slightly lower than those obtained by direct weighing. This is probably due in most cases to free moisture in the monocalcium phosphate monohydrate rather than to flour in the material ignited. If desired, the used carbon

tetrachloride may be re-used several times by filtering before it is necessary to distill.

The assistance of the following collaborators is gratefully acknowledged.

R. A. Barackman and D. L. Gilkey, Victor Chemical Works, Chicago Heights, Ill.

H. K. Parker, Wallace & Tiernan Co., Inc., Newark, N. J.

E. L. Sexton, The Best Foods, Inc., New York City.

E. F. Steagall, Juanita Breit, L. G. Ensminger and O. S. Keener—all of the Food and Drug Administration.

It is recommended* that this method be adopted as official, first action.

REPORT ON MILK SOLIDS AND BUTTERFAT IN BREAD

By V. E. MUNSEY (Food and Drug Administration, Federal Security Agency, Washington 25, D. C.), *Associate Referee*

The tentative method, **20.86,** for determination of the amount of fat and the fat number of the extracted fat for the estimation of the amount of butterfat in bread has been modified, based on further experience with the method and previous collaborative study. A 160 g sample of air dry bread and 10 g of fat was submitted to 8 collaborators with the request that fat and fat number on the bread be determined in duplicate, and the fat number on the sample of fat in triplicate. The details of the procedure are given in *This Journal,* **32,** 85 (1949).

Results from six chemists were obtained, which appear in the following table. The results for fat are very good and the fat numbers are considered satisfactory for this type of determination. A value of 1.5 for maximum variation from average for fat number seems somewhat large, but in actual application it represents only 0.2 per cent butterfat in bread. This bread was made by adding a definite amount of a mixture of 44.5 per cent lard and 55.5 per cent butterfat, and contained on the air dry bread 2.2 per cent butterfat. On the basis of the Associate Referee analysis of the lard and butterfat for fat number, the determined amount of butterfat was found to be 2.4 per cent butterfat. The fat sample consisted of 54 per cent lard and 46 per cent butterfat. This fat mixture was chosen to closely approximate the butterfat content extracted from the bread due to the change caused by the flour fat. The average value of fat numbers reported by the collaborators are essentially the same for the fat extracted from the bread and the sample of fat. These results confirm previous results that the composition of the fat is not changed during extraction and that the agreement in results is similar whether determined on a uniform sample of fat or extracted fat from bread.

* For report of Subcommittee D and action of the Association, see *This Journal,* 32, 62 (1949).

TABLE 1.—*Fat and fat number on air dry bread containing 2.2% butterfat, and fat number on lard-butterfat mixture*

COLLABORATOR	FAT IN AIR DRY BREAD	FAT NUMBER ON FAT EXTRACTED FROM AIR DRY BREAD	FAT NUMBER ON FAT SAMPLE
	per cent		
1	5.53	14.6	14.7
	5.53	14.6	15.0
	5.52	14.4	15.0
	av. 5.5	av. 14.5	av. 14.9
2	5.38	14.6	15.6
	5.37	14.6	15.5
		14.9	15.5
	av. 5.4	av. 14.7	av. 15.5
3	5.5	15.6	16.7
	5.5	15.0	16.9
	5.4	16.3	16.5
	av. 5.4	av. 15.6	av. 16.7
4	5.76	16.6	16.2
	5.65	17.0	15.6
		17.4	15.2
	av. 5.7	av. 17.0	av. 15.7
5	5.30	16.0	15.8
	5.36	16.1	15.9
		16.0	
	av. 5.3	av. 16.0	av. 15.9
6	5.4	14.8	14.7
	5.4	14.9	14.6
		15.0	
		15.0	
	av. 5.4	av. 14.9	av. 14.7
	max. 5.7	max. 17.0	max. 16.7
	min. 5.3	min. 14.5	min. 14.7
	av. 5.5	av. 15.5	av. 15.6

Further work was done on the application of the chromatographic separation of fatty acids on silicic acid column outlined in a report last year. The only variation was the substitution of 0.5 per cent *N* butyl alcohol in chloroform for 1.0 per cent in an attempt to improve the performance of the butyric acid band on the column. The fat sample and pure butterfat were analyzed. The butterfat gave 63.5, 64.8, 44.0 and 48.5 mg/g butyric acid, and the fat sample gave 29.6, 23.1, 27.6, and 18.0 mg/g. Assuming the precision may be improved, it is doubtful whether its application has

enough merit to warrant so much more time in comparison with the fat number. No collaborative work was done on the determination of lactose in bread.

The cooperation of the following collaborators is very much appreciated: E. K. Spotts, Ward Baking Co.; R. T. Bohn, General Baking Co.; and S. Kahan, Harold F. O'Keefe, and E. F. Steagall, all of the Food and Drug Administration.

It is recommended* (1) that the determination of fat and fat number in bread be adopted as official, first action, and (2) that the study on the determination of lactose in bread be continued.

REPORT ON PROTEOLYTIC ACTIVITY OF FLOUR AND OTHER PROTEOLYTIC ENZYME-CONTAINING MATERIALS†

By Byron S. Miller (Associate Chemist, Hard Wheat Quality Laboratory, Manhattan, Kansas), *Associate Referee*

The Ayre and Anderson method(1) for the determination of proteolytic activity and a modification thereof (Landis, 2) have been studied collaboratively by Hildebrand (3) and by Redfern (4). Although Hildebrand (3) found the level of precision to be satisfactory when the original Ayre and Anderson technics were employed, Redfern (4) suggested that work on the semi-autolytic method of Landis (2) be discontinued. Recently Miller (5) made a critical study of the modified Ayre and Anderson method and discussed the conditions which must be maintained to secure a high level of precision. Warshowsky and Geddes (6) performed a comparative study of the Ayre and Anderson method and the Modified Landis Procedure (2) and concluded that better differentiation between samples was afforded by the modified Ayre and Anderson procedure.

The present collaborative study was undertaken to test the applicability of the method as standardized by Miller (5) to several materials possessing a wide range of proteolytic activity and, further, to test the reliability of the method as used in several laboratories.

PROTEOLYTIC ACTIVITY OF FLOUR AND MALTED WHEAT FLOUR

(Applicable to slightly active materials such as patent flour or to diluted extracts of active proteolytic preparations.)

REAGENTS

(a) *Buffer stock soln.*—Make 120 ml of acetic acid and 164 g anhydrous sodium acetate up to 1000 ml with H_2O. Dilute 1:20 before using (*p*H 4.7).

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* For report of Subcommittee D and action of the Association, see *This Journal*, 32, 61 (1949).

† Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the Department of Milling Industry, Kansas State College. Published as Contribution No. 156, Department of Milling Industry, Kansas State College.

(b) *Bacto-hemoglobin substrate.**

(c) *Trichloroacetic acid solns:*

Soln (1).—Dissolve 36 g trichloroacetic acid in 64 ml water. Use 5 ml aliquot.

Soln (2).—Dissolve 36 g trichloroacetic acid in 44 ml water. Use 4 ml aliquot.

(d) *Kjeldahl solns.*—Including 0.0714 *N* sodium hydroxide.

(e) *Pumice or fine sand.*

METHOD

Preparation of enzyme solutions.—For slightly active materials such as flour, weigh as much as 10 g directly into digestion flasks. For active enzyme preparations prepare a water extract or suspension immediately preceding digestion. (The amount of extract or dilutions thereof used in the digestion mixture may vary up to 1 ml. Appropriate activation technics may be applied to the enzyme extract.)

Digestion procedure.—Weigh 0.625 g (moisture-free basis) of Bacto-hemoglobin into each 125 ml Erlenmeyer flask and add ca 3 g of finely divided pumice. Add 5 g sample of flour to each of two flasks and agitate the mixture by rotation until flour and substrate are intimately mixed. Then add 25 ml of reagent (a) previously warmed to 40° (in thermostat-controlled bath within ±0.1°C.) to each flask and agitate its contents to insure uniform suspension. Place the tightly stoppered flasks in a constant temp. (40°) bath and agitate either continuously or at hourly intervals.

Add a 5 ml portion of trichloroacetic acid soln **c(1)** to one flask of each pair at the end of 15 min. digestion and to the second flask of the pair after 5 hours of digestion. Mix contents thoroly and allow flasks to remain in the bath at 40°C. for exactly 30 min. Centrifuge the suspension for 5 min. at 1800 r.p.m. and filter. Pipet duplicate 5 ml aliquots directly into Kjeldahl flasks and determine soluble nitrogen.

Follow essentially the same procedure in determining the enzyme activity of an extract. In place of the solid material, use a total of 1 ml of extract or extract plus water. After zero time and 5 hour digestion periods, add to each flask a 4 ml aliquot of trichloroacetic acid soln **c(2)**. Thoroly mix the contents, allow to remain in the water bath for exactly 30 min. and filter without centrifuging; analyze 5 ml aliquots for soluble nitrogen.

Soluble nitrogen.—Proceed as under **2.24**, **2.25**, or **2.26**. Use a definite volume of water (350 ml) to dilute the cooled digest and add in such a way as to wash down all the trichloroacetic acid which has condensed in the neck of the flask during the digestion process. Also add the concentrated alkali (one and one-half times the usual quantity) in such a manner as to lave the neck of the flask. After distillation, back-titrate the unneutralized standard acid with 0.0714 *N* sodium hydroxide.

Expression of proteolytic activity.—Proteolytic activity is measured by the difference in back-titration volumes for the 15-min. or zero time digestions and the corresponding 5 hour digestion, expressed in ml of 0.0714 *N* sodium hydroxide. This difference may be translated into mg of soluble nitrogen released from a given weight of the enzyme source.

NOTES

(1) A duplicate Kjeldahl determination is made on each digestion filtrate and in most cases there should be enough sample left for a third analysis if one titration should be of doubtful accuracy. Duplicate titrations should vary by no more than 0.05 ml of 0.0714 *N* sodium hydroxide.

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* A suitable quality is obtainable from the Difco Laboratories, Detroit, Mich.

(2) Each still should be previously checked for leaks and duplicability by distilling aliquots of ammonium oxalate solution to which 350 ml water and 10 ml concentrated sodium hydroxide have been added. A 5 ml aliquot containing 25 mg of ammonium oxalate is convenient.

(3) Higher concentrations of hemoglobin substrate should not increase the corrected titration values for the enzyme sources studied.

(4) Careful washing down of the trichloroacetic acid from the neck of the digestion flasks is mandatory.

(5) An enzyme such as papain can be activated by bubbling washed hydrogen sulfide gas through the enzyme suspension and through the digestion mixture itself.

(6) For some materials such as flour a turbid solution may remain after the final filtration. Such turbidity may be removed by boiling the centrifuged digestion mixture for a few seconds prior to final filtration. The liquid lost through evaporation should be replaced by the addition of water.

COLLABORATIVE STUDY

Preparation of Samples and Instructions to Collaborators.—Each of eight collaborators tested a total of four samples which included a patent flour, a malted wheat flour, a commercial fungal amylase concentrate, and a commercial papain preparation. All samples were thoroughly blended; sub samples were taken, sealed, and shipped. Included in the shipment were sufficient Bacto-hemoglobin, pumice, and concentrated buffer solution to complete the analyses.

The collaborators were requested to complete a set of blank and 5 hour digestions on three different days, which were not necessarily consecutive, and to make duplicate Kjeldahl nitrogen analyses on each filtered solution. The amount of sample for each digestion was fixed at 5 g for patent flour and malted wheat flour, 30 mg. for the fungal concentrate and 5 mg. for papain. Since most of the variability in the method appears to be associated with the Kjeldahl procedure it was suggested that someone thoroughly familiar with that technic run the nitrogen analyses and that the same individual be employed throughout the study.

Results and discussion.—Results obtained by two analysts were excluded at their own request. The individual results of each of the remaining six collaborators are summarized in Table 1. The means and standard deviations for each type of material are also included.

The low average values for papain, obtained by collaborators A and E are probably due to inactivation of the enzyme on standing. These data, therefore, were not analyzed statistically.

The analysis of variance shown in Table 2 for the remaining data indicates a significant difference in results obtained by different collaborators for malted wheat flour and the fungal concentrate. A non-significant difference, however, was obtained for patent flour. For digestions within the same laboratory significant differences are shown for all preparations.

Additional knowledge of the precision of the method can be gained by considering the work of one analyst. The results obtained over a period of

TABLE 1.—*Proteolytic activity of four different enzyme sources as determined by six collaborators*
Values expressed in ml of 0.0714 *N* sodium hydroxide

REPLICATE DIGESTIONS		SAMPLE											
		PATENT FLOUR			MALTED WHEAT FLOUR			FUNGAL PREP.			PAPAIN		
COLLABORATOR	DAY	*ml*	*ml*	*Av.*	*ml*	*ml*	*Av.*	*ml*	*ml*	*Av.*	*ml*	*ml*	*Av.*
A	1	1.23	1.15		4.85	4.88		3.43	3.50		2.60	2.55	
	2	1.15	1.25		4.93	4.90		3.55	3.50		2.90	2.90	
	3	1.13	1.08		4.88	4.88		3.55	3.50		2.65	2.60	
				1.16			4.90			3.50			2.70
B	1	1.11	1.12		4.87	4.89		3.52	3.61		3.06	3.04	
	2	1.08	1.08		4.82	4.87		3.66	3.59		3.28	3.23	
	3	1.12	1.11		5.00	4.90		3.65	3.61		3.10	3.05	
				1.10			4.89			3.60			3.16
C	1	1.25	1.20		4.85	4.90		3.30	3.40		3.10	3.15	
	2	1.20	1.20		4.75	4.95		3.35	3.30		3.15	3.15	
	3	1.15	1.10		4.75	5.00		3.35	3.40		3.15	3.70	
				1.18			4.87			3.35			3.14
D	1	1.09	1.08		5.06	4.99		3.21	3.17		3.17	3.16	
	2	1.21	1.17		4.79	4.97		3.02	3.03		3.13	3.19	
	3	1.17	1.12		4.84	4.80		3.11	3.25		3.24	3.09	
				1.14			4.91			3.13			3.16
E	1	1.19	1.30		4.83	4.94		3.36	3.53		2.58	2.56	
	2	1.12	1.15		5.21	5.14		3.63	3.61		2.60	2.65	
	3	1.12	1.12		4.90	4.97		3.68	3.74		3.15	3.08	
				1.17			5.00			3.59			2.77
F	1	1.30	1.20		4.80	4.75		3.10	3.10		2.95	2.95	
	2	0.90	0.90		4.50	4.60		3.20	3.20		3.00	3.00	
	3	1.10	1.15		4.60	4.50		3.10	3.20		2.95	2.95	
				1.09			4.63			3.15			2.97
Mean, all samples				1.14			4.87			3.39			2.98
Standard deviation within laboratory				0.08			0.10			0.08			—

several months by one individual working with a fungal amylase preparation at various concentrations are reported in Table 3. Values for duplicate 5-hour digestions are recorded. The standard deviation of 0 04 ml calculated from these data is approximately one-half that calculated for the preparations analyzed collaboratively. This indicates that experience contributes substantially to a higher degree of precision of the method.

The standard error of a single determination as calculated by Hildebrand (3) from data obtained in a collaborative study of the Ayre and Anderson method was 0.99 mg of nitrogen per 5 grams of flour sample. The value obtained from the data in Table 3 is 0.24 mg for an equivalent

quantity of flour, or less than one-fourth the standard error reported previously. For the collaborative data in Table 1 the standard error for a single determination is approximately one-half as large as the value reported by Hildebrand (3).

TABLE 2.—*Analysis of variance of collaborative data*
(2) compared with (3) and (1) compared with (2).

VARIANCE DUE TO:	DEGREES OF FREEDOM	MEAN SQUARES		
		PATENT FLOUR	MALTED WHEAT FLOUR	FUNGAL CONCENTRATE
(1) Collaborators	5	.006	.0941**	.272***
(2) Between digestions in same laboratory	12	.015**	.0184**	.011**
(3) Between analyses within same digestion	18	.0021	.0058	.003

** Significance exceeds the 1% level.
*** Significance exceeds the 0.1% level.

TABLE 3.—*Replicability of proteolytic enzyme assay within one laboratory*
Values expressed in ml of 0.0174 *N* sodium hydroxide

DIGESTION I	DIGESTION II	ABSOLUTE DIFFERENCE	DIGESTION I	DIGESTION II	ABSOLUTE DIFFERENCE
ml			*ml*		
1.40	1.38	.02	2.80	2.80	.00
0.25	0.20	.05	0.35	0.40	.05
0.20	0.15	.05	0.25	0.40	.15
0.05	0.00	.05	1.75	1.73	.02
0.30	0.28	.02	1.48	1.50	.02
1.60	1.53	.07	1.00	1.10	.10
0.38	0.28	.10	0.30	0.30	.00
1.10	1.10	.00	1.65	1.65	.00
0.60	0.70	.10	1.05	1.05	.00
1.40	1.38	.02	0.90	0.85	.05
0.10	0.08	.02	0.05	0.05	.00
1.45	1.48	.03	1.18	1.20	.02
0.54	0.62	.08			—
			Mean		.04
			Standard deviation		.04

Although significant tendencies were found for different laboratories to obtain different results and for one analyst to obtain significant differences between digestions, the modified Ayre and Anderson method (5) gives a lower level of error than the original Ayre and Anderson method (1). It is

believed that these data justify the recommendation that the method as written be officially adopted for the determination of the proteolytic activity of patent flour and malted wheat flour.

LIST OF COLLABORATORS

The letters assigned the collaborators in Table 1 bear no relation to the alphabetical arrangement in this list.

Allan D. Dickson, U. S. Department of Agriculture, Madison, Wis.
John W. Giertz, Kansas Milling Company, Wichita, Kan.
Rae H. Harris, North Dakota Agricultural Experiment Station, Fargo, N. D.
B. D. Hites, Nebraska Agricultural Experiment Station, Lincoln, Neb.
Eric Kneen, Kurth Malting Company, Milwaukee, Wis.
Byron S. Miller, U. S. Department of Agriculture, Manhattan, Kan.
Hugh K. Parker, Wallace and Tiernan Company, Newark, N. J.
Roland W. Selman, C. J. Patterson Company, Kansas City, Mo.

COMMENTS OF COLLABORATORS

(1) The quantities of alkali and water suggested made the Kjeldahl distillations quite difficult to run. It was necessary to reduce the volume of alkali to 75 ml and the water to 250 ml. By carefully adding these solutions from large burettes no further difficulties were encountered.†

(2) The weighing, transferring and mixing of the 5-g samples of flour were found to be the most tedious requirements of this method.

(3) From our work we would judge the method to be satisfactory for the determination of proteolytic activity and feel no need for major modifications.

(4) Some difficulty was encountered with the 5-hour digestion of patent flour. The filtrate was cloudy after centrifuging and filtering. Replication was poorer on this sample than for the others.

RECOMMENDATIONS*

It is recommended—

(1) That the method as proposed be officially adopted for the determination of proteolytic enzymes in patent flour and malted wheat flour.

(2) That further work be done on the application of the method to the analysis of proteolytic enzymes in materials other than patent flour and malted wheat flour.

(3) That other methods for the determination of proteolytic activity be investigated.

ACKNOWLEDGMENTS

The Associate Referee gratefully acknowledges and appreciates the cooperation manifested by all collaborators and their respective organizations. His thanks are also extended to Mr. B. Marlo Dirks for the use of the data included in Table 3 and to Prof. H. C. Fryer, Head of the Statistical Laboratory, Kansas State College, for consultation on the statistical aspects of the problem.

† The volumes of solutions suggested in the procedure are for 800 ml Kjeldahl flasks.
* For report of Subcommittee D and action of the Association, see *This Journal, 32,* 61 (1949).

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(4) REDFERN, SUTTON, *This Journal*, **29**, 258 (1946).
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REPORT ON ANALYSIS OF SOYBEAN FLOUR

By W. L. TAYLOR (General Mills, Inc., Minneapolis, Minnesota), *Associate Referee*

The changes made in the tentative method for soybean flour* at the 1947 meeting of the Association indicated the need of further collaborative

TABLE 1.—*Collaborative results*

LABORATORY	MOISTURE		PROTEIN (N×6.25)		OIL		ASH	
		per cent				*per cent*		*per cent*
				Defatted Soy Flour				
A	(7.94)		(55.25)		(.69)		(6.02)	
	(7.82)	7.88	(55.15)	55.20	(.72)	.705	(6.09)	6.05
B	(8.02)		(55.38)		(.674)		(6.02)	
	(7.94)		(55.62)		(.658)		(6.09)	
	(7.90)	7.95	—	55.50	—	.666	(6.13)	6.08
C	(7.76)		(54.2)		(.66)		(6.16)	
	(7.73)	7.74	(54.6)	54.40	(.67)	.665	(6.11)	6.13
				Low Fat Soy Flour				
A	(6.23)		(51.45)		(5.21)		(5.77)	
	(6.33)	6.28	(51.45)	51.45	(5.21)	5.21	(5.90)	5.83
B	(6.48)		(51.125)		(5.35)		(5.82)	
	(6.61)		(51.187)		(5.34)		(5.80)	
	(6.52)	6.54	—	51.16	—	5.34	(5.83)	5.82
C	(6.43)		(50.6)		(5.41)		(5.83)	
	(6.41)	6.42	(50.4)	50.50	(5.41)	5.41	(5.82)	5.82
				Full Fat Soy Flour				
A	(6.39)		(44.40)		(20.20)		(4.54)	
	(6.34)	6.37	(44.50)	44.45	(20.07)	20.14	(4.64)	4.59
B	(6.51)		(44.50)		(19.63)		(4.68)	
	(6.52)		(44.87)		(19.67)		(4.73)	
	(6.42)	6.48	—	44.69	—	19.65	(4.83)	4.75
C	(6.16)		(43.8)		(20.3)		(4.68)	
	(6.09)	6.12	(43.8)	43.80	(20.1)	20.20	(4.70)	4.69

work. Supplies of the three leading types of soy flour, namely, defatted soy flour, low fat soy flour, and full fat soy flour were, therefore, obtained from manufacturers. From these supplies one pound samples were prepared and distributed to four collaborators (three of whom completed the work, with one incomplete and too late to tabulate). The collaborators were directed to follow the tentative method for moisture, **20.4,** nitrogen **2.26,** oil, **31.67,** and ash **27.9,** as revised at the 1947 meeting.*

COLLABORATORS

Randall, Fred, Cooperative G. L. F. Mills, Inc., Buffalo, N. Y.
Blanchard, J. F., Department of Health and Welfare, Food and Drugs Office, Winnipeg, Manitoba, Canada.
Kyser, George H., General Mills, Inc., Chemical Division, Belmond, Iowa.

DISCUSSION

One of the reasons for doing the collaborative work reported above is that few laboratories have been called upon to make routine analyses of soy flours. It is, therefore, more necessary than usual to use methods which can be followed exactly by any competent chemist and to prescribe the reagents and equipment needed for making the determinations.

RECOMMENDATION†

In order that the tentative method for the analysis of soy flour may be thoroughly tested, it is recommended that the collaborative work on soy flour be continued and that the assistance of additional laboratories be enlisted.

No reports were given on starch in raw and cooked cereals; fat acidity in grain; sugar in bread, flour, etc.; soybean flour in foods (immunological test); baked products (moisture, ash, protein, fat, and crude fiber); moisture in self-rising flour, etc.; bromates in flour; phosphorus; unsaponifiable matter and sterols in noodles, etc.; and carbon dioxide in self-rising flours.

REPORT ON BAKING POWDER

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

The Associate Referee has submitted a report of his collaborative study on the application of the A.O.A.C. tentative method and 3 modifications of drying oven methods to three samples of baking powder for determination of residual carbonic acid. The results obtained warrant the recom-

* See *This Journal*, 31, 81 (1948).
† For report of Subcommittee D and action of the Association, see *This Journal*, 32, 61 (1949).

mendation of the Associate Referee that the A.O.A.C. tentative method be adopted as official, first action, in which the Referee concurs.

The results obtained by the oven modification No. 1 method (heated 5 hrs. at 100°C. with 20 ml added water) were as good as those obtained by the A.O.A.C. method. In view of the need for a special steam bath for the A.O.A.C. tentative method and the common existence of an accurate electric oven in all laboratories, it is recommended that the oven modification No. 1 method be adopted as an alternate method, official first action, in which the Associate Referee concurs.

In the determination of available carbon dioxide in baking powder, the present official method for total carbon dioxide specifies sulfuric acid (1+5), and the official (first action) residual carbon dioxide methods (recommended above) specify hydrochloric acid (1+2). There are certain definite advantages to the use of hydrochloric acid (1+2), especially for baking powder containing added calcium carbonate. In a collaborative report by J. R. Chittick, *This Journal*, **29**, 259, it is concluded that hydrochloric acid (1+2) be an alternate for sulfuric acid (1+5) in the official method of total carbon dioxide.

There are official methods for starch in baking powder, and there is no general need for the tentative, modified McGill method, **17.21.**

RECOMMENDATIONS*

It is recommended—

(1) That the tentative, modified McGill method, **17.21** be dropped.

(2) That the tentative qualitative test for phosphoric acid, **17.31,** be adopted as official, first action.

(3) That the tentative methods, *This Journal*, **31**, 78, and the oven modification No. 1, as set forth in the Associate Referee report for this year on residual CO_2 in baking powder, be adopted as official, first action.

(4) That HCl (1+2), be adopted as alternate for H_2SO_4 (1+5) in the official method for total CO_2, **17.4** and **17.6.**

(5) That the available CO_2, **17.9,** be determined by subtraction of residual CO_2 (see recommendation 3) from the total CO_2, **17.6.**

(6) That the official gasometric method **17.8** be dropped, final action.

REPORT ON RESIDUAL CARBON DIOXIDE IN BAKING POWDERS

By J. E. TATAR (Standard Brands Inc., Chicago, Ill.),
Associate Referee

This report covers a continuation of the collaborative study of the Quartermaster Corps method (Rewritten Version) which was adopted as

* For report of Subcommittee D and action of the Association, see *This Journal*, 32, 62 (1949).

a tentative method for the determination of residual carbon dioxide in baking powders,[1] and modifications of this method as outlined in the recommendations of the Associate Referee in his last report.[2]

One of the recommendations calls for a study of the tentative method in which the evaporation to dryness is carried out in a drying oven instead of a water bath and at temperatures ranging from 70 to 100°C. The lower temperatures were suggested to effect the evaporation to dryness without gelatinization of the starch which may interfere both in the driving out of the available CO_2 during the treatment of the sample, and the shaking out of the residual CO_2 in the final determination. Preliminary work done in the Associate Referee's laboratory indicated that although gelatinization of the starch takes place on the water bath, it does not proceed in the oven to an extent great enough to interfere until temperatures of over 100°C. are reached. Therefore, the work with the drying oven methods was limited to temperatures of 90 and 100°C.

Another recommendation which was made in the Associate Referee's report and which was tried in his laboratory before submitting it for collaborative study, was the use of a saturated sodium chloride solution as a reaction medium instead of distilled water. It was found that the evaporation to dryness took much too long whether it was done on the water bath or in the oven, and any other treatment short of evaporation to dryness did not drive out all of the available CO_2. It could be accomplished by boiling, but it was difficult to standardize and control the technique. Therefore, no collaborative work was done on this recommendation.

Four methods were submitted for collaborative study:
The A.O.A.C. Tentative method, *This Journal*, **31**, 78 (1948).
Drying Oven Modification No. 1—100°C., with 20 ml. H_2O.*
Drying Oven Modification No. 2—100°C., with 10 ml H_2O.
Drying Oven Modification No. 3—90°C., with 10 ml H_2O.

The four methods were applied to three baking powders, the formulas of which were exactly the same as in last year's work.[3] They were marked D, E, and F, corresponding to A, B, and C, respectively of last year's samples. However, the ingredients used in making up the baking powder samples were not all from the same lots as last year's samples, and for that reason powders D and E had slightly lower residuals.

Collaborators were requested to report on all four methods, running each sample once a day on three different days, instead of in triplicate on the same day, so that the results would reflect a more accurate measure

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[1] See report of Referee, *This Journal*, **31**, 273 (1948).
[2] See report of Associate Referee, *This Journal*, **31**, 274 (1948).
[*] Adopted as an alternate method, *This Journal*, **32**, 83 (1949).
[3] See report of Associate Referee, *This Journal*, **31**, 275 (1948).

TABLE 1.—*Results of collaborators on sample "D" in percent carbon dioxide*

ANALYST	TENTATIVE A.O.A.C. METHOD	DEVIATION FROM MEAN	OVEN MODIFICATION #1	DEVIATION FROM MEAN	OVEN MODIFICATION #2	DEVIATION FROM MEAN	OVEN MODIFICATION #3	DEVIATION FROM MEAN
1	0.80	0.24	1.00	0.23	0.70	0.05	0.90	0.19
	0.71	0.15	.91	0.14	0.81	0.16	0.91	0.20
	0.70	0.14	.80	0.03	0.80	0.15	1.10	0.39
2	0.58	0.02	0.99	0.22	0.71	0.06	0.76	0.05
	0.68	0.12	0.97	0.20	0.68	0.03	0.95	0.24
	0.60	0.04	0.80	0.03	0.70	0.05	0.82	0.11
3	0.52	0.04	0.70	0.07	0.51	0.14	0.56	0.15
	0.52	0.04	0.57	0.20	0.46	0.19	0.51	0.20
	0.40	0.16	0.61	0.16	0.61	0.04	0.61	0.10
4	0.55	0.01	0.83	0.06	0.66	0.01	0.70	0.01
	0.50	0.06	0.85	0.08	0.70	0.05	0.60	0.11
	0.51	0.05	0.78	0.01	0.71	0.06	0.62	0.09
5	0.30	0.26	0.60	0.17	0.50	0.15	0.50	0.21
	0.30	0.26	0.65	0.12	0.49	0.16	0.64	0.07
	0.39	0.17	0.68	0.09	0.44	0.21	0.60	0.11
6	0.74	0.18	0.82	0.05	0.99	0.34	0.97	0.26
	0.78	0.22	0.84	0.07	1.06	0.41	0.96	0.25
	0.77	0.21	0.86	0.09	0.97	0.32	0.98	0.27
7	0.77	0.21	0.73	0.04	0.76	0.11	0.82	0.11
	0.71	0.15	0.73	0.04	0.81	0.16	0.73	0.02
	0.72	0.16	0.77	0.00	0.72	0.07	0.77	0.06
8	0.64	0.08	0.67	0.10	0.43	0.22	0.73	0.02
	0.61	0.05	0.87	0.10	0.31	0.34	0.61	0.10
	0.61	0.05	0.93	0.16	0.77	0.12	0.62	0.09
9	0.40	0.16	0.62	0.15	0.61	0.04	0.51	0.20
	0.62	0.06	0.64	0.13	0.61	0.04	0.62	0.03
	0.49	0.07	0.90	0.13	0.61	0.04	0.69	0.04
10	0.30	0.26	0.59	0.18	0.52	0.13	0.62	0.03
	0.29	0.27	0.65	0.12	0.52	0.13	0.47	0.18
	0.37	0.19	0.65	0.12	0.44	0.21	0.47	0.18
Mean	0.56		0.77		0.65		0.71	
Ave. Dev.		0.14		0.11		0.14		0.14
Max. Var.	0.51		0.43		0.75		0.63	

TABLE 2.—*Results of collaborators on sample "E" in percent carbon dioxide*

ANALYST	TENTATIVE A.O.A.C. METHOD	DEVIATION FROM MEAN	OVEN MODIFICATION #1	DEVIATION FROM MEAN	OVEN MODIFICATION #2	DEVIATION FROM MEAN	OVEN MODIFICATION #3	DEVIATION FROM MEAN
	6.91	0.34	7.01	0.41	6.91	0.16	6.91	0.13
1	6.97	0.40	6.97	0.37	7.07	0.32	7.07	0.29
	6.90	0.33	6.90	0.30	6.90	0.15	6.90	0.12
	6.41	0.16	6.59	0.01	6.50	0.25	6.74	0.04
2	6.59	0.02	6.78	0.18	6.65	0.10	6.90	0.12
	6.60	0.03	6.59	0.01	6.80	0.05	6.83	0.05
	6.45	0.12	6.62	0.02	6.72	0.03	6.75	0.03
3	6.45	0.12	6.59	0.01	6.74	0.01	6.89	0.11
	6.40	0.17	6.51	0.09	6.83	0.08	6.81	0.03
	6.64	0.07	6.53	0.07	6.67	0.08	6.77	0.01
4	6.63	0.06	6.55	0.05	6.65	0.10	6.60	0.18
	6.50	0.07	6.44	0.16	6.75	0.00	6.73	0.05
	6.22	0.35	6.59	0.01	6.58	0.17	6.61	0.17
5	6.29	0.28	6.46	0.14	6.56	0.19	6.80	0.02
	6.42	0.15	6.44	0.16	6.56	0.19	6.56	0.22
	6.61	0.04	6.52	0.08	7.21	0.46	7.09	0.31
6	6.63	0.06	6.55	0.05	7.26	0.51	7.14	0.36
	6.60	0.03	6.54	0.06	7.19	0.44	7.24	0.46
	6.74	0.17	6.64	0.04	6.70	0.05	6.87	0.09
7	6.61	0.04	6.59	0.01	6.70	0.05	6.79	0.01
	6.77	0.20	6.57	0.03	6.72	0.03	6.87	0.09
	6.63	0.06	6.46	0.14	6.55	0.20	6.49	0.29
8	6.49	0.18	6.86	0.26	6.86	0.11	6.72	0.06
	6.60	0.03	6.67	0.07	6.91	0.16	6.87	0.09
	6.64	0.07	6.49	0.11	6.68	0.07	6.73	0.05
9	6.61	0.04	6.52	0.08	6.69	0.06	6.69	0.09
	6.65	0.08	6.60	0.00	6.85	0.10	6.76	0.02
	6.42	0.15	6.49	0.11	6.50	0.25	6.42	0.36
10	6.42	0.15	6.41	0.19	6.38	0.37	6.53	0.25
	6.34	0.23	6.40	0.20	6.55	0.20	6.45	0.33
Mean	6.57		6.60		6.75		6.78	
Ave. Dev.		0.14		0.11		0.16		0.15
Max. Var.	0.75		0.57		0.88		0.82	

TABLE 3.—*Results of collaborators on sample "F" in percent CO_2*

ANALYST	TENTATIVE A.O.A.C. METHOD	DEVIATION FROM MEAN	OVEN MODIFICATION #1	DEVIATION FROM MEAN	OVEN MODIFICATION #2	DEVIATION FROM MEAN	OVEN MODIFICATION #3	DEVIATION FROM MEAN
1	0.50	0.16	0.50	0.15	0.50	0.17	0.50	0.19
	0.51	0.17	0.51	0.16	0.51	0.18	0.51	0.20
	0.50	0.16	0.50	0.15	0.50	0.17	0.50	0.19
2	0.39	0.05	0.52	0.17	0.38	0.05	0.38	0.07
	0.34	0.00	0.39	0.04	0.39	0.06	0.34	0.03
	0.38	0.04	0.40	0.05	0.36	0.03	0.40	0.09
3	0.42	0.08	0.30	0.05	0.10	0.23	0.25	0.06
	0.42	0.08	0.21	0.14	0.31	0.02	0.31	0.00
	0.35	0.01	0.25	0.10	0.31	0.02	0.31	0.00
4	0.39	0.05	0.41	0.06	0.30	0.03	0.23	0.08
	0.38	0.04	0.28	0.07	0.30	0.03	0.20	0.11
	0.40	0.06	0.32	0.03	0.34	0.01	0.33	0.02
5	0.10	0.24	0.30	0.05	0.20	0.13	0.10	0.21
	0.20	0.14	0.30	0.05	0.20	0.13	0.20	0.11
	0.30	0.04	0.39	0.04	0.20	0.13	0.20	0.11
6	0.15	0.19	0.18	0.17	0.34	0.01	0.29	0.02
	0.18	0.16	0.20	0.15	0.32	0.01	0.30	0.01
	0.22	0.12	0.21	0.14	0.38	0.05	0.32	0.01
7	0.31	0.03	0.41	0.06	0.46	0.13	0.41	0.10
	0.31	0.03	0.36	0.01	0.41	0.08	0.36	0.05
	0.36	0.02	0.46	0.11	0.36	0.03	0.36	0.05
8	0.41	0.07	0.33	0.02	0.30	0.03	0.31	0.00
	0.31	0.03	0.46	0.11	0.43	0.10	0.30	0.01
	0.51	0.17	0.49	0.14	0.51	0.18	0.33	0.02
9	0.33	0.01	0.34	0.01	0.40	0.07	0.31	0.00
	0.42	0.08	0.36	0.01	0.33	0.00	0.26	0.05
	0.41	0.07	0.41	0.06	0.36	0.03	0.45	0.14
10	0.16	0.18	0.29	0.06	0.10	0.23	0.15	0.16
	0.19	0.15	0.31	0.04	0.23	0.10	0.18	0.13
	0.32	0.02	0.18	0.17	0.13	0.20	0.19	0.12
Mean	0.34		0.35		0.33		0.31	
Ave. Dev.		0.09		0.09		0.09		0.08
Max. Var.	0.41		0.34		0.41		0.41	

of the precision. The assistance of the following collaborators is gratefully acknowledged:

Barackman, R. A., and Gilkey, D. L., Victor Chemical Works, Chicago Heights, Ill.
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Dick, Ludwig, Standard Brands, Inc., Chicago, Ill.
Holch, R. D., and Martin, W. T., Jaques Mfg. Co., Chicago, Ill.
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Novitsky, Peter, and Matson, Gloria, General Foods Corporation, Chicago, Ill.
Pugsley, L. I., and Kelly, J. T., Food and Drug Division, Ottawa, Canada.
Schilb, T. W., and McKim, Elizabeth, Monsanto Chemical Co., St. Louis, Mo.
Steele, Harold K., Fleischmann Laboratories, New York, N. Y.

(The order in which these collaborators are listed does not conform to the numbers used in the tables of results.)

DISCUSSION

The results shown in Tables 1, 2, and 3 reflect a definite improvement in the agreement among the collaborators when compared to last year's collaborative study. This is particularly true of the results obtained with the tentative method on baking powder E which contained calcium carbonate and which corresponded to baking powder B in last year's work. The maximum variance for this powder with the tentative method last year was 1.95 compared to 0.75 in this year's results and an average deviation of 0.37 last year compared to 0.14.

All four methods were equally good on powder F, the average with the tentative method (0.34%) being a perfect check of last year's average for the same powder, while the averages for this powder with the other three methods were 0.35, 0.33, and 0.31, also excellent checks.

With powders D and E the tentative method and the No. 1 oven modification were superior to the other two in having lower maximum variances and the average deviations and maximum variances obtained with the No. 1 oven modification on those powders gave this method a slight edge in precision over the tentative method. However, this method requires 5 hours of oven drying time compared to 2 hours on the water bath for the tentative method, and the differences in precision are not large enough to compensate for the extra time. Neither of the two methods require much of the operator's time, so that they are equal from that standpoint. The oven modification requires a thermostatically controlled drying oven, whereas the tentative method can be carried out on a simple water bath heated by gas or electricity.

RECOMMENDATIONS*

It is recommended—

That the tentative method and the Drying Oven Modification No. 1, *This Journal*, **32**, 83, (1949), be adopted as official, first action, for the determination of residual CO_2 in baking powders, and that the study be discontinued.

REPORT ON PLANTS

By ELROY J. MILLER (Michigan Agricultural Experiment Station, East Lansing, Michigan) *Referee*

Substantial progress has been made by the Associate Referees on Plants during the past year. A number of new Associate Referees were appointed and have begun work on the methods assigned them for study. Several have reports to present.

The following have submitted reports:

E. J. Benne, Associate Referee on Carotene and Zinc in Plants, has a report on Zinc.

Gordon H. Ellis, Associate Referee, has a report on Cellulose and Lignin in Plants.

Ray L. Shirley, Associate Referee on Sodium, has a report on that subject.

Carroll L. Hoffpauir, Associate Referee on Starch, has a report on Starch in Plants.

L. K. Wood, Associate Referee on Boron, has a report in collaboration with C. M. Austin.

All the Associate Referees expect to continue work on their respective methods for constituents in plants.

RECOMMENDATIONS†

It is recommended—

(1) That the resignation of J. S. McHargue, Associate Referee on Boron and Iodine, be accepted with appreciation for his long and valuable service.

(2) That the resignation of Mrs. Dorothy Waldron be accepted with thanks for past contributions.

(3) That the list of Associate Referees and their assignments as given in *This Journal*, **31**, 284 (1948), be continued.

(4) That the recommendations made by the Associate Referees in their Reports on Zinc, Boron, Cellulose and Lignin, Sodium and Starch, respectively be accepted.

* For report of Subcommittee D and action of the Association, see *This Journal*, 32, 62 (1949).
† For report of Subcommittee A and action of the Association, see *This Journal*, 32, 45 (1949).

REPORT ON ZINC IN PLANTS

By RAY L. SHIRLEY, ERWIN J. BENNE (*Associate Referee*), and E. J. MILLER (Michigan Agricultural Experiment Station, East Lansing, Michigan)*

The tentative A.O.A.C. method for determining zinc in plant materials **(12.24)** (1) depends upon the combination of zinc with dithizone (diphenylthiocarbazone) under controlled conditions. The zinc dithizonate and some excess dithizone are removed from an ammoniacal aqueous solution with carbon tetrachloride, and the zinc is evaluated photometrically by a mixed-color procedure. This method was published by Cowling and Miller (2) and was accepted as a tentative method by the Association in 1941 upon the recommendation of Cowling (3), who was then Associate Referee on zinc in plants.

Shirley *et al.*, investigated this method and a report of the findings was presented at the annual meeting of the Association in 1947 and later published (4). The present authors have continued this investigation, and this report presents a modification of the tentative procedure which possesses the following advantages: (a) one extraction step preliminary to the extraction of the zinc dithizonate is eliminated, (b) two less reagents are necessary, (c) only one separatory funnel is required per determination, and (d) about one-third less time is required for making an analysis.

MODIFIED METHOD

APPARATUS

The same apparatus as is used in the tentative method except that amber or low actinic glassware is preferable for certain operations (4).

REAGENTS

The same as required in the tentative method except that reagent B and 0.02 *N* HCl are no longer used.

PROCEDURE

Ashing and Extraction of Ash

Weigh 5 gms. of finely-ground, air-dried plant material into a platinum dish of suitable size. Mix 25 ml of ca *N* H_2SO_4 soln with the sample in the dish and dry on steam bath until most of the water is removed. Place the dish in hot air oven at 105°C. and complete removal of water. Ash in an electric muffle furnace at 500-550°C. and proceed as directed in the tentative procedure as far as "First Extraction."

Removal of Interferences, Formation of Zinc Dithizonate and Separation of Excess Dithizone

Add first 15 ml of distilled H_2O, then an aliquot of the ash extract containing not more than 30 mmg. of zinc, to an amber glass separatory funnel of 125 ml capacity.

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A 10 ml aliquot is satisfactory for most plant tissues, but if it is necessary to use a different volume, add 1 ml of 0.2 *N* HCl for each 5 ml of ash extract less than, or 1 ml of 0.2 *N* NH_4OH for each 5 ml more than, 10 ml taken, since it is essential that the soln have a *p*H of 2.0–2.3.

Add 10 ml of dithizone reagent to the separatory funnel, stopper funnel, and shake contents vigorously for 1 min. Allow the layers to separate and draw off and discard the CCl_4 layer. (Allow at least 3 min. for phases to separate after each shaking period thruout this procedure.) Add 6–8 ml of CCl_4 to the separatory funnel, stopper, and shake contents vigorously for 15 sec. Allow the layers to separate, shake down the CCl_4 from the surface, and draw off and discard the non-aqueous layer. Repeat this rinsing process twice more. After the last rinsing let the funnel stand long enough for all remaining CCl_4 to evaporate from the surface of the aqueous layer.

To the aqueous soln in the funnel add with a graduated cylinder 40 ml of ammonium citrate buffer soln and follow this with additions of exactly 5 ml of carbamate reagent and 10 ml of dithizone reagent from accurate pipets or burets. Stopper funnel and shake contents vigorously for 1 min. Allow the layers to separate and shake down CCl_4 from the surface. Draw off the aqueous layer thru a glass tube with a fine tip attached with rubber tubing to a water aspirator. Add 50 ml of 0.01 *N* NH_4OH, stopper funnel, and shake contents vigorously for 30 sec. to separate excess dithizone from the CCl_4 soln of zinc dithizonate.

Evaluation of Zinc Present

Draw off 2–3 ml of the CCl_4 soln of zinc dithizonate to flush out the bore of the stopcock and stem of the funnel and discard this portion. Collect the remainder in an amber glass bottle and stopper immediately. Pipet a 5-ml aliquot of this soln into a 25 ml, glass-stoppered volumetric flask of amber glass, make to volume with CCl_4, and mix.

Measure the per cent of light transmitted by the soln of zinc dithizonate with a suitable photoelectric colorimeter (or spectrophotometer) as directed in the A.O.A.C. procedure, and evaluate the quantity of zinc present from a light transmission-concentration curve prepared with known amounts of zinc carried through the modified procedure.

ACCURACY OF THE MODIFIED METHOD

The accuracy of the modified method was tested by: (a) analyzing synthetic solutions containing known amounts of zinc and various other cations, (b) analyzing several plant tissues with it and comparing values for zinc with those obtained by use of the A.O.A.C. method, and (c) analyzing plant tissues with and without added zinc. The results obtained are given in Tables 1, 2, and 3, respectively.

DISCUSSION

The addition of sulphuric acid to the sample prior to ashing was found to be advantageous in three ways: (a) reproducibility of results with some materials was improved, (b) it should help to prevent volatization of zinc and many of its salts, and (c) removal of the ash from the platinum dishes during the extraction process was greatly facilitated.

Extraction of acid-stable dithizonates from an acidified, aqueous solution with a CCl_4 solution of dithizone has been practiced by numerous

investigators, including Fischer and Leopoldi (5), Hibbard (6), and Bendix and Grabenstetter (7). The results of this investigation show that interferences by other ions present in extracts of plant ash are as effectively eliminated by extracting directly from the acidified solution as by using the extra step specified in the A.O.A.C. method.

The data in Tables 1 and 2 indicate good reproducibility of results by

TABLE 1.—*Application of the modified procedure to the determination of zinc in solutions containing known amounts of zinc and various other cations*

Zn	MICROGRAMS							
	CATIONS PRESENT IN ALIQUOTS ANALYZED						ZINC DETERMINED	VARIATION FROM THE THEORETICAL
	Pb	Cu	Hg	Co	Cd	Ni		
—	25	—	—	—	—	—	0	0
14.9	25	—	—	—	—	—	15.0	0.1
14.9	100	—	—	—	—	—	15.0	0.1
—	—	25	—	—	—	—	0	0
14.9	—	25	—	—	—	—	15.1	0.2
14.9	—	100	—	—	—	—	16.2	1.3
9.0	—	—	5	—	—	—	8.8	−0.2
14.4	—	—	100	—	—	—	14.5	0.1
9.6	—	—	—	—	—	5	10.4	0.8
9.6	—	—	—	—	—	10	10.3	0.7
20.2	—	—	—	—	—	25	21.1	0.9
9.6	—	—	—	5	—	—	9.2	−0.4
9.6	—	—	—	10	—	—	9.4	−0.2
9.6	—	—	—	20	—	—	9.7	0.1
9.0	—	—	—	—	2.5	—	9.5	0.5
20.2	—	—	—	—	100.0	—	24.0	3.8

TABLE 2.—*P.p.m. of zinc determined by the A.O.A.C. and modified procedures, respectively*

PLANT TISSUE ANALYZED	PROCEDURE	
	A.O.A.C.[1]	MODIFIED[2]
Alfalfa leaf meal	26.9	28.0
Parsnip roots	22.2	24.6
Spinach leaves	45.1	42.4

[1] Averages of 8 results.
[2] Averages of 2 results.

the modified method. Consideration of the data in Table 3 is of interest. One hundred micrograms of lead in the aliquot analyzed did not interfere with the determination of zinc. Likewise 25 micrograms of copper did not interfere, but 100 micrograms gave a high value for zinc. In the authors' opinion, however, it seems that the latter quantity of copper would seldom

occur in the aliquot of an extract of plant ash suitable for the determination of zinc by this procedure. Out of 39 different kinds of plant materials that have been analyzed for copper in the authors' laboratory, the highest values obtained were 52 and 70 p.p.m. The remaining 37 values ranged from 1 to 30 p.p.m. Even with the tissue that contained 70 p.p.m. of copper, the aliquot normally used for determining zinc would contain only 35 micrograms, an amount that would probably be effectively removed by the modified procedure. In analyzing a sample for zinc that was known to

TABLE 3.—*Zinc determined by the modified procedure in plant tissues with and without added zinc*

PLANT TISSUE ANALYZED	MICROGRAMS OF ZINC IN ALIQUOTS ANALYZED				
	IN TISSUE[1]	ADDED	TOTAL PRESENT	DETERMINED[2]	PER CENT OF TOTAL
Lettuce leaves	14.3	1.2	15.5	15.2	98.1
Lettuce leaves	14.3	2.4	16.7	16.3	97.3
Lettuce leaves	14.3	2.9[3]	17.2	16.6	96.5
Lettuce leaves	14.3	4.8[3]	19.1	19.6	102.4
Lettuce leaves	14.3	6.7[3]	21.0	21.9	104.3
Lettuce leaves	14.3	9.6[3]	23.9	24.5	102.5
Alfalfa leaf meal	11.6	4.8	16.4	15.9	96.7
Alfalfa leaf meal	11.6	9.6	21.2	21.3	100.5
Spinach leaves	11.8	4.8	16.6	17.0	102.4
Spinach leaves	11.8	9.6	21.4	21.2	99.1

[1] Determined by the modified procedure.
[2] Averaged values from replicate determinations.
[3] In these cases zinc was added after ashing, in all others before.

contain an above-average quantity of copper, a prolonged period of shaking during extraction of the acidified solution, as recommended by Bendix and Grabenstetter (7), would probably be effective in removing the copper. In the authors' experience an extra extraction with dithizone solution will achieve the same result.

The difference between the theoretical and determined values for zinc in the presence of 100 micrograms of mercury was within the limits of experimental error. Added nickel increased the determined value for zinc perceptibly; however, Bertrand and Makragnatz (8) determined nickel in a considerable variety of plant materials, and the highest concentration of nickel encountered was 3.5 p.p.m. Hence, it appears that nickel would seldom interfere seriously with the determination of zinc in plant material by the modified procedure. Cobalt up to 20 micrograms did not interfere with the determination. Out of 39 different kinds of plant materials that have been analyzed for cobalt in the authors' laboratory, the highest con-

centration encountered was .55 p.p.m.; therefore, it seems unlikely that cobalt would ever interfere to an appreciable extent in determining zinc.

Even small amounts of cadmium interfered perceptibly. Cholak *et al.* (9) attributed such interference to the instability of cadmium carbamate. Decomposition of this compound permits some of the cadmium to form a dithizonate which is evaluated as zinc. It should be pointed out, however, that this is true of the A.O.A.C. method also. Fortunately, high concentrations of cadmium are not known to occur in plants. Klein and Wichmann (10) analyzed several kinds of plant products and found less than 1 p.p.m. of cadmium. This is in keeping with the authors' experience in this regard.

It is believed that the modification of the tentative A.O.A.C. method presented in this report is definitely more convenient than the original and that it can be used without sacrifice of accuracy.

It is recommended* that the study be continued.

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REPORT ON SODIUM IN PLANTS

By RAY L. SHIRLEY, *Associate Referee*, and ERWIN J. BENNE (Michigan Agricultural Experiment Station, East Lansing, Michigan)†

In 1935 the Association accepted the magnesium uranyl acetate method as a tentative method for the determination of sodium in plant materials, and included it in the Plant Chapter of the Fourth Edition of *Methods of Analysis, A.O.A.C.* This method was proposed by Caley and Foulk (1) and is based on the precipitation of a salt formed between sodium and magnesium uranyl acetate.

* For report of Subcommittee A and action of the Association, see *This Journal*, **32**, 45 (1949).
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In 1934, Butler, then Associate Referee on sodium in plants, compared the method with the direct weight method and the sodium perchlorate method, in the analysis of a standard solution of sodium chloride; and with the latter when applied to the analysis of grasses and soil. At the time recommendation for adoption was made (3) as well as the following year (4) further study of the tentative method was recommended; however, no report of the proposed study has been published. The present Associate Referee was appointed in 1948 to carry out such a study, and the findings to date are described in this report.

EXPERIMENTAL AND RESULTS

To date the investigation has included the following: (A) A comparison of the tentative A.O.A.C. method (5) with the zinc uranyl acetate method of Barber and Kolthoff (6) in regard to (a) the solubility characteristics of the respective precipitates concerned and (b) the results obtained in the analysis of synthetic solutions and various crop plants; (B) The precision of the tentative method when applied to different plant materials and when varying sample weights were used; and (C) Application of the tentative method to the analysis of different plant materials with and without added sodium. Each of the above will be described in turn.

Effects of temperature on the solubility of sodium magnesium uranyl acetate, and the corresponding zinc salt, in the respective precipitating and wash reagents.—In this study approximately 0.1 g samples of sodium magnesium uranyl acetate and the corresponding zinc salt were weighed into 100 ml beakers, and 50 ml of the corresponding uranyl acetate reagent added and stirred at intervals to effect solution. In the case of the magnesium uranyl acetate reagent, studies were made on (a) reagent which was saturated previously at 23°C. with sodium magnesium uranyl acetate, and (b) reagent which was not saturated with the sodium salt. In the case of the zinc uranyl acetate it was saturated with the respective sodium salt but was used (a) with 15 ml of concentrated nitric acid per 100 ml of reagent added according to the recommendations of Broadfoot and Browning (7), and (b) without addition of nitric acid to it. Solubility determinations were made at 4°, 23°, and 35°C. The determinations at 4°C. were allowed to stand 44 hours in an ice box; whereas those made at 23° and 35° were allowed to be in contact with the salts for 30 minutes, after which the solutions were filtered through Selas crucibles. Fifteen ml of 95 per cent alcohol at the respective temperatures were used for transferring and washing the precipitates. The crucibles and contents were dried at 100°C. for 16 hours. The data obtained are shown in Table 1. These data indicate (a) that saturation of the magnesium uranyl acetate reagent with sodium magnesium uranyl acetate has little influence on the results obtained, (b) temperatures appreciably different from 23°C. which was the temperature at which the reagents were prepared should be avoided during the precipi-

tation of the sodium, and (c) the zinc uranyl acetate reagent with nitric acid is more sensitive to temperature changes in effecting solubility than is the magnesium uranyl acetate reagent.

TABLE 1.—*Effect of temperature on solubility of magnesium and zinc sodium uranyl acetate salts in their respective reagent solutions*

TEMP. C°	Mg[1] PER 100 ml			
	MAGNESIUM SALT		ZINC SALT	
	SATURATED[2]	UNSATURATED	WITH HNO_3	WITHOUT HNO_3
4[3]	−8.5	−10.8	−151.6	—
23	0.4	0.6	3.8	2.4
35	12.7	13.6	23.3	—

[1] Values given are averages of duplicate determinations.
[2] Saturated with sodium magnesium uranyl acetate at 23°C.
[3] Negative signs indicate that salts came out of solution.

Solubility of magnesium and zinc sodium uranyl acetate salts in 95 per cent alcohol during the washing process.—To make this study approximately 0.1 g samples of the magnesium or zinc sodium uranyl acetate salts were weighed into tared Selas crucibles and the salts were rinsed with five 5-ml quantities of either (a) 95 per cent alcohol saturated with the respective sodium salt, or (b) 95 per cent alcohol containing no salt. Determinations were made at 25° and 35°C. and the data obtained are shown in Table 2. These data indicate that (a) in the case of magnesium sodium uranyl acetate both the saturated and unsaturated alcohol wash solution effect a loss of approximately 2 mg of salt per 25 ml at 25°C., (b) approximately twice this much, *i.e.*, 4 mg of salt is dissolved at 35°C. using the unsaturated alcohol wash solution, (c) the zinc sodium uranyl acetate is slightly more soluble in the unsaturated alcohol than in the saturated alcohol wash solution, and (d) the zinc salt is approximately twice as soluble in general as the magnesium salt.

TABLE 2.—*Solubility of magnesium and zinc sodium urnayl acetate salts in alcohol wash solutions*

SODIUM URANYL SALT	Mg[1] PER 25 ml		
	25°C.		35°C.
	SATURATED	UNSATURATED	UNSATURATED
Magnesium	2.0	2.1	4.1
Zinc	3.7	5.3	8.8

[1] Average of two or more determinations.

Effect of washing all glassware with dilute nitric acid immediately before use.—Table 3 shows the values of the blanks obtained in the magnesium

TABLE 3.—*Blanks obtained with and without cleansing of all glassware with nitric acid just before use*

Mg OF PRECIPITATE	
HNO_3 CLEANED	NOT HNO_3 CLEANED
2.5	24.6
3.9	5.5
6.6	10.2
6.2	10.4
2.7	32.6
5.4	21.8

uranyl acetate procedure where the usual clean glassware and crucibles were used vs. where all surfaces were rinsed with dilute nitric acid and distilled water just previous to use.

Comparison of the magnesium and zinc uranyl acetate methods for the analysis of sodium in c.p. NaCl solutions.—Table 4 shows data obtained when sodium in c.p. solutions of sodium chloride was determined by the two methods. The data indicate that reasonably satisfactory analysis of known sodium solutions may be made by either method.

TABLE 4.—*Results by the magnesium and zinc uranyl acetate methods with known amounts of sodium*

Mg Na PRESENT	Mg Na DETERMINED	
	MAGNESIUM METHOD	ZINC METHOD
0.235	0.260 0.390	0.360 0.280
0.705	0.790 0.760	0.750 0.770
1.000	1.00, 0.95 1.00	—
1.175	1.250 1.270	1.260 1.210
2.350	2.48 2.44	2.37 2.38
23.500	23.35 23.20	23.52 23.38

Comparison of the magnesium and zinc uranyl acetate methods in the analysis of plant samples for sodium.—Plant samples of rape, sugar beet roots, and sugar beet tops were analyzed for sodium by both methods. Table 5 shows the data obtained. Excellent duplication of values were obtained by both methods and both methods gave essentially the same results.

Precision of the results obtained by the magnesium uranyl acetate method for sodium in plants when different weights of samples were used.—Table 6 shows the precision of the results obtained by the tentative A.O.A.C. method (5) when applied to the analysis of celery and corn using samples of different weights. These results should be considered satisfactory.

TABLE 5.—*Comparison of the magnesium and zinc uranyl acetate methods in the analysis of plant samples*

PLANT SAMPLE	SAMPLE NO.	% SODIUM DETERMINED					
		MAGNESIUM URANYL ACETATE DUPLICATE DETERMINATIONS			ZINC URANYL ACETATE DUPLICATE DETERMINATIONS		
		1	2	AVE.	1	2	AVE.
Rape	1	0.375	0.369	0.372	0.337	0.401	0.369
	2	1.06	1.07	1.07	1.12	1.08	1.10
Sugar beet roots	1	0.176	0.169	0.173	0.169	0.171	0.170
	2	0.061	0.060	0.061	0.057	0.057	0.057
Sugar beet tops	1	1.08	1.06	1.07	1.06	1.06	1.06
	2	0.185	0.185	0.185	0.175	0.166	0.171

TABLE 6.—*Precision of results obtained by the tentative A.O.A.C. method for sodium in plants when different weights of samples were used*

SAMPLE	WEIGHT	SODIUM DETERMINED	
	g	*mg*	*per cent*
Celery	0.5	4.95	0.99
	1.0	9.54	0.95
	1.0	9.51	0.95
	2.0	19.93	1.00
	2.0	19.51	0.98
Corn	1.0	3.34	0.27
	1.0	2.90	0.28
	3.0	8.09	0.27
	3.0	7.66	0.26

TABLE 7.—*Analysis of plant materials with and without added sodium by the tentative A.O.A.C. method*

PLANT MATERIAL[1]	Mg SODIUM			
	PRESENT[2]	ADDED	TOTAL	DETERMINED
Alfalfa	0.40	1.00	1.40	1.45
Soybean oil meal	0.12	1.00	1.12	1.27
Soybean oil meal	0.12	2.00	2.12	2.11
Oats	0.40	1.00	1.40	1.41
Corn	2.69	1.00	3.69	3.71
Celery	9.53	2.00	11.53	11.50
Celery	9.53	3.00	12.53	12.83

[1] One gram samples used.
[2] Averages of results of duplicate determinations.

Application of the magnesium uranyl acetate method to the analysis of sodium in plant materials with and without added sodium.—Table 7 shows the results obtained when the tentative A.O.A.C. procedure (5) was applied to the determination of sodium in various plant materials with and without added sodium.

DISCUSSION AND CONCLUSIONS

The comparisons made in this investigation are not conclusive as to whether the magnesium or the zinc uranyl acetate procedures have any marked advantages over one another. The magnesium reagent shows slight advantage in that the sodium salt has less solubility in the precipitation and wash reagents. These differences are not great and apparently are taken care of in the blank values obtained since both methods gave essentially the same results when applied to the analysis of plant materials. The solubility data obtained in this investigation indicate that the precipitation of the sodium should be made at a temperature near that at which the precipitating reagent was prepared, since at higher temperatures, *i.e.*, at 35°C. it is capable of dissolving appreciable quantities of the sodium salt, and at very low temperatures, *i.e.*, 4°C., appreciable amounts of non-sodium uranyl salts may precipitate out.

The prevalence of sodium salts about the laboratory makes it necessary to exercise extreme care in the cleansing of all glass surfaces before use in the sodium procedures. Washing all surfaces with dilute nitric acid just before use resulted in uniformly low blank values.

The accuracy of both the magnesium and zinc uranyl acetate procedures was demonstrated to be satisfactory on known c.p. sodium chloride solutions in the range of 1 mg. or more of sodium. Caley (8) reported that the magnesium uranyl acetate reagent would not give satisfactory results with samples containing less than 0.2 mg. of sodium. The precision of the magnesium uranyl acetate method was shown to be satisfactory when varying weights of celery and corn samples were analyzed. However, it was found that some plant materials, such as alfalfa leaf meal, soybean oil meal, and oats, gave colloidal-type precipitates during the sodium precipitation if too large samples were taken for analysis. This may have been due to some interfering ion such as phosphorus being present in greater concentration than the method could tolerate.

Caley and Foulk (1), using magnesium uranyl acetate, found that when 10 or 20 mg of sodium were precipitated in either 100 or 200 ml of reagent, 250 mg of potassium did not interfere. Broadfoot and Browning (7) reported that with the zinc uranyl acetate method, 50 mg of potassium, 1.0 mg of sodium, and 10 ml of reagent, the results were about 10 per cent high. They found that by dissolving the precipitate in 2 ml of water and again precipitating the sodium in the usual manner theoretical recovery was obtained. As plants commonly contain as much as 40 mg of potassium

per gram and some may contain as much as 80 mg per gram (9) a sufficiently small sample must be taken for analysis in order that it will not exceed the amount of potassium that can be tolerated, or the potassium must be removed to a tolerable level before the final sodium precipitation is made.

Phosphorus, which forms uranyl phosphate, has been found to be removed satisfactorily by use of calcium chloride and ammonium hydroxide (7). Calcium hydroxide has been used to advantage in the removal of interfering phosphates (7, 10). Caley and Sickmann (11) found that 1 mg of sodium in the presence of 500 mg of aluminum or chromium may be determined within ± 0.2 mg error. They also reported that a concentration of ammonium or sulfuric ions, as can ordinarily occur in 5 ml of test solution, will not result in the precipitation of the salt complex, $(NH_4)_2SO_4 \cdot MgSO_4 \cdot 6H_2O$, where 100 ml of reagent is used. Lithium has been found to interfere markedly in the determination of sodium (2). However, lithium has been reported to occur in extremely low concentrations (12) relative to sodium in plants.

The recoveries of the small amounts of added sodium to the various plant materials should be considered satisfactory.

RECOMMENDATIONS*

The Associate Referee recommends that the magnesium uranyl acetate method should be kept tentative and that the study be continued, especially in respect to tolerance of the procedure to various levels of interfering ions such as occur in plant materials.

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* For report of Subcommittee A and action of the Association, see *This Journal*, **32**, 45 (1949).

REPORT ON LIGNIN AND CELLULOSE IN PLANTS

By GORDON H. ELLIS (U. S. Plant, Soil, & Nutrition Laboratory, Agricultural Research Administration, Ithaca, New York), *Associate Referee*

The estimation of the nutritional value of forages and other feed stuffs has, for many years, been based on their content of ether extract, crude protein (N×6.25), ash, crude fiber, and nitrogen-free extract. The need for revision of this method has long been recognized.[1] The main basis for the Henneberg method is that crude fiber is designed as a measure of the fibrous, relatively poorly digested part of plants, while the nitrogen-free extract is designed to represent the more readily digested fraction. Crampton and Maynard[2] pointed out that crude fiber is often as completely digested by ruminants as is the nitrogen-free extract, thus raising a serious objection to this scheme. Norman[3] showed that lignin, which is relatively indigestible, is present in varying amounts in both the crude fiber and nitrogen-free extract fractions, thus indicating further the inadequacy of the present method. Mitchell[4] has stated the situation satisfactorily, in a review dealing with methods for evaluating feeds, when he stated " . . . it would seem more profitable to study the digestibility of nutrients, or classes of nutrients, better characterized and more accurately determinable chemically than those involved in the old routine method of analysis. Such additional measurements might well include lignin, cellulose, fatty acids . . . "

Crampton and Maynard[5] have proposed that the carbohydrate fraction be defined in terms of lignin, cellulose, and "other carbohydrates" (obtained by difference) rather than in terms of crude fiber and nitrogen-free extract. Studies made with animals along these lines indicate that this proposal is a valuable one. Further study is needed and there is a real need for some agreement as to suitable procedures for lignin and cellulose. The adoption of a uniform method for lignin would be of value if for no other reason than that data from several laboratories could be used in determining the correlation between lignin content and the digestibility of various forages by livestock.

While cellulose is a reasonably well defined entity, lignin is not, and many annoyances arise when attempting a quantitative estimation. This problem has recently been discussed by Phillips.[6] The two most widely employed methods for lignin are the 72 per cent sulfuric and the fuming hydrochloric acid ones. A thorough study of the latter procedure has been made by Phillips and coworkers and has been adopted as a tentative

[1] *This Journal*, **23**, 102, (1940).
[2] *J. Nutrition*, **15**, 383, (1938).
[3] *J. Agr. Research*, **25**, 529, (1935).
[4] *J. Animal Sci.*, **1**, 159, (1942).
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[6] *This Journal*, **23**, 108, (1940).

method by this Association. It is not the purpose of the present study to replace this method, but to initiate work leading to another procedure more adaptable to routine use. Since there is no absolute chemical criterion by which to judge the validity of a lignin method, this will have to be judged finally by its utility in biological studies. As a prior condition, such a method should meet an acceptable standard of reproducibility and convenience. As a starting point, a 72 per cent sulfuric acid method[7] which already has been found of value in nutrition work has been chosen for this study. The objective in this study is to determine the variation in results as obtained by different analysts on a common sample.

A modified Norman-Jenkins method[8] for cellulose was chosen as a starting point primarily because the cellulosans are included along with the "true" cellulose $[(C_6H_{10}O_5)_n]$ in this method and Ferguson[9] has presented evidence that the cellulosans are digested to the same extent by sheep as is "true" cellulose. This method has the additional advantage that a larger proportion of the carbohydrate fraction of plants is included, particularly in the case of the grasses.

COLLABORATIVE WORK

SAMPLES USED

No. 1 (a mature Timothy hay) ground in a Wiley mill to pass a 40 mesh sieve.

No. 2 (an immature Sudan grass) obtained already ground from the Cerophyl Co., Kansas City, Missouri, and all passed a 40 mesh sieve.

No. 3 (sheep feces) ground in a Wiley mill to pass a 40 mesh sieve.

Samples 1 and 2 were submitted to the 8 collaborating laboratories with the request that 6 analyses be made. Sample 3 was submitted to 5 of these laboratories.

METHODS OF ANALYSIS

Lignin.—Extract 1 g of sample with an alcohol-benzene mixture (1:2 by volume) for 4 hours in Soxhlet or comparable apparatus (the extraction vessel may be either a coarse porosity alundum or a paper thimble, closed at the top with filter paper or plug of cotton). Wash the sample in the thimble, with the aid of suction, using two small portions of alcohol followed by two small portions of ether. Heat at 45°C. in a non-sparking oven to drive off the ether and transfer the sample to a 250 ml, wide-mouthed Erlenmeyer flask. Add 40 ml of 1% pepsin (U.S.P. grade) in 0.1 *N* HCl, wetting the sample well by adding a small portion of the soln, stirring or shaking thoroly, and finally washing down the sides of the flask with the remainder of the soln. Incubate at 40°C. overnight. Add 20–30 ml of hot H_2O and filter using a filter stick.[10] Repeat this washing twice and then wash the residue into the flask by forcing 7–8 ml of 5% H_2SO_4 soln (by weight) downward thru the filter stick with the aid of

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[7] *J. Animal Sci.*, **5**, 285 (1946).
[8] *J. Animal Sci.*, **5**, 306 (1946).
[9] *Biochem. J.*, **36**, 786, (1942).
[10] The filter sticks are of the type made with a pyrex fritted glass disk 30 mm. diameter, medium porosity. A thin layer of pre-ashed diatomaceous earth (hyflo supercel, or similar filter aid) is sucked onto the filter disk from a water suspension. This is usually sufficient for easy filtration; if not, add extra supercel to the material being filtered. In the author's laboratory, about one-third of the sticks purchased were found to filter slowly with some samples. It may be advisable, therefore, to purchase more than needed and discard the slow-filtering ones. It is convenient to arrange the filter sticks in a set of twelve attached to a vacuum manifold by rubber tubing.

air pressure. Wash the stick further with the H_2SO_4 soln, finally adding enough to the flask to bring the total volume to ca 150 ml. Reflux vigorously on a hot plate for one hour, adding H_2O occasionally to maintain the original volume. Filter off the acid. Wash the residue three times with 20–30 ml portions of hot H_2O, twice with 15–20 ml portions of alcohol, and twice with 15 ml portions of ether. Leave the vacuum on a few minutes to dry the residue, and transfer from the stick into the flask by tapping and brushing. Heat to drive off any residual ether. If the disk formed upon drying is difficult to break up into a finely divided state, as it sometimes is in the case of immature plant samples, disperse the residue in ether in the flask and then boil off the ether on a steam bath. Add 20 ml of 72% H_2SO_4 (by weight) at 20°C. to the residue and hold at 20°C. for two hours with occasional stirring. Add 125 ml of H_2O, filter, wash once with 20 ml of hot H_2O and again filter. Wash the residue from the filter stick and reflux as before for two hours, using 150 ml of 3% H_2SO_4 (by weight). Filter the residue on a Gooch crucible with an asbestos pad and wash with hot H_2O until free of acid. Dry at 105–110°C., and determine lignin by the loss of weight on ignition at 600°C.

Cellulose.—Extract 1 g of the sample with an alcohol-benzene mixture (1:2 by volume) in a soxhlet apparatus for 3–4 hours, or alternatively, add the solvent to the sample in a 100 ml beaker (tall form), allow to stand at room temp. for one hour with occasional stirring. Filter using a filter stick (see footnote under lignin method). Repeat with two more portions of solvent. To the extracted sample in a 100 ml beaker add 50 ml of 3% Na_2SO_3 soln and bring to a boil. Filter and add 10 ml of 0.25 *N* NaOH in dilute alcohol (3 alcohol to 2 H_2O) washing out the filter stick with this soln. Stir and neutralize to a *p*H of 7–9 with 1 ml of 2.5 *N* H_2SO_4, filter, wash the residue once with 25 ml of hot H_2O, and again filter. Add 43 ml of cold H_2O and 7 ml of NaClO soln (5.25% available chlorine).[11] Allow to stand for 10 min., stirring occasionally with the filter stick. Filter, add 50 ml of 3% Na_2SO_3 soln and boil for at least 5 min., the filter stick serving to decrease bumping. Filter and repeat the 0.25 *N* alcoholic NaOH treatment as before. Suspend the neutralized and washed material in 50 ml of H_2O, add 1.5 ml of NaClO soln and 1 ml of 20% H_2SO_4 (by weight). Chlorine is evolved and the material frequently turns yellow. After standing for 10 min. with occasional stirring and protected from direct sunlight, filter and treat with the Na_2SO_3 soln as before. The intense purple coloration noted indicates the presence of lignin. Continue the alcoholic NaOH washings and the acid hypochlorite treatments as long as a positive reaction for lignin is found upon addition of the sulfite. After the final boiling with sulfite, filter and wash with 50 ml of hot H_2O and transfer the cellulose to an alundum crucible. Wash several times more with hot water. Dry at 105–110°C. and determine the cellulose by the weight loss on ignition.

RESULTS

The results are given in Table 1. A moisture determination in duplicate was made in each laboratory and this value used to calculate the results on a dry basis weight. In addition to the mean value, the standard error of the mean and the extremes for the six determinations are given as an indication of the variability of the results in each laboratory.

It is apparent that a greater magnitude of difference occurs between laboratories as compared to the differences within a single laboratory,

[11] "Chlorox" sold as a household bleach is satisfactory. This will deteriorate on long standing, so the supply should be fresh.

particularly with the cellulose values. These differences could be due to a non-uniformity of the samples, but in view of the close agreement found for the lignin values with sample No. 1, this is probably not the case. In comparing the lignin values, the results from laboratory G indicate that the temperature during the 72 per cent sulfuric acid treatment is an important factor. Omitting the values from laboratory G and H, the only

TABLE 1.—*The lignin and cellulose values for reference samples*

LAB.	LIGNIN (PER CENT DRY MATTER BASIS)		
	SAMPLE NO. 1	SAMPLE NO. 2	SAMPLE NO. 3
A	10.17 ± .075 (10.0–10.3)	5.65 ± .065 (5.39–5.87)	23.48 ± .101 (23.1–23.7)
B	10.27 ± .081 (10.1–10.5)	5.40 ± .120 (5.10–5.72)	
C	10.19 ± .063 (10.0–10.4)	5.38 ± .036 (5.27–5.46)	
D	10.03 ± .021 (9.95–10.1)	5.22 ± .037 (5.10–5.35)	23.10 ± .102 (22.8–23.4)
E	10.31 ± .068 (10.2–10.5)	5.83 ± .038 (5.71–5.96)	
F	10.20 ± .188 (9.40–10.6)	6.08 ± .252 (5.04–6.58)	24.06 ± .188 (23.7–24.5)
G[1]	12.02 ± .120 (11.8–12.4)	6.23 ± .046 (6.12–6.39)	24.05 ± .135 (23.7–24.5)
H[2]	10.65	6.68	24.42
	CELLULOSE (PERCENT DRY MATTER BASIS)		
A	27.32 ± .029 (27.1–27.4)	24.13 ± .053 (23.9–24.2)	37.69 ± .116 (37.2–38.0)
B	31.05 ± .064 (30.9–31.2)	26.47 ± .093 (26.2–26.6)	
D	26.01 ± .685 (25.2–26.5)	25.20 ± .217 (24.9–25.3)	35.86 ± .578 (35.5–36.7)
E	29.67 ± .174 (29.3–30.4)	23.39 ± .286 (22.6–24.5)	
F	29.27 ± .153 (28.8–30.0)	23.20 ± .551 (20.7–24.4)	36.47 ± .100 (36.2–36.9)
G	33.30 ± .374 (29.9–31.6)	28.04 ± .163 (27.6–28.5)	44.41 ± .350 (43.4–45.4)
H	28.3	22.86	37.26

[1] In this laboratory the temperature during the 72 per cent sulfuric acid treatment ranged between 30.5 and 31.5°C.

[2] The individual values were not obtained from this laboratory in time to allow the inclusion of further information in this report.

statistically significant difference (at the 1% level) between laboratories for the lignin values on sample No. 1 is that between D and E, this difference amounting to 3 per cent. For sample No. 2, 4 of a possible 15 differences are significant (between D and ACEF). For sample No. 3, the results from laboratory F are higher than those from either A or D the greatest difference being approximately 4 per cent.

The cellulose values show a much greater variation from laboratory to laboratory even though the range of values for the six determinations within a given laboratory may be fully as limited as for the lignin values.

Consideration of these data indicates that the lignin method is reasonably satisfactory in so far as reproducibility is concerned, although further work, particularly with immature plant samples, would be desirable.

The cellulose method is clearly unsatisfactory and work leading to a closer definition of the conditions for carrying out the isolation of cellulose should be undertaken.

RECOMMENDATIONS*

It is recommended—

(1) That collaborative work on the lignin method be continued for another year using other samples of plant material.

(2) That the cellulose method be studied to determine the causes for the variation in results that have been found.

COLLABORATORS

The Associate Referee wishes to express his appreciation of the fine cooperation shown by the collaborators in this study.

C. E. French, Pennsylvania State College, State College, Pa.
Bartley P. Cardon, University of Arizona, Tucson, Ariz.
John Lawrence, State College of Washington, Pullman, Wash.
R. M. Forbes, University of Kentucky, Lexington, Ky.
R. E. Davis and Ivan Lindahl, Bureau of Animal Industry, U.S.D.A., Beltsville, Md.
Miss Jean Lowe, U. S. Plant, Soil, & Nutrition Laboratory, Ithaca, N. Y.
John K. Loosli and Miss Cornelia Hassan, Cornell University, Ithaca, N. Y.
Lorin E. Harris, Utah State Agricultural College, Logan, Utah.

REPORT ON STARCH IN PLANTS

By CARROLL L. HOFFPAUIR (Southern Regional Research Laboratory,† New Orleans, Louisiana), *Associate Referee*

Where a considerable amount of starch is present, as in cereal grain or tubers, there are several satisfactory methods for its determination. However, when dealing with tissues low in starch but high in accompanying non-starch polysaccharides, the difficulties are accentuated. Preliminary to collaborative work it is found essential to investigate possible methods for the determination of starch in plant materials containing low percentages.

To be generally applicable such a method should meet the two criteria stated by Denny (1), namely, the method must give correct values when pectin, non-starch polysaccharides and protein are present in the sample; and the method should give zero or trace values on samples containing little or no starch by qualitative tests, but containing high amounts of substances which might interfere. Steiner and Guthrie (2) have developed a method which separates starch from the interfering materials usually

* For report of Subcommittee A and action of the Association, see *This Journal*, **32**, 45 (1949).

† One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

present in plant materials. It consists of treatment of the sample at boiling temperature with dilute ammonium carbonate solution, precipitation of the starch with iodine, decomposition of the starch iodide, reprecipitation with iodine, decomposition of the starch iodide, precipitation of the starch with alcohol, dispersion in calcium chloride, precipitation of any remaining protein with uranyl acetate and determination of the optical rotation. It is, however, recommended by the authors only for samples containing 10 per cent or more of starch since when less starch is present the optical rotation is too small to be read precisely. In an attempt to adapt the Steiner-Guthrie method to the determination of low percentages of starch, two methods of evaluating starch in the final calcium chloride dispersion were tried. The first of these was an acid hydrolysis of the starch to glucose followed by a reducing sugar determination. The second was a colorimetric method based on the blue starch-iodine complex.

SAMPLE MATERIAL

The following samples were used to test the method:

1. *Orange rind.*—Composite of many kinds of orange peel that had been dropped into boiling alcohol, ground in a food chopper, Soxhlet extracted with 80% alcohol and ground in a ball mill.
2. *Cottonseed meal.*—Composite of ground cottonseed kernels thoroughly extracted with ethyl ether, and then ground in a ball mill.
3. *Jerusalem artichokes.*—Artichokes were sliced and dropped into boiling alcohol, then ground in a Waring Blendor, dried and ground in a ball mill.
4. *Cotton root bark No. 1.*—Bark was extracted with alcohol and ground in a ball mill.
5. *Cotton root bark No. 2.*—Another sample of bark prepared as above.
6. *Cotton leaves.*—Leaves extracted with alcohol and ground in ball mill.

METHODS

Acid hydrolysis procedure.—Pipet a suitable aliquot (containing ca 10 mg of starch) of the calcium chloride dispersion obtained by the Steiner-Guthrie method into a 125-ml ground-glass stoppered Florence flask. Add water to make volume 50 ml and then add 5 ml of hydrochloric acid, sp. g. 1.125. Attach the flask to a reflux condenser and boil on a hot plate for 2.5 hours.

After cooling, neutralize the soln to the phenol red end point (*p*H 7 to 8) with 20% sodium hydroxide soln. Transfer to a 100-ml volumetric flask, make to volume with water, and mix well. Place a portion of the soln in an Erlenmeyer flask, add dry sodium oxalate and shake for about an hour. Test a drop of the supernatant for calcium on a black spot plate with sodium hydroxide soln. Continue the addition of sodium oxalate followed by shaking until the calcium has been removed. Centrifuge the mixture and analyze 5 ml of the supernatant for reducing sugars by the Somogyi modification of the Shaffer-Hartman method (3). Multiply the value of glucose found by the factor 0.93 to convert to starch.

Colormetric procedure.—Pipet an aliquot (containing ca 10 mg of starch), of the calcium chloride dispersion obtained by the Steiner-Guthrie method, into a 100-ml volumetric flask. Dilute to volume with water. Mix well. Pipet a 25-ml aliquot of the diluted soln into a 50-ml volumetric flask. Dilute to ca 40 ml with water. Add 2.5 ml of 10% acetic acid and 1ml of 5% potassium iodide soln. Mix by swirling. Pipet 5

ml of 0.01 *N* potassium iodate soln into the flask. Dilute to volume and mix thoroly. Read the transmission of the soln in a photoelectric colorimeter, using a filter with maximum transmission at 565 mμ after first setting the instrument at 100% transmission with a reagent blank. Determine mg of starch in the sample aliquot by means of a standard curve obtained from a known starch soln.

DISCUSSION

In Table 1 are shown the values obtained by the methods described together with values obtained by other well known methods on the samples described and used (4, 5).

TABLE 1.—*Comparison of methods for determination of starch*

METHOD	STARCH FOUND[1]					
	COTTON ROOT BARK NO. 1	COTTON ROOT BARK NO. 2	COTTON LEAVES	ORANGE RIND	COTTONSEED MEAL	JERUSALEM ARTICHOKES
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Values obtained on Steiner-Guthrie dispersion						
Acid hydrolysis	4.6 5.3	2.7 3.6	0.9 1.2	1.7 1.7 1.3 1.4	1.1 1.1	0.4 0.3
Colorimetric (potato starch standard)	4.6 4.6	6.0 6.0	2.0 2.0	0.4[2]	0.2[2]	0.0 0.0
Colorimetric (isolated starch standard)	4.1 4.1	5.4 5.4	1.8 1.9			
Values obtained by other methods						
Taka-diastase	4.9[3] 5.1[3]	6.0[3] 6.2[3]	1.7[3] 1.8[3]			
Malt-diastase				12.8[2]	9.8[2]	27.4[2]
Hopkins				22.6[2]	−4.4[2]	−18.0[2]
Steiner-Guthrie	3.1 3.2	4.6 4.8	0.9 0.9	−0.2[2]	−0.6[2]	− 0.3[2]

[1] All values were obtained on the samples used and described.
[2] Values obtained by Steiner and Guthrie (2).
[3] Values obtained by Eaton and Ergle (9).

Qualitative tests with iodine indicated that the first cotton root bark sample contained somewhat less starch than the second sample and that the cotton leaves contained less than the bark samples. All three of these

samples were deeply colored. Nielson (6, 7) has pointed out that the intensity of the starch-iodine color is dependent on the amylose-amylopectin ratio. Consequently the starch dispersions from samples of cotton root bark and cotton leaves were compared to color standards made from starch isolated from the respective samples as well as with those from white potato starch. Because of the difficulty of isolating pure starch from such materials the values so obtained are probably somewhat high. They are, however, slightly lower than those obtained using potato starch as a standard. The starch contents were considerably below the best range of the Steiner-Guthrie method, and the values obtained by it are lower than the colorimetric values while those by the taka-diastase method are slightly higher for the bark samples and in good agreement for the sample of leaves. With each of these methods the starch contents were in the same order as indicated by the qualitative iodine test, and the agreement between duplicate determinations was satisfactory. The acid hydrolysis method on the other hand gave poorly agreeing duplicate values and reversed the order of the starch contents of the cotton root bark samples.

Qualitative test indicated that the orange rind sample contained very little starch. The colorimetric value was in agreement with the qualitative test. The Steiner-Guthrie method gave a low negative value and the acid hydrolysis method a somewhat higher value than the colorimetric method. Both the malt-diastase and Hopkins methods gave values which were absurdly high. These high values are no doubt due to the interference of polysaccharides such as pectin. A similar pattern of results was obtained with cottonseed meal, which qualitative test indicated contained only a trace of starch. In this case the interference of protein gave negative values with both the Hopkins and the Steiner-Guthrie methods.

The sample of Jerusalem artichokes was starch free by qualitative test. The values obtained again emphasize the interference of non-starch polysaccharides. No starch was found with the colorimetric procedure.

While none of these methods are satisfactory for samples low in starch it is believed that the measurement of the light absorbed by the starch iodide complex shows considerable promise. Recent investigations of Swanson (8) suggest the possibility that the difficulties caused by the varying amylose-amylopectin ratio in starches from different natural sources may be overcome by suitable spectrophotometric measurements. It is recognized that the scattering of light by the dispersed starch may be an important factor in such measurements.

RECOMMENDATION*

It is recommended that the spectrophotometric measurement of starch-iodine dispersions be investigated as a possible method for the determination of starch in samples low in starch.

* For report of Subcommittee A, and action of the Association, see *This Journal*, 32, 45 (1949).

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REPORT ON BORON IN PLANTS

By CALVIN M. AUSTIN and L. K. WOOD (*Associate Referee*), Kentucky Agricultural Experiment Station, Lexington, Ky.

Because of personnel changes and other difficulties work on the boron problem was delayed, and results obtained this year are not felt to be appropriate for a report to the A.O.A.C.

At the present time work is underway to further investigate the "Chromotrope B" method of Austin and McHargue,[1] particularly ignition technique. The application of the method to soils and other biological material is also being investigated.

It is requested that those persons interested in collaborating in this work write the Associate Referee. It is hoped that several will respond so that an adequate collaborative program can be set up and a report submitted next year.

RECOMMENDATIONS*

It is recommended that the "Chromotrope B" method for boron be further investigated and that collaborative work be undertaken this year.

No report was given on sampling (plants) sugar, copper and cobalt, carotene, or pectin.

No report was given on spectrographic methods.

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[1] Calvin M. Austin and J. S. McHargue. *This Journal*, **31**, 427 (1948).
* For report of Subcommittee A and action of the Association, see *This Journal*, **32**, 45 (1949).

MONDAY—AFTERNOON SESSION

REPORT ON PROCESSED VEGETABLE PRODUCTS

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

It is recommended—*

(1) That studies of methods for determining quality factors in canned and frozen fruits and vegetables be continued.

(2) That studies of methods for the estimation of the enzymatic activity of frozen fruits and vegetables be continued.

(3) That studies of the determination of moisture in dried vegetables be continued.

REPORT ON PEROXIDASE IN FROZEN VEGETABLES

By M. A. JOSLYN (Food Technology Division, University of California, Berkeley 4, Calif.), *Associate Referee*

A variety of methods have been reported in the literature for both the quantitative and the qualitative measurement of peroxidase activity, and several of these methods have been applied to the determination of peroxidase activity in frozen vegetables with a considerable amount of conflicting data (see review by Joslyn (1)). There has been no critical investigation of the determination of peroxidase since that of Balls and Hale (2) in 1933. Their procedure was subjected to collaborative study chiefly for cereal products, but was applied in modified form by Joslyn *et al* (3) to the determination of peroxidase activity in frozen vegetables. The methods that have been proposed for the determination of plant peroxidase fall into the following categories:

(1) *Purpurogallin procedures.* The quantity of purpurogallin formed by oxidation of pyrogallol by peroxidase in presence of hydrogen peroxide has been repeatedly shown by Willstätter and his coworkers (4) to be directly proportional to the time of reaction and to the quantity of peroxidase present. The purpurogallin formed may be determined gravimetrically or colorimetrically after several extractions with ether. While this procedure has the advantage that it is not affected by the presence of catalase, it is time consuming, not very sensitive, nor sufficiently specific. Pyrogallol under the conditions used (5.0 grams of pyrogallol, 50 mg of hydrogen peroxide in reacting volume of 2 liters without buffer at 20°C; usual reaction time of 5 minutes) is not oxidized specifically by peroxidase as polyphenol oxidase and certain catalytically active metals may interfere.

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* For report of Subcommittee C and action of the Association, see *This Journal*, 32, 51 (1949).

(2) *Titrimetric hydrogen peroxide procedures.* Balls and Hale (2) introduced the iodometric determination of residual hydrogen peroxide as a simple and rapid method of measuring peroxidase activity. In their procedure the quantity of peroxide decomposed in the presence of 0.02*M* phosphate buffer at *p*H 8, 0.0016 *N* hydrogen peroxide, and 0.625 gm of pyrogallol in a reaction mixture of 250 ml, is determined periodically by titrating iodine liberated from 25 ml aliquots after reaction with potassium iodide in acid-pyrogallol solutions with standard thiosulfate (5). The reaction is allowed to proceed in a glass stoppered cylinder at 30°C. Freshly boiled water is used as a diluent, covered with air-free paraffin oil, and stirred with hydrogen or nitrogen just prior to introduction of enzyme solution. They found the decomposition of peroxide to be linear with time, provided the peroxide concentration is low (below 0.002 *N*), and the amount of enzyme is such that not more than one-fourth to one-third of the peroxide is decomposed.

Essentially similar conditions were used in our tests (3), except that paraffin oil was not used and the air in the reaction vessel was not swept out with nitrogen. The end point in the titration, however, was found to be sharper if autoxidation of the pyrogallol did not occur. This was best controlled by vacuumization of the reaction mixture followed by release of vacuum with nitrogen. Difficulty was experienced in obtaining constant P.E. values with the more active samples, even when only one-fourth of the peroxide present was decomposed in 10 minutes. The best results were obtained when the volume of 0.01 *N* thiosulfate used for the titration of the aliquots decreased from about 4.5 ml to 3.9 ml in the course of about 10 minutes.

Morris *et al* (6) further modified the Balls and Hale (2) titrimetric procedure by substituting guaiacol for pyrogallol, using a lower *p*H (5.6) because of change of substrate and the addition of an excess of standard thiosulfate to the mixture in which the unused hydrogen peroxide liberated iodine from potassium iodide. The latter change made it possible to titrate with standard iodine to a sharp end point, even on extracts containing considerable soluble starch.

(3) *Colorimetric procedures.* A large number of indicators, chiefly mono and polyhydroxy benzenes, mono and di-amines, amino phenols and related compounds, have been introduced as reagents for peroxidase. In the early investigations, the activity of peroxidases was investigated almost exclusively with these chromogenic substances. The rate of pigmentation during enzymatic reaction, however, is not necessarily proportional to the rate of oxidation, for pigment development is markedly influenced as to degree and color by *p*H, presence of reducing agents such as ascorbic acid which may retard color development for some time, and other factors.

In recent years, guaiacol (7), p-amino benzoic acid (8), α naphthol and

the Nadi reagent (9) have been used for quantitative assay. Of these guaiacol is more specific for plant peroxidase, since it is not oxidized by the usual plant phenolases and lends itself well to photo-electric-colorimetric measurement of rate of formation of the orange-brown oxidation product. In the procedure developed by Masure and Campbell, a 2 ml aliquot of the filtrate from a *p*H 4.5 citrate buffer extract of vegetable tissue is added to 20 ml of water in a test tube, followed by 1 ml of 0.5 per cent guaiacol in 50 per cent alcohol and 1 ml of 0.085 per cent hydrogen peroxide. The contents of the tube are then mixed, a portion of the reaction mixture transferred into 14 mm Klett-Summerson colorimeter tube, and placed in the colorimeter using the 420 millimicron filter. From the galvanometer readings taken at several time intervals, the divisions of color per minute are calculated and used as an index of peroxidase activity of extract.

In the Morris, *et al* (6) modification of this procedure, the reaction mixture consisted of 2.5 ml of 1 *M* acetate buffer of *p*H 5.6, 1 ml of 10 per cent guaiacol in 95 per cent alcohol, and sufficient water to make 50 ml, when the enzyme extract (usually 1–5 ml depending on activity) and 1 ml of 0.75 per cent hydrogen peroxide solution were added. The completed mixture was immediately stirred and a portion poured into the Klett-Summerson colorimeter tube. The rate of color formation, which was linear at first, was measured, using filter No. 42 (400–465 mμ). Since the *p*H optimum for the oxidation of guaiacol is close to 5.6, this change in *p*H used for measurement is desirable.

(4) *Direct oxidation of iodide.* It has long been known that peroxidase will catalyze the direct oxidation of dilute acid iodide solution by hydrogen peroxide, and recently the iodide oxidation has been proposed as a field test for dehydrated vegetables (10).

(5) *Ascorbic acid oxidation.* Since Szent-Gyorgyi's early demonstration (11) that "hexuronic acid" retarded the rate of color formation of peroxidase reagents, it has been demonstrated that peroxidase in presence of hydrogen peroxide and a suitable phenolic compound will rapidly oxidize ascorbic acid (12). Several methods based on the volumetric or photometric determination of the rate of oxidation of added ascorbic acid have been proposed (13).

It is fairly well established that activity measured by these various methods may differ widely, particularly when different substrates are used in gravimetric, titrimetric or volumetric methods. When the same substrate is used, agreement between colorimetric methods such as that of Morris *et al* (6) and the titrimetric method is good, at least for some vegetables. Not only does the relative activity of the peroxidases of a particular vegetable tissue vary with indicator used, but the apparent rate of thermal inactivation of the vegetable peroxidases in situ also varies with the indicator (1). Consequently, in interpreting peroxidase activity

as a possible index of quality retention in frozen vegetables, it is necessary to select a procedure which will measure that peroxidase activity which has a thermal destruction rate similar to the enzymes involved in off-flavor formation.

In the development of an appropriate peroxidase method we must also have a measure of sampling error due not only to the natural variability in vegetables, themselves, but also due to the unequal distribution of peroxidase in various portions of the plant. As a rule peroxidase activity is greatest in those vegetable parts which are most active in respiration, growth, etc., and is largely concentrated along the vascular bundles. The presence of thermostable peroxidase-like substances in localized regions of vegetable tissue as well as of naturally occurring inhibitors are additional factors involved. Furthermore, difference in degree to which the tissue peroxidases are made available for reaction, as well as the conditions of the test, will influence the results. There are significant differences between the intensity of color produced by test reagents in cut tissues macerates of such tissues, or filtrates prepared from them. Although filtration will remove portions of tissues containing thermostable peroxidase-like substances, it will also remove peroxidase activity that may be significant in testing for adequacy of the scalding procedure.

The proper preparation of tissue extracts for analysis is an important problem. Balls and Hale (2) proposed that 5 grams of material to be analyzed be ground fine in a mortar with clean, sharp sand, and then triturated with 45 ml of 0.1 *M* phosphate buffer (pH = 8) added gradually. The sand and larger particles are settled or centrifuged out and a suitable portion of the supernatant liquid used in enzyme determination. Joslyn, *et al* (3) modified this procedure somewhat by first grinding a representative sample of frozen vegetables in a food chopper while still frozen, then triturating a 5 gram aliquot with 10 grams of sharp quartz sand and 45 ml of 0.1 *M* phosphate buffer of pH 8.0, allowing the mixture to stand 15 minutes, and then separating the aqueous portion from the remaining fibers by pressing through cheesecloth.

Masure and Campbell (7) placed 60–150 gm of tissue in a Waring blendor jar with 3.0 ml of pH 4.5 citrate buffer per gram of tissue, saturated the mixture and displaced air in the jar with nitrogen, and then ground at full speed for 2 minutes with good flow of nitrogen. The extract was then filtered through cotton milk filter, discarding the first 10 or 20 ml of filtrate. Morris *et al* (6) prepared their extracts (of fresh carrots) by comminuting 50 gram portions of diced samples in a Waring blendor for 3 minutes with about 1 gram of calcium carbonate and sufficient cold 2 per cent aqueous sodium-chloride solution to make a total volume of 200 ml. The larger solid particles were then removed by filtration through a gauze-backed cotton milk filter. They reported that the enzyme activities of filtrates prepared as above, unfiltered salt-carbonate suspensions

and aqueous suspensions of carrots were about the same. Filtrates of aqueous extractions, however, exhibited only two-thirds of the total peroxidase activity.

Although Willstätter and his collaborators (4) found that the peroxidase of horseradish was fairly firmly adsorbed upon the tissue particles and readily solubilized by dilute alkalis, there is but little data on the relative solubility of other plant peroxidases. There are indications that incomplete extraction of peroxidases from vegetables such as asparagus occurs when acid buffers are used.

In the light of the problems summarized above, the investigations carried out the past year were purely exploratory to determine the most appropriate test procedure and method of preparation of sample. It was tentatively decided to concentrate on the guaiacol procedure because previous experience has shown that guaiacol-peroxidase activity appears to parallel off-flavor formation in most of the frozen vegetables tested. It is intended, however, to compare this with the ascorbic acid and titrimetric procedures. A suitable method for sampling, preparation of the sample, and extraction of peroxidase, has not yet been developed. Since the accuracy and precision of a given method must be tested in more than one laboratory and by more than one investigator, considerable thought was given to the feasibility of collaborative assay of frozen vegetables which would have to be shipped over long distances. Arrangements are being made to do this, however, in at least four laboratories. Now that the preliminary survey has been made, better progress on this problem is possible.

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No report was given on quality factors, moisture in dried vegetables, or catalase in frozen vegetables.

REPORT ON COLORING MATTERS IN FOODS

By C. F. JABLONSKI (Food and Drug Administration, Federal Security Agency, New York, N. Y.), *Referee*

The Committee of the A.O.A.C. requested the Referee to continue the study of a quantitative estimation of Tartrazine (FD&C Yellow No. 5) in presence of Sunset Yellow F.C.F. (FD&C Yellow No. 6). As a result of the investigation the following method is proposed.

The method is based on the observation that Sunset Yellow F.C.F. is completely destroyed by hydrogen sulphide heated under pressure with the formation of an almost colorless solution. Tartrazine on the other hand being more resistant to this treatment is affected only to a slight extent. Since the amount of Tartrazine destroyed appears to be proportional, results of a quantitative order are possible by applying a correction factor.

METHOD

Prepare 1% soln of the dye or dye mixture. For orientation purposes place 10 ml of a 0.01% soln in a test tube and add 0.5 ml 10% sodium hydroxide soln. The presence of FD&C Yellow No. 6 manifests itself by a more or less brownish red coloration depending on the respective amounts. FD&C Yellow No. 5 is almost unaffected. Add to this alkaline color soln a few crystals at a time of sodium hydrosulphite ($Na_2S_2O_4$) with constant shaking. FD&C Yellow No. 6 becomes colorless, while FD&C Yellow No. 5 remains yellow. This procedure detects less than 0.5% of FD&C Yellow No. 5 in a dye mixture. Conversely, presence of FD&C Yellow No. 6 can be detected by comparing above sodium hydroxide soln of color mixture with standard soln of FD&C Yellow No. 5 of equal strength and alkalinity. Looking thru tubes vertically against white background, FD&C Yellow No. 6 will be discernible by slight brownish coloration.

Withdraw several 20 ml portions of the 1% dye soln of above and estimate total dye by titrating with 0.1 *N* titanium trichloride using sodium citrate as buffer following procedure as outlined in 6th Ed. **21.39(a)**. In case of over-titration, back-titrate immediately with a standard soln of amaranth or other dye. If qualitative tests listed above gave evidence of presence of both dyes pipet that amount of dye soln which is equivalent to ca 15 ml of 0.1 *N* titanium trichloride soln into two or more pear-shaped Pyrex pressure flasks (capacity 110–150 ml). Add to each flask:

Tartaric acid — 5.0 g.
Water —50 ml
Alumina cream—25 ml
1% soln of FD&C Green No. 2—0.5 ml

Total volume should not exceed 100 ml. Introduce into flasks a steady stream of

hydrogen sulphide gas for exactly 5 min. Stopper flask securely and place in sterilizer or autoclave and heat with steam at 15 lbs. pressure for exactly one hour. When cool transfer contents with hot water into a 500 ml Erlenmeyer flask and add 5.0 g sodium bitartrate and water to make to 200 ml and titrate the hot soln slowly with the 0.1 *N* titanium trichloride soln until a very light greenish yellow end point is reached. Treat blank containing the tartaric acid, alumina cream, and FD&C Green No. 2 exactly as above and titrate (ca 0.08–0.10 ml). Subtract blank from above titration. Difference×1.14 (correction factor) = value for Tartrazine in the aliquot. Subtract this corrected volume from the total color titration. Difference×1.013 (buffer correction) = value for Sunset Yellow F.C.F.

This method possesses the advantage of comparative simplicity with reproducible results.

RECOMMENDATIONS*

The Referee recommends—

(1) That the method for the quantitative estimation of ED&C Yellow No. 5 (Tartrazine) in presence of FD&C Yellow No. 6 (Sunset Yellow F.C.F.) be tested collaboratively.

(2) That the rapid method of detection of small amounts of Tartrazine FD&C Yellow No. 5 as published in *Methods of Analysis*, **20.125,** be made official, final action, with slight modification adopted last year.

(3) That investigational work be undertaken to separate and determine quantitatively FD&C Green No. 2 (Light Green S.F. Yellowish), FD&C Green No. 3 (Fast Green F.C.F.), and FD&C Blue No. 1 (Brilliant Blue F.C.F.).

(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 No. 2 (Orange SS), and FD&C Red No. 32 (Oil Red XO).

(5) That investigational work on analytical methods for coal-tar colors certifiable for use in foods be conducted.

REPORT ON DAIRY PRODUCTS

By GUY G. FRARY (State Chemist, Vermillion, South Dakota), *Referee*

Credit is due the Associate Referees who completed work during the year and have given their reports here. I hope sincerely that before another meeting of the Association some other items may have been given sufficient study to justify making additional methods in the chapter official.

RECOMMENDATIONS†

It is recommended—

(1) That the Sanders-Sager Method for phosphatase in dairy products be further studied.

* For report of Subcommittee C and action of the Association, see *This Journal*, 32, 52 (1949).
† For report of Subcommittee C and action of the Association, see *This Journal*, 32, 52 (1949).

(2) That studies be conducted to compare the Sanders-Sager Method for phosphatase in milk or cream with the methods appearing under Nos. **22.43** and **22.57.**

(3) That studies on methods for the detection of chlorine in milk and for the detection of reconstituted milk be discontinued.

(4) That studies be continued on the determinations of the acidity of milk and ash in milk.

(5) That studies of methods for preparation of butter samples, including the use of mechanical shaking and cooling of samples, be continued.

(6) That work be discontinued on methods for preparation of samples of frozen desserts, and on methods for determination of fruits and other characterizing ingredients in such products.

(7) That studies of the Babcock Method for fat in milk be made and with particular attention to homogenized milk.

(8) That further study of the Roese-Gottlieb Method for fat in dairy products be made.

(9) That the recommendation of the Associate Referee as to change of wording of the instructions for digestion of sample in **22.130,** fat in cheese, be adopted.

(10) That the sour serum method for detecting added water in milk, **22.29,** be dropped, first action.

(11) That further study be made of the acetic serum method, **22.28,** and of the copper serum method, **22.30,** for added water in milk.

REPORT ON SAMPLING, FAT, AND MOISTURE IN CHEESE

By WILLIAM HORWITZ (*Associate Referee*) and LILA KNUDSEN (Food and Drug Administration, Federal Security Agency, Minneapolis 1, Minnesota and Washington 25, D. C.)

A more extensive collaborative study of the official and modified methods for fat and moisture[1] has been conducted and analyzed statistically during the past year. Portions of the same cheese used in the previous study (which had been stored in the frozen condition) were prepared in the same manner as described in the previous report.[1] The design of the experiment was extended by submitting two sets of samples to each of five laboratories with the request that each set be analyzed by a different chemist, in duplicate, if possible. With this experimental design, the total variation could be separated into (1) the variation between laboratories (how well two laboratories can check each other), (2) the variation between collaborators within a laboratory (how well two chemists in the

[1] *This Journal,* **31,** 300 (1948).

same laboratory can check each other), and (3) the variation between duplicate samples run by the same collaborator (how well a single chemist can check himself). The data are also used to determine which, if any, of the two methods for fat and moisture yields more reproducible results.

All of the participating laboratories analyzed the samples for moisture in duplicate. Four of the five laboratories analyzed the samples for fat in duplicate; the fifth laboratory made single determinations for this constituent because of a temporary shortage of the required equipment. Determinations of fat and moisture in additional samples numbers 3 and 4 were made, however, by this laboratory.

METHODS

MOISTURE

I. Official Method: Sec. **22.124.**

II. Modified Method: 1.25 hours in the forced draft oven at 130°C. See reference 1.

FAT

III. Modified Method: Direct weighing of sample into and digestion in the Mojonnier tube. See reference 1.

IV. Official Method: Sec. **22.130.**

Fat on the dry basis is to be reported using the average moisture by the official method (I).

RESULTS AND DISCUSSION

Since a table listing over 700 results for fat, moisture, and the derived value of fat on the dry basis would not be informative, the data are presented graphically as differences from the average of each pair of samples. A general average was calculated for all results by the official A.O.A.C. moisture method on samples 1 and 2 (duplicate samples of the same cheese) 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. These differences are plotted together on the same scale and grouped by collaborator and laboratory 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 moisture method. Figure 2 treats similarly the modified method for fat and the official method for fat, while Figure 3 does the same for fat on the dry basis.

In order to assess the significance of the differences between laboratories and between collaborators within the same laboratory and also to obtain estimates of the types of variations mentioned in the first paragraph, analyses of variance were performed on the data for each method separately. In most cases the variation between laboratories contributed a significant amount of variation. The difference between collaborators within a laboratory was also very significant ($P < .01$). Since the duplicate *analyses* on a single sample were made at the same time, they would be

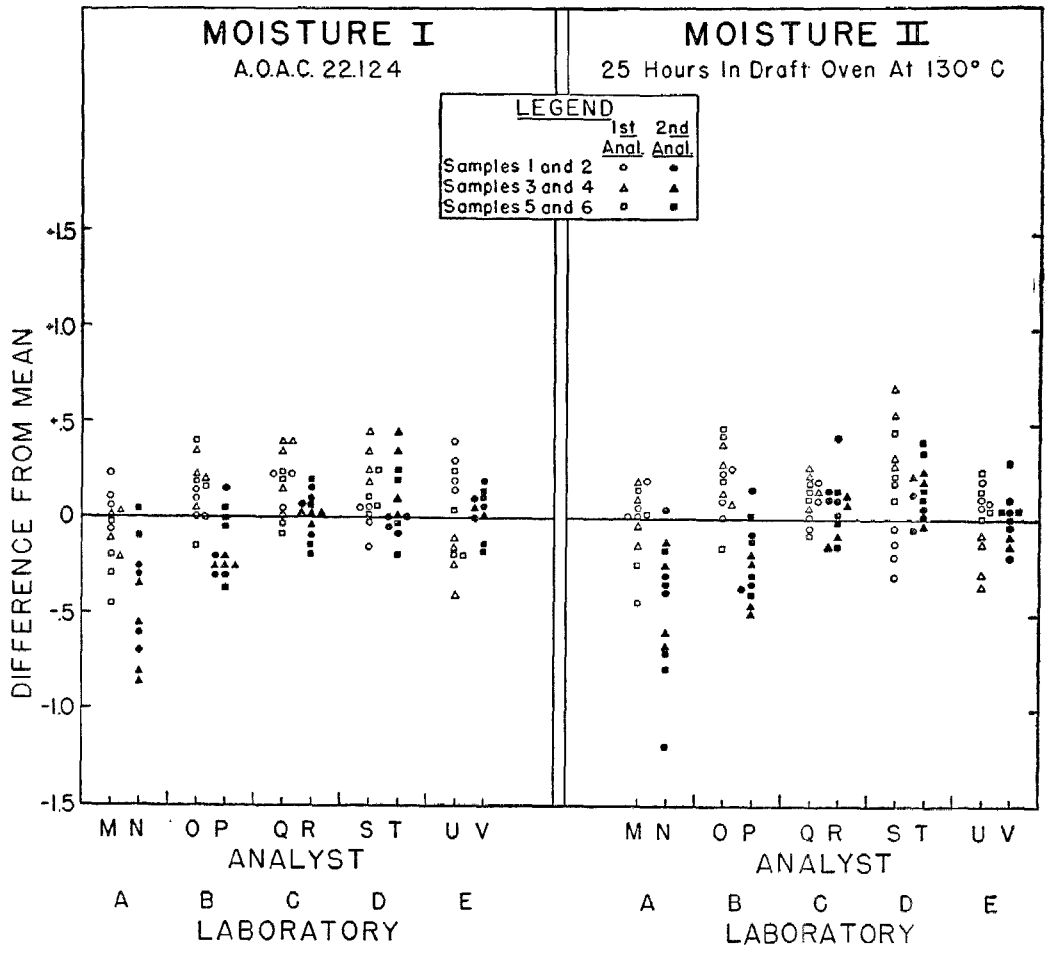

FIG. 1.—Moisture.

expected to check more closely than the analyses on the duplicate *samples* of the cheese (*e.g.*, samples 1 and 2 were duplicate samples from the same cheese mixture and duplicate analyses were made on each sample). This proved to be the case.

The three most important components of variation are given for each method in Table 1; (1) the variation between laboratories, (2) the variation between collaborators within a laboratory, and (3) the variation between duplicate samples run by the same collaborator. These variations are shown in two ways: in terms of the standard deviation and in terms of variations to be exceeded 1 time in 20 (*i.e.*, limits within which one would expect the check determinations to fall 19 times out of 20, here called the P = .05 limits).

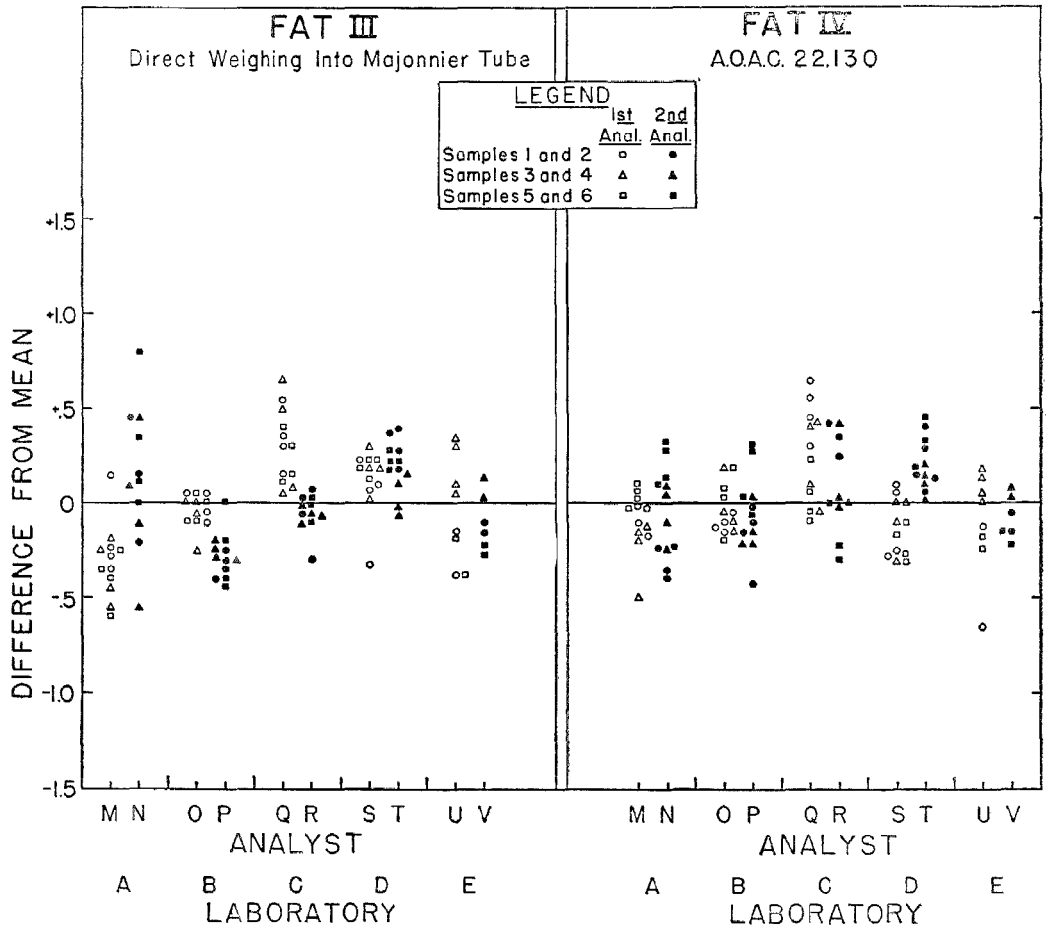

FIG. 2.—FAT.

As an explanation of the use of Table 1, the following illustration can be given. Suppose one chemist analyzes a sample of ground cheese for moisture by the official A.O.A.C. method and finds it to contain 36.50% moisture. One would expect another chemist at the same laboratory to get a result above 36.99 per cent or below 36.01 per cent only one time in 20 (36.50 ± 0.49 per cent). The same chemist should expect to obtain a result at another time on the same sample of cheese between 36.19 per cent and 36.81 per cent (or 36.50 ± 0.31 per cent).

Larger limits could be taken that could be exceeded only one time in one hundred by taking a larger multiple of the standard deviation (*i e.*, 2.58 times the standard deviation for $P = .01$). The $P = .05$ limits in Table 1 were obtained by using 1.96 times the standard deviation.

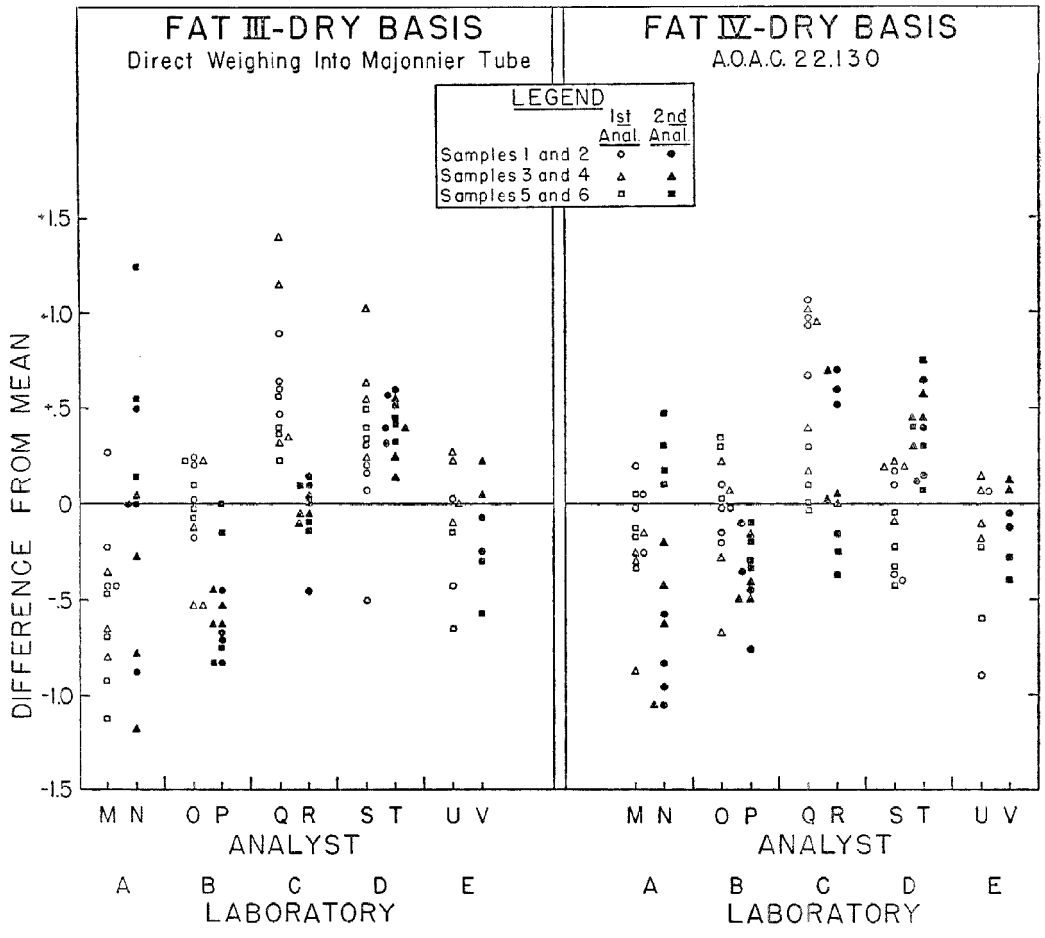

FIG. 3.—Fat—Dry Basis.

From Table 1 it can be seen that the official methods in use at present seem to be slightly more precise than the modified methods since the standard deviations are smaller.

The measures of variation obtained in this study on the moisture methods are not very different from those obtained in the previous study.[1] However, the variations in the fat methods are much smaller in this study. The standard deviations for check results between laboratories were 0.48 per cent and 0.44 per cent on the official method and the modified methods, respectively, in the previous study as compared with the respective figures of 0.24 per cent and 0.32 per cent in this study.

Since the statistical analysis indicated that the official method was somewhat greater in precision, it was not felt advisable to recommend

a change in the official method unless it could be demonstrated that a substantial improvement in technique or convenience would result from the change. A letter was sent to the collaborators requesting their opinion as to whether the modified method offered such a substantial improvement. Four of the collaborators expressed an outright preference for the official method, two preferred the modified method, one stated that he

TABLE 1.—*Components of variation*

CHECK RESULTS TO BE OBTAINED:	METHOD I		METHOD II	
	MOISTURE A.O.A.C. 22.124		MOISTURE 1.25 HOURS IN DRAFT OVEN AT 130°C	
	STANDARD DEVIATION*	P=.05 LIMITS	STANDARD DEVIATION*	P=.05 LIMITS
(1) Between laboratories	± .29	± .57	± .31	± .61
(2) Between collaborators within one laboratory	± .25	± .49	± .27	± .53
(3) By one collaborator	± .16	± .31	± .20	± .39

CHECK RESULTS TO BE OBTAINED:	METHOD III		METHOD IV	
	FAT DIRECT WEIGHING INTO MOJONNIER TUBE		FAT A.O.A.C. 22.130	
	STANDARD DEVIATION*	P=.05 LIMITS	STANDARD DEVIATION*	P=.05 LIMITS
(1) Between laboratories	± .32	± .63	± .24	± .47
(2) Between collaborators within one laboratory	± .28	± .55	± .19	± .37
(3) By one collaborator	± .19	± .37	± .15	± .29

* Obtained by using variance components or corrected variances.

saw no particular advantage of the modified procedure over the official method, and one suggested that the modified procedure be made optional for dry, granular cheese, while no statements were received from the other two collaborators. On the basis of these statements, it would appear that there is insufficient advantage to be gained by the method of direct weighing into the Mojonnier tube to warrant a recommendation to modify the official method for fat.

Several collaborators expressed the opinion that the exclusive use of sand in the official method is too restrictive and that the use of other antibumping agents should be made optional. Glass beads, porcelain chips, and carborundum grains have been employed for this purpose by analysts without noticeable effect on the results. It is therefore recom-

mended that the statement in the cheese method for fat, **22.130,** "Add ca 0.5 g of sand, previously digested with HCl, to prevent bumping . . . " be changed to read "Add a few glass beads, or other inert material, previously digested with HCl, to prevent bumping, . . . "

ACKNOWLEGMENT

Grateful acknowledgment is due the following collaborators (all of the Food and Drug Administration): John H. Bornmann and Leon E. Wener, Chicago; F. J. McNall and Halver C. VanDame, Cincinnati; Harry W. Conroy and Floyd E. Yarnall, Kansas City; Sidney Williams, Minneapolis; N. Aubrey Carson and Frederick M. Garfield, St. Louis. Acknowledgment is also due to William Weiss, Washington, D. C., for drafting the charts.

RECOMMENDATIONS*

It is recommended—

(1) That the statement in the cheese method for fat, **22.130,** "Add ca 0.5 g of sand, previously digested with HCl, to prevent bumping . . . " be changed to read, "Add a few glass beads, or other inert material, previously digested with HCl, to prevent bumping, . . . "

(2) That study of methods for sampling, fat, and moisture in cheese be continued.

REPORT ON THE DETECTION OF ADDED WATER BY THE SERUM TESTS

By H. J. HOFFMANN (Department of Agriculture, Dairy & Food, St. Paul, Minnesota), *Associate Referee*

The Association of Food, Feed and Drug Officials of the South Central States, which met in April, 1946, adopted a resolution which reads as follows:

"The Food Committee recommends that a request be made to the Association of Official Agricultural Chemists for a study of the possibility of using the sour serum test for added water in milk as a quantitative procedure after re-investigating the qualitative limits now applying in that test."

The Committee submitted comments with this resolution which suggested the need of further information as to the value and use of the serum method in detecting added water in milk, in amounts of less than 13 per cent. They also feel that the sour serum limit of 38.3, which is now fixed in the official method, is too low for detecting or sorting out samples which contain added water in amounts of less than 13.5 per cent. They also state that some health departments are using the serum method in a quantitative manner. In response to the above resolution, and the added

* For report of Subcommittee C and action of the Association, see *This Journal*, 32, 52 (1949).

comments, the Associate Referee has completed certain work which it is hoped will serve to clarify this situation.

The tests outlined in the tables which are included in this report illustrate that the work has been performed on authentic samples of milk which were known to be free from added water. Other tests have been made where known amounts of water have been added to authentic samples, and the tests are complete with respect to serum results and detection of actual percentages of added water by the cryoscopic method.

DISCUSSION OF RESULTS

The writer was successful in obtaining only two collaborators to perform the necessary work which was requested for the 1948 meeting of the Association. The work, however, of these two collaborators has been excellent, and I think gives to this meeting the information which is desired. The Associate Referee feels that these results, coupled with the information which may be found in the literature, serve to demonstrate without question that the official serum methods only serve to indicate to the analyst that added water may be present in a milk sample. If all milk samples are reported as containing added water when they fall below the present standards (38.3 sour serum, 36 copper serum, and 39 acetic serum), the only thing the analyst is certain of is that the milk sample does contain added water. No indication is ever given as to any quantitative amount, and one is at a loss to understand the comments of the South Central Association of Food, Feed and Drug Officials, who state that "Some health departments are using the test in a quantitative manner." It would be very interesting to learn exactly who these health departments may be, so that through correspondence perhaps their results would be made available for study.

This department has maintained a great many successful prosecutions in the courts for the addition of added water in milk. In every instance it has been necessary to include as a part of the court records the exact percentage of added water. Courts and juries are loath to convict a law violator when it can not be established to their satisfaction exactly what the wrongdoer has done. In view of these facts, this office would not authorize prosecution based solely on a result obtained by any one of the serum methods, since we could never by testimony state to the court any known percentage of added water. We have had sufficient experience on this score to know that we can not secure a verdict in our favor, therefore do not prosecute unless cryoscopic results are available.

The presence of added water may be indicated to an analyst by several methods. Serum readings may be taken by any one of the three official methods, and if these readings are below the accepted standards of 39, in the case of acetic serum; 38.3 in the case of sour serum; or 36, in the case of copper serum, then the true percentage of water should be determined

TABLE 1.—*Investigation of the serum method for detection of added water in milk*
Collaborator—Donald J. Mitchell, State Chemical Laboratory, Vermillion, S. D.

DESCRIPTION OR NUMBER OF SAMPLE	MILK CONTAINING KNOWN PERCENTAGES ADDED WATER—NONE									
	FAT	S-N-F	IMMERSION REFRACTOMETER READINGS 20°C.				ASH G IN 100 ML		CRYOSCOPIC EXAMINATION	
			SOUR SERUM	ACETIC SERUM	COPPER SERUM	ADDED WATER INDICATED	SOUR SERUM	ACETIC SERUM	FREEZING POINT—0°C.	ADDED WATER
	per cent	*per cent*								*per cent*
A. Holstein Herd (15 cows)	3.10	8.79	42.5	42.0	38.4	None	.7812	.7488	.544	1.09
B. Predominately Brown Swiss, some Holstein (10 cows)	3.60	9.26	43.7	43.2	39.1	None	.7856	.7600	.542	1.45
C. Holstein Herd (8 cows)	2.85	8.46	41.4	41.9	38.1	None	.7780	.7465	.546	0.73
D. Mixed Herd (7 cows)	3.85	8.73	41.4	42.0	38.2	None	.7884	.7428	.557	None
E. Mixed Herd (12 cows)	3.35	9.07	42.7	42.7	38.2	None	.8060	.7636	.546	0.73

Note: In all the tables, the words "none," "probable," or "present" can be used for reporting whether or not added water is indicated by the serum results.

TABLE 2.—*Investigation of the serum method for detection of added water in milk*
Collaborator—Donald J. Mitchell, State Chemical Laboratory, Vermillion, S. D.

DESCRIPTION OR NUMBER OF SAMPLE	MILK CONTAINING KNOWN PERCENTAGES ADDED WATER, 5%					
	IMMERSION REFRACTOMETER READINGS 20°C.				ASH G IN 100 ML	
	SOUR SERUM	ACETIC SERUM	COPPER SERUM	ADDED WATER INDICATED	SOUR SERUM	ACETIC SERUM
A. Holstein Herd (15 cows)	41.1	40.6	37.4	None	.7416	.7108
B. Predominately Brown Swiss, some Holstein (10 cows)	42.1	41.7	38.1	None	.7440	.7108
C. Holstein Herd (8 cows)	39.7	40.5	37.1	None	.7360	.7088
D. Mixed Herd (7 cows)	39.8	40.6	37.2	None	.7460	.7120
E. Mixed Herd (12 cows)	40.9	41.3	37.2	None	.7604	.7296

Note: The words "none," "probable," or "present" can be used for reporting whether or not added water is indicated by the serum results.

TABLE 3.—*Investigation of the serum method for detection of added water in milk*
Collaborator—Donald J. Mitchell, State Chemical Laboratory, Vermillion, S. D.

DESCRIPTION OR NUMBER OF SAMPLE	MILK CONTAINING KNOWN PERCENTAGES ADDED WATER, 10%					
	IMMERSION REFRACTOMETER READINGS 20°C.				ASH G IN 100 ML	
	SOUR SERUM	ACETIC SERUM	COPPER SERUM	ADDED WATER INDICATED	SOUR SERUM	ACETIC SERUM
A. Holstein Herd (15 cows)	39.6	39.3	36.3	Probable	.7028	.6876
B. Predominately Brown Swiss, some Holstein (10 cows)	40.5	40.2	37.0	None	.7052	.6868
C. Holstein Herd (8 cows)	37.7	39.1	36.3	Probable	.6916	.6724
D. Mixed Herd (7 cows)	38.2	39.1	36.2	Probable	.7016	.6660
E. Mixed Herd (12 cows)	39.7	39.8	36.3	Probable	.7196	.7008

Note No. 1. The words "none," "probable," or "present" can be used for reporting whether or not added water is indicated by the serum results.
Note No. 2. Used the word "probable" for indicating added water when the reading of anyone of the three serums was suspicious.

TABLE 4.—*Investigation of the serum method for detection of added water in milk*
Collaborator—Donald J. Mitchell, State Chemical Laboratory, Vermillion, S. D.

DESCRIPTION OR NUMBER OF SAMPLE	MILK CONTAINING KNOWN PERCENTAGES ADDED WATER, 15%					
	IMMERSION REFRACTOMETER READINGS 20°C.				ASH G IN 100 ML	
	SOUR SERUM	ACETIC SERUM	COPPER SERUM	ADDED WATER INDICATED	SOUR SERUM	ACETIC SERUM
A. Holstein Herd (15 cows)	37.6	38.1	35.3	Present	.6628	.6484
B. Predominately Brown Swiss, some Holstein (10 cows)	39.1	38.7	36.0	Probable	.6652	.6524
C. Holstein Herd (8 cows)	36.7	37.7	35.3	Present	.6568	.6332
D. Mixed Herd (7 cows)	36.9	37.8	35.2	Present	.6684	.6304
E. Mixed Herd (12 cows)	38.1	38.3	35.3	Present	.6856	.6580

Note No. 1. The words "none," "probable," or "present" can be used for reporting whether or not added water is indicated by the serum results.
Note No. 2. Used the word "probable" for indicating added water when the reading of anyone of the three serums was suspicious.

TABLE 5.—*Investigation of the serum method for detection of added water in milk*
Collaborator—Donald J. Mitchell, State Chemical Laboratory, Vermillion, S. D.

DESCRIPTION OR NUMBER OF SAMPLE	MILK CONTAINING KNOWN PERCENTAGES ADDED WATER, 20%					
	IMMERSION REFRACTOMETER READINGS 20°C.				ASH G IN 100 ML	
	SOUR SERUM	ACETIC SERUM	COPPER SERUM	ADDED WATER INDICATED	SOUR SERUM	ACETIC SERUM
A. Holstein Herd (15 cows)	36.2	36.7	34.3	Present	.6232	.6108
B. Predominately Brown Swiss, some Holstein (10 cows)	37.5	37.2	35.0	Present	.6256	.6084
C. Holstein Herd (8 cows)	35.1	36.4	34.3	Present	.6128	.5904
D. Mixed Herd (7 cows)	35.4	36 4	34.2	Present	.6240	.5916
E. Mixed Herd (12 cows)	37.1	36.8	34.3	Present	.6336	.6128

Note No. 1. The words "none," "probable," or "present" can be used for reporting whether or not added water is indicated by the serum results.

Collaborator—H. C. Petersen, Department of Agriculture, Dairy and Food, St. Paul, Minnesota

DESCRIPTION AND NUMBER OF SAMPLE	MILK CONTAINING NO ADDED WATER							
	SP. GR. @60°F.	FAT	S-N-F	SOUR SERUM* @20°C.	ADDED WATER INDICATED	SOUR SERUM ASH	FREEZING POINT	ADDED WATER
		per cent	*per cent*				0°C.	*per cent*
Past. Milk No. 183	1.0311	3.5	8.61	40.00	None	.7662	0.535	2.72
Past. Milk No. 78	1.0321	3.5	8.87	40.00	None	.7712	0.545	0.91
Past. Milk No. 79	1.0317	3.7	8.80	39.32	None	.6440	0.537	2.36
Past. Milk No. 80	1.0322	3.6	8.91	40.72	None	.7720	0.547	0.54
Past. Milk No. 81	1.0318	3.6	8.82	40.35	None	.7648	0.545	0.91
Past. Milk No. 82	1.0321	3.5	8.87	41.00	None	.7632	0.540	1.82
Past. Milk No. 83	1.0321	3.6	8.89	41.33	None	.7756	0.550	None
Past. Milk No. 279	1.0321	3.3	8.81	41.52	None	.7708	0.544	1.09
Past. Milk No. 109	1.0322	2.9	8.77	41.03	None	.7684	0.536	2.54

* Immersion Refractometer Readings 20°C.

TABLE 7.—*Investigation of the sour serum method for detection of added water in milk*
Collaborator—H. C. Petersen, Department of Agriculture, Dairy and Food, St. Paul, Minn.

DESCRIPTION AND NUMBER OF SAMPLE	WATERED MILK—SOUR SERUM READING 38.3 OR OVER							
	SP. GR. @60°F.	FAT	S-N-F	SOUR SERUM* @20°C.	ADDED WATER INDICATED	SOUR SERUM ASH	FREEZING POINT	ADDED WATER
		per cent	*per cent*				0°C.	*per cent*
Raw Milk No. 240	1.0302	2.7	8.22	38.71	None	.7042	0.505	8.18
Raw Milk No. 203	1.0313	3.0	8.56	39.48	None	.7362	0.513	6.73
Raw Milk No. 204	1.0319	3.0	8.70	40.00	None	.7346	0.530	3.64
Raw Milk No. 205	1.0317	3.5	8.76	39.75	None	.7314	0.520	5.45
Raw Milk No. 206	1.0311	3.0	8.50	39.31	None	.7310	0.512	6.91
Raw Milk No. 207	1.0311	2.8	8.48	39.82	None	.7302	0.511	7.09
Raw Milk No. 208	1.0321	2.7	8.70	39.79	None	.7328	0.515	6.36
Raw Milk No. 209	1.0312	3.25	8.57	39.18	None	.7310	0.513	6.73
Raw Milk No. 210	1.0314	3.6	8.71	39.46	None	.7344	0.522	5.09
Raw Milk No. 211	1.0294	3.9	8.28	39.80	None	.7320	0.518	5.82
Raw Milk No. 212	1.0313	3.25	8.62	40.00	None	.7420	0.523	4.91
Raw Milk No. 310	1.0285	3.3	7.91	38.41	None	.6964	0.530	3.64

* Immersion Refractometer Readings 20°C.

TABLE 8.—*Investigation of the sour serum method for detection of added water in milk*
Collaborator—H. C. Petersen, Department of Agriculture, Dairy and Food, St. Paul, Minn.

DESCRIPTION AND NUMBER OF SAMPLE	WATERED MILK—SOUR SERUM READING LESS THAN 38.3							
	SP. GR. @60°F.	FAT	S-N-F	SOUR SERUM* @20°C.	ADDED WATER INDICATED	SOUR SERUM ASH	FREEZING POINT	ADDED WATER
		per cent	*per cent*				0°C.	*per cent*
Raw Milk No. 241	1.0280	3.4	7.81	37.80	Present	.6628	0.472	14.18
Raw Milk No. 377-A	1.0276	4.5	7.92	37.52	Present	.6326	0.445	19.09
Raw Milk No. 377-B	1.0274	3.6	7.70	37.31	Present	.6282	0.428	22.18
Raw Milk No. 377-C	1.0264	3.9	7.52	36.89	Present	.6234	0.417	24.18
Raw Milk No. 378-A	1.0262	4.5	7.58	37.00	Present	.6342	0.420	23.64
Raw Milk No. 378-B	1.0269	3.3	7.52	37.22	Present	.6248	0.426	22.54
Raw Milk No. 378-C	1.0271	4.5	7.81	37.25	Present	.6414	0.430	21.82
Raw Milk No. 160	1.0235	2.8	6.58	34.50	Present	.6148	0.378	31.27
Raw Milk No. 170	1.0258	3.3	7.25	37.48	Present	.6210	0.410	25.45

* Immersion Refractometer Readings 20°C.

TABLE 9.—*Investigation of the serum method for detection of added water in milk*
Collaborator—H. C. Petersen, Department of Agriculture, Dairy and Food, St. Paul, Minn.

DESCRIPTION OR NUMBER OF SAMPLE	MILK CONTAINING KNOWN PERCENTAGES OF ADDED WATER										
	SP. GR. @60°F.	FAT	S-N-F	IMMERSION REFRACTOMETER READINGS 20°C.				ASH G IN 100 ML		CRYOSCOPIC EXAMINATION	
				SOUR SERUM	ACETIC SERUM	COPPER SERUM	ADDED WATER INDICATED	SOUR SERUM	ACETIC SERUM	FREEZING POINT	ADDED WATER
		per cent	*per cent*							*0°C.*	*per cent*
Guernsey Herd	1.0326	4.55	9.19	43.3	43.6	38.6	None	.8192	.7880	0.548	0.36
Market Milk Grade A	1.0314	3.50	8.69	40.2	41.6	37.3	None	.7616	.7560	0.538	2.18
Market Milk Grade A	1.0322	3.50	8.89	41.2	42.0	37.6	None	.7908	.7616	0.540	1.82

TABLE 10.—*Investigation of the serum method for detection of added water in milk*
Collaborator—H. C. Petersen, Department of Agriculture, Dairy and Food, St. Paul, Minn.

DESCRIPTION OR NUMBER OF SAMPLE	MILK CONTAINING KNOWN PERCENTAGES OF ADDED WATER (5%)					
	IMMERSION REFRACTOMETER READINGS 20°C.				ASH G IN 100 ML	
	SOUR SERUM	ACETIC SERUM	COPPER SERUM	ADDED WATER INDICATED	SOUR SERUM	ACETIC SERUM
Guernsey Herd	41.2	42.1	37.5	None	.7732	.7476
Market Milk Grade A	38.6	40.3	36.3	None	.6960	.7072
Market Milk Grade A	39.6	40.8	36.7	None	.7496	.7304

TABLE 11.—*Investigation of the serum method for detection of added water in milk*
Collaborator—H. C. Petersen, Department of Agriculture, Dairy and Food, St. Paul, Minn.

DESCRIPTION OR NUMBER OF SAMPLE	MILK CONTAINING KNOWN PERCENTAGES OF ADDED WATER (10%)					
	IMMERSION REFRACTOMETER READINGS 20°C.				ASH G IN 100 ML	
	SOUR SERUM	ACETIC SERUM	COPPER SERUM	ADDED WATER INDICATED	SOUR SERUM	ACETIC SERUM
Guernsey Herd	39.8	40.8	36.5	Probable	.7308	.7260
Market Milk Grade A	37.3	38.7	35.4	Present	.6840	.6720
Market Milk Grade A	38.0	39.3	35.7	Present	.6948	.6924

TABLE 12.—*Investigation of the serum method for detection of added water in milk*
Collaborator—H. C. Petersen, Department of Agriculture, Dairy and Food, St. Paul, Minn.

DESCRIPTION OR NUMBER OF SAMPLE	MILK CONTAINING KNOWN PERCENTAGES OF ADDED WATER (15%)					
	IMMERSION REFRACTOMETER READINGS 20°C.				ASH G IN 100 ML	
	SOUR SERUM	ACETIC SERUM	COPPER SERUM	ADDED WATER INDICATED	SOUR SERUM	ACETIC SERUM
Guernsey Herd	38.0	39.0	35.5	Present	.6860	.6856
Market Milk Grade A	36.0	37.2	34.3	Present	.6508	.6304
Market Milk Grade A	36.6	37.7	34.8	Present	.6708	.6480

TABLE 13.—*Investigation of the serum method for detection of added water in milk*
Collaborator—H. C. Petersen, Department of Agriculture, Dairy and Food, St. Paul, Minn.

DESCRIPTION OR NUMBER OF SAMPLE	MILK CONTAINING KNOWN PERCENTAGES OF ADDED WATER (20%)					
	IMMERSION REFRACTOMETER READINGS 20°C.				ASH G IN 100 ML	
	SOUR SERUM	ACETIC SERUM	COPPER SERUM	ADDED WATER INDICATED	SOUR SERUM	ACETIC SERUM
Guernsey Herd	36.6	37.6	34.4	Present	.6528	.6232
Market Milk Grade A	34.6	35.9	33.4	Present	.6064	.5932
Market Milk Grade A	35.2	36.3	33.7	Present	.6248	.6128

by the cryoscopic method, which will supply the analyst with the exact percentage of added water. The presence of added water may also be indicated to the analyst from the fat and solids-not-fat figure coupled with the graph according to Brown and Ekroth. It has also been found that the specific gravity figure, together with a comparison with the Brown and Ekroth table, is of value in a sorting procedure, and will indicate whether an official test be made by the cryoscopic method. It is very probable, however, that whether the analyst adopt any one of the above enumerated procedures (serum, specific gravity, or Brown and Ekroth table) that any one of these procedures only indicates whether added water may or may not be present. In no instance do any of these procedures give any quantitative results, which may only be obtained by the cryoscope.

From the figures in the tables which give the results for milk containing small (5–10%) percentages of added water, it will be apparent that present serum figures would fail to disclose the presence of such added water. Since the object of any one of the serum procedures is to detect the presence of added water in milk it must be apparent that the figures, 38.3 sour, 39 acetic, and 36 copper, must be at such a level as to give authentic and reliable information to the analyst. A further examination of all of the serum figures discloses that they are not uniform with respect to the presence of added water which has been detected by means of the cryoscope. This would serve to indicate that the serums do show which samples may contain added water, but do not give any true criterion as to the exact percentage present, inasmuch as the serums do not decrease in direct numerical order with respect to the true percentage of added water present in the milk sample. This discrepancy is true with respect to milks from like breeds of cows and becomes more apparent when the serums of milks from various breeds of cows are compared with one another. The sour serum is an analysis which is performed with extreme difficulty today. Most milk which is now offered to consumers is pasteurized, and when the analyst seeks to prepare a sour serum he frequently has the difficulty of having the milk rot, rather than sour. It has been the experience of analysts that sour serums are frequently only obtained when a culture is added to the milk, and this procedure is, of course, not provided for in the official method. This difficulty is not encountered when the copper and acetic serum tests are made.

I wish to take this opportunity to compliment Mr. Donald J. Mitchell, of the State Chemical Laboratory, at Vermillion, South Dakota, and Mr. H. C. Petersen, of the Associate Referee's staff. These two gentlemen, through their collaborative work, have brought to light the errors which probably exist in the present method, and have shown the need for new work which must be performed if these methods are to be used for giving a true picture as to the composition of milk samples.

RECOMMENDATIONS*

The Associate Referee recommends that the serum procedures be confined to their present limitations, *i.e.*, that they serve to be used as a yardstick to indicate the presence of suspected samples. In view of the results obtained, however, it would seem that the present serum figures of 38.3 for the sour serum, 39 for the acetic serum, and 36 for the copper serum are subject to question. It is therefore recommended:

(1) That further study be made with the view in mind of raising these present figures to some higher level, with the object in mind of causing the method to be more effective in screening out suspected watered milk samples. In the light of present information, I do not like to recommend any definite figure, but suggest that a level of 40 for the sour serum, 40 for the acetic serum and 37 for the copper serum be used as a basis for the further study which I recommend in this report.

(2) That efforts be abandoned to make any one of the serum methods applicable to any quantitative procedure, since the results indicate that this goal can not be achieved.

(3) That the present official copper **(22.30)** and acetic serum **(22.28)** methods be made tentative until sufficient new data is available and that the present sour serum **(22.29)** be dropped completely, since it has the same analytical defect as do the other serum methods and is a determination which is extremely difficult to perform due to the change in methods of marketing milk.

No reports were given on phosphatase test in dairy products, ash in milk and evaporated milk, frozen desserts, chlorine in milk, preparation of butter samples, tests for reconstituted milk, acidity of milk, or fat in dairy products.

REPORT ON MICROANALYTICAL METHODS FOR EXTRANEOUS MATERIALS IN FOODS AND DRUGS

By KENTON L. HARRIS (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Referee*

The methods at present in Chapter 42 of the sixth edition of *Methods of Analysis* resulted from an attempt to cover a wide range of products in a relatively new field. In line with this same thought the Association adopted several additional tentative procedures at the 1945, 1946, and 1947 meetings. We now find ourselves in a position where, along with the addition of methods that normally may be expected, we can replace several specific procedures by generalized directions that cover a whole group of products. Such is the case with **42.54** (Candy). In place of eight procedures

* For report of Subcommittee C and action of the Association, see *This Journal*, **32**, 53 (1949).

we now recommend that three more universally applicable methods be applied. So far as Chapter 42 is concerned, a consolidation of methods will be an aid to the interpretation of comparative results from different products. It is planned to continue this trend following out the recommendations of the Committee on Classification of Methods (*This Journal*, Feb. 1948) and work toward the inclusion of generally applicable official methods.

The Associate Referee assignments should be regrouped as follows for 1949:

Extraneous Materials in Baked Products, Cereals, and Confectionery.
Extraneous Materials in Nut Products.
Extraneous Materials in Fruit Products.
Extraneous Materials in Dairy and Egg Products.
Extraneous Materials in Beverage Materials.
Extraneous Material in Vegetable Products.
Extraneous Materials in Drugs, Spices, and Miscellaneous Products.
Sediment Tests for Milk and Cream should be included in this section for Extraneous Materials.

RECOMMENDATIONS*

The new methods for pepper and prepared mustard have been developed to the point where collaborative work is needed. It is recommended that work be done on them this coming year.

The reports of the Associate Referee for Nut Products and Confectionery and the Associate Referee for Baked Products, Cereals, and Eggs contain completely new methods for candy and for baked products, which have been used successfully by analysts of some U. S. Food & Drug Administration stations. It is recommended that collaborative results be obtained on them as soon as possible.

The procedure suggested to replace the present method for starch, **42.38**, clarifies several aspects of the old method and is recommended for tentative adoption.

The recommendations on sections **42.29–42.31** are based upon the work by K. L. Harris, *This Journal*, **31**, 786 (1948) and the observations of many analysts which indicated a need for an alternative HCl-boil flour method and the necessity for several changes in the pancreatin procedure. It is recommended that the collaborative work on these methods be continued.

The two new procedures suggested for popcorn fill a vacancy that has long existed. It is recommended that these methods be accepted as tentative procedures.

Method **42.32** for rodent excreta, in corn meal, is recommended for official, final action.

The methods for mold in cranberry sauces, *This Journal*, **31**, 783 (1948), are recommended for official, first action; and the suggested strawberry

* For report of Subcommittee C and action of the Association, see *This Journal*, **32**, 53 (1949).

procedures of the Associate Referee for Fruit Products and Beverage Materials, are recommended for further study.

It is recommended that the changes in the wording of 42.61(b) and 42.75 be made.[1]

REPORT ON EXTRANEOUS MATERIALS IN DRUGS, SPICES, AND MISCELLANEOUS MATERIALS

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

Because of the undue amount of time involved in picking over pepper berries, two semi-mechanical sorting procedures were studied. The method for "siftings and pickings" was submitted by Eastern District

TABLE 1.—*Comparison of whole black pepper separation by flotation in alcohol and kerosene-petroleum ether*

SAMPLE	PERCENTAGE WEIGHT OF FLOATERS IN—	
	95% ALCOHOL	KEROSENE-PET. ETHER (SP. GR. 0.800)
1	2.40	3.80
2	3.20	2.40
3	3.14	1.86
4	1.50	1.60
5	4.96	6.40
6	7.70	7.60
7	3.30	3.60
8	6.00	4.80
Average	4.03	4.01

of the U. S. Food and Drug Administration, where the method has been in use for many years. The procedure employs a standard pepper sieve known as a No. 9½ round-hole sieve. Because this sieve is not manufactured in this country and is obtainable only in England, a detailed description is appended to the method.

The gravity flotation procedure was submitted by Powers and Mendelsohn from the San Francisco and Los Angeles Stations, respectively, of the U. S. Food and Drug Administration. The method is based on the fact that peppers which are internally moldy, decomposed, or insect infested are lighter than sound peppers and may be separated by flotation in liquids of suitable specific gravity, *i.e.*, about 0.80. Both 95% alcohol and 0.800 sp. gr. kerosene (the latter adjusted to this specific gravity, with petroleum ether or gasoline if necessary) were proposed as suitable

[1] For details of changes, see *This Journal*, 32, 117 (1949).

liquids. Data by Eisenberg and Ungar comparing the separation by the two liquids indicates that there is very little significant difference in the use of either liquid (Table 1).

The gravity flotation method is intended to supplant in part the "dry-cut" procedure which entails cutting open each pepper berry for visual examination. Comparative data by Mendelsohn indicates that the separation by flotation with kerosene-petroleum ether shows no significant difference from the dry-cut procedure (Table 2). The data show that

TABLE 2.—*Comparison of whole black pepper separation by flotation and by dry-cut procedures*

SAMPLE*	WEIGHT DAMAGED—PER CENT		NUMBER DAMAGED—PER CENT	
	DRY-CUT	KEROSENE-PET. ETHER	DRY-CUT	KEROSENE-PET. ETHER
1	4.00	2.80	8.53	7.05
2	4.40	4.84	9.76	8.77
3	2.90	3.50	7.26	8.29
4	3.20	2.40	3.05	3.51
	3.63	3.39	7.15	6.91

* 25 g or about 750 berries in each test.

the two methods are in closer agreement when compared by count than by weight. This is readily understandable because of the lightness of the "floaters" and the inordinate effect of an occasional damaged berry that may be heavy enough to sink in the liquid. The flotation technic is less time-consuming and should therefore make feasible the examination of a larger and more representative sample. The peppers that float are subsequently cut open for examination to determine the kind and extent of damage. Samples involving external damage which has not penetrated the berry, such as surface mold, will not be suitable for examination by the new procedure, and the dry-cut method will be retained for such samples.

The methods for whole black pepper follow:

WHOLE BLACK PEPPER

(*A*) *Siftings and Pickings*

Standard pepper sieve.—Use the so-called "No. 9½ round hole sieve" specified in the Rules for the Standard Arrival Contract of the American Spice Trade Association. It consists of a round screen with frame 18–22″ in diameter and 2¾″ in height (small or "office" size 8 or 9″ in diameter); bottom made of metal, perforated with round holes averaging 0.112″ in diameter (approximately 7/64″); average 5½ holes to the linear inch.

(a) *Siftings.*—Weigh sample to the nearest ounce and sift entire sample, consisting of not less than 5 lbs. Introduce 1½ to 2 lbs. of pepper into the sieve at a time; (when using the small or "office" size sieve, introduce only ¾ to 1 lb. into the sieve at a time). Obtain the siftings by tilting the sieve from side to side, so that the pepper

passes from one side of the screen to the opposite side 10 times. Weigh siftings and report as percentage siftings.

(b) *Pickings.*—Examine sifted sample for foreign material, consisting of stems, stones, mud, and other foreign matter. Add the pickings to the siftings, weigh the combined total, and report as percentage siftings and pickings.

(B) Separation of Damaged Berries by Flotation

Weigh 50 g of pepper, count, and transfer to a tall 800 ml beaker about ¾ filled with 95% alcohol or kerosene-petroleum ether mixture (sp. gr. 0.800). Stir ca 1 min. with spoon. Skim off the floating berries, dry, count, and weigh. Record per cent of floating berries by count and by weight. Cut open representative number of floating berries, determine and report kind and extent of damage.

Method **42.94,** which has been found to be unsuitable for most prepared mustards, should be replaced by a new method submitted by the Cincinnati Station of the U. S. Food and Drug Administration. This new method, which proposes a HCl digestion in place of the pancreatin digestion, serves to simplify the procedure and results in a better filth separation.

The method follows:

PREPARED MUSTARD

Filth

Transfer 100 g to a 2-liter trap flask. Add 500 ml H_2O and 15 ml concd HCl. Boil for 10–15 min., stirring constantly to prevent scorching. Cool. Trap off using gasoline and H_2O.

No report was given on extraneous materials in dairy products.

REPORT ON EXTRANEOUS MATERIALS IN NUT PRODUCTS AND CONFECTIONERY

By W. G. HELSEL (Food and Drug Administration, Federal Security Agency, Washington, C. D.), *Associate Referee*

The present **(42.54)** methods for filth in candy have not been universally applicable to the wide variety of candy products now on the market and it is recommended* that the following procedures which were developed by the analysts of the Baltimore and San Francisco Stations of the Food and Drug Administration be studied.

"*Filtration of hard candy, gum drops, gum, starch, or pectin-base candies.* Dissolve in boiling 0.5% HCl (0.5+35) and filter thru rapid filter paper in suction funnel. Examine filter for filth." **42.54 (b).** Substitute the following for **42.54 (b), (c), (d), (e), (f)** and the paragraphs beginning "In candy containing corn flakes . . . " and "In chocolate candy coating . . . ":

"*Chocolate candy with or without fruit or nuts, fruit candy, etc.*—Weigh 225 g of sample into a 2-liter beaker. Add 1 liter 5–10% $Na_2B_4O_7$ soln. Simmer 10–15 min.

* For report of Subcommittee C and action of the Association, see *This Journal*, 32, 54 (1949).

Pour thru 5–8″ #140 screen. While pouring play a forcible stream of hot water on this material. Wash well with large stream of hot water. Wash twice alternately with alcohol and $CHCl_3$ in that order and give a final alcohol rinse. Transfer material to a filter paper if little residue remains or to a 1–2 liter Wildman trap flask if a larger amount remains. Transfer bulk of material with a spoon. Rinse residue from screen with alcohol from a wash bottle. Wash screen with a forcible stream of hot water, collecting final residue at one edge of screen and transferring to the trap flask with a stream of alcohol as above. Add 200 or 400 ml of 60% alcohol depending on size of trap flask. Boil for 20 min. Cool below 20° and add 20 or 40 ml gasoline. Fill flask with 60% alcohol and trap off in usual manner. Add gasoline and trap off a second time. Filter and examine. If a large amount of peanut testa or similar material floats up into the neck of flask, pour trapped-off material thru a #8 or #10 sieve, rinse thoroly, filter liquid portion, and examine."

(c) "*Chocolate candy coating for insect excreta and other filth.*—Heat 400 ml gasoline in an 800 ml beaker to 40–50° and maintain at this temp. Place a portion of candy in a wire basket (ca 3¼″ diam. ×1″ high) made from #8 screen and with wire handles. Move basket up and down thru the gasoline until chocolate coating is dissolved. Rinse each candy center with a fine stream of gasoline from a wash bottle and save the center. Repeat with balance of sample. Stir the gasoline-chocolate suspension and pour thru a #140 sieve. Transfer residue from the sieve to a filter paper and examine microscopically. Examine the candy centers by **42.54 (a)** or **(b)** as given above

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REPORT ON EXTRANEOUS MATERIALS IN BAKED PRODUCTS, CEREALS, AND EGG PRODUCTS

By J. Frank Nicholson (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

In view of the widespread difficulties encountered in using the **42.26–42.28** methods on baked products it is believed that these present methods should not be used and that the following procedures, which have been used successfully for several years, should be substituted for them and studied with a view to official adoption.

(1) *Sieving for insect fragments and rodent hairs.*—Weigh 225 g of sample into a 2 liter beaker. Add sufficient hot water to soften and saturate the material. If lumps persist or if water is not immediately absorbed uniformly thru the entire mass (*e.g.*, in the case of hard English-type cookies and alimentary paste) estimate volume of mixture and adjust the acidity to 1% by adding sufficient concd. HCl (1 part concd. HCl to 35 parts of mixture). Boil until mixture becomes finely divided and so digested that it will not froth over when covered during boiling. Neutralize to ca *p*H 6 with NaOH soln. Add Na_3PO_4 soln to bring mixture to *p*H 7–8. Stir and break up the material as much as possible. Cool to 40°, add 100 ml of pancreatin soln made as directed in **42.2(c)** and then diluted to 100 ml. Stir thoroly and readjust to *p*H 7–8. Allow to stand 30 min. Stir and readjust *p*H.

For white flour products, add 2 ml of formaldehyde and digest overnight. For products made from whole wheat and rye flours and similar materials of high bran content, digest only 2–3 hours. (The shorter digestion is advisable because the starch and protein are not completely stripped from the bran and gives a cleaner separation on trapping.)

After digestion pour this material thru 5–8″ #140 screen. While pouring play a

forcible stream of hot water from the tap on this material. Wash well with a large stream of hot water. After complete washing (no starchy material visible unattached to bran) wash twice alternately with alcohol and chloroform in that order, and give a final alcohol rinse.

Transfer the material to a filter paper if little residue remains or to a 1–2 liter Wildman trap flask if a larger amount remains. Transfer the bulk of the material with spoon. Rinse residue from the screen with alcohol from a wash bottle. Wash screen with a forcible stream of hot water, collecting final residue at one edge of screen and transferring to trap flask with a stream of alcohol as above. Add 200 or 400 ml of 60% alcohol depending on the size of the trap flask. Boil for 20 min. Cool below 20° and add 20 or 40 ml of gasoline, fill flask with 60% alcohol and trap off in usual manner. Trap off a second time. Use care in stirring and during addition of alcohol to prevent formation of emulsion or inclusion of air. If residue in the flask tends to rise, stir material down 2–3 times. Filter trapped-off material and examine.

(2) *Direct trapping to show insect fragments and rodent hairs contributed by wheat or mill.*—To 1000 ml of boiling 1% HCl add 225 g sample. Continue heating for 30–40 min., or until the mixture becomes a finely divided mass that will not froth over when covered; cool somewhat. Neutralize with NaOH soln and bring to *p*H 7–8 with Na_3PO_4 soln. Cool to 35–40° and digest with pancreatin as in (1) above. Bring to a boil; cool; transfer to 2-liter Wildman trap flask, and extract and examine in the usual manner.

The above method was first used by Baltimore Station (U. S. Food and Drug Administration) and later modified by use and by other stations. This method is an attempt to standardize the bakery products methods so as to insure more uniform results.

RECOMMENDATIONS*

It is recommended—

(1) That method **42.38** for starch, which contains several ambiguities, be deleted and a method, which is essentially a clarified revision of the same procedure, substituted as a tentative method.[1]

Weigh out 225 g of starch into a 1500 ml beaker. Add with stirring 1200 ml of cold water (15–20°). Stir out lumps and pour thru 5–8″ No. 140 sieve. Wash with cold running water. Rinse particles from the sieve to a filter paper, using first water and then 60% alcohol. Examine paper microscopically.

This change was suggested by Mr. E. M. Hoshall of Baltimore Station, U. S. Food and Drug Administration, to clarify certain portions of the procedure and to standardize it with other methods of this same type.

(2) That sentence in parentheses, in method **42.31(b)** for flour, be deleted; and that **42.29** and **42.30** be deleted and the following methods substituted:

Insect fragments and rodent hairs (Two alternates):

(a) *Pancreatin digestion.*—Weigh 50 g of flour into beaker and stir into a thin smooth slurry. Add a filtered aqueous extract of 5 g pancreatin and mix. Adjust to

* For report of Subcommittee C and action of the Association, see *This Journal*, 32, 54 (1949).
[1] Details of the methods are published in *This Journal*, 32, 116 (1949).

*p*H 7–8 with Na_3PO_4 soln. Allow to stand 15 min., and if necessary readjust to *p*H 7–8. Digest overnight. Transfer digested material to 2 liter Wildman trap flask. Trap off twice with gasoline and water in the usual manner. Combine trappings in beaker. Transfer contents of beaker to trap flask and fill with H_2O. Stir and after 30 min. trap off into beaker and filter thru rapid filter paper using suction. Examine microscopically.

(b) *HCl digestion.*—To 50 g flour add water and stir into a thin smooth paste. Add water to make total 400 ml. Add 17 ml concd. HCl. With intermittent stirring bring to a boil and boil 10 min. Cool (if in a beaker transfer to 2-liter trap flask), trap off with gasoline and water in the usual manner. Combine trappings in beaker, filter, and examine.

These changes are based on work to be reported in *This Journal* by Kenton L. Harris, Division of Microbiology, U. S. Food and Drug Administration.

(3) That two new methods on filth in popcorn be adopted as tentative methods.[1]

The method for unpopped popcorn was developed by analysts of Chicago and Minneapolis Stations, U. S. Food and Drug Administration. The method for popped popcorn was developed by Mr. R. T. Elliott of Seattle District, U. S. Food and Drug Administration.

(4) That method **42.32,** which was made official, first action, last year, be adopted as official, final action.

REPORT ON SEDIMENT TESTS IN MILK AND CREAM

By Curtis R. Joiner (Food and Drug Administration, Federal Security Agency, St. Louis, Mo.), *Associate Referee*

In 1929 Hoffmann (1) presented a method for the preparation of standard sediment pads using weathered cow dung suspended in 50 per cent sugar solution. On the basis of a series of pads prepared by each of seven collaborators, he recommended that the method be adopted as tentative. However, Subcommittee C recommended (2) "That the method presented by the associate referee . . . be further studied, and that the associate referee confer with other associations interested in this line of work whose methods are now uniform with those of this association, with a view of preserving the existing uniformity." This recommendation was repeated by Subcommittee C in 1930 and in 1931.

In 1935 Frary (3) recommended "that this association adopt the method for sediment test described in Standard Methods of Milk Analysis, 1934, 6th ed., pages 44–46, as tentative." Subcommittee C concurred in this recommendation. The method as adopted was essentially that proposed by Hoffmann.

J. O. Clarke, then Chief of the Central District, Food and Drug Administration, in 1943 was a member of a committee appointed by the

American Public Health Association to standardize methods of sediment testing. At his request, Samuel Alfend of the St. Louis Station prepared several series of standard sediment pads* using peat moss, dried cow manure, and a mixture of cow manure, soil, and charcoal. Several persons experienced in making milk sediment tests agreed that the pads made from the mixture of materials more closely resembled pads obtained from commercial milk than did those prepared from peat moss and cow manure alone. It was recognized, however, that the experimental pads did not resemble many commercial milk sediment pads at all.

The committee examined the discs prepared by Alfend and some prepared by another member of the committee. In addition, Clarke had six different laboratories make up a series of pads by Alfend's method (except that they were directed to filter the sediment through pads with the aid of suction, whereas Alfend drew the sediment and milk up into a milk sediment pump and forced the milk out through the pad), using materials from local sources, and submit them to the committee for study. The committee finally recommended that the method developed by Alfend (modified to filter the milk by suction) be adopted by the A.P.H.A.

In 1947 Frary (4) recommended that the tentative method for sediment test in milk be dropped, and that the A.P.H.A. method (5) be adopted as tentative. Subcommittee C concurred and recommended that the method be further studied.

In using the method as written, some workers have had difficulty in getting the sediment to remain in uniform suspension long enough to withdraw a representative aliquot with a pipet. The soil and manure particles have a tendency to settle out, while the larger pieces of charcoal rise to the surface. Prior to his appointment as Associate Referee, the writer used a motor stirrer at low speed to stir the sediment suspension while withdrawing aliquots. This stirring appeared to overcome the above difficulties. For measuring aliquots, it is convenient to use a series of four graduated pipets (5, 10, 25, and 50 ml.) with the tips cut off so the openings have a diameter of two to three millimeters. The method as used by the writer is given below, the italicized portions being changes or additions to the present tentative method:

(d) *Preparation of standard sediment discs.*—Make a uniform mixture of oven dried (100°) materials which meet the following screening specifications:

	Per cent
Cow manure, 40 mesh	53
Cow manure, 20 mesh, retained on 40 mesh	2
Garden soil, 40 mesh	27
Charcoal, 40 mesh	14
Charcoal, 20 mesh, retained on 40 mesh	4

* Unpublished work.

Place 2.00 gm. of the above mixture *in a 100 ml volumetric flask and* moisten with *5* ml of 1% aerosol soln or other suitable wetting agent, add 46 ml. of 0.75% gum soln such as gum arabic (*42.2(b) (1)*), and then make up to 100 ml with a 50% by weight sucrose soln. Mix thoroly, *pour into a 250 ml beaker or container of similar size and stir with a small motor stirrer at a speed (ca 200–300 r.p.m.) such that the mixture is thoroly agitated, but so that very little air is whipped into the suspension.*

Transfer (*while stirring*) a 10 ml portion (200 mg of standard sediment) with a large tipped, graduated pipet to a flask and make up to a liter with 50% by weight sucrose soln. When thoroly mixed, each ml contains 0.2 mg of sediment. *Mix contents of flask, pour into 1500 ml beaker and stir with suitable motor stirrer as directed above. While stirring, pipet out* definite volumes of the sediment mixture and add to ¾ pint of filtered *skimmed* milk. Mix thoroly and pass the mixture thru a standard sediment disc *in filtering device* having a filtering area measuring 1⅛ in. in diam. *Pour the milk gently down the side of filtering apparatus and filter with very little or no suction.* Wash the container promptly with ¼ pint of filtered *skimmed* milk. *Let the last portion of the milk flow thru the pad with no suction applied. If sediment does not appear to be evenly distributed over pad, add 15 or 20 ml of milk and let it filter thru without any suction. Repeat this until sediment appears to be evenly distributed. Suck air thru disc for ca 1 min. to remove excess milk.* For a permanent record, mount and spray the disc with 40% formaldehyde or with a soln containing 2.5 g menthol and 2.5 g thymol made up to 100 ml with alcohol. *Alternatively, if most of milk is removed by thoro aspiration, no preservative need be added to pads. A transparent colorless plastic cement may be used for mounting them.* Following the above method, prepare a series of discs containing the sediment remaining from 0.0, 0.2, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 12.0, and 14.0 mg. of the standard mixture. Identify each disc on the permanent record with the amount of sediment in milligrams used to prepare the pad.

(For the purpose of comparison, the entire series of discs may be used. . . .)

EXPERIMENTAL

The experimental work this year was limited to the following objectives:

1. To demonstrate the precision obtainable by one worker with a given sediment mixture.
2. To determine the difference in appearance between two sets of standard discs prepared from materials obtained from different geographical locations.

Two different sediment mixtures, designated A and B, were prepared from materials obtained as follows:

A. Fresh cow manure and soil from a dairy farm in Southeastern Louisiana. The manure was dried in the laboratory a few days before it was used.

B. Fresh cow manure obtained from a dairy farm in the St. Louis area in 1942 and dried in the laboratory. Soil from downtown St. Louis. The same lot of commercial charcoal was used in both A and B.

Twenty-one pads, made up as follows, were prepared from both A and B: 0.5, 3.0, 6.0, and 12.0 mg pads in triplicate, and single 1.0, 2.0, 4.0, 5.0, 7.0, 8.0, 9.0, 10.0, and 14.0 mg pads. The discs were cemented on two 8 by 10 inch cards and photographs were made. The two photographs are submitted as Figures 1 and 2.

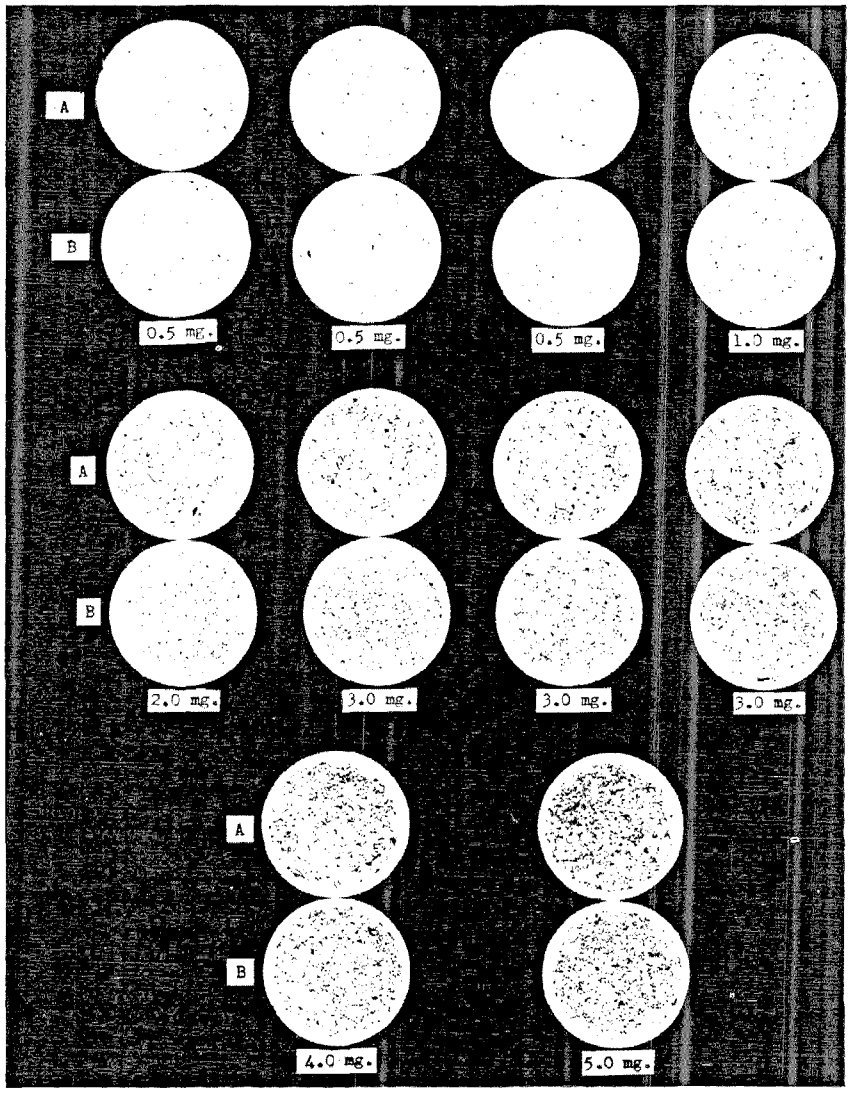

FIGURE 1.

Both the soil and the manure used in B appeared darker than those used in A. This difference in appearance was also apparent on the standard pads. To the eye in daylight, the predominating color of the B discs was gray-black, with some brown, while the A pads appeared to be predominantly brown with some black particles. The particle density on the two sets of discs appeared to be about equal, but the average particle size of A

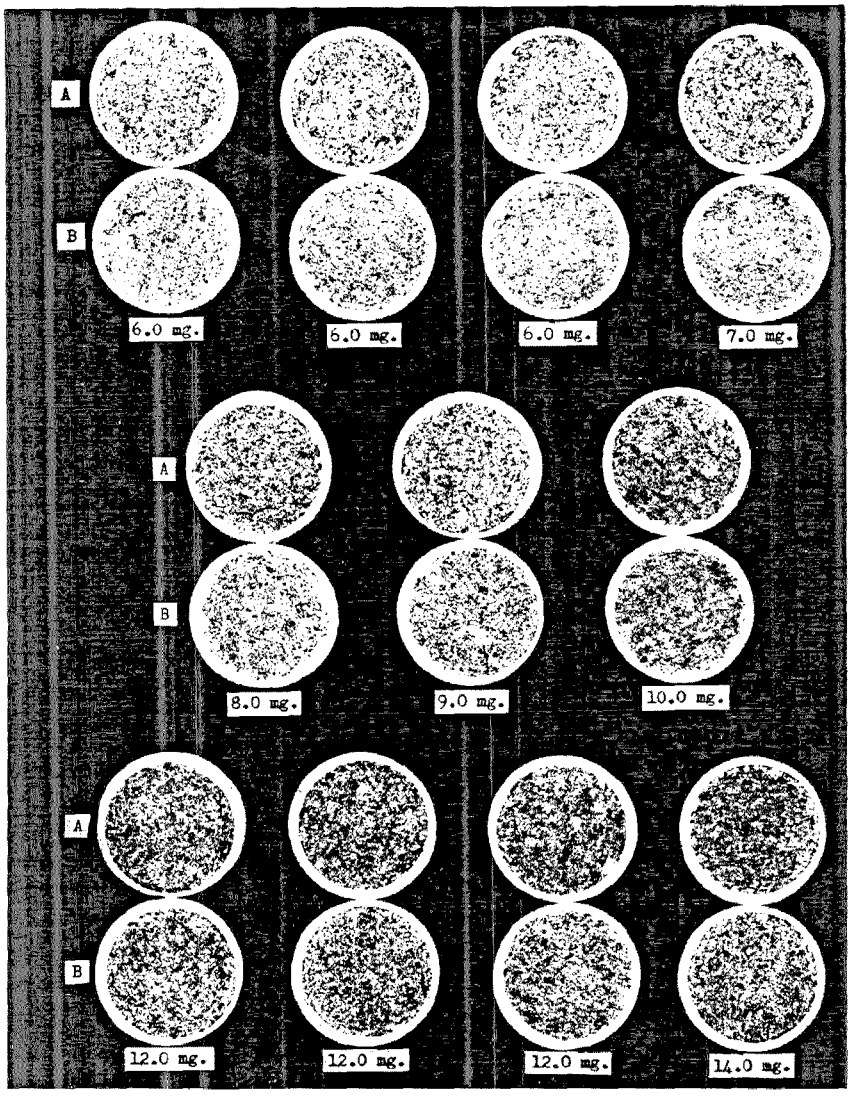

FIGURE 2.

seemed to be somewhat greater than that of B. The differences between the two sets of pads were emphasized in the photographs, and, in addition, the brown sediment on A photographed darker than did the gray-black sediment on the B discs. This difference is not noticeable with the 0.5 mg discs but with the 12.0 and 14.0 mg pads it becomes quite marked. In the photograph, the amount of sediment on the 14.0 mg B disc appears to be

slightly less than that on the 12.0 mg A pad. When viewing the actual pads, the former appears to fall in between the 12.0 and 14.0 mg A standard discs.

The various triplicate sets of pads are, for practical purposes, identical. In both groups of discs, in going from 0.5 mg to 14.0 mg there is, with one exception, approximately the proper proportional increase in the amount of sediment on the pads. The single exception is in the A set. The amount of sediment on the 8.0 mg disc appears to be equal to or slightly greater than the amount on the 9.0 mg pad. (In making these pads, no discs were discarded. In preparing a standard set of pads, any that are obviously out of line should be discarded and remade.)

In addition to the work represented by Figures 1 and 2, the Associate Referee made two diluted suspensions of sediment from one concentrated suspension and prepared a series of pads from each dilution. The two sets of pads were comparable in appearance.

DISCUSSION

The changes in the tentative method for the preparation of standard sediment discs presented in this report appear to have obviated the source of difficulties reported by some workers.* It is probable that standard pads prepared from sediment materials obtained from several different sections of the country would show greater variations than do the two sets of pads represented by Figures 1 and 2. If the variations in appearance of soil and manure from different sections of the country, or even within one area, are great enough to warrant it, it might be desirable for this Association to prepare a large batch of a standard sediment mixture for distribution.

The present tentative method for sediment in fluid milk (5) permits the use of photographic standards as a guide in grading sediment pads but recommends the use of actual standard discs. It is generally agreed by inspectors experienced in making milk sediment tests that pads are difficult to grade with photographic standards. However, it is very doubtful that actual standard pads could be used in the field unless the pads were rendered "permanent" by treating them in some manner that would prevent the sediment particles from falling off the pads. The Associate Referee has seen such a set of standard discs. They apparently had been treated with a solution of a transparent plastic in a volatile solvent. The Associate Referee has done a limited amount of work in the treatment of standard pads in this manner. The appearance of the pads are altered but slightly by this treatment. They are much more realistic appearing than are photographic reproductions.

SUMMARY

1. In the hands of the Associate Referee the method as presented in this report gives reproducible results with a given sediment mixture.

* Unpublished work.

2. There is considerable difference in the appearance of pads prepared from sediment mixtures from two different sections of the country.

3. The differences between standard pads are in some cases accentuated in photographic reproductions.

RECOMMENDATIONS*

It is recommended that—

(1) The method presented in this report be subjected to collaborative study in order to determine (a) the precision of the method in the hands of different analysts using the same sediment mixture, and (b) the variation in appearance of pads prepared with sediment materials from different sections of the country.

(2) Further work be done on the preparation and use of standard sediment pads rendered "permanent" by treatment with a solution of a transparent plastic.

REFERENCES

(1) HOFFMANN, H., JR., *This Journal*, **13**, 237 (1930).
(2) Report of Subcommittee C on Recommendations of Referees, *Ibid.*, 67.
(3) FRARY, GUY G., *Ibid.*, **19**, 377 (1936).
(4) ———, *Ibid.*, **31**, 298 (1948).
(5) Report on Changes in Methods of Analysis, *Ibid.*, 93.

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*

There are included below two new procedures for cranberry sauce which cover a field where previously there has been no established method. This method, *This Journal*, **31**, 783 (1948), has been subjected to collaborative study and it is recommended for adoption as official.

Several difficulties have been reported in the **42.41** and **42.42** procedures for frozen strawberries. As a result of investigations by W. G. Helsel, U. S. Food and Drug Administration, it is now possible to recommend changes in the strawberry procedures The draining of sliced strawberries is unnecessary under present commercial practices in which practically all berries are packed in either 30-pound cans or consumer-sized packages which freeze more uniformly than the larger barrels. Since the sugar is no longer removed, the suction-alcohol treatment is needed to remove air. The larger sieve opening permits the increase in sample size and yet will retain rotten berry tissue well under 6 mm in diameter. The new procedure is given below and should be further studied.

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* For report of Subcommittee C and action of the Association, see *This Journal*, 32, 54 (1949).

42.41 PREPARATION OF SAMPLE

Each sample representing a barrel, 30 lb. can, or other large container, should consist of ca 4 quarts of berries. If the berries are packed in 1 lb. consumer packages, four such packages from the same code should be composited to make one sample. Thaw the entire sample and divide into 2 portions including juice, without damaging berries.

42.42 ROT

(a) *Mold count.*—Pulp one portion of sample thru cyclone with screen openings 0.027" in diam. and mix thoroly (pour juice thru cyclone first). If the pulp contains too many air bubbles for mold counting, mix thoroly ca 50 g of pulp and 1 ml alcohol. Remove most of the air from mixture with suction, mix thoroly, and make mold count as directed under **42.57.**

(b) *Macroscopic separation.*—Drain second portion of sample on No. 8 sieve. Immerse berries in tap H_2O in large white pan. Decant most of H_2O thru sieve, catch and return any strawberry tissue. Repeat washing until H_2O is fairly clear. Examine berries under H_2O and remove all questionable berries to another pan containing deaerated H_2O. Re-examine suspected berries. Confirm all questionable rot spots by examining fragments of berry tissue for mold under compound microscope. Classify tissue as rotten only when a substantial number of mold filaments are present. Class a berry or fragment as rotten if it has rot area 6 mm. or more in diam. Separate berries and fragments with rot areas over 12 mm in diam. Drain separately the good and rotten berries 2 min. on No. 8 sieve and weigh.

REPORT ON EXTRANEOUS MATERIALS IN VEGETABLE PRODUCTS

By FRANK R. SMITH (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

The following changes are recommended* in the methods for extraneous materials in vegetable products.

42.61 (b).—Change separator size to 6 liter. The 5 liter size is not available in stock.

42.75 To clarify an ambiguity, add to the next to the last sentence ending "mushrooms" the words "allowing them to drop through the screen."

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 the Committee on Classification of Methods (*This Journal*, **31**, 63) the Referee has reviewed Chapter 24, Fish and Other Marine Products, in *Methods of Analysis*. As a result the following comments and recommendations for study are made. Discussion of current collaborative problems now under study and appropriate recommendations are also included in this report.

* For report of Subcommittee C and action of the Association, see *This Journal*, 32, 54 (1949).

The established shucked oyster standards and the proposed standards for shucked soft shell clams make it imperative that the applicable analytical methods in the Book of Methods be in conformity with those needed for the official standards. In the following comment we will mention specific points in connection with the revision and extension of such methods.

The paragraph (**24.1**, p. 359) "Apparatus-Tentative" does not completely meet the needs of present laboratory technics. For example: the skimmer described is smaller than that required for the measurements specified in making official oyster standard, drained liquid measurements. There are other descriptions in this paragraph warranting study and revision. It may be considered desirable to delete this paragraph and incorporate pertinent portions in other sections under appropriate methods.

Paragraph 24.2 on "Preliminary Treatment and Preparation of Sample-Tentative" needs to be thoroughly considered as to title and subject matter for possible revision of the wording in several places. For example: the part designated as **(f)** shucked oysters, indirectly suggests it is a method for the determination of free liquid by loss of volume, whereas the official standards require measurements in terms of loss of weight. Consideration should be given to eliminating the ambiguity of including what appears to be a method where it does not conform to the title of the paragraph. The method for determination of free liquid for shucked oysters and clams should be considered for preparation of a new separate paragraph in which could be included most of the description (which should be revised) now in paragraph **24.1.** The method for drained liquid as outlined in the present oyster standard may warrant inclusion, as is, with appropriate changes in wording to fit the style of *The Journal* and the *Methods of Analysis*.

Although designated by Subcommittee C for study during the past year (*This Journal*, **31**, 52) no work has been possible on Total Solids in Fish and Other Seafoods by the Associate Referee because of the press of other work.

Although no adverse reports have been received by the Referee on the method for Salt II (paragraph **24.7**) "With Calcium Acetate as Fixative—Tentative," it is believed that the method should be studied for deletion if not needed, otherwise it should be considered with or without further collaborative study for official, first action. The method, if retained, could be condensed by omitting the last part and referring to the identical portion of paragraph **24.6.**

The Associate Referee on Ether Extract in Fish has during the past year followed the suggestions of Committee C (*This Journal*, **31**, 52) and has made studies of the composition of substances extracted from fish by ether, and by ether-petroleum ether mixture after acid hydrolysis. The report by Associate Referee Voth is being submitted.

RECOMMENDATIONS*

It is recommended—

(1) That paragraph **24.1** titled "Apparatus—Tentative" of *Methods of Analysis* be reviewed and studied for the purpose of revision.

(2) That paragraph **24.2** titled Preliminary Treatment and Preparation of Sample—Tentative" of the *Methods of Analysis* be likewise reviewed and studied for the purpose of revision.

(3) That in connection with Recommendations No. 1 and No. 2 a study be made of methods for determining drained liquid in oysters and clams.

(4) That the subject, "Total Solids in Fish and Other Seafoods" be continued as a subject of study with the end in view of revising paragraph **24.3**, titled "Total Solids—Tentative," which is now limited to oysters and scallops.

(5) That Method II for Salt (Chlorine as Sodium Chloride) paragraph **24.7**, titled "With Calcium Acetate as Fixative-Tentative" in the *Methods of Analysis*, be studied for deletion (if no longer of value) or for revising and consideration of changing the "tentative" designation to "official, first action."

(6) That the extract obtained by using a mixture of ethyl ether and petroleum ether by the method described in *This Journal*, **31**, 334 (1948), be designated as "Crude Fat (acid hydrolysis method)" and that the method so designated be adopted as official, first action.

REPORT ON ETHER EXTRACT IN FISH

By MENNO D. VOTH (Food and Drug Administration, Federal Security Agency, Boston, Mass.), *Associate Referee*

The recommendation of Subcommittee "C" (*This Journal*, **31**, 52, 1948) was that some further studies be made on ether extract in fish with the idea of determining whether the material, extracted by the solvents etbyl ether or a mixture of ethyl ether and petroleum ether, is largely fat.

Two separate quantities of extract were prepared. In both cases the fat was extracted from canned mackerel of identical origin and the fish flesh was digested with HCl on the steam bath for 90 min. One portion of this digested material was then extracted with ethyl ether in the manner previously described.[1] The second portion was then extracted by a mixture of ether and petroleum ether as described in the last report.[2]

The two quantities of extract were quite similar in appearance although the ethyl ether extract was slightly darker. Various constants of the two portions of the extracts were then determined (see Table 1).

* For report of Subcommittee C and action of the Association, see *This Journal*, **32**, 54 (1949).
[1] *This Journal*, **29**, 46, 1946.
[2] *Ibid.*, **31**, 334, 1948.

TABLE 1.—*Constants of crude fat (extracts) from canned mackerel*

DETERMINATION	WT. OF FAT USED	ETHYL ETHER EXTRACT			MIXTURE OF ETHYL ETHER AND PETROLEUM ETHER EXTRACT		
		SUB 1	SUB 2	AVE.	SUB 1	SUB 2	AVE.
Saponification Number (Methods of Analysis, 1945, 497, **31.24**)	5 g	187.6	—	187.6	189.1	—	189.1
Unsaponifiable Residue (Methods of Analysis, 1945, 506, **31.43,** 1st paragraph)	5 g	0.61%	—	0.61%	0.61%	—	0.61%
Lipoid Phosphoric Acid (Methods of Analysis, 1945, 348, **23.12(b)**)							
As P_2O_5	Approx. 1 g	0.045%	0.048%	0.047%	0.025%	0.029%	0.027%
As Lecithin		0.51%	0.55%	0.53%	0.28%	0.33%	0.31%
Hanus Iodine Number (Methods of Analysis, 1945, 494, **31.18**)	Approx. 1 g	140.8	141.5	141.1	120.1	119.1	119.6
Free Fatty Acids (Method: 25 ml neutral ethanol added, heated almost to boiling, titrated with 0.1 *N* NaOH to phenolphthalein)	Approx. 10 g						
Acid Number		18.0	—	18.0	18.6	—	18.6
As Oleic Acid		9.1%	—	9.1%	9.4%	—	9.4%
Free Fatty Acids* (Method as above, fat extracted by ethyl ether in continuous extractor, no digestion.) Acid Number	Approx. 5 g	3.8*	—	3.8			

* This value obtained in order to determine amount of fatty acids due to acid digestion.

DISCUSSION

The additional work on the ether extract of canned mackerel shows that the composition of this crude fat is normal and involves no unusual problems.

The fish fat obtained by extracting with ethyl ether alone and with a mixture of ethyl ether and petroleum ether are identical for all practical purposes.

REPORT ON SPICES AND CONDIMENTS

By SAMUEL ALFEND (Food and Drug Administration, Federal Security Agency, St. Louis, Mo.*), *Referee*

The Referee has studied the recommendations of the Committee on Classification of Methods, and if the suggested changes are adopted by the Association at this meeting, he expects to submit recommendations in 1949 to bring the chapter into conformity with the new classification.

VINEGAR

Associate Referee Loughrey submitted the tentative permanganate oxidation number to collaborative study on a distilled vinegar, a fortified wine vinegar, and two colored dilute acetic acid solutions. The titrations obtained on the dilute acetic acid solutions were nil. Those on the distilled vinegar were fairly uniform at approximately 4 ml. The results on the fortified wine vinegar were very high and erratic. The method obviously is suitable for the purpose for which it is proposed—to distinguish dilute acetic acid solutions from distilled vinegar. It is believed that adoption of the method as official need not wait for the accumulation of data on the actual oxidation numbers of commercial distilled vinegars from various sources.

The Associate Referee made a comparative study of the official method for tartrates in vinegar and the bitartrate method for tartrates in fruits. The results indicate a clear advantage for the bitartrate method. The Referee agrees with the recommendation to continue the studies.

Loughrey has studied the various tests for caramel in vinegar, but is not yet ready to make a recommendation. The studies should be continued.

MAYONNAISE AND SALAD DRESSINGS

No report was submitted by the Associate Referee. The work suggested for this year should be continued.

MUSTARD

Associate Referee Garfield has developed a procedure for determination of sugars in prepared mustard. The recoveries are slightly too high. The studies he has outlined for next year should be carried out.

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* Present address, Kansas City Station, Kansas City, Mo.

In studying the methods of Terry and Corwin for the pungent principles in different varieties of mustard seed, Garfield has found volatile oil in so-called "white" or "yellow" mustard seeds, contrary to the statements of the English workers. He has therefore taken steps to obtain authentic seeds of known botanical classification, and plans to work on these samples next year.

VOLATILE OIL IN SPICES

No report has been submitted by the Associate Referee for several years. The topic should be reassigned and studies should be continued.

RECOMMENDATIONS†

It is recommended—

(1) That studies on the application to vinegar of the Mathers test for caramel, and the test for caramel in wine **(15.39)** be continued.

(2) That the tentative permanganate oxidation number, with the slight changes described in the Associate Referee's report, be made official, first action).

(3) That methods for the quantitative determination of free mineral acids be further studied.

(4) That studies of the determination of tartaric acid and tartrates in vinegar be continued.

REPORT ON VINEGAR

By JAMES H. C. LOUGHREY (Food and Drug Administration, Federal Security Agency, Boston, Mass.), *Associate Referee*

Subcommittee C, (*This Journal*, **31**, 54, 1948), made the following recommendations for work on Vinegar:

(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, such as Mather's 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 in order to ascertain if it has value in differentiating this type of vinegar from a dilute acetic acid.

(3) That the method for the quantitative determination of free mineral acids in vinegar be further studied.

(4) That studies of the determination of tartaric acid and tartrates in vinegar be continued.

RECOMMENDATION (1)

The Associate Referee has conducted tests on a number of vinegars, comparing the present tentative method **(33.78)** with Mather's test and

† For report of Subcommittee C and action of the Association, see *This Journal*, **32**, 58 (1949).

with the confirmatory test for caramel in wines **(15.39)**. Mather's test involves coprecipitation of the caramel with pectin from an acidified alcohol solution. The caramel is confirmed by the use of 2,4-dinitrophenylhydrazine, which reagent appears to offer several advantages over the present reagent, phenylhydrazine hydrochloride.

The results of this comparison are given in Table 1. The cider vinegars used were received from two New England firms which are deemed to be reliable and which guaranteed the authenticity of the samples.

TABLE 1.—*Comparative results*

VINEGAR	PRESENT TENTATIVE METHOD	MATHER'S TEST	A.O.A.C. CONFIRMATORY
New (1947) Cider	Positive	Doubtful	Negative
Old (2½ years) Cider	Negative	Negative	Negative
Old (3½ years) Cider	Positive	Negative	Negative
Old (2 years) Cider	Negative	Negative	Negative
New (1½ years) Cider	Positive	Negative	Negative
Cider-Distilled Vinegar (50-50; Cider Vinegar 2 years old)	Positive	Negative	Negative
Very light caramel solution	Positive	Positive	Positive

It will be noted that the present tentative method **(33.78)** gave positive results on four of the six cider vinegars tested, which confirms previous findings.

The two methods with which the tentative method was compared appear to be more reliable However, both methods will need further study before a recommendation is made. Of these two methods, the A.O.A.C. confirmatory test, in which the caramel is coprecipitated with zinc hydroxide, appears easier in manipulation. However, it suffers from the handicap that only a small proportion of the caramel actually present is precipitated by the zinc hydroxide; on known solutions of caramel, about 15 per cent of the caramel present is recovered. The figure is quite constant.

RECOMMENDATION (2)

The Associate Referee was able to obtain one distilled vinegar of known history. This vinegar was made from formula No. 35A of the Bureau of Internal Revenue, which calls for 5 gallons of ethyl acetate (not less than 85 per cent ethyl acetate upon saponification) to be added to each 100 gallons of ethyl alcohol. This formula was made into vinegar by the "closed generator" process. As received, the acidity was 5.5 per cent as acetic.

This sample of distilled vinegar was colored with amaranth and caramel, to resemble wine vinegar, and was submitted to collaborators as Sample A for determination of the permanganate oxidation number. Also submitted

were dilute acetic acid, colored with caramel, as Sample B; dilute acetic acid colored with amaranth and caramel as Sample C; and a wine vinegar to which ethyl acetate and a small amount of amyl acetate were added (to increase the oxidation number) as Sample D.

The method was substantially the same as that submitted in 1947. A few changes, in line with recommendations of collaborators, were made. Details of the method are published in *This Journal*, **32**, 102 (1949).

The number of ml of the 0.5 *N* sodium thiosulfate 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).

If the permanganate oxidation number is more than 15, repeat the determination, using 25 ml of the adjusted vinegar plus 25 ml H_2O. Repeat this reduction by half until the ml of potassium permanganate used is less than 15. Calculate permanganate oxidation number to basis of 50 ml of adjusted vinegar.

The results reported by the various collaborators are given in Table 2.

TABLE 2.—*Collaborative results*

	PERMANGANATE OXIDATION NUMBER			
COLLABORATOR	SAMPLE A	SAMPLE B	SAMPLE C	SAMPLE D
1	4.24	0	0	44.55 (10 ml)
2	4.46	0	0	—
3	4.40	0	0	36.25 (10 ml)
4	3.92	0.10	0.20	—
5	3.82	0	0	38.40 (10 ml)
6	4.47	0	0	38.12 (10 ml)
7	4.43	0	0	53.30 (5 ml)
8	3.84	0.05	0.14	48.85 (10 ml)
Assoc. Ref.	4.39	0	0	45.98 (10 ml)

The average of all nine results on Sample A, the "known" distilled vinegar, was 4.22. The results on the two samples, B and C, of dilute acetic acid, are in line with what was to be expected. Sample D was prepared purposely to give high values, inasmuch as some vinegars, such as wine vinegar, may give high values.

The method is an empirical one and is affected by time, temperature, and most of all by the concentrations of the oxidizing and reducing substances present in the reaction mixture. For that reason, it is recommended that, if the oxidation number is higher than 15, the determination be repeated, using smaller quantities of the vinegar. Two collaborators used 25 ml samples of Sample D and obtained permanganate oxidation numbers of 30.72 and 33.64, respectively; these values were not included in the above table. Using 25 ml samples, collaborator No. 1 obtained a

value of 31.21 for Sample D; the Associate Referee obtained 31.05 by the same procedure. It is obvious, therefore, that the aliquot taken for this determination must be small enough to allow for an excess of permanganate.

The method is of value *only* in differentiating between true vinegars and dilute acetic acid. The actual oxidation number when the value is high is not pertinent.

The Associate Referee wishes to thank the following collaborators for their prompt assistance: Paul Somerville, of H. J. Heinz Company; G. A. Michael, of the Massachusetts Dept. of Public Health, Division of Food and Drugs; and C. G. Cunningham, F. M. Garfield, E. H. Grant, R. E. O'Neill, P. B. Rokita, and S. Shendleman, all of the Food and Drug Administration.

RECOMMENDATION (3)

No work was done this year on the quantitative determination of free mineral acids in vinegar.

RECOMMENDATION (4)

The present official method for tartaric acid and tartrates in vinegars **(33.84)** is the same method as that for wines **(15.28)**. This method appears to offer several opportunities for error. The method calls for evaporation of the vinegar to a sirupy consistency to remove excess of acetic acid, determination of the residual acidity, and the addition of a weighed amount of tartaric acid proportional to this residual acidity. The Associate Referee has found it difficult to drive off all the acetic acid; even as many as three evaporations will require as high as 3.0 ml of *N* alkali per 100 ml of vinegar. This would call for the addition of as much as 225 mg of tartaric acid to be added in the present method, which is an obvious defect because most vinegars will contain far less than that amount of tartaric acid. The occlusion of acetic acid by vinegar solids is well known in the determination of total solids in vinegar.

On the other hand, the bitartrate method for tartaric acid in fruits **(26.32, 26.33)** appears to be less subject to error. The Associate Referee has made a few analyses comparing the two methods, and the results are given below in Table 3.

TABLE 3.—*Comparison of present and bitartrate methods*

SAMPLE	TARTARIC ACID—MG PER 100ML VINEGAR	
	WINE METHOD	BITARTRATE METHOD
Wine Vinegar A	46.5 (225 mg added)	99.3
Wine Vinegar B	77.1 (168.8 mg added)	105.5, 105.5
Distilled Vinegar	6.7, 6.7 (18.8 mg added)	4.7 4.7
Distilled Vinegar and Tartaric Acid (1 mg per ml)	28.5 (112.5 mg added)	88.2, 100.8

The figures in parenthesis under the wine method show the amount of tartaric acid it was found necessary to add, following the directions given in that method. In every case, the vinegars were evaporated three times to the consistency of a sirup. The amounts of tartaric acid reported have been corrected for the added tartaric acid.

It is to be noted that in the distilled vinegar containing added tartaric acid (100 mg per 100 ml) the amount recovered was only 28.5 mg. In another determination by the wine method on this "known," the total amount recovered was 25.5 mg, despite the fact that a total of 212.5 mg (100 mg plus 112.5 mg added following directions) were present.

The Associate Referee recommends*—

(1) That further study be made of Mather's test and of the test for caramel in wine **(15.39)** to determine their applicability to vinegar.

(2) That further study be made of the permanganate oxidation number, as applied to distilled vinegars of known history.

(3) That the method for the quantitative determination of free mineral acids in vinegar be further studied.

(4) That further study be made of the applicability to vinegars of the bitartrate method for total tartaric acid.

REPORT ON SUGAR, ASH, AND PUNGENT PRINCIPLES IN MUSTARD

By FREDERICK M. GARFIELD (Food and Drug Administration, Federal Security Agency, St. Louis, Mo.), *Associate Referee*

It was recommended by the Referee[1] and approved by Subcommittee C[2] and the Association that studies be made of a suitable method for the determination of sugars in prepared mustard. There appeared to be a real need for such a method to supplement the one for starch in prepared mustard. Previously, sugars and starch were lumped together under copper reducing substances by direct inversion. (This method, **33.40**, was dropped, final action.[3])

Clarification of the prepared mustard was first studied. After addition of calcium carbonate to take care of free acidity, clarifying agents such as lead acetate, lead carbonate, and lead acetate and carbonate were tried. Flocculent precipitates formed which could be easily filtered. A combination of lead carbonate (in place of calcium carbonate) and normal lead acetate seemed to give the best clarification. Difficulty was encountered in removing the excess lead. Both anhydrous potassium oxalate and

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* For report of Subcommittee C and action of the Association, see *This Journal*, **32**, 58 (1949).
[1] *This Journal*, **31**, 340 (1948).
[2] *Ibid.*, **31**, 55 (1948).
[3] *Ibid.*, **31**, 108 (1948).

sodium carbonate were tried, but the resulting precipitated lead would not settle or filter. This was overcome by centrifuging the material. The clear supernatant liquor was then poured through a filter. Anhydrous potassium oxalate seemed to work best.

Reducing sugars before and after inversion were determined by the Munson-Walker procedure. The resulting cuprous oxide was somewhat off color, tending toward the brown. The copper content was then checked by titration with thiosulfate **(34.41)**. The sugars calculated from both the weighed cuprous oxide and the titrated copper showed that the weighed results were slightly high, but probably not high enough to warrant the additional work required to titrate the copper. For tabulation of results see Table **1.**

DISCUSSION OF RESULTS

The recoveries of added sucrose are somewhat high, but they are promising. Further work will be undertaken in an attempt to account for these high recoveries. A correction for error due to volume occupied by insoluble mustard and lead carbonate in the clarification procedure should account for about 2 per cent of the high results.

Apparently the presence of starch has no effect on the sugar recoveries.

The results obtained by titration of the cuprous oxide are lower than those obtained by weight. The differences are slight and the additional work required to titrate the copper may be unwarranted.

Further work appears to be in order to account for the 1 per cent of reducing substances before inversion. Two or three authentic prepared mustards will be put up and the ingredients and the final product will be assayed in order to find the source of the copper reducing material. Examination of a sufficient number of commercial prepared mustards should give data to establish a figure for the normal content of reducing sugars in prepared mustard; values higher than this demonstrating the presence of added sugars.

Some work will be carried out on the effect of vinegar on the inversion of sucrose in prepared mustard under normal storage conditions.

METHOD USED

Approximately 10 g of prepared mustard were weighed into a 200 ml volumetric flask and clarified by the addition of 2 g of lead carbonate and 1 ml of 30 per cent normal lead acetate. The contents were made to volume and filtered. The excess lead was precipitated by the addition of anhydrous potassium oxalate, the lead oxalate settled by centrifuging and the supernatant liquid poured through a filter. Reducing sugars before inversion were determined in an aliquot by **34.39.** A second aliquot was inverted by **34.24(c)** and reducing sugars were determined by **34.39.** Sucrose was calculated by **34.30.**

TABLE 1.—*Tabulation of results—ounces*

DETERMINATION	1	1A	2	2A	3	3A	4	4A
Wt. prepared mustard—g.	10.124	10.113	10.282	10.062	10.150	10.098	10.047	10.316
Starch added	—	—	—	—	0.40	0.40	0.40*	0.40*
Sucrose added	—	—	0.4000	0.4000	0.4000	0.4000	0.4000*	0.4000
Reducing sugars before inversion as invert—								
from weighed Cu_2O	0.115	0.116	0.115	0.127	0.116	0.119	0.110	0.119
by titration	0.110	0.108	0.112	0.118	0.112	0.114	0.106	0.114
Reducing sugars after inversion as invert—								
from weighed Cu_2O	0.108	0.110	0.562	0.560	0.560	0.566	0.554	0.573
by titration	0.113	0.107	0.553	0.553	0.550	0.553	0.545	0.557
Sucrose—								
from weighed Cu_2O	—	—	0.425	0.411	0.422	0.425	0.422	0.431
by titration	—	—	0.419	0.413	0.416	0.417	0.417	0.420
% Recovery—								
from weighed Cu_2O	—	—	106.3	102.8	105.5	106.3	105.5	107.8
by titration	—	—	104.8	103.3	104.0	104.3	104.3	105.0

* Starch gelatinized.

VOLATILE OIL AND OTHER PUNGENT PRINCIPLES IN MUSTARD

Work on this subject was initiated last year. A literature study was made on available methods. The most promising appeared to be one by Tercy and Corran in the "Analyst."[1]

The present A.O.A.C. method 33.26 can be used to determine the pungent principle (allyl isothiocyanate, which is volatile with steam) in black or brown mustard. According to Tercy and Corran the pungent principle (p-hydroxybenzyl ester of isothiocyanic acid, which is not volatile with steam) in white or yellow mustard cannot be determined in the same manner. They propose methods that will determine either principal alone, or in mixtures of the two.

When, in early experiments, so-called white or yellow mustards gave allyl isothiocyanate by the A.O.A.C. procedure in contradiction to numerous literature references, work was stopped.

It was not possible for the Associate Referee to identify botanically the mustard seeds on hand or to have them identified in St. Louis. Efforts were then directed toward obtaining true black (*Brassica nigra*) and white (*Sinapis alba*) mustard seeds. Work will be continued when these seeds are obtained.

RECOMMENDATIONS*

It is recommended that this subject be continued.

No work was done on "Ash in Prepared Mustard." It is recommended that this subject be continued.

It is recommended that the method for "Starch in Prepared Mustard and Mustard Flour" (now official, first action) be made official, final action.

No report was given on volatile oil in spices, preparation of sample, or fat in mayonnaise and salad dressing.

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*

CADMIUM

Previous work having demonstrated that certain grades of carbon tetrachloride will not produce accurate results for cadmium in the presence of 5 per cent alkali, the Associate Referee was faced with the necessity of

[1] Analyst, 64, 164 (1939).

* For report of Committee C and action of the Association, see *This Journal*, 32, 58 (1949).

either reducing the alkalinity of the sample solution or else especially treating the carbon tetrachloride before use. The first alternative might easily result in the incomplete removal of zinc, to which any dithizone cadmium method is particularly sensitive. Therefore, he adopted the other possibility and purified his carbon tetrachloride by treatment with hot sodium hydroxide solution and redistillation to remove the impurities which caused the disturbances, particularly the fading, later on in the determination. Certain brands of carbon tetrachloride or perhaps, certain deliveries of these brands, did not need purification, while others did. To be on the safe side purification may be required unless previous trial of the carbon tetrachloride with known quantities of cadmium demonstrates its purity. The Associate Referee used relatively large amounts of interfering metals, particularly zinc, in the collaborative samples to provide a most rigorous test of the method. Under ordinary circumstances samples would not contain so much zinc, but this metal is almost universally present in food or biological material and is in some instances extraordinarily high in quantity. The Referee has in mind the 400 p.p.m. reported in some maple syrups, the 1000–2000 p.p.m. in some oysters (wet basis), and 5000–10,000 p.p.m. (dry basis) in some plants grown on high zinc soils. The Associate Referee wished to devise a method that would take care of these exceptions as well as the usual run of samples and, therefore, introduced into it a double extraction with 5 per cent alkali. Apparently these precautions are necessary on samples containing micro amounts of cadmium and relatively large amounts of zinc, because all three collaborators obtained slightly high results on the sample with only 5 micrograms of cadmium.

The Referee believes these results are encouraging, and that collaboration should continue next year with the object of obtaining sufficient basis for inclusion in the seventh edition, *Methods of Analysis*.

LEAD

No work has been done on lead methods for a number of years because there seemed to be no necessity for it, the methods that had been developed appearing to be adequate. In the interim, what may perhaps be best described as improvements appeared in the literature. Before the publication of the 7th edition of *Methods of Analysis*, the Referee believes that the Association should have a report on these improvements, so that if found worthy they may be incorporated in the next revision. The Referee has in mind particularly the Bambach and Bourky method of separating bismuth and lead at *p*H 3.4, and the Synder extraction of lead at *p*H 11.5 instead of at 9.5. If this proposed change produces as good results as those at the lower *p*H, certain worthwhile advantages might accrue. The associate refereeship on lead has been allowed to lapse for the last several years, subject to the call of the Referee, who believes that this

refereeship should be resumed next year for a report on these improvements. Elaborate collaboration is not necessary perhaps to justify these proposed changes, which do no great violence to the principles of the lead dithizone method but rather seek to streamline them. A favorable report based on actual trial by an expert in the field may be all that is necessary to get the lead methods ready for promotion to official status in time for the next revision.

MERCURY

In the Referee's opinion the two most important items developed in connection with a mercury method in the last two years is the use of selenium to help in the oxidation of the organic matter and the new apparatus designed to effect complete oxidation of organic matter without loss of mercury even in samples containing a large proportion of water. The way is now cleared for the necessary collaboration leading to the adoption of a new mercury method to replace the one now in the Book of Methods, which has sometimes been described as an illustration of "wash-tub" chemistry. Such methods so aptly described may be condoned if we have nothing better, but when the chances for improvement are as good as at present, the Referee recommends that these new ideas be embodied in a method and subjected to immediate collaboration in time for revision.

DDT

The Associate Referee on the determination of DDT has prepared a set of directions for the sample preparation of most of the widely varying plant and animal material likely to contain this insecticide, followed by its determination by the two most promising methods tried last year. The Referee notes, however, that the Associate Referee is silent on the enzymatic method of sample preparation for fats and fatty materials. The Association should have a report by next year on the comparative efficiency of the enzymatic versus the acid method described by the Associate Referee. The same may be said of the purification of benzene extract of plant material containing large amounts of chlorophyl or carotine by absorption methods. No collaborative results were offered. The Referee strongly urges that such results are essential and should be on hand at the next meeting. Certainly the Association must include one or two methods for DDT, if not some of the other chlorinated insecticides, in the next revision of the Book of Methods. In order to do so the Referee urges that the Associate Referee start the collaborative work as soon after this meeting as possible.

No report has been received on the determination of DDT in canned foods. The Referee recommends that this subject be reassigned and that the next Associate Referee investigate the fate not only of DDT but of some of the other newer insecticides as well, in the canning process. If decomposition of these insecticides occurs inside the can, how does it

affect the methods used for their determination? This Association should have the facts.

BENZENE HEXACHLORIDE (BHC)

The determination of benzene hexachloride residues is in an unsatisfactory state. The infra red, chromatographic, or biological methods for the determination of the gamma or other isomers in the commercial insecticide seem to be well advanced, but these methods are impracticable for the determination of residues. The chlorine methods with their nonspecificity defect are about the only ones that can be recommended at the present time for quantitative determinations. Colorimetric methods so far tried are based on the nitration followed by color production of three isomeric tri-chlor-benzenes and suffer from their volatility, which results in 5–15 per cent losses before or during nitration. In addition, interfering color production results from the other organic materials of the samples which require absorption or chromatographic separation. Hence there are low recoveries and compensatory errors. Much more work will be required to devise a satisfactory method along this line. The Referee understands that a method based on the rather specific absorption of the 1-2-4 trichlorbenzene at a particular wave length is a prospect. Whether it will be sensitive enough for spray residues is still to be determined.

The Referee's attention has been called by the Beechnut Company to the possibility that the disagreeable and characteristic odor of commercial benzene hexachloride can be made the basis for a qualitative test for such residues. When the evaporated benzene extract of benzene hexachloride residues are oxidized by nitrating mixtures and then made alkaline, the odor of the insecticide remains predominant, while most of the natural odors have been destroyed or have disappeared. Whether this idea can be worked into a good qualitative method suitable for all kinds of benzene hexachloride residues remains to be determined. It appears at least to have a considerable degree of usefulness.

A most sensational result from the use of benzene hexachloride in agricultural crops occurred in connection with the 1947 crop of potatoes. Benzene hexachloride mixed with soil to kill wire worms, or even sprayed on the growing plants, resulted in a large crop of fine looking U. S. No. 1 potatoes, but unfortunately they were unpalatable to consumers. No benzene hexachloride was found inside the potatoes by any method, qualitative or quantitative, now available. The unpleasant flavor of the potatoes therefore is believed to be the result of secondary reactions in the potato started by the benzene hexachloride. Other agricultural crops have been affected more or less in the same way. Potato crops produced in 1948 on the same soil have been likewise affected. It may be several years before the ill effects wear out. This unfortunate result will naturally curtail the use of benzene hexachloride on food crops and perhaps the energies de-

voted towards devising methods for determining this insecticide in spray residues can be turned into other directions.

CHLORDANE

One method for the detection or determination of chlordane in insecticides or residues has just been published in *Analytical Chemistry*. A paper describing another is on the program. It is too early to assess the possibilities of these methods or the demand for them just now. Appointment of a Referee may be postponed for a year to allow the methods to be shaken down to practical realities.

TOXAPHENE

The Referee knows of no method for the determination of this insecticide unless it is the chloride methods. We had some experience during the year with a chlorine-containing insecticide on cabbage that did not respond to tests for any of the other newer insecticides. Presumably it was toxaphene, but that fact could not be demonstrated. Some specific method for the determination of this insecticide in residues is needed, but there is no promise for one at this time.

PARATHION

Parathion is the name given to perhaps the newest insecticide to appear on the horizon in commercial quantities. It is an ethyl-p-nitro phenyl ester of thiophosphoric acid. It has great insecticidal activity, but unfortunately it also is very toxic to man and other warm-blooded animals. Apparently only small quantities are needed to protect a crop and its reasonable volatility is expected to result in low residues. It has been used only in limited commercial quantity on the 1948 crop but much greater usage is expected next year. It is thought to be the best insecticide developed to combat the mites that have increased so tremendously as the result of killing their enemies with DDT. A combination of DDT and parathion may therefore be expected in future spray residues.

Heretofore spray residues have been found mostly on the surface of fruits and vegetables, if the catalytic action (if it may be described as such) of benzene hexachloride on potatoes is omitted from consideration. In the case of parathion there is definite evidence that it may be transported through the root and stem system to the leaves and edible portions of plants. Besides evidence of translocation of parathion we believe it can also penetrate the skins of fruits or vegetables into their interiors. The factors of time, concentration, weathering, and volatilization that govern translocation or penetration have not yet been fully assessed but we undoubtedly have been presented with a new problem in sample preparation. The old method of "stripping" an insecticide from the surface of fruits or vegetables by acids or organic solvents may no longer be adequate.

Fortunately, a method for the determination of parathion was released by the manufacturer simultaneously with its experimental and commercial usage. The method is based on the reduction of the nitro group attached to the phenyl ring in the para position followed by diazotization and color production with another amine hydrochloride. The efficiency of the results has been in the neighborhood of 85 to 90 per cent. This accuracy has been good enough for experimentation and preliminary assessment of commercial residues. To us it is a challenge for further efforts for increased efficiency. Another method based on different principles for check purposes is also highly desirable. Therefore, the Referee believes the Association should appoint a new Associate Referee to carry forward the work designed to improve the method presented to us and get it ready for adoption and perhaps include it in the next revision of the Book of Methods.

COMPOUND 118

Your Referee has just learned of a new toxicant which has not yet been given a "manageable" chemical name; hence its provisionable numerical name. It is in about the same position that parathion was last year, *viz*, ready for experimental trial generally but not for commercial distribution. Its structure is said to be that of a double bridged naphthalene with six chlorine atoms confined to one of the phenyl rings. It is understood that work on a method for its determination is well on the way and possibly will be ready by the time material amounts of the toxicant become available. The Referee is pleased to see that manufacturers of new insecticides are giving more attention to methods for the determination of their products.

RECOMMENDATIONS*

It is recommended—

(1) That the study of methods for the determination of cadmium, copper, lead, mercury, and zinc in foods be continued, with collaboration where necessary.

(2) That the study on the determination of DDT in foods in general be continued with sufficient collaboration to support adoption and inclusion of a DDT method in the next edition of *Methods of Analysis*.

(3) That the effect of the canning process on the decomposition of the newer insecticides be studied, with respect to (1) the nature of possible decomposition products and (2) their effect on the methods of analysis of the insecticides.

(4) That a new Associate Referee on the determination of parathion be appointed for study of that subject.

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* For report of Subcommittee C, and action of the Association, see *This Journal*, 32, 55 (1945).

REPORT ON CADMIUM

By A. K. KLEIN (Food and Drug Administration, Federal Security Agency, Washington 25, D. C.), *Associate Referee*

The micro cadmium method described in *This Journal*, 28, 257 (1945) was submitted for this year's collaborative study. In the 1947 report, the Associate Referee called attention to the fact that the purity of the carbon tetrachloride, used as solvent for the extraction reagent, dithizone, is of utmost importance. During the past year it was observed that certain reagent grades of the solvent require no further purification. A collaborator in the Pharmacology Division of the Food and Drug Administration obtained excellent cadmium results in the range 0–25 micrograms using reagent grades of carbon tetrachloride and dithizone, neither of which was subjected to any further purification. The uniformly good results were obtained even in the presence of 1000 micrograms of added zinc. The Associate Referee, however, sought to devise a procedure of purification which would insure a satisfactory quality of all supplies of the solvent. Subsequent good results were obtained when the solvent was first refluxed for 1–2 hours with 5% sodium hydroxide, washed thoroughly with water, dried with calcium chloride, filtered, and then distilled over calcium oxide. The Associate Referee then felt assured that collaborative study of the method was merited.

Each collaborator was supplied with two unknown samples marked I and II. They consisted of 25 grams of comminuted tomato pulp, previously tested and found to be free of cadmium, to which were added 5 and 20 micrograms of cadmium, respectively. In addition the following interfering metals were added as soluble salts.

	Sample I *Micrograms*	*Sample II* *Micrograms*
Cu	250	500
Pb	250	500
Bi	50	100
Hg	50	100
Zn	1000	2000
Co	50	100
Ni	50	100

A practice sample of the tomato pulp containing no cadmium was also submitted to familiarize the collaborators with the method. The same interfering metals were added as in the unknowns in the amounts such that 25 g, the recommended weight for practice, would contain 88 per cent of the amount added to sample II. Collaborators were instructed to use the entire contents of the containers of I and II.

Their results are as follows:

Collaborator	*Sample I*	*Deviation*	*Sample II*	*Deviation*
	mmg	*mmg*	*mmg*	*mmg*
A	5.4	+0.4	19.6	−0.4
B	6.8	+1.8	20.7	+0.7
C	6.7	+1.7	19.0	−1.0

Collaborator A offered no comments in his report.

Collaborator B stated that the method appeared satisfactory. In trial runs with the practice sample he obtained a blank of 0.2 mmg, and 9.9 mmg and 19.6 mmg when 10 and 20 mmg, respectively, were added. He experienced some difficulty in matching the optical fields in the visual evaluation of the cadmium dithizonate. This difficulty is due, no doubt, to a deficiency of his photometer, for no other collaborator has spoken of any difficulty in the visual evaluation of the optical density. Another reason for believing that the instrument was at fault is that he subsequently made standards of 6.8 and 20.7 mmg cadmium and the optical densities agreed, as well as he could read them, with those of the unknowns.

Collaborator C stated that the method seemed straightforward but has the weakness of lack of stability of the dithizone-carbon tetrachloride reagent. This latter statement, while true, is not an inherent fault of the cadmium method, because all dilute dithizone solutions are somewhat unstable and for that reason must be freshly prepared.

Two other collaborators were supplied with samples. One has not yet reported his results other than to state that Beer's Law was not followed in the development of the standard curve post 15 micrograms. He was using untreated Merck Reagent grade carbon tetrachloride and was therefore advised by mail to follow the recommended procedure of purification. The last collaborator experienced such erratic recoveries in trial runs that it was obvious that he too was working with an impure reagent. He too was advised to follow the directions for purification.

As a whole the submitted results, when judged from the standpoint of absolute recoveries, with the exception of those of Collaborator A, are not so good as had been expected. However, when we consider the relatively large proportion of metals, the results are not disquieting. For instance, Sample I contained 5 micrograms of cadmium in the presence of 1700 micrograms of interfering metals, any one of which if not completely removed would read in part as cadmium. The same comment may be made for Sample II, which contained but 20 micrograms of cadmium in the presence of 3400 micrograms of interfering metals. Since such samples are not at all likely to be encountered in ordinary analysis, the method was probably submitted to a very rigorous test, especially as none of the collaborators had had previous experience with the method.

The Associate Referee, therefore, feels assured that further collaborative study is merited.

REPORT ON MERCURY

By A. K. KLEIN (Food and Drug Administration, Federal Security Agency, Washington 25, D. C.), *Associate Referee*

The micro mercury method devised by Laug,[1] has been used by him and his associates in the successful determination of mercury in hundreds of samples of various biological materials and animals' diets. Prior to 1947 it had not been applied to determining residual mercury in apples. However, the use of phenyl mercuric ammonium lactate or acetate as a control measure for the fruit disease, apple scab, obviously requires a method for the quantitative estimation of small amounts of residual mercury. Some of the difficulties encountered in the application of Laug's method to apple analyses were described by F. M. Kunze.[2] In brief, erratic recoveries, always on the low side, were obtained unless selenium, either as the metal or as the selenite, was present during the preparation of the sample, by oxidation and digestion with concentrated nitric and sulfuric acids under reflux.

In the analyses of biological materials sufficient mercury was present so that an aliquot of the liquid digest could be used for the analysis, a dithizone method so manipulated that it is specific for mercury. Laug was well aware of the fact that the entire acid digest can not usually be used for the determination. The high acidity and the presence of oxidizing materials decompose the dithizone chloroform reagent so that untrustworthy results were obtained. When an aliquot of the digest was diluted as outlined in the written procedure, no decomposition of dithizone occurred.

The Associate Referee, however, was requested to establish the fact whether or not any mercury at all is present in apples which have never been subjected to any mercury treatment. The amounts of mercury found in this instance would be very small, if present even at all, and would require, therefore, the analysis of the entire apple digest in order to obtain a sufficient quantity of mercury which would not be confused with a small reagent blank. Accordingly, 50 g portions of whole apples were digested under reflux in the conventional manner in the presence of 0.1 g selenium as selenite. The resulting acidity was then 95 per cent neutralized with 50 per cent sodium hydroxide and then extracted with the dithizone reagent. Almost invariably the dithizone was decomposed so completely that no mercury would be extracted had any been present. Control runs with added known amounts of mercury to the apples prior to digestion, even in the presence of selenium, usually led to low recoveries because of this decomposition. Sulfites added to the digest after neutralization with alkali prevented decomposition of the dithizone but combined with the

[1] *This Journal*, 25, 399 (1942).
[2] *This Journal*, 31, 438 (1948).

mercury to form so stable an inorganic complex that no mercury at all could be extracted with dithizone. The final alternative was to digest the sample so completely that no oxidizing organic material would be left to destroy the dithizone. Since apples contain 80 to 90 per cent of water, so complete a digestion could not be obtained unless the water is removed during the digestion. As mercury salts are volatile when heated, a special apparatus was designed which would trap the water and condense any volatilized mercury compounds. This was accomplished by affixing a Soxhlet apparatus devoid of the syphon to the digestion flask. To condense the acid vapors from the digest a reflux condenser was affixed to the top of the modified Soxhlet unit. In this manner, the water and acid vapors liberated during the digestion collect in the Soxhlet unit. The resulting concentrated digest may then be digested as completely as necessary by the portionwise addition of nitric acid through a standard taper dropping funnel leading to the second vent of the digestion flask. When the digestion is finished, the liquid which collected in the Soxhlet unit is drained back into the digestion flask by opening the stop-cock. The entire solution is then refluxed in the usual manner and the vapors and condensate thus formed completely rinse the upper portions of the apparatus so that any mercury which had collected there is restored to the flask. In this manner a digest is obtained which does not decompose the dithizone reagent even though the entire sample be used. A preliminary survey of untreated apples indicates that very little, if any, mercury is present in the fruit.

In Laug's procedure a standard volume of dithizone reagent is added to the final solution in estimating the mercury and in preparing the working standard reference curve. Since the mercury dithizonate is developed in an acid solution, a very large proportion of the optical density is due to unreacted dithizone. This obviously restricts the range of mercury which can be measured by any of the usual photometers. Greenleaf surmounted this difficulty ingeniously in his copper method.[3] Instead of adding a fixed volume of dithizone reagent, he added this reagent portionwise until only a slight excess was present. Then a volume of carbon tetrachloride was added to bring the total to a fixed amount. By measuring the optical densities of the resulting solution at wave lengths maximum for both copper and dithizone he was able to evaluate copper in larger amounts than if the conventional dithizone procedure had been employed. However, a preliminary application of Greenleaf's technique to mercury was not successful. In the presence of only a slight excess of dithizone the mercury complex was too sensitive to light. When the densities were evaluated by the neutral wedge photometer, the color shifted so rapidly that no accurate optical density could be evaluated. It is quite likely that the Beckmann photometer, which employs a much less intense light source, will overcome this defect.

[3] *This Journal*, 30, 144 (1947).

The Associate Referee therefore feels justified in submitting the modified method to collaborative study during the forthcoming year.

REPORT ON DDT AS SPRAY RESIDUE ON FOODS

By ROSCOE H. CARTER (Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, U. S. Department of Agriculture, Beltsville, Md.), *Associate Referee*

It is recommended that two methods be adopted as tentative for the determination of DDT residues in plant and animal materials, (1) the determination of the total organic chlorine content by the sodium and isopropanol method, and (2) the colorimetric determination based on the nitration of the compound and the development of a blue color by the use of sodium methylate.

Method 1 is applicable to cases where the spray history of the material is known. It is simpler and requires less equipment than Method 2. It is not specific, since other organic halogen compounds are determined by the same procedure. Method 2 is quite specific and should be relied on for determinations where only very small amounts are present and as a confirmatory test for the presence of DDT. Since the compound known as TDE (or DDD) gives the same colorimetric reaction, the history of the samples is of importance.

Determination of "blank" values on untreated materials from the same source is of extreme importance, especially when only small amounts of DDT are present.

SAMPLE PREPARATION

The first step in preparing the sample is to extract or strip the material with an organic solvent such as benzene, pentane, or petroleum ether. An apparatus similar to the churn-type washer described by Fahey, Cassil, and Rusk (1) is very useful for stripping operations on apples, pears, leafy vegetables, forage crops, and similar materials which are firm enough to withstand tumbling for short periods of time. Extraction by soaking, with occasional shaking, is satisfactory for some types of material; extractions in Soxhlet apparatus may also be employed. Bulky wet materials, such as forage crops and leafy vegetables such as cabbage and lettuce, must be dried before extraction. Drying at 70° to 80°C. in a circulating air drier has been found very satisfactory. This prevents contamination of the solvent with water and allows a larger sample to be extracted. Care must be taken to prevent entrainment of inorganic chlorides.

Sample preparation for apples and pears and the precautions to be observed have been discussed by Wichmann *et al.* (2).

For animal fats, organs, and flesh samples the following method of preparation has been found satisfactory: A 100 g sample is macerated

with 100 ml of benzene. This mixture is transferred to an evaporating dish, and the benzene is evaporated on a steam bath. The residue is mixed with sufficient anhydrous sodium sulfate to form a crumbly mass, which is then dried in an oven at approximately 70°C and again extracted with benzene. Suitable aliquots of the benzene solution are taken, filtered if necessary, and analyzed by chlorine or colorimetric methods.

Milk samples are treated as follows: To a sample of 200 g or more an equal volume of 95 per cent ethyl alcohol is added, and the mixture is extracted with 250 ml of a mixed solvent (75 per cent of ethyl ether and 25 per cent of a hydrocarbon fraction with b.p. 60–70°C. (Skellysolve B) in a separatory funnel. After separation of the two layers, the aqueous phase is extracted again with three 100 ml. portions of the mixed solvent. The ethyl ether-Skellysolve B solutions containing the DDT and butter fat are then combined and suitable aliquots taken for chlorine or colorimetric determinations. Precautions must be taken here also to prevent entrainment of inorganic chlorides.

I. ORGANIC CHLORINE DETERMINATION (3,4,5)

REAGENTS

Concentrations are expressed as weight per unit volume throughout this paper.

Sodium.—Metallic, C. P.

Silver nitrate soln.—0.1 *N* or other suitable concentration.

Nitric acid.—Concentrated, C. P.

Isopropanol.—99 per cent, C. P.

Ammonium or potassium thiocyanate.—0.1 *N* or other suitable concentration.

PROCEDURE

(Applicable to strip solutions from fruits, vegetables, and forage crops)

Evaporate aliquots of the strip soln from fruits, vegetables, or forage crops on steam bath until most of solvent is removed. It is not desirable to evaporate to complete dryness, as DDT may decompose with loss of hydrochloric acid. Add 25–50 ml of 99% isopropanol, then add 2.5 g of metallic sodium in the form of ribbon or cut in small pieces, and shake flask to mix sample with isopropanol. Connect to reflux condenser and boil gently for at least ½ hour. Shake flask occasionally. Eliminate excess metallic sodium by cautiously adding 10 ml of 50% isopropanol thru the condenser at the rate of 1–2 drops per second. Boil for an additional 10 min., and then add 100 ml of water.

Cool, add 2 or 3 drops of phenolphthalein soln, neutralize by adding nitric acid (1-1) dropwise, and then add 10 ml in excess. If the soln is colored, cool to room temp., transfer contents of flask and aqueous washings to small separatory funnel, and shake with 15 ml of a mixture of equal volumes of isoamyl alcohol and ethyl ether. Draw off aqueous layer into second separatory funnel and extract again with 15 ml of the isoamyl alcohol-ethyl ether mixture. Draw off aqueous layer into 250 ml beaker. Wash the two extracts successively with 10 ml of water, and repeat with second washing with another 10 ml of water. Combine aqueous wash solns with the aqueous soln in the beaker. The chlorine may then be determined by one of following methods:

(a) Add a slight excess of 0.1 *N* silver nitrate and coagulate precipitated silver chloride by digesting on steam bath for ½ hour with frequent stirring. Cool, filter

thru a fast qualitative paper, and wash thoroly with distilled water. Add 5 ml of saturated ferric alum soln, and determine excess silver nitrate in filtrate by titration with 0.1 *N* potassium thiocyanate. Subtract the quantity of silver nitrate found in filtrate from that originally added. The difference will be that required to combine with chlorine in DDT. One ml of 0.1 *N* silver nitrate is equivalent to 0.0035457 g of chlorine. To obtain the amount of DDT multiply the chlorine value by 2.

(b) Add 0.1 *N* silver nitrate from a buret, in excess of amount necessary to precipitate all the chloride. Then add 5 ml of nitrobenzene and 0.5 g of ferric sulfate. Swirl flask to coagulate the precipitate. Back titrate excess silver nitrate with 0.1 *N* potassium thiocyanate until faint pink color appears. Cross-titrate with both standard solns, crossing the end point in each direction. (The end point, which is not too sharp, is more easily perceived in this way.) Calculate amount of DDT as in (a) from amount of silver nitrate required for titration.

(c) Cool the flask to room temp. and then transfer contents to 400-ml beaker. The volume of soln should be 200–350 ml. Titrate with standard silver nitrate using silver-silver chloride electrodes on an electrometric titrimeter. Calculate the amount of DDT as in (a).

(d) Make alkaline to phenolphthalein by addition of 1 *N* sodium hydroxide. Cool the flask and transfer contents to platinum dish. Evaporate to dryness and ignite as thoroly as possible at a temp. not exceeding dull redness. Extract with hot water, filter, and wash. Return the residue to the platinum dish and ignite to ash; dissolve in nitric acid (1+4), filter from any insoluble residue, wash thoroly, and add this soln to the water extract. Add 10% silver nitrate soln, avoiding more than slight excess. Heat to boiling, protect from light, and allow to stand until precipitate is coagulated. Filter on weighed Gooch crucible, previously heated to 140–150°, and wash with hot water, testing filtrate to prove excess of silver nitrate. Dry the silver chloride at 140–150°, cool, and weigh. Calculate the percentage of DDT from weight of silver chloride and weight of sample used.

NOTE (1): If original strip solution is highly colored, it may sometimes be cleared up by adding decolorizing carbon and filtering. Test decolorizing carbon for presence of chlorides by heating with dilute nitric acid (1+4), filtering, and adding silver nitrate soln to the filtrate. If chloride is present, wash with warm dilute nitric acid until the washings no longer give a positive test.

NOTE (2): If free sulfur is present, add 10 ml of 30% hydrogen peroxide to reaction mixture after it has been diluted with water immediately after refluxing, and heat to boiling for 10–15 min.

NOTE (3): If an electrometric titrimeter is to be used, removal of colors from solns is not important.

II. COLORIMETRIC DETERMINATIONS

REAGENTS

Nitrating acid.—A mixture of C. P. fuming nitric acid (sp. gr. 1.49–1.50) and C. P. concentrated sulfuric acid (sp. gr. 1.84), 1 to 1 by volume.

Sodium hydroxide soln.—2%.

Sodium chloride soln.—Distilled water saturated with C. P. sodium chloride. Technical salt is unsatisfactory because of dirt and colored impurities extractable by ether.

Cotton.—Extracted with acetone in a Soxhlet extractor, dried for several hours at 105°–110°C., and stored in a tightly-stoppered bottle.

Ether.—U.S.P. grade distilled before use. Ether that has been standing long enough to accumulate peroxides and aldehydes, or has been recovered after use in this method, is unsatisfactory, and should be purified before it is used again.

Benzene.—C. P., dry. It is conveniently dried by distilling thru a straight con-

denser until no more water distills over with the benzene, and then replacing the condenser with a dry one and continuing the distillation. Benzene that has been used in this method to dissolve the nitrated residues or to make dilutions thereof may be accumulated and recovered for reuse by distillation.

Sodium methylate soln.—10.0 ±0.1% of sodium methylate in dry C. P. methanol (10.0 g per 100 ml of soln). An excellent method of drying the methanol is to reflux with magnesium turnings (5–10 g per liter of methanol) and a small amount of iodine until the magnesium has completely dissolved, and then to distill with the exclusion of moisture. The soln is prepared by dissolving requisite amount of perfectly clean sodium or good grade of powdered sodium methylate (available commercially) in the dried methanol with cooling, using stirrer and reflux condenser protected by soda-lime tube. An aliquot of clear portion of this soln should be diluted with water and titrated with standard hydrochloric acid, phenolphthalein being used as the indicator. The concentration of the soln should be adjusted to 10.0 ±0.1% by the addition of sodium or sodium methylate, or by dilution with dry methanol.

(The sodium methylate soln that is added to the benzene to develop color should be colorless and optically clear. If sediment does not settle completely on standing, soln should be filtered or centrifuged. Occasionally a turbidity or precipitate of crystalline material (probably sodium carbonate) will form when the centrifuged sodium methylate reagent is added to the benzene solns. This difficulty can be obviated largely by cooling standardized soln in refrigerator for day or two, centrifuging while cold, and decanting into another container.)

Sodium sulfate-sulfuric acid.—Dissolve 100 g of C. P. anhydrous sodium sulfate (oven-dried) in 1 liter of C. P. concd. sulfuric acid (sp. gr. 1.84) with aid of heat, and cool to room temp.

Fuming sulfuric acid-concentrated sulfuric acid.—A mixture of equal volumes of fuming sulfuric acid (20–30% sulfur trioxide) and concd. sulfuric acid (sp. gr. 1.84).

Sodium bicarbonate soln.—5%.

Technical acetone, technical chloroform, and petroleum ether boiling at 60°–70°C. (Skellysolve B). These solvents should be redistilled before use.

PROCEDURE (6, 7)

(Applicable to strip solutions from fruits, vegetables and forage crops and extracts of animal products).

Evaporate aliquots of strip soln from fruits, vegetables, forage crops or extracts of animal products to dryness on steam bath. Remove last traces of solvent by using tube connected to a vacuum. If any water is present, add isopropanol and again evaporate to dryness.

Quantitatively wash residue from evaporation into a 500 ml separatory funnel with 150 ml of chloroform. For the analysis of butter or other fat, substitute for this residue a 5 g sample or an extract thereof from which the solvent has been removed. Place 100 ml of chloroform in second 500 ml separatory funnel, and extract chloroform solns successively with (1) 50 ml of sodium sulfate-sulfuric acid (concd), (2) 50 ml of sodium sulfate-sulfuric acid, (3) 50 ml of fuming sulfuric acid-concd sulfuric acid, and (4) 50 ml of sodium sulfate-sulfuric acid. (If this last wash is not light in color, it is advisable to use still another sodium sulfate-sulfuric acid wash.) Drain each acid wash (lower layer) from first funnel into second funnel and finally into a 250 ml cylinder. (The extraction in second funnel is used to minimize loss of DDT by slight emulsification of chloroform in acid washings. The funnels should be shaken vigorously each time and then allowed to stand for 10–15 min. before draining off acid layer. In the rare case where emulsion forms and does not

separate in 30 min., mixture may be centrifuged and poured gently back into separatory funnel. It is well to keep a small beaker under each funnel and to have a wet cloth handy to wipe any acid which may drip.)

After acid extractions are completed, filter chloroform from both funnels thru a 5 cm tightly packed plug of cotton in glass Gooch crucible holder into third 500 ml separatory funnel. Pipet off any chloroform that has risen to surface from combined acid washings in the cylinder, and run it thru the plug of cotton. Rinse the two funnels and the cotton with chloroform, using ca 50–100 ml. Add enough 5% sodium bicarbonate soln (about 40 ml) to combined chloroform filtrate so that it will remain alkaline when tested with litmus paper after vigorous shaking. After allowing ca 10 min. for reasonably clear separation, filter only chloroform layer thru 5 cm plug of tightly packed cotton in glass Gooch crucible holder into a 500 ml Erlenmeyer flask with standard joint. Wash sodium bicarbonate soln remaining in funnel with two successive 30 ml portions of chloroform, and run mixture thru cotton into Erlenmeyer flask. If filtrate is not clear, filter again.

Add a glass bead to Erlenmeyer flask and recover chloroform on steam bath, using an all-glass system, until only about 10 ml of soln are left. Wash this soln quantitatively into large test tube (25×200 mm or larger) with acetone, add glass bead, and cautiously evaporate solvent on steam bath, removing last traces by inserting tube connected to vacuum line.

Nitration of sample.—Cool test tube in beaker of cold water and with pipet add 2.0 or 5.0 ml of the nitrating acid. Immerse test tube one-third to one-half its length in steam bath and heat for 1 hour. Since nitrations of even small quantities of matrials may sometimes be violent, safety precautions should be observed. If there is much extraneous material, it is advisable to place test tube in ice-cold water, add cooled nitrating acid, and warm the tube cautiously to prevent sudden or violent nitration. When initial reaction has subsided, tube may be heated at 100° with safety. After 1-hour nitration, cool test tube in beaker of cold water, add 25 ml of ice-cold distilled water, and mix by gentle swirling. This procedure stops nitration, and test tube may be left overnight if desired.

Extraction of nitrated product.—Rinse contents of the test tube quantitatively thru small funnel into 125 ml separatory funnel with ca 25 ml of water from wash bottle and 50 ml of ether. A small, irregularly shaped piece of glass placed in funnel used for transfer will prevent the glass bead from falling into separatory funnel. Shake vigorously for at least 1 min. After layers have separated clearly, draw off and discard the lower layer. Wash ether with 10 ml portions of 2% aqueous sodium hydroxide until the washings are alkaline; one washing may be sufficient. Then wash ether with two 10 ml portions of salt soln. The final salt wash should be drawn off as completely as possible. Pack 0.75-inch plug of cotton tightly in glass Gooch-crucible holder, moisten it with ether, and allow ether soln from separatory funnel to filter slowly into 125 ml Erlenmeyer flask. Rinse the separatory funnel with 50 ml of ether in 4 or 5 portions, passing this ether thru the cotton in the Gooch funnel. If salt crystallizes in neck of separatory funnel, press stopper funnel in place firmly with rotating motion to prevent leakage of ether. Add glass bead to Erlenmeyer flask, warm the flask on steam bath with a gentle swirling motion until the bead starts bouncing, and recover or evaporate the ether completely. While flask is still being heated, insert a glass tube connected to a source of vacuum two-thirds of the way into the flask for at least half a min.; then remove the flask and stopper it. The analysis may be interrupted at this point if desired.

(The whole extraction procedure must be done carefully to avoid any loss, such as ether sprayed from the separatory funnel when the stopcock is opened to release pressure or when the glass stopper is removed. This type of loss can be minimized

by allowing time for the ether to drain away from the stopcock or the stopper before performing these operations.)

Development of color.—At this stage there is a choice of procedures, depending on the amount of DDT expected, the amount of soln necessary for use in making the photometric measurements, and whether it is desired to have some soln left to repeat the photometric measurements.

Procedure 1.—Add an accurately measured amount of benzene (for example, 5.00 ml) to residue in Erlenmeyer flask and swirl gently until it is dissolved. Use volume of benzene at least equal to one-third the volume necessary for use in absorption cell or tube of photometer. With pipet add 2 volumes (10.00 ml for 5.00 ml of the benzene soln) of sodium methylate reagent to 1 volume of benzene soln. Swirl gently until soln is homogeneous, pour into absorption cell or tube of photometer, and prepare to make the most important measurements 15 min. after the sodium methylate reagent has been mixed with benzene. (This procedure should be used only when it is known that the amount of DDT is very low and in the range where the color developed will be suitable for direct measurement in the photometer. If there is a possibility that the color developed will be too dark for direct measurement, it is preferable to use Procedure 2 rather than add more benzene and sodium methylate to the colored soln to dilute it.

Procedure 2.—Add a measured amount of benzene (for example, 25.00 ml) to the Erlenmeyer flask, and swirl gently until residue is dissolved. To an aliquot (for example, 5.00 ml) add twice its volume of sodium methylate reagent, mix thoroly by gentle swirling, and pour into absorption cell or tube. (In some cases it is possible to mix the solutions directly in the absorption cell or tube. If the color is too deep, a photometric measurement may he made to obtain a rough estimate.) Dilute part or all of remaining benzene soln to a more suitable volume before removing new aliquot for development of color. If color is too light for good photometric measurement, rinse pipet used for first transfer with benzene into the Erlenmeyer flask, evaporate all solvent on steam bath, swirling flask gently to start bead bouncing, and when all the benzene is evaporated remove last traces by inserting glass tube attached to source of vacuum. This residue in Erlenmeyer flask should now be treated as in Procedure 1.

APPLICATION OF THE METHOD

Photometric measurements.—Spectrophotometric or photometric measurements should be made at the most important wave lengths or with the most important filters as close as possible to 15 minutes after the sodium methylate solution has been mixed with the benzene. Measurements at other wave lengths or with other filters can be made just before or after the most significant readings have been taken.

Absorption cells or tubes should be stoppered tightly. Absorption cells usually have glass covers or stoppers, but if test tubes are used, as in many routine photometric measurements, rubber stoppers washed free of sulfur are preferable to cork stoppers, contact with which will turn the solution yellow. Since the solutions on which optical measurements are made are strongly alkaline, absorption cells constructed with alkali-resistant cement should be used. The solutions should be left in the cells no longer than is necessary to make photometric measurements, after which the cells should be cleaned immediately. Although it might be expected that the

alkaline solutions would attack and etch glass cells, no such difficulty has been experienced during several months of use.

In any application of the method it is important to run a blank analysis on a sample of the same type of material being analyzed which has not been treated with DDT. The results, in terms of DDT or extinction values (never in terms of per cent transmission), should be applied as corrections to the values obtained at each wave length or filter used in the analysis of the DDT-treated samples. If appropriate blanks are not run, the results of the analysis may be high. Blank analyses should be made by diluting the blank runs in the same manner as the DDT-treated samples, or the corrections should be calculated to the same weight of untreated material as used in the analysis of the treated material.

A standard curve should be constructed by carrying through the nitration procedure and subsequent operations amounts of 25, 50, 75, and 100 micrograms of technical DDT. The photometric readings plotted on semilogarithmic paper against micrograms of DDT should lie in a straight line.

NOTE.—Samples containing considerable amounts of animal fat or highly colored extracts of plant material sometimes require several treatments with the sulfuric acid-sodium sulfate reagent before treatment with the fuming sulfuric-concentrated sulfuric reagent. A longer time of treatment is also of value in many cases.

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REPORT ON COPPER IN FOODS

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

The last report (1) by this Associate Referee dealt with the laboratory appraisal of the three most promising all-dithizone methods (2, 3, 4) for the determination of copper in foods. The results presented at that time showed no significant differences between the Bendix-Grabenstetter, Morrison-Paige, or Greenleaf methods. The objections to the Bendix method (conversion to enol tautomer) and the Morrison method (lack of sensitivity) did not affect the results as might have been expected.

TABLE 1.—*Results of collaborative samples*

p.p.p. Copper

COLLABORATOR NO.	BENDIX-GRADENSTETTER METHOD							GREENLEAF METHOD					
	SAMPLE A			SAMPLE B		SAMPLE C & A		SAMPLE A		SAMPLE B		SAMPLE C & A	
		DUPLICATES	AVE.	DUPLICATES	AVE.	DUPLICATES	AVE.	DUPLICATES	AVE.	DUPLICATES	AVE.	DUPLICATES	AVE.
1	1	12.9		16.4		41.0							
		13.5	13.2	17.3	16.8	39.0	40.0						
	2	13.6		15.5		45.0							
		14.0	13.8	14.9	15.2	43.5	44.3						
2	1	16.0		12.2		45.9		15.2		15.1		45.1	
		16.0	16.0	12.1	12.2	47.6	46.8	14.4	14.8	10.7	12.9	45.1	45.1
	2	15.9		11.4		46.9		14.9		9.8		46.6	
		15.7	15.8	11.1	11.3	45.3	46.1	14.5	14.7	10.5	10.2	47.3	47.0
3	1	17.5		14.4		50.1		15.7		14.0		44.1	
		16.5	17.0	14.4	14.4	48.1	49.1	15.2	15.5	13.1	13.6	43.6	43.9
	2	17.1		16.8		49.8		15.9		11.8		43.8	
		15.6	16.4	15.1	16.0	44.8	47.3	16.6	16.3	11.8	11.8	43.8	43.8
4		15.5[1]		15.3[1]		51.8[1]							
		15.6		14.8		54.7							
		15.7		14.4		51.8							
		15.7	15.6	15.3	14.5	52.8	52.1						
Average			15.4		12.9		48.0		15.3		12.1		45.0
Max. Deviation			2.2		3.2		8.0		1.0		1.9		2.1
Ave. Deviation			1.1		1.4		3.0		0.6		1.1		1.1

[1] Separate ashings.

In accordance with the recommendations of the last report the "two-color" method of Greenleaf and the "one-color" method of Bendix and Grabenstetter were submitted to collaborative study. A modification of the Bendix method was introduced for this work. The extraction of copper was made at *p*H of 3.2 rather than at *p*H 2.3 in order to reduce the shaking time of the extraction from 10 to 4 minutes.

Sample A consisted of regular Ovaltine powdered and thoroughly mixed to insure homogeneity. Sample B consisted of commercially canned peas, dried, powdered, and homogenized. Sample C was a standard copper solution containing 1.036 mg copper per ml to be added to Sample A for recoveries. Samples A and B were to be oven dried before weighing out the suggested sample sizes. Determinations were to be made in duplicate aliquots from each of two separately ashed samples per the Bendix and Greenleaf methods. An aliquot of solution C equivalent to 30 p.p.m. copper was added to Sample A for recoveries.

DISCUSSION OF RESULTS

The results presented in Table 1 indicate that the Bendix and Greenleaf methods produce comparable results on the selected samples. The Bendix method showed an average deviation of 1.1 and 1.4 p.p.m. copper in Samples A and B, and a deviation of 3.0 p.p.m. in Sample C & A, or in the latter case a recovery of 109 per cent. It should be noted that an average deviation of 1.1 to 3.0 p.p.m. copper over a range of 15–50 p.p.m. copper on the basis of a $\frac{1}{10}$ aliquot on a 2 gram sample will be equivalent to an average deviation of 0.2 to 0.6 micrograms of copper.

The Greenleaf method, although more difficult to carry out, gave an average deviation of 0.6 and 1.1 p.p.m. copper on Samples A & B, and 1.1 p.p.m. copper on Sample C & A, amounting to 99 per cent recovery. In terms of micrograms as above, the Greenleaf method average deviation is equivalent to 0.12 and 0.22 micrograms of copper.

COLLABORATORS' COMMENTS

(1) Changes in laboratory personnel necessitated the use of an inexperienced operator for these analyses and subsequent difficulties with the Greenleaf method produced erratic results which were not reported.

(2) "With respect to the copper methods, the Greenleaf procedure is undoubtedly more elegant since it does avoid the difficulties inherent in one-color methods. The additional manipulations, however, probably cancel out any possible advantage. Our personal opinion is that the procedure earlier published by Greenleaf is a much more satisfactory method if a theoretically perfect method is required for referee work. For routine work and for all practical purposes, however, the loss of copper inherent in the single-color method is probably sufficiently reproducible in unknowns and standards to reduce errors from this source to insignificance."

(3) No difficulties were reported. Samples were ashed as per Greenleaf method.

(4) This collaborator experienced difficulties with the Greenleaf method and felt the results obtained were not suitable to report.

COLLABORATORS

W. C. Stammer—Continental Can Company, Inc., Chicago, Illinois
O. R. Alexander—American Can Company, Inc., Maywood, Illinois
C. A. Greenleaf—National Canners Association, Washington, D. C.
Richard Coleman—Q.M.C., Chicago, Illinois.

RECOMMENDATIONS*

It is recommended—

(1) That the "two-color" method of Greenleaf be accepted as a referee method pending official adoption.

(2) That the "single-color" method of Bendix be accepted as a routine method pending official adoption.

(3) That the Bendix and Greenleaf methods be submitted to further collaborative study to reconcile some of the difficulties encountered in this work prior to accepting as official A.O.A.C. procedures.

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No report was given on zinc, or on DDT in canned foods.

A contributed paper was presented, entitled "Spectrophotometric Method for the Estimation of Chlordane" by Bernard Davidow.

REPORT ON OILS, FATS, AND WAXES

By J. FITELSON (Food and Drug Administration, Federal Security Agency, New York 14, N. Y.), *Referee*

Reports on unsaponifiable matter and on peanut oil have been submitted. Difficulties in technique prevented collaborative work on the chromatographic purification of the unsaponifiable matter but current studies indicate that slight modification in this procedure may produce a satisfactory method. The collaborative results on the modified Bellier method for peanut oil demonstrate the usefulness of this test as a sorting procedure.

There will be no formal report on antioxidants, although a considerable

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* For report of Subcommittee C and action of the Association, see *This Journal*, 32, 55 (1949).

amount of preliminary work has been done. As a result of a questionnaire survey, it has been found that the antioxidants most commonly used in fats are nordihydroguaiaretic acid (N.D.G.A.), Propyl or Lauryl gallate, tocopherols and gum guaiac. Methods for the determination of N.D.G.A in fats are now being studied.

RECOMMENDATIONS*

It is recommended—

(1) That the Modified Bellier test **(31.47–31.48)** with the change suggested by the Associate Referee be adopted as official, first action, and that further collaborative studies be conducted.

(2) That the chromatographic purification of the unsaponifiable matter be further studied.

(3) That methods for the determination of antioxidants in fats be investigated.

REPORT ON PEANUT OIL

By G. KIRSTEN (Food and Drug Administration, Federal Security Agency, New York 14, N. Y.), *Associate Referee*

Five samples of oil were submitted to collaborators for the detection of peanut oil by the Modified Bellier Test. Collaborators were instructed to make tests in duplicate as directed in the A.O.A.C. *Methods of Analysis* 6th Ed., **31.48**, with the exception that the alcohol used in the preparation of the alcoholic potassium hydroxide **31.47(a)** need not be purified if the solution was to be used within a few days. Collaborators were also requested to repeat the tests, if time permitted, using a pipetted one ml sample in place of the weighed sample. The following technique was recommended for measuring the one ml sample of oil: "A short Mohr pipette with a fairly large opening at the tip should be used. Measure the oil by draining to the mark, holding until the meniscus in the pipette stops rising and again draining to the mark."

Collaborators who were requested to report turbidity temperatures and whether the test for peanut oil was positive or negative, obtained the following results:

All collaborators reported positive tests for peanut oil on Samples 1, 2, 3, and 4. Collaborator 2 reported Sample No. 5 as doubtful since his results coincided with the borderline temperature specified in the method. The others reported negative tests on Sample 5.

Sample No. 1 consisted of straight peanut oil; No. 2 of 10 per cent peanut oil in olive oil; No. 3 of 20 per cent peanut oil in soya oil; No. 4 of 15 per cent peanut oil in No. 5; No. 5 was a commercial oil labeled to contain 90 per cent corn oil and 10 per cent olive oil.

* For report of Subcommittee C and action of the Association, see *This Journal*, 32, 56 (1949).

TABLE 1.—*Weighed samples*
Turbidity Temperatures °C

COLLABORATOR	SAMPLE NO. 1	NO. 2	NO. 3	NO. 4	NO. 5
1	36.5	15	21.5	17	9
	37*	14.5*	20.5*	18.5*	9*
2	38	18	24	21	13
3	34.5	15.5	21.5	18.5	11.5
	35.0	15.5	21.5	18.5	11.5
4	36.9	16.0	24.2	20.2	10.5
	37.3	16.0	24.2	20.2	10.5
5	35.5	13.7	22.0	18.5	10.2
	35.8	13.8	22.2	18.7	10.3
6	36.4	15.5	23.0	19.5	10.2
	36.8	15.5	22.8	19.4	10.4
Average	36.5	15.6	22.4	19.3	10.8

* Probably best results according to collaborator.

The results reported, while showing considerable variation in some instances, are in good general agreement. The results on the measured 1 ml samples agree very closely with those on weighed samples for the same chemist, indicating that there is no advantage in the use of weighed samples.

In order to see whether the turbidity temperature could be used as a rough indication of the amount of peanut oil present in mixtures, a curve was prepared by plotting turbidity temperature against per cent peanut

TABLE 2.—*1 ml samples*
Turbidity temperatures °C

COLLABORATOR	SAMPLE NO. 1	NO. 2	NO. 3	NO. 4	NO. 5
2	38	18	24	22	13
3	35.0	15.5	22.0	19.0	11.5
	35.0	15.5	21.5	18.5	11.0
4	37.4	15.9	24.4	20.5	10.6
	37.3	16.0	24.6	20.8	10.5
6	37.0	15.5	22.6	19.8	10.2
	36.5	15.3	23.0	19.5	10.2

oil for mixtures of peanut oil with various proportions of corn, cottonseed and soya oils. Samples of corn and peanut oil containing from 0 to 100 per cent of peanut oil were prepared and the turbidity temperature for each sample determined. The same procedure was repeated with cottonseed and peanut oil, and with soya oil and peanut oil. The turbidity temperatures for the same concentration of peanut oil in the three different oils

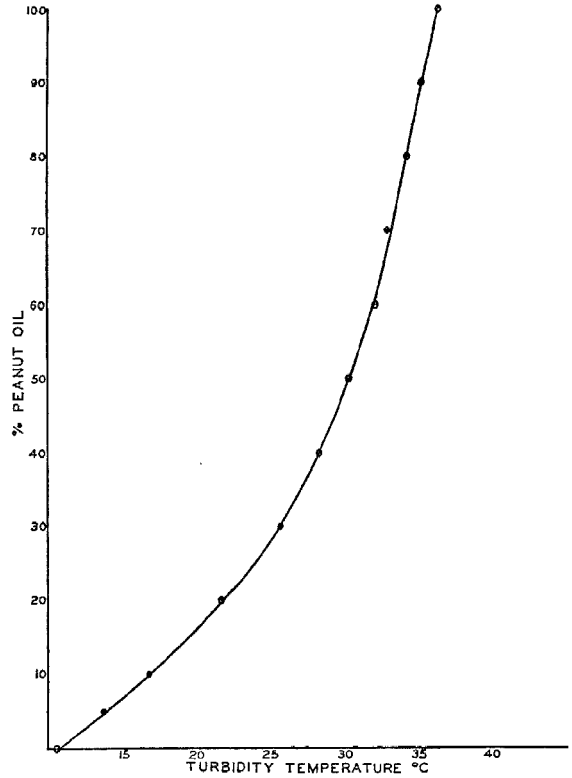

FIG. 1.—Average Turbidity Temperature—% Peanut Oil curve for mixtures of peanut oil with corn, cottonseed, and soya oils.

were averaged and the curve prepared by plotting the average turbidity temperature against per cent peanut oil.

Since the curve was prepared from average turbidity temperatures of different oils it can only be used to give a rough estimate of the amount of peanut oil present. However, since the turbidity temperatures for the mixtures containing the same concentration of peanut oil in the different oils did not vary more than the values obtained by different collaborators on the same sample, the preparation of separate curves did not appear to be justified.

Using the average curve and the average of turbidity temperatures reported by collaborators, the following results were obtained:

TABLE 3.—*Collaborative results*

SAMPLE	PEANUT OIL PRESENT	PEANUT OIL ESTIMATED FROM TURBIDITY TEMPERATURE
	per cent	*per cent*
1	100	100
2	10	7
3	20	22
4	15	15
5	0	0

While the individual turbidity temperatures do vary considerably, these results show that the test can be useful in obtaining an approximation of the amount of peanut oil present.

COLLABORATORS

The Associate Referee wishes to express his grateful appreciation to the following chemists who collaborated in this work:

A. B. Karasz, State of New York, Department of Agriculture and Markets.
George A. Michael, Department of Public Health, Boston, Mass.
H. P. Eiduson, Buffalo Station, Food and Drug Administration.
Frederick M. Garfield, St. Louis Station, Food and Drug Administration.
Sylvia Shendleman, New York Station, Food and Drug Administration.

RECOMMENDATIONS*

It is recommended—

(1) That the first sentence of **31.48**, page 508, *Methods of Analysis*, be changed to read: "Weigh 0.92 g, or measure 1 ml of the oil into 125 ml Erlenmeyer flask with standard taper outer joint. If the oil is measured, use a short Mohr pipet with fairly large opening at tip, drain to lower mark, hold until meniscus stops rising in pipet and drain to mark again. Add 5 ml of the alcoholic KOH soln and heat for 5 min on steam bath, using air condenser to avoid loss of alcohol."

(2) That the modified Bellier Test, **31.47–31.48**, *Methods of Analysis*, 6th Ed., be made official, first action.

(3) That further collaborative work be done on the method.

No report was given on antioxidants.

* For report of Subcommittee C and action of the Association, see *This Journal*, **32**, 56 (1949).

REPORT ON UNSAPONIFIABLE MATTER

By G. KIRSTEN (Food and Drug Administration, Federal Security Agency, New York 14, N. Y.), *Associate Referee*

Preliminary experiments showed the necessity for further study of the chromatographic purification of unsaponifiable matter before initiating collaborative work as recommended at the 1947 meeting.

The method using the chromatographic technique was published by Sylvester, Ainsworth, and Hughes[1] and consists essentially of the S.P.A.[2] extraction followed by purification of the unsaponifiable matter by passing the acidified ether extract through an alumina adsorption column to remove free fatty acids.

The publication reported good agreement between this method and the S.P.A. method. The Associate Referee has confirmed this agreement on several oils.

However, several technical details in this procedure required additional study. Thus, the specified amount of HCl was found to be insufficient in some instances, the method of drying the extract with anhydrous sodium sulfate is somewhat awkward, and some elution of the fatty acids occurs when ordinary reagent ether is used to wash the adsorption column, probably due to presence of alcohol. The specified quantity of HCl was found adequate if a preliminary water wash was used. Studies to improve the technic for drying the extract are continuing. No elution of fatty acids was observed if the column was washed with anhydrous ether or ether dried over calcium chloride.

Some preliminary results obtained using a sample of shark liver oil are given in the following table.

TABLE 1.—*Preliminary results*

METHOD	UNSAPONIFIABLE (CORRECTED FOR ACIDITY)		TITRATABLE ACIDITY (AS OLEIC ACID)	
	per cent	*per cent*	*per cent*	*per cent*
1. S.P.A.	2.54	2.52	0.08	0.08
2. Chromatograph-reagent ether	2.50	2.52	0.15	0.16
3. Chromatograph-$CaCl_2$ dried ether	2.51	2.67	0.06	0.06
4. Chromatograph-anhydrous ether	2.54	2.56	0.04	0.04

The chromatographic purification of unsaponifiable matter appears promising as a modification to shorten the S.P.A. method.

[1] *Analyst*, 70, 295 (1945).
[2] *Methods of Analysis*, A.O.A.C., 6th Ed., 31.40, p. 504.

It is recommended* that the chromatographic purification of unsaponifiable be further studied and be subjected to collaborative study.

REPORT ON SOILS AND LIMING MATERIALS

By W. H. MacIntire (The University of Tennessee Agricultural Experiment Station, Knoxville), *Referee*

Because of changes in personnel among the Associate Referees, the findings upon soils and liming materials have been chiefly those reported currently by the Associate Referee on Liming Materials in a recommendation relative to the "2-point" barium hydroxide-barium acetate titration (as in (9) of the 1947 report, *This Journal*, **31**, 43) and in a paper titled, "The Determination of the Sulfide Sulfur Content of Calcium Silicate Slags, in Relation to their Neutralization Value," as in (14) of the 1947 report, *Ibid*, **31**, 44. Communications from the other Associate Referees express interest and willingness to pursue the severally assigned objectives.

RECOMMENDATIONS†

It is recommended—

(1) That 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-dihydroxy naphthalene-3, 6-disulphonic acid, or "chromotrope-B" be studied as a suitable reagent for the determination of boron in soils.

(4) That further studies on *p*H in soils of arid and semi-arid regions be based upon soil systems of moisture content representative of an air-dry conditions.

(5) That the analytical technic previously proposed for ignition of charge and distillation of fluorine therefrom be studied collaboratively.

(6) That a study be made as to the adequacy of calcium hydroxide as a fixative for fluorine in soil charges of 1 to 1 proportion with calcination at 500°C. in 5 to 60-minute periods.

(7) That the direct distillation of unignited soil with sulfuric acid at 165°C, followed by distillation of an aliquot at 135°C, be studied collaboratively.

(8) That the "2-point" barium hydroxide-barium acetate titration procedure for the determination of exchangeable hydrogen in soils, as re-

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* For report of Subcommittee C and action of the Association, see *This Journal*, **32**, 56 (1949).
† For report of Subcommittee A and action of the Association, see *This Journal*, **32**, 46 (1949).

ported upon at this meeting, be studied further in relation to calcite equilibria in a variety of soils.

(9) That the survey and comparison of methods for the determination of phosphorus (a) that fraction in "available" state and (b) the proportions of organic-inorganic forms therein, be continued (*This Journal*, **30**, 43).

(10) That the survey and comparisons of methods for the determination of exchangeable potassium in soils be continued. (*This Journal*, **30**, 44).

(11) That the tentative procedures for neutralization value of calcium silicate slags, **3.11(a)** be annotated by the statement "without correction for sulphide content." This constitutes a clarification of (13), **31**, 44.

(12) That the procedure for the determination of sulfide sulfur content of calcium silicate slags as reported by the Associate Referee be adopted as tentative.

(13) That the Associate Refereeship on exchangeable calcium and magnesium be maintained.

REPORT ON EXCHANGEABLE HYDROGEN IN SOILS AND LIMING MATERIALS

By W. M. SHAW (University of Tennessee Agricultural Experiment Station, Knoxville, Tenn.), *Associate Referee*

In conformity with last years' recommendations, the Associate Referee made a study of the sulfide content in a large number of slags, and developed a method for its determination, as given in a paper submitted to the 1948 meeting of this Association.

The Associate Referee also made a study of titration procedures for exchangeable hydrogen in soils, utilizing ammonium acetate, calcium hydroxide, barium hydroxide and resultant values were compared with calcite decompositions in a number of soils under natural conditions of contact. The subject matter of this study is incorporated in a paper on Exchangeable Hydrogen of Soils presented to the 1948[1] meeting of the Association.

Upon basis of these two studies,

It is recommended*—

(1) That the procedure for sulfide sulfur in calcium silicate slags as given in the paper presented by the Associate Referee at the 1948[1] meeting, be adopted as tentative.

(2) That the "2-point" titration procedure for exchangeable hydrogen

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* For report of Subcommittee A and action of the Association, see *This Journal*, 32, 46 (1949).

[1] *This Journal*, **31**, 715 (1948).

in soils by means of barium hydroxide additions to 0.5 *M* barium-acetate soil suspensions as given in the paper presented by the Associate Referee be continued in relation to the soil-calcite equilibrium obtained under natural conditions of exposure.

REPORT ON EXCHANGEABLE POTASSIUM IN SOILS

By IVAN E. MILES (North Carolina Department of Agriculture, Raleigh, N. C.), *Associate Referee*

In continuation of the study of exchangeable potassium in soils, a further report is submitted this year, covering completed collaborative results to this date.

Detailed methods have been worked out in two laboratories for submission to each collaborator. Dr. Mehlich, of North Carolina State College, used a volumetric-ceric sulfate method, and Dr. Attoe and Prof. Truog of Wisconsin, a flame-photometric method. Collaborators were instructed to use any other preferred procedure, on condition that they use one of these methods as a basis of comparison.

The ceric-sulfate method is not new; and details of the Attoe-Truog method were published, under the title "Rapid Photometric Determination of Exchangeable Potassium," in *Soil Science Soc. Proceedings*, **11**, 221–226 (1947).

Fourteen samples were submitted to each collaborator. The even numbers were check samples on the odd numbers. For instance, Nos. 1 and 2 were duplicate samples. So were all the other subsequent pairs through No. 14. Samples 1 and 2 were Cecil soil coming from the Piedmont area of North Carolina. Sample 3 through 8 were Norfolk soil from the coastal plain area of North Carolina. Samples 3 and 4 were untreated, 5 and 6 had one increment of potash added, and 7 and 8 had two increments of potash added. Sample 9 through 14 were Portsmouth soil from the coastal plain area of North Carolina. Samples 9 and 10 were untreated, 11 and 12 had one unit of potash added, and 13 and 14 had two units of potash added. This made a range of potash content from very low to relatively high, and involved both the Kaolinitic or 1:1 type of colloid in the Norfolk soils and the Montmorillonitic or 2:1 type of colloid in the Portsmouth soils.

These soils were air dried and mixed thoroughly, boxed, and labeled merely as 1 through 14, and sent to each collaborator. The results are given in Table 1. As will be seen from the table, the results agree better than in last year's collaborative work. However, there is still much variation in the extremes. Procedure A seems to have some advantage over Procedure B, as far as accuracy is concerned and is recommended for further study.

TABLE 1.—*Collaborative results of exchangeable potassium in soils*
All data reported as m.e. of potassium per 100 grams of soil

SAMPLE NUMBER	PROCEDURE A					PROCEDURE B				PROCEDURE C	PROCEDURE D	PROCEDURE E	PROCEDURE F		
	COLLABORATORS					COLLABORATORS				COLLABORATOR	COLLABORATOR	COLLABORATOR	COLLABORATORS		
	I	II	III	VI	AVE.	II	V	VI	AVE.	II	I	IV	IV	VI	AVE.
1	0.958	0.890	0.950	0.878	0.919	0.890	0.936	0.985	0.937	0.920	0.931	0.904	0.895	0.920	0.908
2	.961	.870	.910	.894	.909	.880	.944	.960	.928	.925	.916	.870	.909	.910	.910
3	.091	.074	.090	.100	.089	.080		.090	.085	.095	.091	.110	.102	.090	.096
4	.095	.083	.080	.102	.090	.085	.094	.090	.090	.090	.092	.104	.100	.090	.095
5	.182	.170	.190	.194	.184	.172	.167	.177	.172	.190	.161	.182	.179	.179	.179
6	.186	.176	.190	.189	.185	.164	.163	.177	.168	.180	.156	.184	.180	.174	.177
7	.308	.300	.330	.298	.309	.302	.326	.298	.309	.290	.320	.289	.291	.312	.302
8	.300	.308	.330	.298	.309	.302	.325	.300	.309	.310	.307	.295	.298	.312	.305
9	.076	.064	.080	.080	.075	.070	.095	.077	.081	.075	.080	.095	.092	.078	.085
10	.063	.071	.080	.070	.071	.070	.093	.070	.078	.075	.079	.094	.090	.078	.084
11	.141	.148	.160	.141	.148	.150		.147	.149	.160	.145	.160	.148	.146	.147
12	.137	.143	.160	.145	.146	.140	.171	.151	.154	.150	.155	.166	.154	.147	.151
13	.251	.275	.280	.247	.263	.257	.273	.250	.260	.280	.235	.270	.257	.256	.257
14	.253	.267	.280	.247	.262	.250	.258	.253	.254	.270	.243	.258	.252	.264	.258

Procedure A—Volumetric-Potassium cobaltinitrite-Ceric sulfate procedure. Details of procedure prepared by Mehlich.
Procedure B—Flame Photometric. Details of procedure by Attoe and Truog. *Soil Sci. Soc. Proceedings*, 11, 221–226 (1947).
Procedure C—Flame Photometric-$BaCl_2$-triethanolamine. *Soil Sci.*, 66, 429–445 (1948).
Procedure D—Volumetric. Extractions of potassium *Soil Sci.*, 27, 123–136 (1939), and estimation of potassium, *This Journal*, 16, pp. 137–1940 (1933).
Procedure E—Volumetric—U.S.D.A. Circular 757, 1974.
Procedure F—Flame Photometric.—Extraction accomplished same as U.S.D.A. Circular 757, but amounts of potassium determined by use of Perkin-Elmer flame photometer.

The fine spirit of cooperation manifested on the part of collaborators and by the Association is appreciated.

COLLABORATORS

Brown, I. C., in Reitemeier's Laboratory, U.S.D.A.
Holmes, R. S., in Reitemeier's Laboratory, U.S.D.A.
Hallock, in Attoe and Troug's Laboratory, University of Wisconsin.
Martin, J. C., University of California.
Mehlich, A., North Carolina State College.
Sterges, A. J., in MacIntire's Laboratory, University of Tennessee.
York, E. T., Jr., in Peech's Laboratory, Cornell University, N. Y.

No reports were given on hydrogen-ion concentration of soils, boron and fluorine, zinc and copper, exchangeable calcium and magnesium, or phosphorus.

The contributed paper "A Volumetric Method for the Determination of Magnesium," by L. J. Hardin and W. H. MacIntire, was published in the preceding number, *This Journal*, page 139. Contributed paper entitled "The Determination of the Sulfide-sulfur Content of Calcium Silicate Slags in Relation to Their Neutralization Value," by W. M. Shaw, was published in *This Journal*, **31**, 715 (1948).

REPORT ON ECONOMIC POISONS

By J. J. T. GRAHAM, (Insecticide Division, Livestock Branch, Production and Marketing Administration, U. S. Department of Agriculture, Washington, D. C.), *Referee*

In planning the work on economic poisons for this year an effort was made to increase the number of projects to be studied. As a result of this effort the following chemists agreed to act as Associate Referees:

John W. Elmore, Bureau of Chemistry, State of California Department of Agriculture, Sacramento, California.

A. B. Heagy, Maryland Inspection and Regulatory Service, College Park, Maryland.

L. G. Keirstead, Agricultural Experiment Station, New Haven, Connecticut.

C. V. Bowen, E. E. Fleck, and S. A. Hall, Division of Insecticide Investigations, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, Beltsville, Maryland.

F. A. Spurr, Insecticide Division, Livestock Branch, Production and Marketing Administration, U. S. Department of Agriculture.

The Associate Referees have done a good job and their efforts are appreciated by the Association. Their reports speak for themselves and need no further comments or elaboration by the Referee.

In addition to the work of the Associate Referees, the Referee distributed two samples of pyrethrum powder to four collaborators for study of the Mercury Reduction method, the Seil method, and the Ripert

method. This work was in collaboration with the Consultative Committee on Insecticide Materials of Vegetable Origin, of the Imperial Institute, London. It was mentioned in the report to the Referee for 1947, and was undertaken in an attempt to obtain a uniform method for determination of pyrethrins, for world-wide use. The final results have not as yet been received, and a report for our Association on this work will be held over until next year.

Looking forward to next year, your Referee wishes to point out that there is an abundance of work that may be undertaken if enough chemists can be found who are willing to act as Associate Referees.

In the analysis of insecticides containing organic thiocyanates, the determination is usually based on a determination of nitrogen. It has been suggested that a more specific method should be studied.

Coal tar disinfectants have been used for a long time, and more recently the quaternary ammonium compounds have become very important in the disinfectant field. We have no official methods for analysis of these disinfectants and a study should be made of methods for their analysis as soon as possible.

The Referee has received a suggestion that a study be made of methods for determination of naphthalene in insecticides for control of lice on poultry; and of sabadilla alkaloids in preparations that contain ground sabadilla seed.

Other important economic poisons that offer an interesting field for study are aerosol insecticides, pyrethrins in presence of interfering substances, and products that contain chlordane, piperonyl butoxide, piperonyl cyclonene, or ferric dimethyl carbamate.

The Referee concurs in the recommendations of the Associate Referees as follows:

RECOMMENDATIONS*

It is recommended—

(1) That a study be made of the determination of rotenone in presence of other insecticidal or fungicidal ingredients or of diluents.

(2) That work be continued on the analysis of oil emulsions that are prepared with non-soap emulsifiers, giving consideration to the use of chromatography for determination of the oil.

(3) That the work on methods for analysis of "Antu" and "1080" be continued.

(4) That Methods No. 20 and No. 21 for determination of 2,4-Dichlorophenoxy acetic acid in herbicides be continued, giving consideration to the amount and manner of adding the indicator with a view to improving the end-point in the titration.

(5) That method No. 23 be further studied with emphasis placed upon a broader survey of ester mixtures and upon methods for breaking emulsions formed with this type of product.

* For report of Subcommittee A and action of the Association, see *This Journal*, 32, 44 (1949).

(6) That the study of methods for analysis of tetraethyl pyrophosphate be continued.

(7) That the methods for determination of DDT based on the total chlorine, which were adopted as tentative methods in 1947, be adopted as official methods, first action.

(8) That the work on benzene hexachloride be continued, placing emphasis on determination of the gamma isomer in various formulations.

(9) That study be started on organic thiocyanates and dimethyl dithio carbamates.

REPORT ON RODENTICIDES

By JOHN W. ELMORE (Bureau of Chemistry, State of California, Department of Agriculture, Sacramento 14, California), *Associate Referee*

During the past year more specific methods have been sought for determination of "1080," (sodium fluoroacetate) and "Antu" (alpha naphthylthiourea.)

"ANTU"

Gibb's phenol reagent (1) and Folins's reagent (2) were found to give colors with dilute solutions of antu but reproducible results suitable for quantitative determinations were not obtained.

The method for determination of antu in rat poisons, suggested in last year's report (3) would be more specific if some further purification of the acetone extract were possible. It was found that aqueous alkaline solutions or suspensions of antu could be obtained which could be filtered. Precipitation of the antu with cuprous chloride then served further to isolate the material from accompanying contamination. Occasionally rat poisons are encountered containing acetone soluble nitrogenous material other than antu and in such cases this procedure may be applied as follows:

Obtain an acetone extract of the rat poison equivalent to 0.2 g antu, and evaporate all but 20 ml of the acetone. Add 100 ml 7% KOH (10 ml 1:1 diluted with water to 100 ml) and dilute to 300 ml with water. Filter on a Büchner funnel if a precipitate occurs. Add a few drops of methyl orange to the filtrate and make slightly acid with hydrochloric acid. Add 50 ml cuprous chloride soln (10 g CuCl, 50 g NaCl, 10 ml HCl, dilute to 400 ml with water). Filter on a small Büchner funnel and wash with water. Transfer the filter pad and precipitate to a 500 ml Kjeldahl flask and determine total nitrogen as usual (4).

NOTE—In carrying out this process some preciptation of antu occurs on acidification prior to addition of cuprous chloride. This is incomplete however, and the cuprous chloride must be added to obtain complete recovery.

"Antu" solutions in 95% ethyl alcohol were examined by spectrophotometer. A dip in the curve at 2210 Angstroms was the most significant feature found.

Facilities for investigation of absorption in the infra red were not available.

"1080"

The use of lanthanum as a qualitative colorimetric reagent for detection of acetate ions (5) has been used for detection of fluoroacetate by Dr. John O. Hutchens, University of Chicago. (6) Various modifications of this method were investigated by the writer and a series of colors obtained in water solutions containing 0.015 per cent, 0.02 per cent, 0.03 per cent, and 0.05 per cent of "1080."

Since the test is subject to interference by acetic, lactic, propionic, citric, formic, and other similar acids it would not be applicable to determination of "1080" in rat baits without an initial isolation of the fluoroacetate.

Solutions of "1080" in water were examined by spectrophotometer for ultraviolet absorption but nothing of significance was found between 2000 and 3500 Angstroms.

"Antu"—Millimicrons against per cent transmission

Concentration: 0.01 mg. per ml. Solvent: Alcohol (95%)

mμ	PER CENT TRANS.	mμ	PERCENT TRANS.	mμ	PER CENT TRANS.	mμ	PER CENT TRANS.	mμ	PER CENT TRANS.
200		4	0.33	8	26.1	2	45.0	6	40.4
1		5	0.48	9	27.2	3	44.7	7	41.8
2		6	0.81	250	28.3	4	44.6	8	43.1
3		7	1.39	1	29.7	5	44.2	9	44.6
4		8	2.31	2	31.1	6	43.7	300	46.2
5		9	3.70	3	32.5	7	42.9	1	
6		230	5.28	4	33.9	8	42.1	2	49.3
7	5.5	1	7.13	5	35.3	9	41.3	3	
8	4.49	2	9.04	6	36.8	280	40.4	4	52.2
9	3.94	3	10.9	7	38.1	1	39.4	5	
210	3.19	4	12.7	8	39.3	2	38.7	6	55.1
1	2.53	5	14.3	9	40.5	3	38.2	7	
2	2.00	6	15.8	260	41.4	4	38.0	8	58.1
3	1.55	7	17.1	1	42.2	5	38.2	9	
4	1.15	8	18.1	2	43.2	6	38.3	310	61.4
5	0.85	9	19.1	3	44.1	7	38.3	1	
6	0.63	240	20.0	4	45.0	8	38.2	2	64.3
7	0.52	1	20.7	5	45.8	9	38.0	3	
8	0.38	2	21.4	6	46.1	290	38.0	4	67.1
9	0.30	3	22.1	7	46.3	1	38.0	5	
220	0.23	4	22.8	8	46.5	2	38.0	6	70.2
1	0.19	5	23.5	9	46.2	3	38.2	7	
2	0.22	6	24.2	270	45.8	4	38.5	8	73.0
3	0.24	7	25.2	1	45.3	5	39.3	9	
								320	76.0
								330	88.7

"1080"—Millimicrons against per cent transmission

Concentration: 2 mg. per ml. Solvent: Water

mμ	PER CENT TRANS.	mμ	PER CENT TRANS.	mμ	PER CENT TRANS.	mμ	PER CENT TRANS.	mμ	PER CENT TRANS.
200	54.3	4	93.0	8		2		6	97.0
1		5		9	97.6	3		7	
2	60.4	6	93.9	250	97.2	4		8	
3		7		1		5		9	
4	65.9	8	94.3	2		6	96.5	300	
5		9		3	97.1	7		1	97.2
6	71.2	230	95.2	4		8		2	
7		1		5		9		3	
8	75.8	2	96.0	6	96.7	280		4	
9		3	96.3	7		1	96.6	5	
210	79.9	4		8	96.6	2		6	97.3
1		5		9		3		7	
2	83.2	6	97.1	260		4		8	
3		7		1	96.1	5		9	
4	86.0	8		2		6	96.9	310	
5		9		3		7		1	97.4
6	88.0	240		4	96.1	8		2	
7		1	98.1	5		9		3	
8	89.6	2		6	96.0	290		4	
9		3		7		1	97.0	5	
220	90.9	4		8		2		6	97.5
1		5		9		3		7	
2	92.1	6	98.0	270		4		8	97.5
3		7		1	96.3	5		9	
								320	

RECOMMENDATIONS*

It is recommended that infra red absorption spectra of these compounds be examined for characteristics suitable for use in quantitative analysis.

REFERENCES

(1) *Official and Tentative Methods of Analysis*, 6th Ed., page 321, **22.50 (c).**
(2) SNELL, *Colorimetric Methods of Analysis*, page 183.
(3) ELMORE, J. W., *This Journal*, **31**, 366 (1948).
(4) *Official and Tentative Methods of Analysis*, 6th Ed., page 27, **2.25.**
(5) D. KRUGER and E. TSCHIRCH, *Chemical Abstracts*, Vol. 25, page 894.
(6) DR. JOHN O. HUTCHENS and BEATRICE M. KASS, Private Communication.

* For report of Subcommittee A and action of the Association, see *This Journal*, 32, 44 (1949).

REPORT ON DDT

By ELMER E. FLECK (Bureau of Entomology and Plant Quarantine, Beltsville, Md.), *Associate Referee*

It is recommended* that methods (1) through (6) as published in the Journal of the Association of Official Agricultural Chemists, **30,** 64–66 (1947) and amended, *ibid.*, **31, 73** (1948), be adopted as official, first action.

It is also recommended* that the method for determining DDT in emulsions as published, *ibid.*, **31, 72** (1948), be adopted as official, first action.

REPORT ON TETRAETHYL PYROPHOSPHATE ANALYSIS

By S. A. HALL (Department of Agriculture, Bureau of Entomology and Plant Quarantine, Division of Insecticide Investigations, Beltsville, Md.), *Associate Referee*

Tetraethyl pyrophosphate is a colorless, odorless, high-boiling, hygroscopic liquid of extraordinary toxicity. It is the principal biologically active ingredient of so-called hexaethyl tetraphosphate, which is a mix-

TABLE 1.—*Chemical assays for tetraethyl pyrophosphate*
Comparative results in various laboratories (indicated by number) and different methods (M, V, H, and W)

SAMPLE	PER CENT TETRAETHYL PYROPHOSPHATE										
	1		2	3			4	5	6	U.S.D.A.	AVERAGE
	M	V	H	H	M	W	M	M	M	H	
A	99.1	93.8	93.5	—	—	—	97.6	98.4	98.5	97.9	97.0
B	38.7	39.4	39.8	36	38	36.8	35.9	40.0	36.6	36	37.7
C	38.7	37.3	35.6	33	38	35.4	34.2	36.5	35.9	35	36.0
D	8.1	11.0	10.8	10	7	7.4	7.0	8.3	7.4	8	8.5
E	34.8	35.4	31.5	33	35	32.9	34.2	35.5	34.9	33	34.0
F	42.1	41.4	39.0	38	43[a]	39.9	43.8	42.9	41.0	40	41.1
G	36.4	36.7	40.1	34	33	35.0	36.8	36.0	36.1	36	36.0

[a] Selected result. Assays on this sample ran 51, 21, and 43 per cent.

ture (1). When first produced in this country on a commercial scale, soon after the war, batches of hexaethyl tetraphosphate contained about 8 to 20 per cent of tetraethyl pyrophosphate; the insecticidal potency varied accordingly. Industry has in recent months shifted to a higher grade product, containing 34 to 41 per cent of tetraethyl pyrophosphate, together with relatively inactive ethyl phosphates and polyphosphates.

* For report of Subcommittee A and action of the Association, see *This Journal*, **32,** 45 (1949).

Analysis for tetraethyl pyrophosphate in the products of commerce is based upon selective hydrolysis of the sample followed by separation of the tetraethyl pyrophosphate from acidic constituents. The separated ester is most readily determined by completely hydrolyzing it to diethyl phosphoric acid, which may be readily determined by titration with standard alkali (2). Four separate analytical methods—all of them based upon the principle just described—were recently developed and applied by several collaborators to six samples of commercial products and a sample of purified tetraethyl pyrophosphate which served as a standard (3). Concordant results were obtained, as shown in Table 1, by the four methods, M, V, H, and W, which differ only in technics of separation or hydrolysis.

METHODS

Method M—Dvornikoff and Morrill (4)

APPARATUS

Dropper weighing bottle (5-ml) with ground-glass joint. Two 60-ml separatory funnels, pear-shaped, with stem cut off about ⅜ inch below stopcock and ground at 45° angle. Erlenmeyer flask, 250 ml.

REAGENTS

(1) Solution containing 9 g of sodium chloride per 100 g.

(2) Benzene, nitration grade.

(3) Sodium hydroxide, 0.5 *N*, and hydrochloric acid, 0.5 *N*, accurately standardized.

(4) Bromothymol blue indicator soln, 0.1%, in water, solubilized as sodium salt.

PROCEDURE

1. Selective Hydrolysis

Place ca 3–5 ml of sample in weighing bottle, making the transfer with pipet or with the dropper itself. Avoid spilling material on ground-glass portion of the weighing bottle, and replace the dropper immediately to avoid moisture pick-up by the sample. Weigh the bottle and sample to the nearest milligram. Place 20 ml of 9% sodium chloride soln in a separatory funnel, marking this funnel "A" and the second funnel "B." Adjust the temperature of the salt soln to 30°C. by running warm or cold water over the surface of the funnel.

Add ca 1.0–1.5 g (for samples containing 50% of tetraethyl pyrophosphate or more) to 2.0–2.5 g (for samples containing less than 50% of tetraethyl pyrophosphate) of the sample to the salt soln in separatory funnel "A." Stopper the separatory funnel, shake until the soln is homogeneous, and let stand exactly 5 min. from the time the sample was added. Reweigh the weighing bottle to the nearest milligram to determine the exact sample weight.

2. Extraction

After five min. add 20 ml of benzene to the soln in separatory funnel "A" and shake vigorously for 30 seconds. Allow the layers to separate and draw off the aqueous (lower) layer of separatory funnel "B," taking care that none of the benzene enters the stopcock bore. Wash the benzene layer in "A" with 5 ml of 9% sodium

chloride by shaking for 10 seconds, separate the layers, and draw off the aqueous layer to funnel "B," this time allowing the benzene interface to pass just thru the stopcock bore.

Extract the combined aqueous layers in separatory funnel "B" with 10 ml of benzene for 10 seconds, separate the layers, and draw off and discard the aqueous (lower) layer, keeping the benzene interface above the stopcock bore. Wash the benzene in "B" with 5 ml of 9% sodium chloride by shaking for 10 seconds, separate the layers, and draw off and discard the aqueous layer, allowing the interface to pass just thru the stopcock bore. Drain the benzene layer in separatory funnel "B" into funnel "A."

Wash the combined benzene layers in funnel "A" with 20 ml of ice-cold, distilled water by shaking for 10 seconds. Separate the layers and drain the aqueous (lower) layer into a 250-ml Erlenmeyer flask, keeping the interface above the stopcock bore. Add 5–10 drops of bromothymol blue indicator soln and neutralize immediately with 0.5 *N* sodium hydroxide to the bromothymol blue end point, taking the first definite blue as the end point.[1]

3. Assay

Run the benzene layer in funnel "A" into the cold-water wash previously neutralized in the Erlenmeyer flask. Rinse the walls of funnel "B" with 5 ml of benzene, drain into funnel "A," use this benzene to rinse the walls of funnel "A," and drain into the Erlenmeyer flask.

Add to the benzene-water mixture in the Erlenmeyer flask ca twice as much 0.5 *N* sodium hydroxide as is required for the expected amount of tetraethyl pyrophosphate (use 27 ml per gram of tetraethyl pyrophosphate expected), and stir for 1 hour vigorously enough to obtain good mixing of the two phases. A simple golf-club-type stirrer will give ample agitation. If desired, however, a shaking machine can be used with a ground-glass stoppered flask. After 1 hour, titrate to the bromothymol blue end point[2] with 0.5 *N* hydrochloric acid, swirling the flask to mix the benzene and water layers. Record the net volume of 0.5 *N* sodium hydroxide consumed (not including the volume required for the neutralization of the water wash.)

4. Calculations

Tests with pure tetraethyl pyrophosphate indicate that 97.8 per cent of the tetraethyl pyrophosphate present is recovered in the analysis. Of the remaining 2.2 per cent, 1.3 per cent is lost by hydrolysis during the 5 minute standing period before extraction, and 0.9 per cent remains unextracted. This loss of 2.2 per cent is taken into account in the following calculation:

$$\frac{\text{Net ml of 0.5 } N \text{ NaOH} \times 7.255}{\text{Wt. of sample} \times 0.978} = \% \text{ tetraethyl pyrophosphate}$$

or simplifying,

$$\frac{\text{Net ml of 0.5 } N \text{ NaOH} \times 7.42}{\text{Wt. of sample}} = \% \text{ tetraethyl pyrophosphate}$$

[1] Any diethyl acid phosphate present in the benzene soln is extracted by the cold-water wash and then neutralized. The end point should be reached as rapidly as possible consistent with accuracy. Some tetraethyl pyrophosphate is also extracted, but its rate of hydrolysis in cold water is so slow that only a negligible amount is hydrolyzed and titrated. The unhydrolyzed tetraethyl pyrophosphate in the water layer is not lost in the assay, since the water and benzene layers are recombined for the assay.

[2] The benzene layer will occlude droplets of water. It is therefore necessary to let the layers separate completely after each addition of standard acid or base when close to the end point. Since the first definite blue is taken as the end point, more accurate results are obtained by overtitrating with 0.5 *N* hydrochloric acid to a definite yellow and then titrating back to the blue end point with 0.5 *N* sodium hydroxide. The end point is sharp and stable.

Method V—Wreath and Zickefoose (5)

Weigh accurately ca 2.5 g of the material to be tested into 75 ml of 1–3 acetone-water mixture, temp. 25°C., and allow to stand 15 min. At the end of this time pour the soln containing the sample into a 250-ml Erlenmeyer flask containing 50 g of resin (Amberlite IR-4B) that has been washed with two 50-ml portions of the 1–3 acetone-water. Rinse the original flask quickly with 25 ml of 1–3 acetone-water and pour into the flask containing the sample and resin. Agitate for 5 min. Allow to stand for several seconds until the resin has settled, and then decant the liquid thru a coarse filter paper into a 500-ml volumetric flask. Give the resin five 30-second washes with 50 ml of 1–3 acetone-water, allowing the resin to settle after each wash before decanting the liquid into the flask. After the washing of the resin is complete, dilute to 500 ml with water and mix thoroly. This extraction procedure should take slightly less than one-half hour.

Pipet a 100-ml aliquot of the sample into a 400-ml beaker, add 50 ml of 0.1 *N* sodium hydroxide, stir, and allow to stand 1 hour at 50°C. This period of time allows complete hydrolysis of all tetraethyl pyrophosphate. After one hour cool to room temp. and back-titrate with 0.1 *N* hydrochloric acid to *p*H 6.0, using the glass electrode.

Calculations

$$\frac{500 \times \text{Net titration 0.1 } N \text{ NaOH}}{\text{Sample wt.} \times 69} = \% \text{ tetraethyl pyrophosphate}$$

69.0 ml of 0.1 *N* sodium hydroxide is the calculated amount required for complete hydrolysis of 1 g of pure tetraethyl pyrophosphate.

This method has the following advantages:

1. The resin is an efficient agent in the separation of the acidic materials from tetraethyl pyrophosphate.
2. All extractions are run at room temperature. No ice water is required.
3. As solutions used are miscible with water in all proportions, no agitation is required in the hydrolysis stage of the procedure.
4. No factor is involved in the calculations, because tests show that less than 1.0% of the tetraethyl pyrophosphate present is hydrolyzed during the extraction.[3]

Method H—Hall and Jacobson (2)

With a small weighing buret weigh 2 g of sample to the nearest milligram into a dry 125-ml Erlenmeyer flask; stopper the flask. Remove the stopper to add ca 30 g of crushed ice. At this moment take note of the time or turn on an interval timer set for 60 min. Immediately after introducing the ice, drop in a strip of Congo red paper and, while swirling the flask, add rapidly from a buret or large medicine dropper a saturated soln of sodium bicarbonate. Place the flask in the refrigerator. Remove at the end of the 60-min. hydrolysis period and add at once 20 ml of chloroform. Mix by swirling the flask and then transfer the contents thru a funnel (to catch small ice crystals) into a 125-ml pear-shaped separatory funnel. Use ca 10 ml of chloroform to effect a quantitative transfer and to wash the ice crystals caught

[3] The authors of Method V have recently modified their method by using a column of the acid absorbing resin. By suitable staggering of the samples in the improved method it is claimed that ten determinations may be run in 3½ hours.

in the funnel. When the lower chloroform layer becomes clear, in about 30 seconds, draw it off into a 250-ml Erlenmeyer flask. Do not allow any of the acidic aqueous layer to get to the bore of the stopcock. Then quickly extract (using only moderate shaking) the aqueous portion successively with two 10-ml portions of chloroform. Add two glass beads to the flask containing the combined extracts, place on the steam bath, and distill off the chloroform. Add 10 ml of ethyl alcohol, washing down the sides of the flask during the addition. Then insert a short glass tube (attached to a water aspirator or other source of vacuum) about half way into the flask to effect turbulence of the vapors while continuing the heating for about a minute longer. Remove the flask from the steam bath, add ca 100 ml of warm distilled water, washing down the sides of the flask. Cover the neck of the flask with a small inverted beaker and place in an oven at 50°C. (±2°) overnight (17 hours). In the morning titrate the accumulated samples with standard sodium hydroxide solution (0.1 to 0.15 *N*) using phenolphthalein indicator. No error is introduced if the samples are left in the 50°-oven for more than 17 hours.

Two moles of diethyl orthophosphoric acid (a strong monobasic acid) are formed for every mole of tetraethyl pyrophosphate, which has a molecular weight of 290.2. Per cent tetraethyl pyrophosphate may therefore be expressed as follows:

$$\frac{\text{Ml of NaOH} \times \frac{1}{2}N \times 290.2 \times 1.036 \text{ (correction factor)} \times 100}{\text{Wt. of sample in mg.}}$$

The correction factor 1.036 compensates for a loss of 2.0% in the selective hydrolysis and a further loss of 1.5% in the chloroform extraction of the aqueous solution of tetraethyl pyrophosphate. The equation reduces to:

% Tetraethyl pyrophosphate = Ml of NaOH × N × 15.04/Wt. of sample in grams

NOTE: The results shown in Table 1 by the use of Method H are based upon its earlier and less refined form. As previously described above, the method has given results as follows at the Beltsville laboratory:

Sample	*% Tetraethyl Pyrophosphate*
A	97.9
B	36.3
C	35.0
D	7.4
E	33.9
F	40.2
G	36.4

Method W—Olson and Williams (6)

REAGENTS

Chloroform, technical grade; methyl orange indicator, modified with xylene cyanole F F; sodium hydroxide, 0.1 *N*.

APPARATUS

The apparatus listed below is in the most part standard laboratory equipment. For ease in handling, flasks and condensers with standard-taper ground joints are used.

Burette, 50 ml; condenser, Liebig; dropping bottle with ground-glass joint,

30-ml capacity, Erlenmeyer flask, 250 ml; glass beads; graduate, 100 ml; heater, Precision electric; separatory funnel, 250 ml with glass stopper, pear-shaped; Variac, type 200-C.

PROCEDURE

Weigh the dropping bottle after it has been filled about half full with tetraethyl pyrophosphate. With the dropper place 1.5–2 g of the sample in the Erlenmeyer flask, and reweigh the dropping bottle and contents. Record the weight to the nearest milligram. The difference between the initial and final weights is the weight of sample taken.

Add 20 ml of iced distilled water (0 to 5° C.) to the flask (note the time at which the water was added), swirl 5–10 seconds to insure soln, add 4–5 drops of the modified methyl orange indicator, and titrate immediately with 0.1 *N* sodium hydroxide. The first definite green color is taken as the end point. The amount of this titration is not important and need not be recorded. The color will probably change back to the red fairly quickly. However, no attempt should be made to obtain a permanent green color.

Allow the neutralized soln to stand 15 min. at room temp., counting time from the addition of the ice water. During this time clean the separatory funnel thoroly and grease the stopcock with stopcock grease. To prevent water from coming in contact with this stopcock grease any more than is necessary, pour 20 ml of chloroform into the separatory funnel.

After the soln has stood for 15 min., pour it into the separatory funnel on top of the chloroform. Rinse the flask three times using ca 5-ml portions of water, and add these rinsings to the soln in the funnel. Place the wet flask beneath the funnel.

Stopper the funnel and agitate its contents vigorously for 30 seconds. Release any pressure build-up by inverting the funnel and opening the stopcock carefully. Replace the funnel in its rack and allow the chloroform layer to settle. When two distinct layers have formed, draw off the lower layer into the Erlenmeyer flask, closing the stopcock when all but 1 or 2 ml of the chloroform layer has been withdrawn. Repeat the extraction procedure with three 15-ml portions of chloroform, leaving 1 or 2 ml of chloroform in the funnel each time when withdrawing the lower layer. Combine all the chloroform layers in the Erlenmeyer flask. After the last extraction discard the soln left in the separatory funnel.

Add 100 ml of distilled water to the chloroform extract in the flask and drop in 3 or 4 glass beads to prevent bumping. Place the flask on the electric heater and boil off the chloroform. When all the chloroform has evaporated, as evidenced by the presence of steam at the mouth of the flask, lower the condenser and reflux the solution for 2 hours. Adjust the Variac, controlling the heater voltage to about 80 volts so that only a mild boiling is obtained.

After this 2-hour reflux, rinse the condenser with about 20 ml of water and remove the flask. Cool the flask to room temp. by immersing it in an ice bath or by holding it under the cold-water tap. Add 4 or 5 drops of modified methyl orange indicator and titrate with 0.1 *N* sodium hydroxide to a definite green color.

Calculations

Calculate the tetraethyl pyrophosphate content from the following equation:

$$\frac{\text{Ml NaOH} \times N \text{ NaOH} \times 0.145 \times 1.025 \times 100}{\text{Wt. of sample in g}} = \% \text{ tetraethyl pyrophosphate}$$

or

$$\frac{\text{Ml NaOH} \times N \text{ NaOH} \times 14.86}{\text{Wt. of sample in g}} = \% \text{ tetraethyl pyrophosphate}$$

SUMMARY

Four methods for the determination of tetraethyl pyrophosphate in the products of commerce have been described. All methods are based upon the selective hydrolysis and separation of the tetraethyl pyrophosphate, which is then completely hydrolyzed to diethyl phosphoric acid and determined by titration.

The Associate Referee recommends* that studies to compare the different methods be continued so that the best procedure or modifications may be embodied in a tentative official method for collaborative testing.

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REPORT ON 2,4-D HERBICIDES

By A. B. HEAGY (Maryland Inspection Service, Univ. of Maryland, College Park, Md.), *Associate Referee*

HISTORY

New methods for the estimation of 2,4-Dichlorophenoxy acetic acid, its salts, esters, and amides were considered desirable, in view of objections to the total chlorine methods previously in use. Among these objections are included inaccuracies arising from the presence of chlorides or other chlorine compounds, and the need for water-free samples when the sodium-alcohol method is used. The total chlorine procedure published in Soap & Sanitary Chemicals in October 1947 is open to the same objection that the presence of other chlorine materials will vitiate the results. Furthermore, any titration procedure must provide for elimination of all other organic acids to avoid high results.

Macro methods for the titration of 2,4-D and its compounds were first developed for use in the Bureau of Chemistry, Sacramento, California, and published in *Analytical Chemistry*, **19**, page 179, July 1947. In effect, the procedure and its modifications consist in titration of the acid groups when in the form of the free acid. In 2,4-D derivatives the samples are treated to produce the acid before titrating.

At the 1947 meeting the Association recommended that a study of these methods be made for the purpose of obtaining suitable procedures for the analysis of 2,4-D weed killers to be included in the chapter on Insecticides

* For report of Subcommittee A and action of the Association, see *This Journal*, 32, 45 (1949).

and Fungicides, in A.O.A.C. *Methods of Analysis*. Copies of the procedures studied have been distributed for reference. It was also requested that collaborators examine the materials by the Parr Bomb method for the purpose of comparing results.

TABLE 1.—*Sample No. 1, Method No. 20*

COLLABORATOR	RESULTS	OTHER METHODS
	FEDERAL AND STATE CONTROL LABORATORIES	
	per cent	*per cent*
A	50.78	
B	51.12	
C	50.69	37.58[1,5]
D	51.58	46.13[5]
E	51.59	45.79[5]
F	52.38	
G	50.60	
H	50.13	
I	50.99[3]	50.59[5]
I	50.49[4]	50.76[2]
J	50.11[3]	50.70[6]
J	48.07[4]	
K	50.49	50.37[6] 50.70[5]
L	53.90	
M	49.99	
N	47.95	
Average	50.68	48.78[5]
	INDUSTRY LABORATORIES	
	per cent	
A	50.81	
B	50.92	
C	50.52	
D	50.28	
E	50.34	
F	50.27[3]	
F	49.83[4]	
G	50.25	49.88[5]
Average	50.40	
General Average	50.59	48.97[5]
High	53.90	
Low	47.95	

[1] Not included in average.
[2] Combustion Chamber Method.
[3] Method #20 part 1.
[4] Method #20 part 2.
[5] Parr Bomb Method.
[6] Corrected for inorganic chlorides.

TABLE 2.—*Sample No. 2, Method No. 21*

COLLABORATOR	RESULT	OTHER METHODS
FEDERAL AND STATE CONTROL LABORATORIES		
	per cent	*per cent*
A	91.81	
B	90.43	
C	91.44	91.69[1]
D	91.11	90.95[1]
E	91.04	91.12[1]
F	95.08[2]	
G	93.50	
H	9.08[2]	
I	91.85	91.58[1]
J	90.28	
K	91.68	91.69[3] 92.65[1]
L	96.70[2]	
M	91.36	
N	90.08	
Average	91.33	91.60[1]
INDUSTRY LABORATORIES		
	per cent	
A	92.34	
B	—	
C	93.63	
D	92.29	
E	91.77	
F	96.44[2]	
G	91.38	91.67[1]
Average	92.28	
General Average	91.62	91.63[1]
High	96.70	
Low	9.08	

[1] Parr Bomb method.
[2] Not included in average.
[3] Corrected for inorganic chlorides.

The Associate Referee in pursuing this work sent out to collaborators three samples of materials to be examined by these methods. Sample No. 1 was a 50% 2,4-D acid mixed with 50% talc, supplied by the J. T. Baker Chemical Company; No. 2 was a sodium salt of 2,4-D purchased from the consumer market; No. 3 was an isopropyl ester of 2,4-D supplied by the duPont Company.

Included in the list of collaborators are eight industry and sixteen federal and state laboratories. Most laboratories completed the work;

TABLE 3.—*Sample No. 3, Method No. 23*

COLLABORATOR	RESULT	OTHER METHODS
	FEDERAL AND STATE CONTROL LABORATORIES	
	per cent	*per cent*
A	19.66[1]	
B	48.04	
C	44.45	48.84[2]
D	45.87	49.63[2]
E	45.76	48.66[2]
F	45.60	
G	50.16	
H	1.34[1]	
I	51.02	51.16[2]
I	51.60	51.76[3]
J	—	
K	48.91	50.05[2]
L	45.30	
M	25.24[1]	
N	46.56	
Average	47.57	49.67[2]
	INDUSTRY LABORATORIES	
A	47.27[4]	
A	47.45[5]	
B	—	
C	48.65	
D	47.65	
E	46.44	50.77[5]
E	—	51.33[6]
F	42.65[1]	
G	48.94	51.77[2]
Average	47.73	51.27[2]
General Average	47.63	50.13[2]
High	51.60	
Low	1.34	

[1] Not included in average.
[2] Parr Bomb Method.
[3] Combustion Furnace Method.
[4] Emulsions Present.
Emulsions Avoided.
[5] Experimental Laboratory Method.

a few, however, examined only one or two of the samples.

Tables of results have been distributed from which a comparative picture of these methods can be drawn.

TABLE 4.—*2,4-Dichlorophenoxy acetic acid*
Commercial samples—Analyzed in Maryland Inspection Laboratory

SAMPLE NO.	GUARANTEE	TITRATION NO. 20 (2)	PARR BOMB
	2,4-DICHLOROPHENOXY ACETIC ACID		
	per cent	*per cent*	*per cent*
1	9.60	10.20	11.00
2	10.00	9.12	9.42
3	50.00	51.67	49.74
4	70.00	78.72	78.77
	SALTS OF 2,4-DICHLOROPHENOXY ACETIC ACID		
		Titration No. 21	
1	14.00	14.58	16.71
2	14.00	14.98	15.06
3	14.00	15.02	15.40
4	77.00	81.42	75.90
5	20.00	21.16	21.73
6	19.80	21.18	21.57
7	2.00*	3.80	4.52

* Ethyl ester of 2,4-D (powder form).

A digest of the collaborators' comments is included to focus attention on those points of the methods requiring special care.

DISCUSSION

Results on samples No. 1 and No. 2 show a sufficient degree of consistency to prove the value of the procedures. Unquestionably, more familiarity with the manipulations involved would make for still closer agreement.

Sample No. 3 gave rise to the formation of very persistent emulsions, and caused general difficulty in applying the procedure. It was expected that results would vary widely. Most of the collaborators expressed the opinion that the method was unsatisfactory. Further investigation seems to be indicated here.

Results on all samples reported by the Parr Bomb method were somewhat erratic and not uniformly high, as expected. The inference can be drawn that incomplete combustion was an influencing factor.

Whether or not the end point in the titration is subject to further refinement is still to be learned. Since five collaborators reported it to be unsatisfactory it is possible that an optimum amount of the thymolphthalein indicator will effect an improvement. Further study will be recommended in this regard.

COLLABORATOR COMMENTS
Methods No. 20 & No. 21

COLLABORATOR	AMT. OF INDICATOR	END POINT	NO. OF WASHINGS
FEDERAL AND STATE CONTROL LABORATORIES			
A	0.5–1.0 ml	Unsatisfactory	3
B	5 drops 1%	Satisfactory	5
C	5–7 drops	Satisfactory	3
D	1–2 ml 1%	Unsatisfactory	3–5
E	1–2 ml 1%	Unsatisfactory	3–5
F	0.5 ml	Satisfactory	4
G	1 ml .05%	Satisfactory	3
H	10 drops	Satisfactory	4
I	8 drops	Satisfactory	3–4
J	5 drops	Satisfactory	3–4
K	10 drops	Unsatisfactory	3–4
L	1–2 ml	Satisfactory	3
M	5 drops	Satisfactory	3
N	4 drops	Satisfactory	10
INDUSTRY LABORATORIES			
A	12 drops	Satisfactory	3
B	1 ml .05%	Satisfactory	3
C	18 drops 0.2%	Unsatisfactory	3
D	4 drops	Satisfactory	3
E	6 drops 1%	Unsatisfactory	3
F	20 drops	Satisfactory	3 (30 ml. ea.)
G	15 drops 0.1%	Unsatisfactory	5

It is recommended*—

(1) That methods No. 20 and No. 21 be subjected to another year's study, with indicator added in 3 different quantities with the purpose of improving and standardizing the end point.

(2) That method No. 23 be subjected to another year's study with emphasis placed upon a broader survey of ester mixtures and upon methods of breaking the emulsions formed with this type of product.

LIST OF COLLABORATORS

INDUSTRY

J. M. F. Leaper, Chief Chemist, American Chemical Paint Company.
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A. W. Beshgetoor, Director, Main Lab., The Dow Chemical Company.
J. F. Garrett, Process Section, E. I. duPont de Nemours & Company.
W. R. Flach, Chief Chemist, Eastern States Farmers' Exchange.
J. W. Zabor, Director of Development, Pittsburgh Coke & Chemical Company.
R. W. Towne, Development Dept., Monsanto Chemical Company.

* For report of Subcommittee A and action of the Association, see *This Journal* 32, 44 (1949).

FEDERAL AND STATE OFFICIALS

Clemens Olsen, Arizona, Inspection Laboratory.
Herbert A. Rooney, California, Bureau of Chem. Dept. of Agriculture.
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E. R. Winterle, Florida, Chemical Div., Agricultural Dept.
Giichi Fujimoto, Territory of Hawaii, Comms. of Agr. and Forestry.
A. C. Keith, Kansas, Control Div., Board of Agriculture.
John E. Schueler, Maryland, Inspection & Regulatory Service.
Albert B. Heagy, Maryland, Inspection & Regulatory Service.
Percy O'Meara, Michigan, Department of Agriculture.
Albert L. Weber, New York, Food & Drug Administration.
Howard Hammond, North Dakota, State Laboratories Dept.
T. L. Ogier, Texas, Agricultural Experiment Station.
Miss Edith Lawrence, Washington, Agricultural Experiment Station.
Mrs. Edith Huey, Washington, Agricultural Experiment Station.

Methods for the Collaborative Study of 2,4-D

Tentative No. 20

Determination of 2,4-Dichlorophenoxyacetic Acid

(1) Transfer 1 g of 2,4-D acid to 250 ml Erlenmeyer flask. Dissolve in 75 ml neutral ethyl alcohol and titrate with 0.1 *N* sodium hydroxide, using thymolphthalein as an indicator. Calculate percentage of 2,4-D on basis that 1 ml of 0.1 *N* NaOH is equivalent to 0.0221 g of acid.

(2) If sample contains insoluble carriers, transfer a sample equivalent to 1 g of 2,4-D acid to 250 ml beaker, add 25 ml of 1 *N* sodium hydroxide, and 50 ml of water. Stir to dissolve the 2,4-D. Filter and wash any insoluble matter that may be present and transfer to 250 ml separatory funnel. Proceed as directed in tentative method No. 21, par. 1, beginning "Neutralize with 10% sulphuric acid."

Tentative No. 21

Determination of Salts of 2,4-Dichlorophenoxyacetic Acid

(1) Dissolve sample equivalent to ca 1 g of 2,4-D acid in 50 ml of water (filter and wash samples containing insoluble carriers), transfer to 250 ml separatory funnel, neutralize with 10% sulfuric acid, and add 10 ml excess acid.

(2) Extract aqueous phase twice with 75 ml portions of ether. Wash combined ether extracts free from mineral acid with 10 ml portions of water until washings remain alkaline with addition of one drop of 0.1 *N* sodium hydroxide and phenolphthalein (2–3 washings).

(3) Transfer ether soln to 400 ml beaker, rinsing separatory funnel with ether. Add 25 ml of water, a few boiling chips, and evaporate ether layer on steam bath.

(4) Dissolve the aqueous mixture in 100 ml of neutral ethyl alcohol and titrate with 0.1 *N* sodium hydroxide, using thymolphthalein as an indicator.

(5) Each ml of 0.1 *N* sodium hydroxide is equivalent to 0.0221 g of dichlorophenoxyacetic acid.

Tentative No. 23

Determination of Esters of 2,4-Dichlorophenoxyacetic Acid in Presence of Soap, Acids, Alcohols and Oils

(1) Reflux sample of weight equivalent to 0.7 g of the ester with ca 1 g of potassium hydroxide and 90 ml of 95% ethyl alcohol for one hour in a 250 ml S/T

Erlenmeyer flask. Transfer contents of flask to 250 ml beaker, add 50 ml of water, and evaporate on steam bath to about 50 ml to remove the alcohol. Transfer aqueous soln to 250 ml separatory funnel and extract with 75 ml of petroleum ether to remove unsaponifiable oils.

(2) Draw off aqueous phase into a 200 ml volumetric flask, add a few drops of 1% phenolphthalein soln and a few drops of 1 to 1 soln of hydrochloric acid, to disappearance of the pink color, and then add 1 to 1 ammonium hydroxide soln until slightly alkaline. Add sufficient water to give volume of about 150 ml. Add slowly sufficient 10% barium chloride soln to precipitate the fatty acids, make to volume, shake, and filter. The soln must be alkaline after the addition of barium chloride; otherwise the 2,4-D will precipitate.

(3) Transfer 100 ml aliquot in 250 ml separatory funnel, and acidify with hydrochloric acid. Proceed as in tentative method No. 21, beginning "Extract the aqueous phase twice."

Total Chlorine by Parr Bomb Method

Mix thoroly 14 g of powdered sodium peroxide and 0.75 g of powdered potassium nitrate in dry fusion cup, then mix in 0.4 g of finely-powdered cane sugar, and finally 0.2–0.25 g of sample, also in the form of a fine powder. If a larger amount of sample is used decrease amount of sugar in accordance with the content of carbon. Finely-powdered benzoic acid may be used in place of the cane sugar. Thoro mixing is essential. Take care to avoid spontaneous ignition when the organic matter comes in contact with sodium peroxide. Assemble the bomb and ignite. Cool bomb, open, and note whether there has been complete combustion. Rinse cover with distilled water into 400 ml beaker, set the fusion cup on its side, cover, and add enough water to cover about two-thirds of cup. Gentle heating on steam bath may be necessary to start decomposition reaction. After complete decomposition, remove cup and rinse, and heat the soln for few minutes to insure complete removal of all peroxide. Acidify with conc. nitric acid, boil for several minutes, cool, and precipitate with a measured excess of 0.1 *N* silver nitrate. Cool again, filter, and wash. Determine excess silver nitrate by titration with standard thiocyanate. Calculate percentage of chlorine in sample, and convert to the 2,4-D compound present, according to one of factors given below.

NOTES: (1) An alternate, and more rapid titrating procedure, used with complete success in this laboratory, consists in adding the excess silver nitrate, stirring to induce flocculation, then adding 5 ml of nitrobenzene, and stirring until all silver chloride particles are coated with the nitrobenzene (vigorous stirring is necessary). Titration with the thiocyanate can be done immediately, with no filtering.

(2) To avoid violent spontaneous ignition when liquid samples are analyzed in this manner it is advisable to use a weighing bulb. A light, round glass bulb, 1–1.5 cm in diameter, and having a short capillary neck is weighed, charged with sample by alternate warming and cooling, carefully wiped, sealed in a flame, weighed, and imbedded in the fusion mixture. Other operations are the same as above.

FACTORS:

Cl→2,4-D (acid) = 3.1172
Cl→Sodium Salt of 2,4-D = 3.4268
Cl→Isopropyl ester of 2,4-D = 3.7086

OTHER COLLABORATOR COMMENTS

Dow Chemical Company:

Sample No. 1 was first analyzed according to the prescribed procedure, *i.e.*, using three washes. Results were as follows: 50.96, 50.86, 50.95, and 50.90, for an average of 50.92. Then, it was analyzed by the procedure using six washes giving the

following results of 50.59 and 50.50. The water washes were titrated with 0.1 *N* NaOH giving the following titration: (1) 0.49 ml, (2) 0.11 ml, and the next four 0.10 ml. This indicated that after the third wash there was no reduction in the amount of NaOH to titrate the acid in the water washes.

Using a slight excess of mineral acid, (.5 ml sulfuric acid). Three washes were used and the 2,4-D acid found was 50.48%. The washes titrated as follows: first, 0.10 ml, and the second and third washes, 0.09 ml.

Conclusion: A solubility factor is involved and the method is quite empirical. Therefore, the number of washes should be closely standardized. The difference between the figures using 3 and 6 washes become serious in good manufacturing practice. A standard correction might be advantageous.

In reply to the question regarding the practicability of methods No. 20 and No. 21, 15 cooperators reported these procedures satisfactory with more experience by the operators. Other comments were as follows:

(1) Ether extracts should not be combined until after washing.
(2) Use of filter paper may cause the difficulty in getting check results.
(3) Difficulty encountered in washing sample off the filter paper.
(4) In part (2) solution heated 50–60°C. to facilitate solution, and advocated the use of phenolphathalein.

Method No. 23

Three cooperators found the method satisfactory, partially because they had previous experience with it, but had not encountered trouble with emulsions before. The remainder declared the procedure unsatisfactory because of the difficulty in breaking emulsions that were formed. Comments from collaborators were as follows:

(1) Suggested addition of ethanol took 65 hours to break emulsions.
(2) Used modified method under investigation in experimental laboratory.
(3) No method found to break emulsions.
(4) Method unreliable, questioned possibility of 2,4-D being held by Ba salt.
(5) Added 10 ml isoamyl alcohol to first extraction.
(6) One day to a week required to break emulsions.
(7) Emulsions centrifuged.

The following suggestions for breaking and preventing emulsions were submitted:

(1) Transfer the alcoholic solution after saponification and cooling to a 250 ml separatory funnel with 80 ml of water and extract with 75 ml of petroleum ether. Draw off the alcohol-water phase into another 250 ml separatory funnel, extract with another 75 ml portion of petroleum ether. Draw off the alcohol-water phase, wash the petroleum ether a few times with 10 ml portions of water, add the washings to the alcohol-water solution and evaporate down to about 50 ml on a steam bath. Make the residue to about 100 ml with water, cool the solution, and transfer to a 200 ml volumetric flask. After making to 150 ml with water, follow the remainder of Method 23 (2) in the precipitation of fatty acids.

(2) After refluxing sample for one hour with potassium hydroxide, transfer the contents to a 600 ml beaker. Add 50 ml of water and evaporate to 25 ml, swirl to aid particles to redissolve. Add 25 ml of water and evaporate to 25 ml. Transfer the warm solution to a separatory funnel with the aid of warm water. Add 75 ml of petroleum ether. Shake well for one minute. Let stand for several minutes. Draw off the bottom yellowish layer leaving the ether and emulsions in the funnel. Wash the contents of the funnel with successive 10 ml portions of a 10% salt solution, each time making sure that the emulsion remains in the funnel. After the third washing the

emulsion will be completely broken. The combined salt washings are collected separately from the yellow layer first drawn off. Treat the first layer drawn off which will contain practically all the fatty acids with mineral acid and proceed with the barium chloride precipitation as described in Method No. 23. Treat the salt washings in identical fashion. Combine the two solutions, make to 200 ml. Using filter cell collect 100 ml of clear filtrate. Proceed from this point as described in Method No. 21.

REPORT ON INSECTICIDES CONTAINING DERRIS OR CUBÉ (ROTENONE DETERMINATION)

By F. A. SPURR (Insecticide Division, Livestock Branch, Production and Marketing Administration, U. S. Department of Agriculture, Washington, D. C.), *Associate Referee*

In the collaborative work that preceded the adoption of the present official method for rotenone determination, only the application of the method to samples of powdered derris and cubé roots was studied. In practice, however, the method has been adapted for use with products consisting of derris or cubé root powder mixed with other insecticidal and fungicidal ingredients and diluents.

No difficulty has been experienced in applying a modification of the official method to products in which, in addition to the derris or cubé root powder, the usual diluents were the only other ingredients.

Sulfur interferes in the determination of rotenone by the official procedure, but it has been found that a modification of the procedure by which the rotenone and resins are separated from the sulfur by means of acetone, has given satisfactory results.

Other substances, such as organic thiocyanates, oils, and various wetting or spreading agents, have interfered to such an extent that reliable results could not be obtained.

This year it was our intention to study the extent of the interference caused by several of the ingredients that are frequently incorporated in rotenone-bearing insecticides, with a view to determining the modifications to the official method that might be necessary to overcome such interferences. However, because the of large amount of extra work that was required of the personnel of the Insecticide Division in connection with the registration of products under the new Federal Insecticide, Fungicide, and Rodenticide Act, we were unable to conduct any study of methods for rotenone determination.

RECOMMENDATIONS*

It is recommended that a study be made of the determination of rotenone in presence of other insecticidal or fungicidal ingredients and diluents.

* For report of Subcommittee A and action of the Association, see *This Journal*, 32, 44 (1949).

REPORT ON OIL EMULSIONS

By L. G. KEIRSTEAD (Agricultural Experiment Station, New Haven, Conn.), *Associate Referee*

The simplicity and proven usefulness of chromatography for the separation of organic materials led to an investigation of its possibilities for the quantitative estimation of petroleum oil in self-emulsifying oil mixes. The following technique seems to have merit:

REAGENTS

Benzene
Petroleum ether
Sodium sulphate (anhydrous)
Absorptive powdered magnesia No. 2642. (Westvaco Chlorine Products Company, Newark, California)
Hyflo Super-Cel

EQUIPMENT

Chromatograph tube inside diameter 2 cm, height above joint 20 cm, with standard taper fittings.

Fisher Filtrator or equivalent for applying suction and catching solvent.

PROCEDURE

Prepare column by first putting wad of cotton in bottom of tube, turning on suction, and adding mixture of powdered magnesia and Super-Cel (1+1 by weight) tamping firmly occasionally until height of absorbent reaches six to seven cm. Place a 1 cm layer of anhydrous sodium sulphate on top to take up traces of moisture.

Weigh about 2 gm of the self-emulsifying mix into small beaker containing short glass rod. Place a 100 ml beaker beneath the column and turn on suction pump. Decant as much as possible of the oil mix into the column and reweigh, obtaining sample weight by difference. When material is drawn into column wash thru with small increments of a mixture (1+4) of benzene and petroleum ether, continuing until 75 ml have been used. Continue suction until column is nearly dry. Remove the 100 ml beaker and evaporate in a warm place, such as the top of an oven, until the solvent is nearly gone—overnight is convenient. Heat on steam bath ca two hours, then in an oven at 100° for 20 min., cool in desiccator, and weigh.

TABLE 1.—*Recovery of Shell Superior oil after washing through column*

HEATING	SAMPLE A	SAMPLE B	AVERAGE
	per cent	*per cent*	*per cent*
Overnight, top of oven	98.92	98.73	98.83
Plus 2 hrs. on steam bath and 20 min. at 100° in oven	98.65	98.40	98.53
Plus 16 hrs. at 100°	97.25	97.52	97.39
Plus 68.5 hrs. at 100°	95.36	95.81	95.59

TABLE 2.—*Loss of Shell Superior oil on heating*

HEATING	RECOVERY		AVERAGE RECOVERY
	SAMPLE A	SAMPLE B	
	per cent	*per cent*	*per cent*
Overnight, top of oven	99.95	99.97	99.96
Plus 2 hrs. on steam bath and 20 min at 100° in oven	99.32	99.60	99.46
Plus 16 hrs. at 100°	98.47	98.73	98.60
Plus 68.5 hrs. at 100°	96.81	97.08	96.95

TABLE 3.—*Recovery of oil from mixture of 90% Shell Superior oil and 10% Triton X-100*

PERCENTAGE RECOVERY	DEVIATION FROM AVERAGE
101.27	+0.63
103.50	+2.86
98.53	−2.11
100.59	−0.05
99.31	−1.33
Average 100.64	

TABLE 4.—*Recovery of oil from mixture of 90% Shell Superior oil and 10% Igepal 300*

PERCENTAGE RECOVERY	DEVIATION FROM AVERAGE
103.50	−1.22
102.82	−1.90
105.20	+ .48
106.71	+1.99
105.35	+0.63
Average 104.72	

TABLE 5.—*Recovery of oil from commercial mixes*

COMMERCIAL MIX	OIL FOUND, PER CENT		AVERAGE
A	74.53	76.53	75.53
B	82.99	82.68	82.84
C	95.12	94.98	95.05
D	84.59	84.88	84.74
E	97.51	97.71	97.61

The recovery of Shell Superior oil from the column is given in Table 1, together with data showing the effect of continued heating. Table 2 shows the effect of continued heating on Shell Superior oil which had not been put through the column. If the oil may be considered to be anhydrous as received, there is a loss of about 0.5 per cent in evaporating the solvents according to the procedure outlined above. Approximately one per cent of the oil is retained by the column as two fluorescent bands. This material is not eluted by the benzene-petroleum ether mixture but is readily washed out with alcohol. It is a gummy substance. The benzene-petroleum ether soluble fraction is lighter in color than the original oil.

This method of oil determination was applied to (a) a mixture of 90 per cent Shell Superior oil and 10 per cent Triton X-100 and (b) a mixture of 90 per cent Shell Superior oil and 10 per cent Igepal 300. Recoveries are given in Tables 3 and 4, respectively. They were somewhat high for both mixtures.

The method was also applied to five different commercial mixes whose actual oil content was unknown. Results are given in Table 5.

All of the above experiments yielded oils that would not emulsify on vigorous shaking with water, proving that the column did separate the oils from the emulsifiers. The high recoveries of oil from the known mixtures shown in Tables 3 and 4 would appear to indicate that both the Triton X-100 and Igepal 300 contained small amounts of oil. An attempt to separate oil from Triton X-100 and Igepal 300 by passing 2 gram quantities of each of these materials through the column failed, however, as the recovered material emulsified with water.

CONCLUSIONS AND RECOMMENDATIONS*

The idea of using chromatography for the determination of oil in emulsifiable oil mixes has enough merit to warrant investigation. The problem now is to hit on an adsorbent or solvent or some combination thereof that will remove all the oil, including the gums, without taking the emulsifying agents with it. When this is accomplished, the dream of the analytical chemist of putting a sample in one end of an apparatus and collecting the desired component at the other will have been realized. A comparison with existing procedures will then be in order.

No report was given on benzene hexachloride.

No report was made on disinfectants or on leathers and tanning materials.

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* For report of Subcommittee A and action of the Association, see *This Journal*, 32, 44 (1949).

ANNOUNCEMENTS

Referee Assignments, Changes, and Appointments

MALT BEVERAGES, SIRUPS AND EXTRACTS, AND BREWING MATERIALS:

Robert I. Tenney, Wahl-Henius Institute, 64 East Lake Street, Chicago, Ill., has been appointed Associate Referee.

ECONOMIC POISONS:

Fred I. Edwards, Bureau of Insecticide Investigation, Bureau of Entomology and Plant Quarantine, U. S. Dept. of Agriculture, Beltsville, Md. has been appointed Associate Referee on Parathion.

FEEDING STUFFS:

Richart T. Merwin, Agricultural Experiment Station, New Haven, Conn., has been appointed Associate Referee on Sulfa Drugs in Feeds.

SOILS AND LIMING MATERIALS:

Adolph Mehlich, North Carolina State College, Raleigh, N. C., has been appointed Associate Referee on Exchangeable Potassium in Soils, in place of J. Fielding Reed.

COSMETICS:

Henry Kramer, Food and Drug Administration, Baltimore 2, Md., has been Appointed Associate Referee on Deodorants and Anti-perspirants, in place of S. H. Newburger.

George McClellan, Food and Drug Administration, New Orleans 16, La., has been appointed Associate Referee on Depilatories, in place of S. H. Newburger.

SPICES AND OTHER CONDIMENTS:

A. N. Prater, Gentry Inc., Los Angeles 54, Calif., has been appointed Associate Referee on Seeds and Stems in Ground Chili Peppers.

Samuel Alfend, Referee, has been appointed as Chief of the Kansas City District, with address at Kansas City, Mo.

CORRECTIONS IN FEBRUARY JOURNAL

Report on Changes in Methods, Vol. 32, No. 1

Page 89. The method for "Lead in Lakes (Aluminum) of Coal-Tar Colors" should include paragraph under "Apparatus" given in *This Journal*, **31**, 678 (1948).

Pages 94, 95. Fruit and Fruit Products, item (3) and (4) should read: "The following method was adopted as tentative," instead of "official, first action."

Page 95, last line of method for Water-insoluble Solids (rapid method), change "+" to "÷".

Page 104. Preparation of Assay Solution, line 8, change "30 min." to "15 min."; line 15, change "to" to "of."

Page 109, footnote (6), insert "not" before "necessary."

CONTRIBUTED PAPERS

LIMITATIONS OF THE "MODIFIED KJELDAHL METHOD" FOR DETERMINING THE NITRATE NITROGEN IN NITRATE-CHLORIDE MIXTURES*

By H. K. WHITE and O. W. FORD, *Associate Referee* (Purdue University Agricultural Experiment Station, West Lafayette, Indiana)†

The official method for determining the nitrate nitrogen in fertilizers (1) has been criticized because of reported losses of nitrogen in this procedure when chlorides are present. It has been reported that these take place through a series of reactions ending with the formation of nitrosyl chloride (2, 3, 4).

Some investigators have developed new and modified procedures because of this failure of the Kjeldahl method (2, 3).

A review of the literature does not reveal the limits of the official method, or what ratio of nitrate to chloride it can satisfactorily determine. In the first phase of our work this ratio has been determined. Since significant losses do occur as reported (2, 3, 4), further work was undertaken to establish where these losses take place. Because chlorine is evolved from some mixtures, indicating the presence of a redox reaction which could possibly cause losses of nitrogen, some work was undertaken to determine the existence and extent of these losses.

EXPERIMENTAL

To establish the limits of the official method (1) for nitrate-chloride mixtures, a series of determinations were made on mixtures of analytical grade sodium nitrate and potassium chloride in decreasing ratios, by the official method (2.27 part 2) as revised by Ford (5). This procedure, used for the routine determination of total nitrogen in fertilizers in our control laboratory, is as follows:

(1) Prepare, at least ½ day in advance, the special sulfuric acid reagent: a solution of salicylic acid in concentrated sulfuric acid, containing 2 g per 30 ml. (Low results are obtained if solution is not complete.)

(2) Place 0.7 to 3.5 g of the sample in a long neck Kjeldahl flask and add 40 ml of special sulfuric acid. Let stand at least one-half hour; or, in case of nitrate salts, until the salts are dissolved. (When analyzing fertilizer mixtures containing nitrate salts and limestone, keep mixture cool until the action has ceased and solution is complete. Otherwise loss of oxides of nitrogen may result.)

(3) Gradually add 2 g of zinc dust, shaking flask at the same time.

* Purdue University Agricultural Experiment Station Journal Paper No. 377.
† Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 11–13, 1948.

(In the case of nitrate salts, mixture should be kept cool by holding flask under cold water tap until action is complete.)

(4) Heat the flask over low flame or on an electric rack for 5–10 minutes. Turn off heater, add 0.7 g HgO and 10 g K_2SO_4, and reheat until liquid in the flask is colorless or nearly colorless ($1\frac{1}{2}$–2 hours, in the case of electric digestion).

(5) Cool and, just before caking, add ca 200 ml of water. Precipitate mercury with 25 ml of 4% K_2S soln.

(6) After cooling, distill as in **2.24,** using 100 ml of 40–45% NaOH.

(7) Conduct blank test on the reagents, following same procedure as outlined above.

TABLE 1.—*Total nitrogen found in nitrate-chloride mixtures*

RATIO $NaNO_3$:KCl	FORD'S METHOD	DEVARDA METHOD	RATIO $NaNO_3$:KCl	FORD'S METHOD	DEVARDA METHOD
1:0	16.41	16.42	5:2	16.37	16.43
	16.34	16.42		16.37	16.47
	16.34	16.42		16.32	16.42
	16.38			16.25	
	16.38				
	16.35				
	16.37				
Average	16.37	16.42		16.33	16.44
10:1	16.38		2:1	16.24	16.49
	16.43			16.24	16.49
	16.24			16.14	16.41
	16.43			16.02	
Average	16.37			16.16	16.46
5:1	16.37		1:1	16.13	16.47
	16.38			16.13	16.41
	16.25			16.09	16.46
	16.27			16.07	
				16.06	
Average	16.32			16.10	16.45
10:3	16.35	16.41	1:2	15.39	16.47
	16.24	16.42		15.44	
	16.39			15.43	
				15.41	
				15.43	
				15.40	
Average	16.33	16.42		15.42	16.47

Compounds of the types formed as intermediates in the official procedure were analyzed, with and without chloride added, to establish where the losses of nitrogen in the presence of chlorides occur. No significant differences were found (Table 2). The results of the work on the various mixtures are shown in Table 3.

DISCUSSION OF RESULTS

As shown in Table 1 the losses of nitrogen increased as the concentration of nitrate with respect to chloride decreased. However in ratios in excess of 5 to 2, the loss of nitrogen was less than 1 per cent.

The work with the intermediate type compounds definitely establishes

TABLE 2.—*Total nitrogen found in intermediate type compounds with and without potassium chloride*

SAMPLE	WITHOUT ADDED CHLORIDE	WITH ADDED CHLORIDE RATIO (SAMPLE: KCl) 1:2
NH_4Cl (Analytical Grade)	26.12 26.15 26.11	26.15 26.18 26.15
p-aminobenzoic acid (MP 187–188)	10.21 10.21 10.25	10.21 10.21 10.25 10.21
5-nitrosalicylic acid (MP 228°C.)	7.48 7.49 7.45 7.47	7.41 7.47 7.47

that these losses occur in the nitration step. The ability to obtain consistent results, both with and without chloride, with (1) an ammonium salt, (2) an aromatic amine, and (3) an aromatic nitro compound, shows that these losses take place before digestion, before reduction, and before nitration, respectively. Good recovery with the 5-nitrosalicylic acid indicates that the loss occurs before the nitration is completed, and establishes the fact that, once the salicylic acid is nitrated, the subsequent reduction can be performed without loss of nitrogen.

A chloride would not be expected to interfere with this step unless it were oxidized to chlorine. Chlorine could compete with the nitrate ions in the nitration reaction, with the formation of chlorosalicylic acid. However, in the presence of an excess of salicylic acid, the losses from this competitive reaction are insignificant compared to those incurred in the oxidation of chloride to chlorine. The redox reaction between the chloride and nitrate ions is evidently developed when the salicylic-sulphuric

TABLE 3.—*Total nitrogen found in high nitrate-chloride complete fertilizer mixtures*

	PER CENT	PROCEDURE			
		DATE OF DETERMINATION			
		FORD'S METHOD			DEVARDA
		5/11/48	5/23/48	9/9/48	9/10/48
MF 530 (Prepared 5/11/48)					
Composition					
NH_4NO_3	33.3	10.92	10.99	10.79	10.95
KCl	33.3	10.99	11.06	11.00	11.14
Treble superphosphate	33.3		10.06	11.89	11.03
				11.09	
				11.10	
	Average	10.96	11.04	10.98	11.04
MF 531 (Prepared 5/11/48)*					
Composition					
NH_4NO_3	25	8.47	8.75	8.83	8.88
KCl	25	8.33	8.75	8.81	8.92
$CaCO_3$ (ppt).	25		8.68	8.81	9.04
Treble superphosphate	25			8.82	
				8.79	
	Average	8.40	8.73	8.81	8.95
MF 532 (Prepared 5/11/48)					
Composition					
NH_4NO_3	33.3	10.78	10.78	10.67	10.86
KCl	33.3	11.16	11.06	10.64	10.90
20% superphosphate	33.3		10.85	10.79	10.86
				10.99	
				11.14	
	Average	10.97	10.90	10.85	10.87
MF 538 (Prepared 5/23/48)					
Composition					
$NaNO_3$	33.3		5.32	5.21	5.57
KCl	33.3		5.32	5.24	5.56
20% superphosphate	33.3		5.32	5.36	
				5.28	
				5.22	
				5.34	
	Average		5.32	5.35	5.56

* No chlorine detected.

acid mixture is added, and results in the evolution of various oxidation and reduction products. This would seem to indicate that, in fertilizer containing nitrates, any substance which can be oxidized by the nitrate ion is a potential source of nitrogen losses when nitrogen is determined by the modified method.

The results of the study of mixtures indicate that no appreciable losses of nitrogen occur during storage for extended periods (see Table 3). In the sample containing calcium carbonate as well as treble superphosphate (MF 531), an apparent gain in nitrogen occurred which was probably due to the evolution of carbon dioxide from the mixture. However, in the other samples losses are shown, when the modified and Devarda procedures are compared, which are of the order expected in the presence of chloride and nitrate ions.

SUMMARY

If the ratio of nitrate ($NaNO_3$) to chloride (KCl) is less than 5 to 2, an appreciable loss of nitrogen may be expected when analysis is made by the official Kjeldahl method as modified to apply to nitrates. This loss occurs during nitration, and is believed to be due to the oxidation of the chloride by the nitrate ion. These ions are formed in the first step of the procedure, the addition of the sulphuric-salicylic acid mixture, and react to form volatile oxides which escape or cannot be reduced by the official method. No appreciable losses take place in nitrate-chloride mixtures when these are stored for an extended period.

LITERATURE CITED

(1) *Official and Tentative Methods of Analysis*, **2.27**, part 2, p. 27, 1945.
(2) DYER and HAMENCE, *Analyst*, **63**, 866 (1938).
(3) SHUEY, P. McG., *Ind. Eng. Chem., Anal. Ed.*, **19**, 882–883 (1947).
(4) PRINCE, A. L., *This Journal*, **30**, 235 (1947).
(5) ETHEREDGE, M. P., Associate Referee for Nitrogen, Instructions to Collaborators, March, 1948. (page 241, this issue).
(6) *Official and Tentative Methods of Analysis*, **2.31**, p. 28, 1945.

SEMI-MICRO PHENOL COEFFICIENT METHODS FOR TESTING QUATERNARY AMMONIUM DISINFECTANTS*

By GEORGE S. WARNER and MICHAEL J. PELCZAR, JR. (Department of Bacteriology, University of Maryland, College Park, Md.) and L. S. STUART (Production and Marketing Administration, U. S. Department of Agriculture, Washington, D. C.)

The subject of the appraisal of bactericidal efficiency of quaternary ammonium compounds has been excellently reviewed by Rahn and Van

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Eseltine (1). Among other tests, modifications of the official A.O.A.C. phenol coefficient method have been proposed to eliminate the random sampling error of the 4 mm transfer loop used in that method, and thus furnish a more accurate index to the actual killing concentration of such disinfectants. Of these, the method recently proposed by Klarmann and Wright (2) for the evaluation of quaternary ammonium germicides has received considerable attention. This method employed 1/10 of the quantities of medicant and culture used in the A.O.A.C. method; and sub-culture was made of the entire volume of disinfectant-culture mixture at the conclusion of the exposure time interval. It is obviously a more critical test than the A.O.A.C. phenol coefficient test insofar as the determination of the actual killing concentration is concerned, since the sub-culture sample is some 25 times larger and the ratio of apparatus surface to medicant volume is appreciably higher. In general, therefore, lower results might be expected depending among other things on the degree of surface activity of the disinfectant being tested. For this reason results secured with methods of this type are not strictly comparable with results secured by the official A.O.A.C. method.

Such methods may, however, prove to be especially useful in determining actual end points for complete kill with disinfectants of the type that kill a very high proportion of the test culture cells within a very short exposure interval, but which kill the few remaining viable cells at a progressively slower rate. With disinfectants of this type, the random sampling error with the 4 mm loop sub-sampling procedure is great enough at the longer exposure intervals to make it exceedingly difficult to determine exact concentration, death time, and end points with any degree of accuracy. The behavior of quaternary ammonium germicides in this respect has led to a great deal of criticism of the official A.O.A.C method, and to considerable conjecture relative to their true killing or disinfectant concentrations.

A representative number of these compounds were studied, therefore, by both the official phenol coefficient procedure, employing trypicase broth and a modified semi-micro method embodying the general principles as set forth by Klarmann and Wright (2) to establish the relationship of results secured by the two methods and to establish more definitely the actual 10 minute killing concentration of such compounds.

EXPERIMENTAL

TEST ORGANISMS

Eberthella typhosa Hopkins and *Staphylococcus aureus* 209 were employed as the test organisms. Since the above names have been in common usage, this older nomenclature has been retained in preference to that of *Salmonella typhosa* and *Micrococcus pyogenes* var. *aureus* proposed in the Sixth Edition of Bergey's Manual of Determinative Bacteriology (3).

MEDIA AND METHODS

The official A.O.A.C. phenol coefficient procedure was employed exactly as outlined in the official methods except that trypticase broth was substituted for F.D.A. broth. The composition of the trypticase broth was:

Trypticase	10 g
Beef extract	5 g
NaCl	5 g
Distilled water	1000 ml

In the semi-micro method, trypticase broth was also used for propagating the test cultures and a trypticase-Tween 80-Azolectin broth was used for subculturing. The latter was designed and employed in sufficient volume to neutralize both the bactericidal and bacteriostatic effects of the entire quantity of the quaternary ammonium germicide used for each exposure period. The lecithin acted as a neutralizing agent or antidote, and the Tween 80 as a dispersing agent. The formula for this medium was:

Trypticase	10 g
Beef extract	5 g
NaCl	5 g
Azolectin	1.5 g
Tween 80	10 g
Distilled water	1000 ml

Preparation of the medium was accomplished in the following manner: The Azolectin and Tween 80 were added to 400 ml of hot distilled water, and the mixture was boiled 10 minutes to effect a clear solution. The remaining ingredients were dissolved by boiling in 600 ml of distilled water and were added to the clear Azolectin-Tween 80 dispersion. The final solution was boiled for 10 minutes, made to a volume of 1000 ml with distilled water, and adjusted to *p*H 7.1, tubed in 10 ml amounts and autoclaved for 20 minutes at 15 lbs (121°C).

This medium is essentially that initially employed by Quisno, Gibby, and Foter (4). It contains, however, a greater concentration of lecithin and Tween 80. This was found to be necessary to completely neutralize the large quantity of quaternary ammonium germicide present in the sub-culture sample employed. The adequacy of the exact level was determined in a series of preliminary tests. An example of the type of results secured in these tests is given in Table 1.

In this particular study, alkyl dimethyl benzyl ammonium chloride[1] was added to culture tubes containing 10 ml of sterile trypticase-Tween 80-Azolectin broth to give the dilutions indicated (Table 1). The tubes were then conditioned in a 20°C. water bath for 5 minutes and inoculated with 0.1 ml of a 24 hour broth culture of *S. aureus* 209 held for 10 minutes at this temperature and then incubated at 37.5°C. for 48 hours. Condition-

[1] The alkyl radicals in this compound range from C_8H_{17}–$C_{18}H_{37}$.

ing and holding of these tubes at 20°C. for 10 minutes seemed necessary, since this one study was used to check on both bactericidal and bacteriostatic effects. Growth in the tubes, as evidenced by turbidity, was recorded at 24 and 48 hour intervals. At the 48 hour interval, 0.1 and 1.0 ml sub-samples were plated in trypticase agar and these plates incubated at 37°C. for 48 hours to confirm readings based on turbidity evaluations.

Inasmuch as *S. aureus* 209 is an organism especially susceptible to the bactericidal and bacteriostatic effects of alkyl dimethyl benzyl ammonium chloride,[1] such results indicated that the trypticase-Tween-80-Azolectin sub-culture broth would act as an effective antidote for quantities of quaternary ammonium germicides up to .000167 gm where .015 gm of Azolectin and 0.1 gm of Tween 80 were present. It should be noted,

TABLE 1.—*Effect of 1 percent Tween 80 and 0.15 per cent Azolectin on growth of Staphylococcus aureus 209*

DILUTION OF ALKYL DIMETHYL BENZYL AMMONIUM CHLORIDE IN SUB-CULTURE MEDIUM	TRYPTICASE TWEEN 80 AZOLECTIN BROTH GROWTH AT 37.5°C. AS EVIDENCED BY TURBIDITY IN SUB-CULTURE BROTH		AGAR PLATE COUNTS OF 48 HOURS BROTH CULTURES	
	24 HRS	48 HRS	1.0 ML	0.1 ML
1:3000	−	−	0	0
1:5500	−	−	0	0
1:6000	−	−	0	0
1:6500	−	+	TNTC*	TNTC
1:7000	+	+	TNTC	TNTC
1:7500	+	+	TNTC	TNTC
1:10,000	+	+	TNTC	TNTC

* TNTC = Colonies too numerous to count.

however, that the neutralizing effect of this medium appeared to be completely lost when the quaternary salt concentration passed a certain level, and that this level was so sharply defined as to indicate direct quantitative relationships between the neutralizing agent present and the quaternary ammonium salt.

The technique of the semi-micro method employed in these studies varied from that used by Klarmann and Wright (2) in the amount of medicant mixture, in the amount and composition of the sub-culture medium, and in the method of preparation of the medicant mixture. The details are as follows: In preparation for the actual test, an excess quantity (5 ml) of each dilution of medicant, selected to cover the killing range, was pipetted into separate sterile (20×150 ml) test tubes and tempered for 5 minutes in the 20°C. water bath. For the actual 5 minute test interval, 0.1 ml of a 22 to 26 hour broth culture of the test organism, previously shaken for fifteen minutes to break up clumps, was added to each of a series of 10 sterile test tubes labelled to indicate the selected dilution of

medicant contained. This series of medicant tubes was placed in the water bath parallel to the tubes containing the corresponding dilutions of medicant. One ml was pipetted from each medicant dilution into the corresponding medicant tube containing the test culture aliquot, a 30 second interval being employed between each dilution transfer. Five minutes after the dilution of medicant was added to the medicant tube, 10 ml of sterile trypticase-Tween 80-Azolectin broth was added aseptically, the tube shaken briefly and placed in a suitable rack. As soon as this process was completed with the entire dilution series, all tubes were vigorously shaken and placed in the incubator at 37°C. for 48 hours. The foregoing procedure was repeated in its entirety for both the 10 and 15 minute exposure intervals.

For comparative purposes the A.O.A.C. technique using trypticase broth was carried out concurrently each day that the semi-micro studies were made.

In both the semi-micro method and the A.O.A.C. method with trypticase broth, the maximum lethal concentration or the minimum lethal dilution of the test compound or of phenol was established as that dilution which killed the test organism in 10 minutes, but not in 5 minutes.

RESULTS

Phenol coefficient values have been obtained for four quaternary ammonium compounds by the A.O.A.C. method using trypticase broth and a semi-micro method described.

In Tables 2 and 3, the minimum lethal concentrations of the four quaternary ammonium germicides studied for *E. typhosa* and *S. aureus* 209 determined by the A.O.A.C. method with trypticase broth, and by the semi-micro method, are compared. Phenol coefficient values have been calculated from results obtained with both techniques.

It can be seen that the minimum lethal concentration found in the semi-micro procedure was considerably lower, with both test organisms, for all four quaternary ammonium germicides than that found by the A.O.A.C. method using trypticase broth. The minimum lethal concentration with phenol was the same by both the semi-micro and the modified A.O.A.C. method in the case of both test organisms. Obviously, therefore, the phenol coefficient values secured by the semi-micro method are considerably lower than those secured by the A.O.A.C. technique.

If the phenol coefficient values secured by the A.O.A.C. technique for the quaternary ammonium germicides against *E. typhosa* are multiplied by 20, as is the presently accepted standard procedure in determining the safe use dilution for disinfectants, it can be seen that the safe use dilution indicated is higher in 2 instances and lower in the other 2 instances than the minimum 10 minute lethal concentration determined by the semi-micro method. The average safe use dilution, indicated by phenol coeffi-

TABLE 2.—*Phenol coefficient values of four quaternary ammonium compounds determined by the A.O.A.C. method using Trypticase broth and a modified semi-micro technique using Eberthella typhosa Hopkins at 20°C.*

DISINFECTANT	A.O.A.C. TECHNIQUE WITH TRYPTICASE BROTH		MODIFIED SEMI-MICRO TECHNIQUE	
	MINIMUM CONCENTRATION LETHAL IN 10 MIN.	PHENOL COEFFICIENT	MINIMUM CONCENTRATION LETHAL IN 10 MIN.	PHENOL COEFFICIENT
Phenol	1:90		1:90	
Alkyl-dimethyl benzyl ammonium chloride	1:15,000	166.6	1:2,000	22.2
Cetyl trimethyl ammonium bromide	1:15,000	166.6	1:2,300	25.6
p-di iso butyl phenoxy ethoxy ethyl dimethyl benzyl ammonium chloride	1:10,000	110.8	1:2,800	30.8
Cetyl pyridinium chloride	1:6,500	72.2	1:1,500	16.6

TABLE 3.—*Phenol coefficient values of four quaternary ammonium compounds determined by the A.O.A.C. method using Trypticase broth and a modified semi-micro technique using Staphylococcus aureus 209 at 20°C.*

DISINFECTANT	A.O.A.C. TECHNIQUE WITH TRYPTICASE BROTH		MODIFIED SEMI-MICRO TECHNIQUE	
	MINIMUM CONCENTRATION LETHAL IN 10 MIN.	PHENOL COEFFICIENT	MINIMUM CONCENTRATION LETHAL IN 10 MIN.	PHENOL COEFFICIENT
Phenol	1:70		1:70	
Alkyl-dimethyl benzyl ammonium chloride	1:17,500	250	1:3,500	50
Cetyl trimethyl ammonium bromide	1:20,000	285.7	1:3,500	50
p-di iso butyl phenoxy ethoxy ethyl dimethyl benzyl ammonium chloride	1:22,000	314.4	1:2,800	40
Cetyl pyridinium chloride	1:20,000	285.7	1:3,500	50

cient values by the A.O.A.C. method (except that trypticase broth was used), was 1:2581, whereas the critical killing dilution indicated by the semi-micro technique was 1:2150. It would seem, therefore, that the semi-micro method yields results more comparable with those secured by "Use dilution methods" than does the A.O.A.C. phenol coefficient method. For the present, at least, this seems to be the only practical interpretation which can be made; for there is not sufficient evidence available to intelligently use the lesser phenol coefficient values secured by this method in the calculation of safe use concentrations. Data must be compiled from practical use studies, comparing effects with 5% solutions of phenol and varying concentrations of quaternary ammonium germicides, to determine the proper conversion factor to employ before this would be feasible.

It is of interest to note that the phenol coefficient values secured by the A.O.A.C. method employed were considerably lower than those usually claimed or reported on the basis of tests by the official A.O.A.C. method when the original F.D.A. broth was used. This is attributed to the use of trypticase broth in the propagation of the test cultures employed, and tends to confirm the early observation of Brewer (5) that differences in the peptones employed in the culture media will cause significant differences in results secured with quaternary ammonium germicides by the A.O.A.C. phenol coefficient procedure. On the other hand, the trypticase broth used for sub-culturing did not contain Tween 80 and lecithin to neutralize the bacteriostatic effects of the quaternary ammonium germicides, and there is considerable evidence to indicate that if this had been done the results, with *S. aureus* 209 at least, would have been even lower. It is also possible that those secured with *E. typhosa* might have been low enough so that the phenol coefficient values secured by the A.O.A.C. technique would have indicated safe use dilutions considerably less in all instances than the critical killing dilutions found in the semi-micro method.

Both "time skips" and "dilution skips" were occasionally encountered in the semi-micro method tests. They were, however, much less frequently encountered than in tests using the A.O.A.C. technique. Thus, characteristic time-concentration killing end-points were somewhat easier to establish and duplicate. In this connection, it is especially interesting to report that the work sheets for the semi-micro tests generally indicated lower killing concentrations for the 15 min. exposure interval than for the 10 min. exposure interval. This seems to indicate that the small percentage of cells in test cultures, which survive the initial exposure to a given concentration of quaternary ammonium germicides are eventually killed on prolonged contact.

SUMMARY AND CONCLUSIONS

A semi-micro phenol coefficient method for determining the germicidal potency of quaternary ammonium disinfectants has been described. The

maximum lethal killing dilutions found by this method were not appreciably different from the safe use dilutions indicated by the A.O.A.C. phenol coefficient method, using trypticase broth, when the standard formula of 20 times the phenol coefficient found with *E. typhosa* was employed to determine the number of parts of water to add to one part of disinfectant for preparing a safe use dilution. These results suggest that the conditions of the semi-micro test described may have been severe enough to warrant its classification as a "use dilution test," but additional correlations with actual use studies will be necessary to determine whether this is true. If this is the case, then the results secured would indicate that the basic procedure of the official A.O.A.C. test may provide a reasonably reliable presumptive index to the germicidal potency of quaternary ammonium germicides if the sub-culture broth employed contains a neutralizer for bacteriostatic quantities of these germicides which may be carried over.

Critical quaternary ammonium germicide concentration killing times could be more easily established with the semi-micro method described than with the A.O.A.C. technique. For this reason, it may have practical value as a test method for confirming results secured by the A.O.A.C. phenol coefficient procedure or for initial assays when a fixed relation between the critical killing concentrations, or phenol coefficient values found therewith, and safe use dilutions can be established.

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VARIATIONS IN RESISTANCE IN E. TYPHOSA AND S. AUREUS WHEN MAINTAINED AS SPECIFIED ACCORDING TO THE OFFICIAL A.O.A.C. PHENOL COEFFICIENT METHOD*

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INTRODUCTION

The ability to maintain and propagate selected test cultures at standard and uniform resistance levels to phenol, is a fundamental necessity for the

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correct evaluation of chemical germicides by the official A.O.A.C. phenol coefficient method.

This method prescribes the maintenance of 30-day stock cultures of F.D.A. agar slants; and the preparation therefrom of 24-hour broth test cultures by employing 3 successive 24-hour serial transfers using F.D.A. broth.

It occasionally happens that test cultures prepared in this manner are found to possess levels of resistance outside the limits prescribed by the official test. This results not only in invalidating the results secured in the particular tests in question, but also disrupts the entire testing program until a procedure is worked out to re-establish the test cultures at the resistance levels desired.

The proper culture method to employ to re-establish a given resistance level in a test culture, is a moot question. It is well known that changes in culture methods, involving such environmental conditions as temperature, *p*H, and culture composition, may possibly result in permitting the establishment of permanent variant cells; so that sub-cultures occasionally possess characteristics varying widely from those of the mother culture. Therefore, it would seem essential to avoid procedures that employ such variables. If this is done, and the method employed is restricted to use of the specific media and temperatures stipulated in the official test, the only variations which could be encountered would be those, normal to the culture under the condition specified, that result from increases in population density or age.

Inasmuch as no actual data are available on the degree or permanency of variation in the specified cultures; namely, *Eberthella typhosa* (Hopkins Strain) and *Staphylococcus aureus* 209, resulting solely from culture age, this study was conducted to ascertain:

(1) The normal variations in the resistance to phenol of F.D.A. broth test cultures prepared from 30-day agar stock cultures of the two test organisms as prescribed by the A.O.A.C. method.

(2) The degree of permanence of the variation encountered in these cultures.

(3) If methods, based on the investigation of culture variants resulting solely from culture age, are adequate for re-standardizing resistance to the desired level.

EXPERIMENTAL

TEST ORGANISMS

Eberthella typhosa (Hopkins) and *Staphylococcus aureus* 209 were employed as the test organism. As the above names are employed in the present official A.O.A.C. Method, this older nomenclature has been retained in preference to *Salmonella typhosa* and *Micrococcus pyogenes* var. *aureus* proposed in the Sixth Edition of Bergey's Manual of Determinative Bacteriology.

MEDIA, METHODS, AND RESULTS

The only media employed were F.D.A. Agar and F.D.A. nutrient broth prepared exactly as stipulated in the A.O.A.C. method.

Individual 30-day agar slant stock culture of *E. typhosa* and *S. aureus* were washed off and suspended in sterile physiological saline solution. Serial dilution plates were made from these suspensions in F.D.A. Agar and incubated for 48 hours at 37°C. Plates containing between 30 and 100 discrete colonies were then selected from each series of dilutions, and all others discarded. From these plates 30 typical colonies were picked at random and transferred to individual tubes of sterile F.D.A. broth. These tubes were incubated at 37°C. for 24 hours, after which transfers were made to fresh tubes of broth incubated in the same manner, and thence into a third set of broth tubes incubated at 37°C. for 24 hours. The 24-hour cultures resulting from this third set of transfers were then examined for their resistance to dilutions of phenol by the procedure outlined in the official A.O.A.C. method. The highest dilution which killed the test organism within 10 minutes, but not in 5, at 20°C. was considered to be the critical killing dilution.

This procedure was employed with three consective 30-day agar slant stock cultures of both test organisms, providing in each case 90 selected and experimentally standardized sub-cultures for examination relative to phenol resistance. It is believed that these sub-cultures provided a representative sampling of the stock cultures maintained according to the specifications given in the official A.O.A.C. method. The results are shown in Table 1.

TABLE 1.—*Critical killing dilutions of phenol for standardized 24-hour broth cultures of E. typhosa and S. aureus*

PHENOL DILUTION USED	NUMBER OF CULTURES OF *E. typhosa* KILLED IN 10 MIN. BUT NOT IN 5 MIN.				NUMBER OF CULTURES OF *S. aureus* KILLED IN 10 MIN. BUT NOT IN 5 MIN.			
	SERIES			TOTAL	SERIES			TOTAL
	1	2	3		1	2	3	
1–40	0	0	0	0	0	0	0	0
1–45	0	0	0	0	0	0	0	0
1–50	0	0	0	0	1	0	0	1
1–55	0	0	0	0	0	3	0	3
1–60	0	0	0	0	8	18	9	35
1–65	1	1	0	2	7	9	14	30
1–70	1	1	0	2	13	0	7	20
1–75	0	1	0	1	1	0	0	1
1–80	17	4	0	21	0	0	0	0
1–85	3	15	1	19	0	0	0	0
1–90	8	8	15	31	0	0	0	0
1–95	0	0	8	8	0	0	0	0
1–100	0	0	4	4	0	0	0	0
1–105	0	0	1	1	0	0	0	0
1–110	0	0	1	1	0	0	0	0
Total No. of Cultures	30	30	30	90	30	30	30	90

The results in Table 1 indicate that fresh 24-hour broth test cultures of *E. typhosa* prepared as prescribed by the A.O.A.C. method may possess resistances to phenol dilutions varying from 1–65 to 1–110, and that similar test cultures of *S. aureus* may show resistant levels varying from dilutions of 1–50 to 1–75. The frequency of the occurrence of cultures

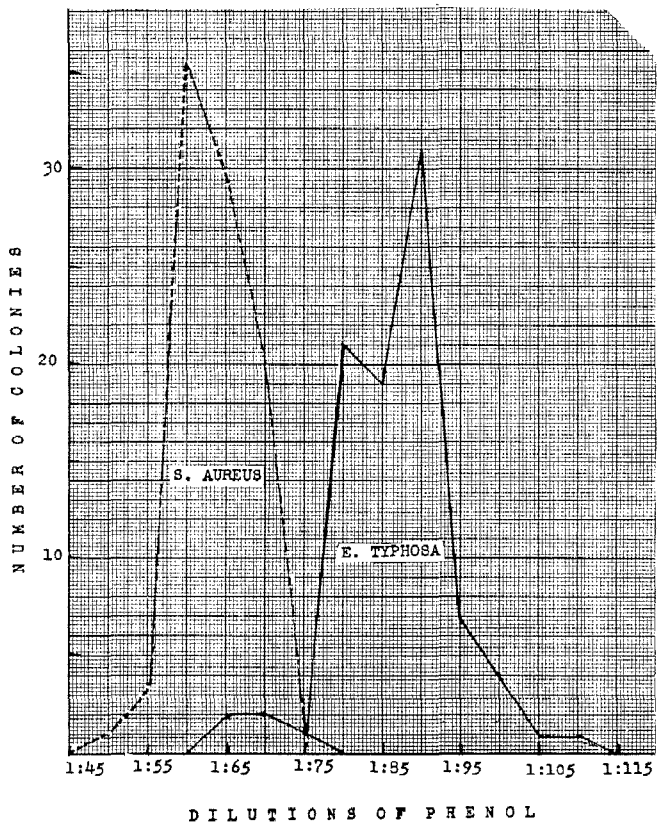

FIG. 1.—Frequency distribution curves on the critical killing dilutions of phenol for *E. typhosa* and *S. aureus*.

outside the prescribed resistance levels of 1–60 to 1–65 for *S. aureus* is small. With *E. typhosa*, however, quite a large number of cultures were obtained with resistances somewhat greater than the greatest concentration or lowest dilution specified in the test, and the range in dilution resistance was considerably broader than with *S. aureus*. These results seem to indicate that cultures of this organism contain cells which vary widely in resistance, and have a tendency to possess a slightly greater resistance in freshly prepared broth test cultures than is specified in the official test. In Figure 1, the data in Table 1 are presented in the form of distribution curves for both organisms.

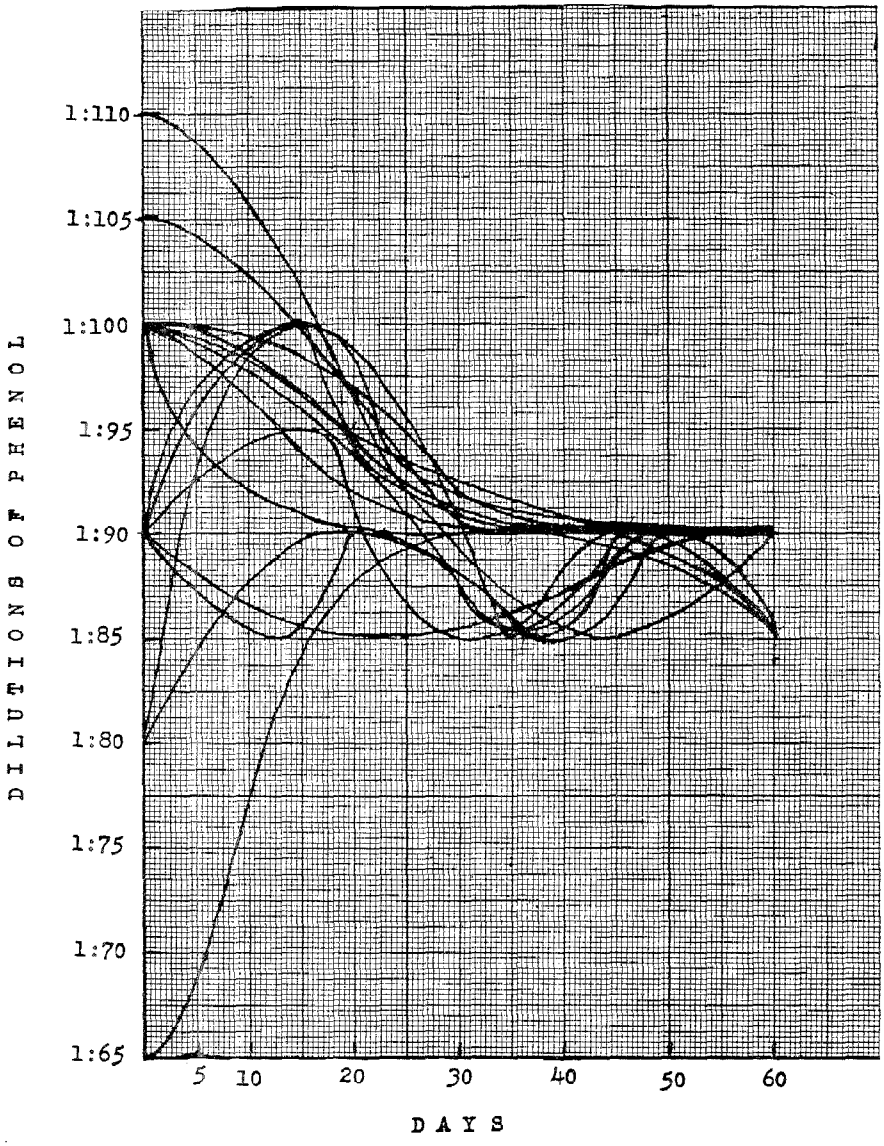

FIG. 2.—Changes in resistance levels at 20°C. of experimentally standardized test cultures of *E. typhosa* in broth maintained with 24-hour serial transfers.

All 24-hour test cultures were maintained in broth at 37°C., and daily transfers were made until the initial resistance level had been ascertained. Broth cultures representative of each resistance level recorded were then selected for further study, to determine the influence of continued propagation in F.D.A. broth at 37°C. (with 24-hour transfers) on the constancy

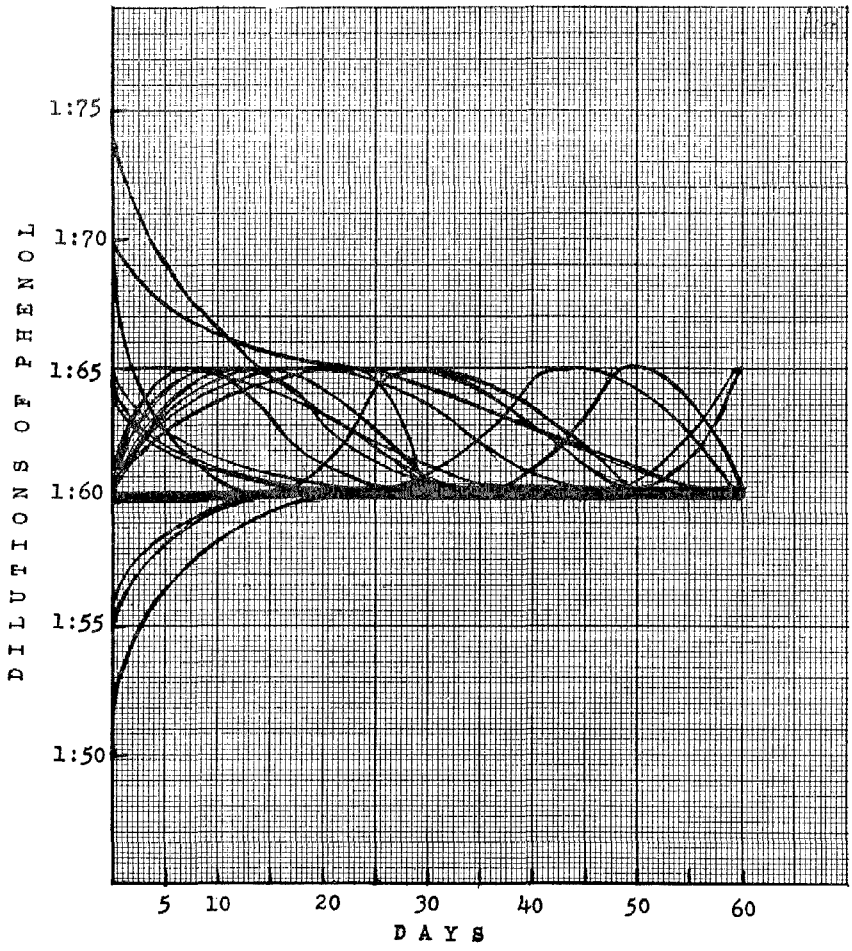

FIG. 3.—Changes in resistance levels at 20°C. of experimentally standardized test cultures of *S. aureus* in broth maintained with 24-hour serial dilutions.

of the initial resistance levels recorded. All other experimental test cultures were discarded.

Phenol resistance levels were determined at various intervals on each test culture so maintained for a period of 60 days. The results of these studies with *E. typhosa* are summarized in graphic form in Figure 2 and those for *S. aureus* similarly presented in Figure 3.

From study of the 16 curves in Figure 2, representing the resistance levels found with 16 individual selected cultures of *E. typhosa* of varying initial resistance levels maintained in F.D.A. broth at 37°C. in the 24-hour transfers over a 60-day period, it can be seen that all 16 cultures eventually

developed resistance levels to dilutions of from 1–85 to 1–95. These levels are within the range of resistance to phenol prescribed for this organism in the test. One culture with an initial resistance level of 1–65 decreased in resistance to 1–85 within 16 days. Two with an initial resistance of 1–80 decreased to the desired level within one week. On the other hand, cultures with low initial resistance levels of 1–100, 1–105 and 1–110, all increased in resistance to the 1–95 dilution level within 26 days. Some cultures, which on initial standardization possessed the 1–90 dilution resistance, temporarily lost this resistance but regained it eventually after continued 24-hour transfers. On the whole, then, it appears that these findings tend to substantiate the conclusion indicated in Table 1 and Figure 1: that cultures of this organism, when maintained as specified in the test, contain a predominantly large number of cells within the resistance range of 1–85 to 1–95.

Examination of the 21 curves representing the resistance levels of the selected strains of *S. aureus* (Figure 3) shows that all 21 cultures developed resistances to phenol dilutions of 1–60 or 1–65, as specified for this organism in the official test. One strain with an initial resistance level at a phenol dilution of 1–50 decreased in resistance in the 1–60 dilution level within 21 days. Two with an initial resistance level at 1–55 decreased to the 1–60 level within 16 days. Five with an initial resistance level at 1–60 remained at this level for the entire 60 days while 6 others vacillated between the levels of 1–60 to 1–65 over this period. None of the 4 cultures which possessed an initial resistance level at 1–65 remained constantly at this level for the entire 60 day period, but all 4 retained a resistance at either 1–60 or 1–65 over the entire period. The 2 cultures possessing an initial resistance level at 1–70 and the 1 culture with an initial resistance at 1–75, rapidly increased in resistance to levels at 1–65 and 1–60 when transfers at 24–hour intervals were continued. These findings confirm the results indicated in Table 1 and Figure 1, that the mean resistance level of cells of this organism to phenol is at the 1–60 dilution.

Results with 24-hour cultures of both organisms derived from cultures maintained in F.D.A. broth at 37 C. for 60 days, with transfers at 48 and 72-hour intervals, were also secured. The resistance levels to phenol with such cultures were not constant, and many possessed resistance levels outside the ranges prescribed in the official method.

DISCUSSION

From the results secured in this study, it appears that the procedure described for maintaining and propagating test cultures in the official A.O.A.C. method, will provide test cultures of the prescribed resistance in at least 64 out of 100 cases of *E. typhosa* and 70 out of 100 cases of *S. aureus*. It is quite clear, however, that some cultures may be obtained possessing an initial resistance in each instance outside of the resistance

ranges tolerated. On the other hand, if these cultures that do not comply in resistance to the standards stipulated in the methods are continually maintained, with 24-hour transfers, in F.D.A. broth at 37°C., they will eventually develop the resistance levels prescribed. On the whole then the official procedure for maintaining and propagating the test culture as now outlined seems to be both fundamentally sound and practical.

Although a large number of experimentally standardized subcultures were employed in these studies, it is probable that the number used actually was too small from a statistical standpoint to yield frequency distribution curves of absolute accuracy. However, the curves established do probably give a fairly representative picture of the general character of the resistance frequency distribution in each instance. They indicate that resistance with *S. aureus*, when propagated according to the standard procedure, is highly characteristic at dilutions of phenol of 1–60 and 1–65. *E. typhosa* does not appear to possess the sharply defined characteristic resistance level possessed by *S. aureus* 209, as quite large numbers of test cultures of *E. typhosa* were secured at resistance levels of the 1–80, 1–85 and 1–90 dilutions of phenol and some at such widely variable dilutions as 1–65 to 1–110, in the initial standardizations. This was apparently appreciated by the authors of the test, since the tolerance with *E. typhosa* is greater than with *S. aureus* 209.

The results of continued propagation, with 24-hour transfers in F.D.A. broth at 37°C., of selected experimentally standardized cultures over the entire resistance ranges observed, together with the initial standardizations for both cultures, tend to emphasize the fact that the resistances indicated as characteristic by the frequency distribution curves are characteristic and fixed properties of the 2 test cultures.

No permanent variation from these characteristic resistance levels was encountered in these studies with either culture. However, attention should be called to the fact that this does not eliminate the possibility that such permanent variation might occur if such variable environmental conditions as changes in *p*H, media composition and temperature, were employed. It should also be reported here than no R (rough) variants of *S. aureus* 209 were encountered in these studies, and that all experimentally standardized test cultures with this organism were propagated from S. or smooth type colonies.

Continuous propagation by the use of uninterrupted 24-hour transfers in F.D.A. broth at 37°C., would appear to be the most simple and convenient method for re-standardizing these 2 specified test cultures. If time does not permit this procedure to be followed, dilution plates by the use of F.D.A. agar may be made from the stock cultures and incubated for 48 hours at 37°C., from which 10 single colony isolates can be transferred to individual tubes of F.D.A. broth. If the resulting cultures are transferred serially at 24-hour intervals for 3 successive days, and phenol

resistance is then determined, a number of test cultures which possess the exact resistance desired will be obtained. It would appear that the latter procedure, from the standpoint of avoiding permanent variations from the mother culture, can be considered as a relatively safe one for the restandardization of test culture resistance with both *E. typhosa* and *S. aureus* 209. No permanent variants were encountered in these studies, in which a technique similar to that in the initial standardizations of experimental test cultures was employed.

Constancy of behavior is particularly important in the selection of a test culture. With respect to phenol resistance, the specific cultures of *E. typhosa* and *S. aureus* 209 used in the studies reported herein and selected for use in the official A.O.A.C. phenol coefficient method, appear to possess relatively constant characteristics. This undoubtedly accounts in part for the successful use of this test for such a long period of time.

Many other cultures have been, and are being, suggested for use as test cultures in the evaluation of chemical germicides. It would seem essential that each culture proposed for this use be studied carefully to determine procedures of maintenance and propagation which will prevent the development of permanent variants. The procedure outlined in the A.O.A.C. phenol coefficient method for maintaining and propagating *E. typhosa* (Hopkins strain) and *S. aureus* 209 appears to be adequate in this respect.

SUMMARY AND CONCLUSION

1. The procedure as now outlined in the official A.O.A.C. phenol coefficient method for maintaining and propagating test cultures of *E. typhosa* (Hopkins) and *S. aureus* 209 at standard resistance levels to phenol has been studied and found to be adequate in most respects.[1]

2. The normal variations in cultures of *E. typhosa* (Hopkins) and *S. aureus* 209 when maintained and propagated according to the prescribed procedure have been recorded. With 90 freshly prepared 24-hour broth cultures from 30-day stock slant cultures employing 3 successive F.D.A. broth serial transfers at 37°C., the resistance to phenol dilutions varied with *S. aureus* 209 from 1–50 to 1–75, and with *E. typhosa* from 1–65 to 1–110. These resistance levels are somewhat wider than the 1–60 and 1–65 dilutions specified for *S. aureus* 209 and 1–85 to 1–95 dilutions specified for *E. typhosa* (Hopkins) in the test. However, with continued serial transfers at 24-hour intervals all experimental test cultures rapidly developed a resistance to phenol within these specified levels.

3. The phenol dilution resistance levels of 1–60 and 1–65 for *S. aureus* and 1–85 to 1–95 for *E. typhosa* specified in the test appear to be fairly constant and fixed characteristic properties of the two test cultures specified when maintained according to the A.O.A.C. procedures.

[1] *Official and Tentative Methods of Analysis*, Sixth Ed., 86–91, 1945.

4. Methods for restandardizing test cultures of *E. typhosa* (Hopkins) and *S. aureus* 209 to the phenol resistance levels desired have been described.

DETECTION OF TARTARIC ACID AND TARTRATES IN WINES

By ALEX P. MATHERS (Alcohol Tax Unit Laboratory, Bur. of Internal Revenue, Washington 25, D. C.)

A review of the literature indicates some divergence of opinion as to the presence of tartaric acid and tartrates in fruits and berries other than grapes. Bigelow and Dunbar (1) state, "The burden of evidence in the literature seems to indicate that tartaric acid is not an ingredient of fruit juices except, of course, of grape juice." Winton (2) lists numerous references in discussing fruit acids, many of which are controversial with regard to the presence of tartaric acid in fruits other than grapes.

The procedure as outlined below is suitable for ordinary laboratory examination of wines, as ten per cent of grape wine in the presence of other fruit wines is easily detectable.

The selective oxidizing action of periodic acid on α, β-glycols to convert tartaric acid into glyoxylic acid is employed. Sprinson and Chargaff (3) in a study of periodic acid oxidations report the conversion of tartaric acid into glyoxylic acid, and the conditions for arresting the oxidation at this point.

The glyoxylic acid is converted into a p-nitrophenylhydrazone through the agency of p-nitrophenylhydrazine. The rearrangement of the p-nitrophenylhydrazone of glyoxylic acid in alkaline solution to give an intensely red-colored compound (4) (5), provides a delicate means of detecting small amounts of this compound.

$$\begin{matrix} \text{CHOH}\cdot\text{COOH} \\ | \\ \text{CHOH}\cdot\text{COOH} \end{matrix} + HIO_4 \rightarrow 2\ \text{CHO}\cdot\text{COOH} + HIO_3 + H_2O$$

$$\text{CHO}\cdot\text{COOH} + \text{p-}NO_2C_6H_4NHNH_2 \rightarrow \text{p-}NO_2C_6H_4NHN{:}CH\cdot COOH + H_2O$$

$$\text{p-}NO_2C_6H_4NHN{:}CH\cdot COOH + NaOH \rightarrow \text{Red Compound}$$

The p-nitrophenylhydrazone of glyoxylic acid is easily obtained in crystalline form for microscopic identification, by evaporating the ethereal solution or alcoholic eluate containing the compound. The compound can be obtained in quite pure form by dissolving the crude residue in ether, extracting the ethereal solution with aqueous (2%) sodium bicarbonate solution, acidification of this aqueous solution with 5% sulfuric acid, and re-extraction with ether. The p-nitrophenylhydrazone of glyoxylic acid crystallizes in a fairly pure state upon evaporation of the ether.

REAGENTS

Activated Carbon (Darco S-51)
Neutral lead acetate (5% aqueous solution)
Neutral lead acetate (3% in glacial acetic acid)
Ammonium acetate (20% in glacial acetic acid)
Glacial acetic acid
Periodic acid (1% aqueous solution)
Sodium bisulphate (10% aqueous solution)
Sodium bisulphite (powdered)
Ethyl ether
Ethyl alcohol (anhydrous)
Sodium hydroxide (solid)
Powdered glass (Eimer and Amend) G-17
p-Nitrophenylhydrazine reagent: one gram of p-nitrophenyl hydrazine, or one of its salts, dissolved in 7.5 ml of concentrated sulfuric acid and diluted to 75 ml with ethyl alcohol (95%)
Asbestos fibers

Adsorption Tube:

Any small chromatographic column is satisfactory. Column is packed to height of 40 to 50 mm. with glass powder held in place by asbestos fibers. Tube may be made by sealing about 1″ of 6 mm o.d. tubing to the bottom of a 16×150 mm test tube.

METHOD

Dilute 10 ml of wine to 50 ml with distilled water; add approximately one-half gram of activated carbon, warm on steam bath about ten minutes and filter into a centrifuge bottle. To the filtrate add 5 ml of 5% neutral lead acetate solution, centrifuge and decant the supernatant liquid. Wash the precipitate with 40 ml of distilled water again centrifuge and decant. Dissolve the precipitate in 5 ml of ammonium acetate solution, warming if necessary to effect solution. If a clear solution is not obtained due to coagulated material or inorganic salts, filter into a second centrifuge bottle, washing the filter paper with 5 ml of glacial acetic acid. Add 5 ml of 3% lead acetate solution in glacial acetic acid, plus 25 ml of anhydrous ethyl alcohol. Centrifuge and decant the supernatant liquid. To the precipitate add 4 ml of 10% sodium bisulphate solution, 10 ml of 1% periodic acid solution, and 10 ml of water. Allow oxidation to take place at room temperature for 20 minutes. Destroy the excess oxidizing agent by addition of powdered sodium bisulphite. Add 4 ml of p-nitrophenylhydrazine reagent and place the centrifuge bottle in boiling water for ten minutes. Filter the solution into a separatory funnel, cool and extract the aqueous layer with 50 ml of ethyl ether. Wash the ether extract with 5 ml of distilled water and pass the washed extract through the packed adsorption column. Wash the column with 30 ml of ethyl ether and discard the washings. Elute with 20 ml of anhydrous ethyl alcohol. Dissolve about 0.1 g of solid sodium hydroxide in the eluate. A brilliant pink to red coloration is obtained if tartrates were present in the original sample. The color is stable upon dilution with an equal quantity of water.

DISCUSSION

Table 1 shows the results of the test on a number of wines and fruit acids. The wines utilized in these tests were made by Peter Valaer in the laboratory of the Alcohol Tax Unit, Washington, D. C. No question exists as to their authenticity or chance contamination, as might be the case

TABLE 1.—*Results of color tests*

SAMPLE	COLOR (ALKALINE ELUATE)	TARTRATES
Grape:		
Scuppernong	Red	Positive
Muscat	Red	Positive
Concord	Red	Positive
Muscadine	Red	Positive
Raisin	Red	Positive
Blackberry	Colorless—Faint Yellow	Negative
Strawberry	Colorless—Faint Yellow	Negative
Loganberry	Colorless—Faint Yellow	Negative
Orange	Colorless—Faint Yellow	Negative
Rhubarb	Colorless—Faint Yellow	Negative
Peach	Colorless—Faint Yellow	Negative
Honey	Colorless—Faint Yellow	Negative
Apple (Often no precipitate with 5% lead acetate)	Colorless—Faint Yellow	Negative
Elderberry (Often no precipitate with 5% lead acetate)	Colorless—Faint Yellow	Negative
Cherry	Colorless—Faint Yellow	Negative
10% Grape—90% Blackberry	Red	Positive
1% Grape—99% Blackberry	Pink	Positive
Fruit Acids: (10 mg. in 50 ml. of aqueous solution)		
Tartaric Acid, 10 mg.	Red	Positive
Citric Acid, 10 mg.	Colorless	Negative
Malic Acid, 10 mg. (No precipitate with lead acetate)		
Maleic Acid, 10 mg. (No precipitate with lead acetate)		
Isocitric Acid, 10 mg.	Colorless	Negative
Aconitic Acid, 10 mg.	Colorless	Negative

when fruit or berry juices are fermented in vats previously used to ferment grape juice. The organic acids used were of Reagent Grade.

ADSORBENTS

A number of adsorbents have been found useful for packing the adsorption column.

Powdered glass adsorbs the p-nitrophenylhydrazone of glyoxylic acid excellently from ethereal solution, offers ease of elution with anhydrous alcohol, and the solutions pass through the column rather quickly. Suction may be used with this adsorbent to speed the passage of the liquids. Powdered glass purchased from Eimer and Amend, ground glass prepared from whiskey bottles and glass wool "Corning" Brand Fibre Glass No. 008 were used with equally good results. The glass wool, however, does not give as uniform packing as the powdered glass.

Calcium carbonate, reagent grade, is a good adsorbent and may be used alone or in combination with filtercel to speed the process of adsorption and elution. Exhaustive washing with ether must be avoided as the p-nitrophenylhydrazone of glyoxylic acid is gradually eluted thereby.

Activated alumina is too powerful an adsorbent and the same may be said of calcium hydroxide, though the latter may be used with some modification of the procedure.

Anyhdrous potassium carbonate provides a good column packing.

For the separation of a mixture of p-nitrophenylhydrazones, U.S.P talc is an excellent adsorbent. Roberts and Green (6) separated the 2,4-dinitrophenylhydrazones of low molecular weight aliphatic aldehydes by chromatographic adsorption on a column of silicic acid-Super Cel. When using a column of talc, the p-nitrophenylhydrazones are adsorbed from a solution of ethyl ether-petroleum ether. The chromatogram is developed by increasingly greater concentrations of ethyl ether. For tartrate detection by this method, talc is not as suitable as powdered glass or calcium carbonate, as it must be handled with some skill to effect separations without complete elution of the desired p-nitrophenylhydrazone of glyoxylic acid.

INTERFERING SUBSTANCES

α,β-Dihydroxy acids will give positive tests under the prescribed conditions but no such acids, other than tartaric, have been reported in wines.

Polyhydroxy compounds (such as sugars, glycerine, proteins, dextrins, caramel and coloring materials) are removed by treatment with activated carbon and washing of the lead precipitate. Such compounds, if not removed, are oxidized to a variety of products which will react with p-nitrophenylhydrazine to produce p-nitrophenylhydrazones and osazones, including the p-nitrophenylhydrazone of glyoxylic acid (7). Alkaline solutions of such compounds produce powerfully colored solutions.

Tannins are very nearly quantitatively removed by the charcoal treatment. Traces remaining which form p-nitrophenylhydrazones, seem to be held on the adsorbent more firmly than the glyoxylic acid derivative, and thus do not interfere.

Amino acids from protein degradation appear the most difficult interfering substances to remove. Martin and Synge (8) have applied periodic acid oxidations to some hydroxyamino-acids of protein hydrolyzates. Any amino acid containing adjacent $-NH_2$ and $-OH$ groups is oxidized by periodic acid to aldo-acids, and as such will follow the oxidation product of tartaric acid throughout the test. Serine, for example, is oxidized to glyoxylic acid as follows:

$$HOCH_2 \cdot CHNH_2 \cdot COOH + HIO_4 \rightarrow CHO \cdot COOH + NH_4IO_3 + HCHO$$

Some p-nitrophenylhydrazones have been produced from wines which

chemically behave very similarly to the glyoxylic acid derivative, but these have been separated therefrom on a column of talc. Dakin and Dudley (9) report the slow conversion of 2-amino and 2-hydroxy acids to ketonic aldehydes.

Pectin degradation products, if not removed, will form aldo- and keto-hydroxy acids upon oxidation, and their p-nitrophenylhydrazones will give positive tests. Apple wines containing large amounts of pectin, and solutions prepared from powdered citrus pectin, have been tested by this method with negative results.

OTHER REAGENTS

In addition to p-nitrophenylhydrazine reagent, a 2,4-dinitrophenylhydrazine reagent has been employed in a similar manner. The 2,4-dinitrophenylhydrazone is more sensitive to hydroxyl ion concentration (10), but the color produced by the glyoxylic acid derivative is not as distinctive as that from the p-nitrophenylhydrazone of glyoxylic acid.

SUMMARY

A satisfactory method is presented for the detection of tartrates in quantities as small as 0.1 mg. per ml. of solution, in the presence of other fruit acids and wine ingredients. Tartaric acid has been found only in grape wine and wines produced from some grape product.

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DETERMINATION OF BORAX IN MIXED FERTILIZERS*

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The present official A.O.A.C. methods for determination of borax in mixed fertilizers have met with general dissatisfaction in recent years. In many instances a sizable fraction of the borax known to have been added has not been found, resulting in unsatisfactory reports by testing laboratories and necessitating "overdosing" with borax by the manufacturer. It is difficult to assign reasons for these errors. The situation has sometimes been confused by the non-uniform mixing of added borax, and oftentimes by the use of two different analytical methods, neither of which gives consistently good results. Usually the method employed has not been designated, so a statistical study of results which have been obtained is not feasible. It is difficult to understand why methods showing good results when first developed some years ago should now seem unreliable. One factor may be that the amounts of borax involved were usually lower in earlier use of the methods, since the primary concern was then with borax contaminated materials rather than with deliberate borax additions. Also the wide range of materials which can be present in fertilizers makes it impossible to be sure all conditions have been encountered in preliminary testing of new methods.

The A.O.A.C. methods in current use are known as the "water soluble" and "acid soluble" methods. The "water soluble" method (2) makes use of solution in water or dilute hydrochloric acid and precipitation of interfering substances by treatment with barium chloride and barium hydroxide. The "acid soluble" method (1) involves treatment of sample with phosphoric acid and distillation with methanol to remove boron from interfering substances.

It was our belief that under present conditions no useful purpose is served by having a method for "water soluble" boron. Slightly soluble borates which might form on storage of mixtures should be decomposed, dissolved and included in the analytical results, since these represent some of the added borax and presumably are essentially available for plant use. Accordingly, all work on revised methods was designed to insure solution of all borates, and no statement of "water soluble" or "acid soluble" etc., is included in the names of the methods.

It was also our belief that methods involving precipitation removal of interfering substances could be developed to adequate accuracy and precision for the purposes involved, and that such methods are more suited to intermittent use in laboratories principally concerned with other types of analyses. The methods being reported are of the precipitation

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removal type, and have been shown to be adequate in all cases tried. By adequate we mean accuracy within less than 0.10% borax (or 2 pounds borax per ton fertilizer) and precision substantially better than that ($\pm$0.03% average deviation). Errors of this amount do not seem serious with borax added in amounts ranging from 10–100 pounds per ton, though better accuracy would of course be desirable. It is possible that methods involving methyl borate distillation could ultimately be developed to give greater accuracy and precision.

PRELIMINARY STUDIES

There are two major problems involved in determination of boron oxide content of fertilizer by methods involving precipitation removal of interfering substances. Unless both of these problems are satisfactorily solved there can be no hope of accurate results. These problems are:

(a) Complete solution of boron oxide in the sample.

(b) Complete removal of interfering supstances (mainly phosphate) by precipitation without serious loss of boron oxide. (If the phosphate ion is not removed, the titrations for boron oxide will of course be high.)

These two problems are described in more detail in the following:

(a) *Complete solution of boron oxide* is not difficult to accomplish. Though water is adequate if all boron oxide is present as borax it was felt the possibility of forming slightly soluble calcium borates or borophosphates on storage prevents its general reliability. Therefore any method should insure solution of such borates, even though by so doing more interfering substances (phosphate mainly) are also put in solution. Several means for dissolving slightly soluble borates suggest themselves, and we have confined ourselves to use of (1) dilute hydrochloric acid or (2) sodium carbonate solutions.

(b) *Complete removal of interfering substances by precipitation without boron oxide loss* is more difficult. Iron and aluminum are readily precipitated in neutral solution, and phosphate is readily removed by barium hydroxide. However, when much phosphate is precipitated by barium hydroxide as in the present A.O.A.C. method there is much danger of loss of boron oxide. Our earliest work showed that if the A.O.A.C. water soluble method (for inorganic compounds) is used as directed on some fertilizers containing carbonates (slight excess of hydrochloric acid employed in dissolving) the results are very unsatisfactory. Much phosphate is dissolved and a very sticky precipitate which holds boron oxide is obtained when barium hydroxide is added. The amount of boron oxide lost seems to vary widely and erratically, and is not solely dependent on the amount of phosphate present in the solution treated. In fact simultaneous presence of much calcium ion (which is common) seems to greatly increase the difficulty.

It thus appeared that the major problem was to find some means for precipitating all the phosphate without these boron oxide losses. Attempts

to accomplish this by use of barium chloride and limited amounts of barium hydroxide or ammonium hydroxide so that the final *p*H was not so high, were not successful. Lower *p*H tended to decrease boron oxide loss but phosphate was not so adequately removed. Trial of several other possibilities uncovered one promising method, consisting of the addition of excess solid barium carbonate to an acid solution containing considerable barium chloride. The use of barium carbonate alone added to acid extracts from borate ores to precipitate impurities before boron oxide titration has been common (3). It is a very convenient way of establishing a *p*H near 7 without tedious adjustment (excess reagent merely remains undissolved). The *p*H obtained does not appear high enough to result in precipitation of all phosphoric acid, and trials demonstrated that using barium carbonate alone resulted in small amounts of phosphate left in solution. If however a considerable concentration of barium ion was already present in the solution substantially all the phosphate was precipitated. Conceivably this barium ion could be obtained by using much acid and barium carbonate, but we have preferred to add a measured amount of barium chloride to the solution (in excess of amount reacting with sulphates).

This technique which we might call the barium chloride-barium carbonate method was first tried on solutions obtained by digesting samples in dilute hydrochloric acid. On some samples very good results were obtained but on others an unexpected and curious disadvantage was discovered. Large amounts of magnesium ion in the solution being treated in some way to prevent the barium carbonate acid reaction from proceeding to completion. In extreme cases the barium carbonate will not react sufficiently to make the solution basic to methyl red (*p*H 5–6). In other cases the indicator changes but the *p*H is insufficient to quite remove the phosphate. The reason for this behavior is not known, but since dolomite is widely used in fertilizers, dissolving methods must be employed which minimize magnesium content of the solution. Use of a sodium carbonate solution is proposed. The methods required are somewhat more tedious than would be possible if the magnesium interference did not exist.

All precipitation methods tend to result in a final solution for titration which has a rather high salt content. This tends to produce a less satisfactory end point. Variations in salt concentration may also result in a variable blank which may be responsible for somewhat lower precision than might be desired. However, in general, results are believed adequate for purposes in mind.

When organic matter is present in the sample being analyzed the method which must be used is analogous but also includes a carbonizing step to destroy interfering organic matter. Also physical properties of the sample dictate some variations in manipulation, so the method has been written out in full for such cases.

METHODS

Reagents—Same as in present A.O.A.C. Method.

I. *Materials Free From Organic Matter*

Weigh a 5 g sample into a 250 ml beaker, add 40 ml of 5% Na_2CO_3 soln, 5 drops phenolphthalein (**2.10d**), and ca 35 ml of water. Boil, adding water as needed to keep volume at about 70–80 ml. Boil at least 15 min., keeping color of phenolphthalein decidedly red by adding more Na_2CO_3 soln if required. If NH_3 is present boil ½ hour after the smell of NH_3 has disappeared. Filter the hot soln and wash solids well with hot water. Add a few drops of methyl red to the soln in a 250 ml beaker, and boil until the volume is about 80–100 ml, acidify cautiously with conc. HCl (pink to methyl red), and boil a few minutes longer. Add slowly 10% $BaCl_2 \cdot 2H_2O$ soln, until it is evident that sulfates have all precipitated (probably 20–30 ml), then add 10 ml in excess. Heat mixture a few minutes near boiling and cautiously add powdered $BaCO_3$ with stirring until the soln shows the yellow of methyl red. Add a small excess and boil a few minutes. If enough $BaCO_3$ has been added a very persistent foam is obtained. Filter into a 500 ml Erlenmeyer flask, wash solids well with hot water. Cautiously acidify the filtrate with HCl to the pink of methyl red and boil a few minutes to expel CO_2. Cool, and neutralize exactly, using 0.5 *N* NaOH at first and finally 0.1 *N* NaOH, reacidifying with 0.1 *N* HCl if at first the neutral point (orange) is exceeded. Add several g of neutral mannitol, a few tenths of a ml phenolphthalein (**2.10d**) and titrate with 0.1 *N* NaOH to the first appearance of orange color due to the phenolphthalein red. (Color after mannitol addition is red, goes through yellow, to orange and red again as alkali is added.) Add a little more mannitol and if the phenolphthalein color disappears titrate carefully until it reappears. Repeat this procedure until addition of mannitol has no further action on the color. Note volume of 0.1 *N* NaOH used. Run a blank using the same amount of reagents and subtract the amount of 0.1 *N* NaOH required for the blank from the gross titration. Calculate the borax content of the sample.

$$\% \ Na_2B_4O_7 \cdot 10H_2O = \frac{\text{net ml } 0.1 \ N \text{ NaOH} \times 0.954}{\text{g sample}}$$

II. *Materials Containing Organic Matter*

Weigh a 5 g sample into a 250 ml beaker, add 40 ml of 5% Na_2CO_3 soln, 5 drops of phenolphthalein, and about 35 ml of water. Boil the mixture gently for a few minutes. (Care is required to prevent frothing over.) If mixture is not alkaline to phenolphthalein (external spot test if necessary) add more Na_2CO_3 soln and heat a very short time longer. Filter the hot soln and wash solids well with hot water. Place filtrate and washings in a 250 ml beaker, boil until volume is ca 80 ml, acidify cautiously with HCl until acid to methyl red (external spot test may be necessary), and boil a few minutes longer. Add 10% $BaCl_2 \cdot 2H_2O$ soln slowly until sulfates are precipitated (perhaps 10–20 ml) and then 10 ml in excess. Heat mixture near boiling and add enough $BaCO_3$ to make alkaline to methyl red (external spot test may be required) followed by a small excess. Boil briefly and filter the hot soln, washing solids well with hot water. Put filtrate and washings in a large casserole, add $Ba(OH)_2$ solid to keep alkaline to phenolphthalein, and evaporate to dryness. When dry, carbonize the organic matter thoroly just below redness (500–550°C. in muffle furnace preferably). When cool, add 50 ml hot water, methyl red indicator, and just enough HCl to make acid; heat to dissolve. When soln is complete and hot soln is acid, add excess solid $BaCO_3$, boil briefly, and filter into a 500 ml Erlenmeyer flask, wash solids well with hot water. Cautiously acidify the filtrate with HCl and boil a

few minutes to expel CO_2. Cool and neutralize exactly using 0.5 *N* NaOH at first and finally 0.1 *N* NaOH, reacidifying with 0.1 *N* HCl if at first the neutral point (orange) is exceeded. Add several grams of neutral mannitol, a few tenths of a ml phenolphthalein **(2.10d)** and titrate with 0.1 *N* NaOH to the first appearance of the phenolphthalein red. Add a little more mannitol and if the phenolphthalein color disappears titrate carefully until it reappears. Repeat this procedure until addition of mannitol has no further action on the color. Note volume of 0.1 *N* NaOH used. Run a blank using the same amount of reagents and subtract the amount of 0.1 *N* NaOH required for the blank from the gross titration. Calculate the borax content of the sample.

$$\% \ Na_2B_4O_7 \cdot 10H_2O = \frac{\text{net ml 0.1 } N \text{ NaOH} \times 0.954}{\text{g sample}}$$

NOTES: (1) In this laboratory it is preferred to use suction with a platinum cone for all filtrations.

(2) Throughout these procedures care should be exercised to avoid more reagents than necessary. Over additions of acid and alkali build up the salt content of final solution with some effect on accuracy of titration.

(3) In this laboratory methylene blue is often added to the solution during titration as the color changes (purple to green to purple) are somewhat easier to observe. The amount to add can be found by trial, and should not be so great that it tends to mask the indicator color changes.

SAMPLES USED

Fertilizer No. 1—*F. S. Royster Guano Company 0–12–12 Fertilizer*, said to contain KCl, superphosphate, and about 4% borax. Seemed to contain dolomite also.

Fertilizer No. 2—*Magruder Check Sample No. 9, 2–12–2 Fertilizer*, prepared by F. S. Royster Guano Company. Borax content 4.40% (88 pounds per ton).

Fertilizer No. 4—*Laboratory Mixture as Follows*:

19% Ammonium Sulfate (Ford Motor Company)
16% KCl } (Supplied by F. S. Royster
35% Superphosphate } Guano Co., Norfolk, Va.)
30% Pulverized Dolomite (U. S. Lime Products Corp., Los Angeles, California)

Fertilizer No. 5—*Laboratory Mixture as Follows*:

19% Ammonium Sulfate (Ford Motor Company)
16% KCl } (Supplied by F. S. Royster
40% Superphosphate } Guano Co., Norfolk, Va.)
25% Ground Limestone

Fertilizer No. 6—*Made by Adding 10% Royster Superphosphate to "Red Star Gro-Master V"* (4–10–2). Said to contain blood meal, bone meal, cottonseed meal, superphosphate, KCl and K_2SO_4. Manufactured by Downey Fertilizer Company, Downey, California.

RESULTS

Test results on mixtures using four different inorganic fertilizers and one organic fertilizer are shown in Table 1. In most cases comparative results

by the A.O.A.C. water soluble method were obtained and are shown. The data include results obtained by three different operators.

Where not otherwise indicated, the borax additions were made just before analysis. In all such cases the agreement with the amount added is good by the new methods, and generally very poor by the A.O.A.C. water soluble method.

Some samples with borax were stored six months at 35–40°C. before

TABLE 1.—*Test results*

FERTILIZER USED, NO.	% BORAX ADDED	SPECIAL TREATMENT	RESULTS BY A.O.A.C. WATER SOLUBLE METHODS			RESULTS BY PROPOSED METHODS		
			% BORAX FOUND	DEVIATIONS, % BORAX	AV. % BORAX FOUND	% BORAX FOUND	DEVIATIONS, % BORAX	AV. % BORAX FOUND
1	0	—	1.90			3.45	0.00	3.45
			1.47			3.45	0.00	
			1.60	?	?	3.40	−0.05	
			2.10			3.45	0.00	
			1.90			3.45	0.00	
			2.03			3.40	−0.05	
						3.47	+0.02	
						3.48	+0.03	
						3.48	+0.03	
	2.00	—	—			5.42	−0.03	5.45
						5.42	−0.03	
						5.43	−0.02	
						5.50	+0.05	
						5.48	+0.03	
						5.47	+0.02	
2	0	Contains 4.40% Borax				4.28	−0.03	4.31
						4.32	+0.01	
						4.33	+0.02	
4	0	—	0.03		0.04	0.07		0.06
			0.04			0.05		
						0.06		
	0.50					0.54	−0.03	0.57
						0.60	+0.03	
						0.58	+0.01	
	2.00		0.98	+0.05	0.93	2.03	−0.03	2.06
			0.90	−0.00		2.01	−0.05	
			0.85	−0.08		2.04	−0.02	
			1.01	+0.08		2.06	0.00	
						2.08	+0.02	
						2.05	−0.01	
						2.11	+0.05	

TABLE 1—(*continued*)

FERTILIZER USED, NO.	% BORAX ADDED	SPECIAL TREATMENT	RESULTS BY A.O.A.C. WATER SOLUBLE METHODS			RESULTS BY PROPOSED METHODS		
			% BORAX FOUND	DEVIATIONS, % BORAX	AV. % BORAX FOUND	% BORAX FOUND	DEVIATIONS, % BORAX	AV. % BORAX FOUND
4	0	Stored 6 months 35–40°C.	0.04 0.05		0.04	0.07 0.03 0.02	+0.03 −0.01 −0.02	0.04
	0.50	Stored 6 months 35–40°C.	0.64 0.25 0.20	+0.28 −0.14 −0.16	0.36	0.59 0.54 0.59	+0.02 −0.03 +0.02	0.57
	1.00	Stored 6 months 35–40°C.	0.34 0.38 0.22	+0.03 +0.07 −0.09	0.31	1.07 1.09 1.13	−0.03 −0.01 +0.03	1.10
	2.00	Stored 6 months 35–40°C.	0.79 0.78 0.85	−0.02 −0.03 +0.04	0.81	2.19 2.12 2.08	+0.06 −0.01 −0.05	2.13
	6.00	Stored 6 months 35–40°C.	3.64 3.24 3.32	+0.24 −0.16 −0.08	3.40	6.50 6.55 6.46	0.00 +0.05 −0.04	6.50
5	0	—	—	—	—	0.03 0.06		0.05
	1.00	—	—	—	—	0.99 1.08 0.99 1.08	−0.05 +0.04 −0.05 +0.04	1.04
	2.00	—	—	—	—	2.04 1.99 2.06 2.02	+0.01 −0.04 +0.03 −0.01	2.03
	4.00					4.00 3.99 4.03 3.95	+0.01 0.00 +0.04 −0.04	3.99
	0	Stored 6 months 35–40°C.	0.16 0.09 0.16	+0.02 −0.05 +0.02	0.14	0.11 0.11 0.07	+0.01 +0.01 −0.03	0.10

TABLE 1—(*continued*)

FERTILIZER USED, NO.	% BORAX ADDED	SPECIAL TREATMENT	RESULTS BY A.O.A.C. WATER SOLUBLE METHODS			RESULTS BY PROPOSED METHODS		
			% BORAX FOUND	DEVIATIONS, % BORAX	AV. % BORAX FOUND	% BORAX FOUND	DEVIATIONS, % BORAX	AV. % BORAX FOUND
5	0.50	Stored 6 months 35–40°C.	0.27 0.25 0.28	0.00 −0.02 +0.01	0.27	0.76 0.60 0.57 0.63 0.44 0.51	+0.17 +0.00 −0.02 +0.04 −0.15 −0.08	0.59
	1.00	Stored 6 months 35–40°C.	0.75 0.46 0.51	+0.18 −0.11 −0.06	0.57	1.05 1.03 1.14	−0.02 −0.04 +0.07	1.07
	2.00	Stored 6 months 35–40°C.	1.03 0.93 0.33	+0.27 +0.17 −0.43	0.76	2.16 2.06 2.06	+0.07 −0.03 −0.03	2.09
	6.00	Stored 6 months 35–40°C	4.42 4.15 2.78	+0.64 +0.37 −1.00	3.78	6.26 6.35 6.60 6.36 6.21	−0.10 −0.01 +0.24 0.00 −0.10	6.36
6 (Organic)	0.00	—	0.05 0.07 0.08	−0.02 0.00 +0.01	0.07	0.07 0.06 0.08	0.00 −0.01 +0.01	0.07
	1.00		0.95 1.00 1.02	−0.04 +0.01 +0.03	0.99	—	—	—
	2.00	—	—	—	—	2.00 2.02 2.03 2.04 2.06 2.06 2.08 2.09 2.07	−0.05 −0.03 −0.02 −0.01 +0.01 +0.01 +0.03 +0.04 +0.02	2.05
	4.00	—	3.89 3.94 4.03	−0.06 −0.01 +0.08	3.95	4.10 4.10 4.13 4.14 4.14	−0.02 −0.02 +0.01 +0.02 +0.02	4.12

analysis. The results by the proposed method are somewhat higher than expected (presumably due to water loss from samples) but show satisfactory reproducibility. Results by the A.O.A.C. method were erratic and low in all cases (HCl was used since carbonates were present and this may have increased difficulty with the method.)

The average deviation of all results shown by the proposed methods is $\pm 0.03\%$ borax which seems entirely satisfactory.

The result obtained (4.30%) by the new method on the Magruder Check Sample is close to its reported borax content (4.40%).

SUMMARY

Methods for the determination of borax in mixed fertilizers which involve dissolving in sodium carbonate solution and removal of interferences by barium chloride and barium carbonate addition to the acidified extract have been developed. The procedures have been tried on a number of samples and found reasonably accurate and precise. Analysis of the same samples by the A.O.A.C. "water soluble" methods were generally very unsatisfactory.

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STUDY OF THE ENZYMATIC OXIDATION OF THIOUREA IN FROZEN PEACHES

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INTRODUCTION

Early in 1946, the author obtained incomplete recoveries of known amounts of thiourea added to frozen fresh peaches, although good recoveries had been obtained on orange juice by a similar method of analysis (1). Good recoveries could be obtained if the frozen peaches with added thiourea were immersed immediately in boiling water or in a solution of sodium sulfite. These findings led to the conclusion that the enzyme systems of the peach destroy the thiourea. The nature of the reaction between

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the enzyme systems and the thiourea was then studied, and the results of the investigations are reported herein.

HISTORICAL

Investigators (2, 3, 4, 5, 6) have shown that the oxidation of thiourea with chemical oxidizing agents takes place in at least four stages, depending on the agents and the conditions of oxidation. The following successive oxidation products have been identified:

First. Formamidine disulfide salts
Second. Formamidine sulfinic acid (Dioxide of thiourea).
Third. Formamidine sulfonic acid (Trioxide of thiourea).
Fourth. Urea and sulfuric acid.

VARIABLE ACTIVITY OF THE OXIDIZING SYSTEMS OF PEACHES ON THIOUREA

The frozen peaches were cut into sections and a sample of 200–400 grams, consisting of alternate sections, was weighed in a beaker. To this sample was added one-half its weight of a water solution containing a known quantity of thiourea. From the remaining alternate sections a control sample of equal weight was prepared to which was added one half its weight of water containing no thiourea. The cold material was then thoroughly comminuted in a Waring blendor and allowed to stand for some time at room temperature to permit the oxidizing reaction to proceed.

The samples to which were added varying amounts of thiourea, and which had stood for varying lengths of time, were examined for residual thiourea by a published (1) procedure. A wide variation was found in the activity of the oxidizing systems of different peach samples. Many samples did not destroy thiourea in amounts greater than 25 or 30 p.p.m., and quantities added in excess of this remained unchanged, or were oxidized extremely slowly. Other samples were found to oxidize 80–100 p.p.m. of added thiourea, while a few were capable of readily destroying 130–140 p.p.m. The latter peaches were chiefly used in the search for formamidine disulfide salts and other oxidation products of thiourea as described below.

Search for sulfate and urea.—As stated above, sulfuric acid and urea may be final products of the oxidation of thiourea. In order to determine if the sulfate normally occurring in peaches can be uniformly removed by an aqueous extraction, several sulfate analyses were made. The results showed that it was not uniformly extracted. Therefore, any sulfate which might be formed from thiourea could not be accurately measured and no further effort was made to use sulfate as an index of the progress of oxidation of the added thiourea.

In order to establish definitely whether the oxidation of thiourea in peaches proceeds to the urea stage, tests for urea were made by various methods. The xanthydrol method (7) was modified so that it could be applied to the peach solutions after (a) clarification with ethyl acetate or ether, (b) precipitation with acetone, and (c) evaporation of the acetone. Fifteen ml portions of the solutions were usually taken, and the quantity of reagents was correspondingly increased. The amounts of thiourea added were 79, 110, and 178 p.p.m. Controls with and without added urea were also run. The test gave only an opalescent solution on samples, with or without added thiourea, indicating little or no urea present. The control with 0.5 mg added urea gave a positive test in 10–15 minutes.

Preliminary tests for urea by the urease method were inconclusive, but the effects of the sugar and salt concentration on the urease were not fully appreciated at that time. Preparatory to applying the method to frozen peaches with their natural content of sugars, acid, etc., a study of these effects was conducted and some of the results are given in Table 1.

TABLE 1.—*Urease activity in various solutions of sugars and salts, using ½ grain of urease per determination*

TEST NO.	COMPOSITION OF AQUEOUS TEST SOLUTION	UREA ADDED	TOTAL VOLUME OF SOLUTION	TIME OF UREASE ACTION	ALKALI ADDED FOR DISTILLATION NH_3	0.02 *N* HCl CONSUMED
		mg		*minutes*		*ml*
1	Water only	10	100	30	13 ml 15% NaOH	16.4
2	10% sucrose	10	100	40	13 ml 15% NaOH	13.5
3	8% sucrose 2% dextrose	10	100	40	2 g. MgO	12.21
4	6% sucrose 1.5% dextrose	10	100	90	2 g. MgO	16.17
5	2.5% dextrose 5% sucrose 0.33% malic acid NaOH added to *p*H 6.9	5	120	105	2 g. MgO	6.01
6	Water and urease only	0	100	45	2 g. MgO	0.32

Tests 1, 2, 3, and 4 show that 10 per cent, but not 7.5 per cent, of sugars reduces the activity of the urease. Test 5 shows that sodium malate formed by the neutralization of malic acid with sodium hydroxide also reduces the activity of the urease.

Finally, a series of tests was conducted where there could be no possibility of the inhibition of urease by sugar, salt, etc., and where the urease was acting at the optimum *p*H.

A carton of commercial frozen peaches containing no thiourea was divided into two portions as in previous work, and 100 p.p.m. of thiourea were added to one portion. Both portions were held at room temperature until the thiourea portion gave a negative Grote test. (The test was also negative on the control portion.) Fifty gram samples of each portion were diluted to 220 ml and distilled with magnesium oxide instead of sodium hydroxide to minimize breakdown of organic matter. The distillate from the control had a titer of 1.75 ml of 0.02 *N* HCl; that from the thiourea portions had a titer of 2.0 ml. The experiment was repeated on similar pair of samples which had been treated with urease at *p*H 6.8–6.9 before distillation. The titers of the distillates were 2.5 and 2.8 ml. respectively. Thus any difference in the ammonia content between control and thiourea samples was no greater with the urease treatment than without it. This furnishes definite evidence that urea is not present in frozen peaches as an oxidation product of thiourea.

Search for formamidine disulfide cleavage products.—The formamidine disulfide salts, particularly the salts of organic acids, are unstable and suffer disintegration by molecular cleavage. At least two disintegration patterns have been suggested in the literature. Boeseken's mechanism for the breakdown would produce free sulfur, sulfocyanic acid, cyanamide, and ammonia. (2) Werner claims that the products of the molecular disintegration are thiourea, free sulfur, and cyanamide. (4) To determine which if any, of these products were formed by cleavage of the disulfide salts in solution, two salts (sulfate and hydrochloride) of formamidine disulfide were prepared (2) The reactions of these salts were studied, and tests were made for the products of decomposition. The disulfide salts gave a positive reaction with Grote's reagent similar to thiourea. This is in accord with Grote's (8) original findings that compounds having the S—S linkage give a positive reaction with the reagent.

Aqueous solutions of the prepared salts after standing some time were examined for sulfocyanic acid and cyanamide. Tests for sulfocyanic acid proved negative on the solution of the sulfuric acid salt and only a very faint positive test was obtained on the solution of the hydrochloride salt. The test (see below) for cyanamide showed an abundance of this degradation product to be present in the solution from both of the salts of the disulfide. The test used (bright yellow amorphous precipitate in ammoniacal silver nitrate solution) is characteristic of cyanamide. (2)

The formamidine disulfide salt solution continued to give a strong positive Grote reaction on further standing. These findings suggest that the breakdown is probably along the Werner pattern. The breakdown in this manner would reproduce one-half of the original thiourea. In peaches this

reformed thiourea would in turn be acted on by the enzyme systems of the peaches. Eventually, according to this theory, all residual thiourea in peaches would be converted to cyanamide and free sulfur.

In order not to overlook any possible cleavage products, the fruit samples, with and without added thiourea, were examined for ammonia, sulfocyanic acid, and cyanamide. Tests for sulfocyanic acid were negative on all samples. Results of ammonia tests on thiourea-treated samples were not significantly different from those of the controls.

The tests for cyanamide on all of the fruit samples, were negative. As a check on the method, cyanamide was prepared in the laboratory and a small amount (2–3 mg per cent) was added to control samples. Strong positive cyanamide reactions were obtained.

The test for cyanamide was made by the following method devised by the author and based on some of the known properties of cyanamide.

METHOD FOR DETECTION OF CYANAMIDE

Place 100 ml of sample soln in a 500 ml separatory funnel. Extract liquid by shaking with 300 ml of ether (washed to remove alcohol), allow layers to separate and draw off aqueous layer into second 500 ml separatory funnel; then pour off the ether layer thru filter into a distillation flask. Rinse first funnel with 300 ml of washed ether and run into second funnel. Extract sample as before, and repeat extraction a third time in similar manner. Combine three ether extracts in distillation apparatus and distil off most of ether until only about 30–50 ml remain. Transfer remainder to a 250 ml beaker marked at 40 ml, rinsing the distillation flask 2 or 3 times with 10–15 ml portions of ether. Evaporate to 40 ml mark and pour ethereal liquid into 125 ml separatory funnel. Add 10 ml of water to beaker, rinse, and pour into funnel containing ether extract.

Extract ether solution with water and repeat with a second 10 ml portion of water. Combine water extracts and test 5 ml portion with Ca 1 ml of weakly ammoniacal soln of $AgNO_3$. An immediate bright yellow amorphous precipitate indicates cyanamide. For additional confirmation, determine nitrogen by micro-kjeldahl in the remaining 15 ml of aqueous extraction of ether extract.

According to the literature (9), cyanamide hydrolyzes to urea; but this hydrolysis proceeds very slowly, if at all, at room temperature in neutral or weak acid solution, as shown by the fact that dilute solutions remained for weeks in the laboratory with little if any noticeable change in the strength of the positive test.

It is therefore apparent that the oxidation of thiourea proceeds rapidly through and beyond the disulfide stage and also that the disulfide does not break down into molecular cleavage products.

SEARCH FOR OXIDES OF THIOUREA

Since previous experiments had shown that the thiourea oxidation product produced by the fruit enzymes is neither formamidine disulfide nor urea, it seemed reasonable to suppose that it must be an oxide, intermediate between these two degradation products.

The following test showed that the oxidation product is one or both of the oxides of thiourea:

In establishing absence of urea, it had been shown that little if any ammonia is formed when thiourea is acted on by the enzyme systems of peaches. Previous work has shown that hydrogen peroxide in alkaline solution oxidizes thiourea or its intermediate oxidation products to urea and sulfuric acid. An experiment was conducted in which alternate sections of the same lot of frozen peaches, with and without added thiourea, were allowed to stand until the test for thiourea on the treated sample was negative. The resulting sample solution was then oxidized with hydrogen peroxide in alkaline sodium hydroxide solution, adjusted to *p*H 6.9, treated with urease and subsequently distilled over magnesium oxide. A 50 gram sample containing no added thiourea showed a titer of 2.85 ml of 0.02 ammonium chloride, and a 50 gram sample containing 100 p.p.m. of added thiourea showed a titer of 6.15 ml. This experiment, together with the previous tests showing absence of urea in the enzymatically oxidized solution, of portions of the same sample, conclusively showed that the increase in ammonia came from the intermediate oxidation products *i.e.*, oxides of thiourea.

Test for dioxide of thiourea.—Boeseken (2, 3) states that, in alkaline solution, the dioxide of thiourea is a very powerful reducing substance; it reduces solutions of numerous metallic salts to the free metal with the application of but little heat; that solutions of the dioxide are unstable on standing, dilute solutions becoming acid due to the formation of sulfurous acid; that alkaline solutions sodium hydroxide of the dioxide break down with the formation of cyanamide.

The dioxide of thiourea was prepared following the method of Barnett (5). No detectable amount of sulfurous acid or cyanamide was found in very dilute solutions of this chemical, comparable in strength to those which would be obtained in the examination of frozen peaches. Reactions of the dioxide with metallic salts were tried but could not be applied effectively under the conditions existing in the peach solutions. Although these tests are not sensitive enough to detect the decomposition products of the dioxide in the quantities in which they would be formed in peaches from added thiourea, it is believed, however, that because of its strong reducing properties, no significant amount of the dioxide would exist as such in the peaches for any appreciable length of time.

Test for trioxide of thiourea.—The literature (3) states that the trioxide of thiourea, when treated with barium hydroxide, breaks down immediately to give barium sulfite and cyanamide. This was confirmed when a 10 mg per cent solution of the trioxide (prepared according to Boeseken's directions (3) gave an immediate precipitate with barium hydroxide. This precipitate was soluble in hydrochloric acid with a strong odor of sulphur dioxide. The filtrate gave the bright yellow precipitate with ammoniacal silver nitrate characteristic of cyanamid.

As in the other tests for urea, divided samples of frozen peaches were prepared, and both the control and the thiourea portions were extracted

with ether as in the tests for disulfide. The ether extracts were tested for cyanamid with ammoniacal silver nitrate both before and after treatment with barium hydroxide. Nitrogen determinations were also made on the acidified ether extracts by a micro-Kjeldahl method. The results are shown in Table 2.

TABLE 2.—*Comparative results*

	MODIFIED GROTE TEST	CYANAMID TEST		NITROGEN Ml 0.01 *N* HCl[2]
		BEFORE $Ba(OH)_2$	AFTER $Ba(OH)_2$	
Control No. 1	Negative	—	Negative	0.23
Control No. 1+100 p.p.m. thiourea	Negative	Negative	Positive	0.8[3]
Control No. 2	Negative	—	Negative	0.25
Control No. 2+119 p.p.m. thiourea	42 p.p.m.	Faint Positive	Strong Positive	2.23

[1] 0.05 mg cyanamid contained nitrogen equivalent to 0.91 ml 0.01 *N* HCl.
[2] Only about one-fourth of the ether extract used for determination.

It thus appears that the trioxide of thiourea is the principal decomposition product of the enzyme oxidation of thiourea in peaches.

SUMMARY AND CONCLUSIONS

The reactions of thiourea with the enzyme oxidizing systems of frozen peaches were studied. No evidence was obtained of the presence of formamidine disulfide, dioxide of thiourea, or of significant amounts of ammonia or urea, although these substances have been reported in the literature as being products of the oxidation of thiourea, with chemical oxidizing agents. Strong tests were obtained for trioxide of thiourea, and it is concluded that the reaction of thiourea with the oxidizing systems of peaches proceeds rapidly to the stage where the trioxide of thiourea is formed, and that the end product is chiefly, if not entirely, that compound.

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DETERMINATION OF EXCHANGEABLE HYDROGEN AND LIME REQUIREMENT OF SOILS

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Of late the term "exchangeable hydrogen" has assumed the connotation that was formerly assigned to such topics as "soil acidity" and "lime requirement." The problem of quantitative determination of exchangeable hydrogen in soils may, therefore, be viewed as essentially the same problem that has engaged the attention of soil investigators for more than half a century. During the past decade the *p*H determination by means of the glass electrode has been employed almost universally as an indication of soil acidity. Investigators (2, 5, 16, 18) who had wide experience with this type of determination agree that the *p*H, without detail consideration of physical and chemical properties of a soil, renders an incomplete picture as to the lime requirement of such soil. There is yet a need for a simple but reliable method for the determination of the quantitative lime requirement, or exchangeable hydrogen at some definite level of saturation.

CONCEPTS OF "LIME REQUIREMENTS"

In 1903 Veitch (24) posed two alternatives as to the fundamental concepts of lime requirement: "To neutralize soil so that it shows faintly alkaline reaction, or to neutralize only to the extent as to give satisfactory yield of acid-sensitive plants, like red clover." He concluded that "A soil faintly alkaline from carbonate of lime furnishes the best conditions for the economic production of crops, and the use of any method for estimating the acidity or the lime requirements of soils should be based on this hypothesis." The question was raised again in 1917 in a report on "the status of the problem of lime requirement" by MacIntire (14), wherein he queries, "By lime requirement do we mean its maximum ability to fix CaO through decomposition of applied lime compounds in the laboratory alone or do we intend that the laboratory practice shall approach at least a correlation to crop response under field practice?" He then proposes a definition of " lime requirement" as "the soil's maximum capacity to decompose $CaCO_3$ in the laboratory." In 1933, under the caption "Criteria of Base Saturation of Soils," Bradfield and Allison (3) proposed the definition of a "base saturated soil" as "one which has reached equilibrium with a surplus of $CaCO_3$ at the partial pressure of CO_2 existing in the atmosphere and at a temperature of 25°C." The above citations from three outstanding authorities on liming show agreement in principle as to what should constitute a "base saturated" soil, namely, one that will not react further with an excess of calcium carbonate. The definition proposed by Bradfield and Allison is obviously a departure from the concept of exchange neutrality as enunciated by Gedroiz (7)

and by Kelley (12) which is to the effect that a soil is "base-saturated" when it will not release hydrogen ions when in contact with a neutral salt solution. Although Bradfield and Allison recognized this difference, they saw no cogent reason why the additional hydrogen between *p*H 7 and that of the soil-calcium carbonate equilibrium value should not be included in the calculation of base-absorption capacity of soils. At present there is scarcely any knowledge as to actual quantitative difference in base absorption as arrived at by these two standards for base saturation. The soil-calcium carbonate equilibrium concept has the advantage that it is defined by soil contact with natural liming materials and under natural conditions of contact.

Two distinct problems arise from the adoption of calcium carbonate-equilibration as a standard for a base-saturated soil:

(1) There must be provided a feasible procedure for attainment of the soil calcium carbonate equilibrium in accordance with the above definition.

(2) There must be established a correlation between this saturation standard and the degree of saturation necessary for optimum crop production obtained on various types of soils and as recognized in field practice.

LABORATORY PROCEDURES

Because the reaction of the soil with solid calcium carbonate is too slow as a laboratory procedure there had been a number of methods proposed which were thought by their proponents to simulate the soil-calcium carbonate reaction equilibrium, and which would qualify as laboratory procedures. The Veitch calcium hydroxide serial titration procedure (24) is one of the oldest of such methods. It was used extensively in the past and continues in use with some modification in more recent times (6). The calcium bicarbonate titration procedure proposed by MacIntire (13) and a similar procedure by Hutchinson and McLennan (10) have not found wide application. Bradfield and Allison (3) proposed a procedure which depends on the preliminary reaction of the soil with an excess of calcium hydroxide followed by carbon dioxide passage and equilibration with out-door air and determination of the residual carbonate. This method was tested and found by these investigators to represent equilibrium, similar to the system: soil-calcium carbonate-water-air obtained under natural conditions. In a recent review of "chemical methods for estimating lime needs of soils," Peech and Bradfield (18) refer to this method as standard for comparison in the determination of exchangeable hydrogen, as well as in the rapid determination of the lime requirement of soils, but as one not suited for routine work. The serial potentiometric titrations with calcium hydroxide described by Dunn (6) and the titration with calcium hydroxide in the presence of calcium chloride as described by Hardy and Lewis (8) are given in the same paper as types

of the better lime requirement procedures. The Bradfield and Allison *N* ammonium chloride-0.01 *N* ammonium hydroxide titration method, and the ammonium acetate method of Brown (4) in which the exchangeable hydrogen is read from the change in *p*H in relation to ammonium acetate-acetic acid calibration curve, are cited as rapid routine methods. The most serious objection to the two last-mentioned procedures is that the equilibrium *p*H value is a variable quantity and the extent of hydrogen replacement will in each case depend upon the hydrogen content of the soil sample under test. Thus, among the various chemical methods proposed, the Bradfield and Allison calcium carbonate equilibration procedure is the only method that has been subjected to extensive laboratory tests and found to conform to the calcium carbonate-soil equilibration concept of a "base-saturated" soil.

Examination of the data presented by Bradfield and Allison (3, p. 77) on the comparative hydrogen replacement efficiencies of several procedures shows that, on the two soils tested, the barium-acetate method of Parker (17) has produced hydrogen replacements equal to those indicated by the "residual carbonate" method. The significance of these results was not commented upon by Bradfield and Allison, and one is bound to draw the conclusion that the barium-acetate solution of *p*H 7 is capable of replacing hydrogen ions from soils to an extent equal to that of the calcium carbonate equilibration at *p*H 8.4. If that conclusion is correct, it is surprising that so little attention has been given to the barium-acetate procedure with the view of adopting it to the rapid determination of exchangeable hydrogen in soils.

TITRATION OF EXCHANGEABLE HYDROGEN IN Ba- AND Ca- ACETATE

This possibility was investigated with the use of both calcium-acetate and barium-acetate. A portion of the data was presented by us (21) in a report on exchangeable hydrogen at the A.O.A.C. meeting in 1941. It was shown that 1 *N* calcium-acetate solution in contact with soils in the ratio of 1:5 has effected a hydrogen replacement from 50 to 60 per cent of that attained by titration to *p*H 7. The extent of the initial hydrogen replacement varied with time of contact, frequency of agitation, and hydrogen content of charge. A 2-hour continuous agitation has produced a hydrogen replacement from 60 to 70 per cent, and as much as 80 per cent in soils of high organic matter content. A method then proposed consisted of preliminary 2-hour contact with agitation of 10 g. of soil, in duplicate marked A and B, with 50 ml 1 *N* calcium-acetate of *p*H 7 and the determination of the initial hydrogen replacement from the determined *p*H of the system in relation to similar readings on a calcium acetate-acetic acid calibration curve. To A was added twice the quantity of calcium hydroxide that was indicated in the initial replacement, and

TABLE 1.—*Exchangeable hydrogen, as determined by ammonium acetate, by titration with $Ca(OH)_2$ and $Ba(OH)_2$ in the respective acetate solutions, in millequivalents per 100 g of soil*

NO.	SOIL TYPE	BY 1 *N* NH_4 ACETATE			BY $Ba(OH)_2$ Ba-ACETATE	BY $Ca(OH)_2$ Ca-ACETATE	Ca- & Ba-ACETATE AVERAGE	DIFFERENCES Ca & Ba AVERAGE-NH_4-ACETATE
		EXCHANGE CAPACITY	REPLACEABLE BASES	H, BY DIFFERENCE				
1.	Apison silt loam	5.0	2.9	2.1	2.4	2.6	2.5	+ 0.4
2.	Bolton silt loam	12.1	7.7	4.4	5.4	5.1	5.3	+ 0.9
3.	Conasauga silt loam	10.1	7.4	3.7	4.0	4.0	4.0	+ 0.3
4.	Fullerton silt loam	5.9	2.3	3.6	4.2	4.2	4.2	+ 0.6
5.	Hartsells sandy loam	6.1	1.8	4.3	5.2	5.7	5.5	+ 1.2
6.	Sequoia silt loam	8.8	3.1	5.7	6.0	6.4	6.2	+ 0.5
7.	Tellico sandy loam	12.4	5.8	6.6	7.9	7.6	7.8	+ 1.2
8.	Cumberland clay subsoil	10.8	3.6	7.2	5.0	5.2	5.1	− 2.1
9.	Miami sandy loam	13.2	1.0	12.2	14.6			+ 2.4
10.	Wooster silt loam	11.3	4.0	7.3	7.8			+ 0.5
11.	Drummer clay loam	27.4	22.6	4.8	8.3			+ 4.5
12.	Carrington silt loam, B	23.5	21.0	2.5	3.4			+ 0.9
13.	Peat	97.0	21.1	76.0	123.0			+47.0

to B, 20 per cent more than that added to A. After 24 hours' contact with agitation the *p*H values were determined, and recorded on cross section paper that gives *p*H against m.e. of base added, and the base requirement at *p*H 7.0 was read from the intersection of the 2-point line with that of *p*H 7. It may be noted that the rapid ammonium acetate method proposed by Brown (4) follows that part of our titration technique which was designated by us only as "preliminary test." By the Brown procedure this equilibrium hydrogen replacement, however, is taken as the total exchangeable hydrogen.

Our additional work consisted of the determinations of exchangeable hydrogen by ammonium acetate, and by titration with calcium hydroxide and barium hydroxide in the respective acetate solutions on a number of soils. A comparison of these results is given in Table 1. The exchangeable hydrogen values as obtained by barium hydroxide titrations to *p*H 7 in barium acetate were compared further with calcium absorptions in soil systems that contained an excess of 100-mesh calcite.

The data in Table 2 indicate that there is little difference as to the exchangeable hydrogen values, whether obtained by titration in barium-acetate or in calcium-acetate. In every instance the titration to *p*H 7 gave higher results for exchangeable hydrogen than those obtained by the ammonium acetate. The disparities become increasingly high with increase in organic matter content, as is shown by soils Nos. 9, 11, and 13. The barium hydroxide titration in barium-acetate has the advantage over that with calcium hydroxide in calcium-acetate in that the barium hydroxide can be prepared in moderate concentrations and also because the barium-acetate stock solution is less subject to spoilage thru mold growth. The titrations of exchangeable hydrogen by barium hydroxide were carried out in a manner similar to that described (21), except that 5 experimental points were obtained for plotting the titration curves about *p*H 7.

$CaCO_3$ DECOMPOSITIONS BY SOILS IN RELATION TO TITRATION BY $Ba(OH)_2$ IN Ba-ACETATE TO *p*H 7

Seven soils and a clay subsoil representative of East Tennessee soil types were mixed with 100-mesh calcite in quantities that approximated 80, 100, 120, 140, and 160 per cent of the base requirement indicated by the titration curve to *p*H 7. These soil mixtures were incubated in an oven at 32°C. and at a moisture content of about 80 per cent of water holding capacities. After 90 days, the soils were air-dried, ground to pass 0.5-mm sieve and the carbonate-carbon dioxide determined on the untreated as well as the calcite treated soils by the steam distillation procedure of Shaw and MacIntire (22). The *p*H values were also determined by the glass electrode in water suspensions of a pasty consistency.

The quantities of calcite applied residual carbonate after 90 days, the

TABLE 2.—*Calcium absorptions and resultant pH values of soils treated with calcite, 90 days after addition*

NO.	SOIL AND BASE REQUIREMENT AT pH7*	$CaCO_3$—M.E. PER 100 G			pH	NO.	SOIL AND BASE REQUIREMENT AT pH7*	$CaCO_3$—M.E. PER 100 G			pH
		APPLIED	RESIDUAL	ABSORBED				APPLIED	RESIDUAL	ABSORBED	
1.	Apison silt loam	0	.30	—	6.0	5.	Hartsells sandy loam	0	.35	—	5.2
	m.e.	2.24	.30	2.2	6.9		m.e.	4.56	.35	4.5	6.9
	2.5	2.80	.55	2.5	7.2		5.5	5.70	.60	5.4	7.0
		3.36	.75	2.9	7.3			6.84	1.15	6.0	7.4
		3.92	1.10	3.1	7.5			7.98	1.75	6.6	7.5
		4.48	1.45	3.3	7.6			9.12	2.75	6.7	7.6
2.	Bolton silt loam	0	.25	—	6.2	6.	Sequoia silt loam	0	.25	—	5.3
	m.e.	4.00	.60	3.6	6.9		m.e.	5.60	.55	5.2	6.9
	5.3	5.00	.85	4.4	7.1		6.2	7.00	.85	6.4	7.2
		6.00	1.30	5.0	7.2			8.40	1.65	7.0	7.4
		7.00	1.50	5.6	7.3			9.80	2.35	7.7	7.6
		8.00	2.25	6.0	7.5			11.20	3.75	7.7	7.7
3.	Conasauga silt loam	0	.35	—	6.0	7.	Tellico silt loam	0	.25	—	5.7
	m.e.	3.36	.80	2.9	6.6		m.e.	5.92	.80	5.4	6.8
	4.0	4.20	1.20	3.6	6.95		7.8	7.40	1.40	6.3	7.0
		5.04	1.35	4.0	7.05			8.88	1.65	7.5	7.2
		5.88	1.75	4.5	7.14			10.36	2.45	8.2	7.3
		6.72	2.40	4.7	7.20			11.84	3.35	8.7	7.4
4.	Fullerton silt loam	0	.25	—	5.5	8.	Red clay	0	.20	—	5.1
	m.e.	3.52	.35	3.4	6.5		m.e.	4.32	.55	4.0	6.6
	4.2	4.40	.50	4.1	6.8		5.1	5.40	.60	5.0	7.0
		5.28	.85	4.7	6.9			6.48	.90	5.8	7.2
		6.16	1.25	5.2	7.2			7.56	1.30	6.5	7.4
		7.04	1.95	5.4	7.3			8.64	2.30	6.5	7.5

* Obtained from titrations to pH 7 with $Ba(OH)_2$ in 1 *N* Ba-acetate, after 48 hours' contact.

quantities decomposed from each addition, and the resultant *p*H values are given in Table 2. These data may be examined for two specific points of interest:

First, what *p*H did the soils attain when the calcium absorption from calcium carbonate was equal to the base-requirement indicated at *p*H 7 by the laboratory procedure?

Second, how much greater was the Ca-absorption at soil-calcium carbonate equilibration than that at *p*H 7 (if it may be assumed that such state had been attained in these experiments).

The answer to the first query is found by selecting in the "absorbed" column, Table 2, that value for each soil nearest to "base requirement" indication of that soil and reading the corresponding *p*H in the next column. Such comparison shows that in 4 instances the *p*H was 7.2, in 1 instance, 6.8, in 2 instances, 7.0, and in the other instance, 7.1; with a median of 7.1. From the generally observed effects of electrolytes it would be expected that the *p*H values corresponding to those points of neutralization should have been higher, since the laboratory indications were obtained in the presence of normal barium-acetate concentration, whereas the *p*H reading in the soil-calcium carbonate systems were made in aqueous suspension. The explanation for this phenomenon probably lies in the compensating factor of biochemical activities such as nitrification and sulfofication in the soil-calcium carbonate systems as against the laboratory determinations where such activities are practically nil. The over-all effect of the 100-mesh calcite additions, following the indications by the proposed titration procedure was to produce soils of *p*H 7 to 7.2 as determined after 90 days' contact.

As to the second point, it is obvious from the same data that the soils continue to react with the excess calcium carbonate beyond *p*H 7. This is not surprising, since it was shown by investigations at this Station as far back as 1915 that soils continue to decompose calcium carbonate considerably in excess of the Veitch requirement, and it was this phenomenon that led to the distinction between *immediate* and *continued* lime requirement of soils (14, p. 147). The residual calcium carbonate at the 90 day period was in between 1000 to 3000 pounds per 2,000,000 pounds of soils, and the maximum calcium absorptions by the soils, beyond *p*H 7, ranged from 700 to 1300 pounds. In several instances, namely 5, 6, and 8, of Table 2, the calcium absorptions from the two maximal additions appear to have reached a constant value. However, this may be due to the slow rate of reactivity which failed to produce distinct differences for the variable, but small, excesses of calcite present from the two largest additions. Nevertheless, the average additional calcium absorption from the soils having excess calcium carbonate beyond *p*H 7 was about 1000 pounds per 2,000,000 pounds of soil, and the highest *p*H attained was about 7.6. This value is considerably below the *p*H 8.3

indicated by Bradfield and Allison (3) for calcium-saturated soils in equilibrium with calcium carbonate at the partial carbon dioxide pressure of the atmosphere.

SOIL-CALCITE EQUILIBRATION

Because of the uncertainty attached to the equilibrium status of the 100-mesh calcite treated soils of the preceding experiment, it was thought desirable to prepare soils in which the saturation with calcium

TABLE 3.—*Carbonate decompositions by Fullerton and Hartsells soils from increasing additions of −200+325 calcite and resultant pH values after 90 days incubation at 20°C*

CALCITE ADDED PER 100 g. OF SOIL	FULLERTON				HARTSELLS			
	RESIDUAL CARBONATE	CARBONATE DECOMPOSITION	TOTAL BASES*	*pH*	RESIDUAL CARBONATE	CARBONATE DECOMPOSITION	TOTAL BASES*	*pH*
m.e.	*m.e.*	*m.e.*	*m.e.*		*m.e.*	*m.e.*	*m.e.*	
0	0	0	3.5	4.76	0	0	3.5	4.67
1	0	1.0	4.5	5.10	0	1.0	4.5	4.90
2	0	2.0	5.5	5.43	0	2.0	5.5	5.22
3	0	3.0	6.5	5.70	0	3.0	6.5	5.63
4	0	4.0	7.5	5.98	0	4.0	7.5	6.06
5	0	5.0	8.5	6.37	0	5.0	8.5	6.33
6	0.3	5.7	9.2	6.66	0.3	5.7	9.2	6.70
8	1.0	7.0	10.5	7.14	1.0	7.0	10.5	7.12
10	2.8	7.2	10.7	7.26	2.5	7.5	11.0	7.40
12	4.6	7.4	10.9	7.28	4.3	7.7	11.2	7.46
14	6.6	7.4	10.9	7.30	5.8	8.2	11.7	7.50
16	8.6	7.4	10.9	7.35	8.1	7.9	11.4	7.53
18	10.5	7.5	11.0	7.32	10.0	8.0	11.5	7.55
20	12.5	7.5	11.0	7.40	11.9	8.1	11.6	7.50

* These values represent the sums of each calcite decomposition plus a constant, the bases found in the control soils by extraction with ammonium acetate.

carbonate would be established more positively. A Hartsells fine sandy loam and a Fullerton silt loam that were previously used as controls in the greenhouse were available for this experiment. Into samples of one hundred grams, calcite of −200+325 fineness was mixed at rates up to 20 m.e. per 100 g. of air-dried soil. The soils were placed in 150-ml covered beakers and kept at about 80 per cent of water holding capacity in a constant temperature room at 20°C. The moisture loss was replaced once a week. At the expiration of 90 days the soils were dried, ground to pass 0.5 mm screen, and analyzed for carbonate content. The residual carbonate, carbonate decompositions, and resultant *p*H values are given in Table 3. The calcite decompositions, as affected by rate of addition, are shown graphically in Figure 1. In both soils the decompositions had

reached a virtual maximum at the 12 m.e. addition. In the range between the 12 and 20 m.e. additions, the Hartsells soil shows a possible 0.3 m.e. increase in calcite decompositions, whereas the Fullerton soil shows an increased decomposition of only 0.1 m.e. According to the equilibrium

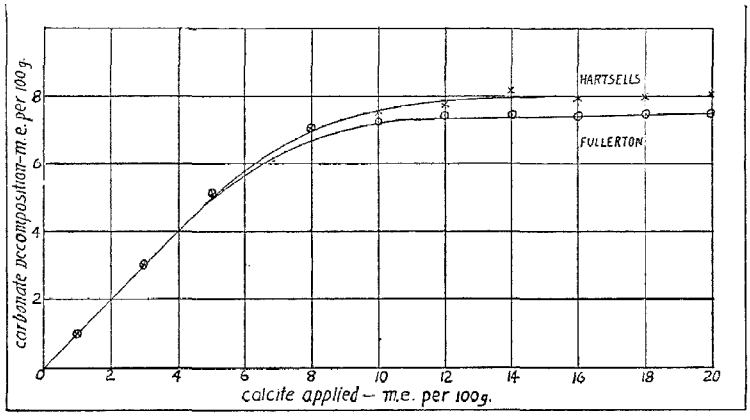

FIG. 1.—Effect of increasing additions of calcite upon extent of carbonate decomposition by Fullerton Silt Loam and Hartsells Fine Sandy Loam, after 90 days' contact.

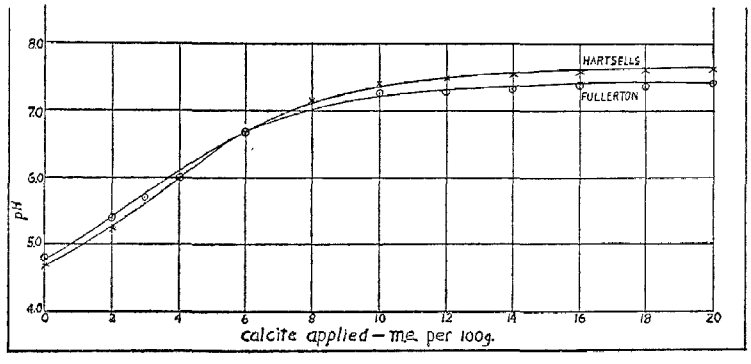

FIG. 2.—Effect of increasing additions of calcite upon *p*H values of Fullerton Silt Loam and Hartsells Fine Sandy Loam after 90 days' contact.

values the exchangeable hydrogen contents are 7.5 and 8.0 m.e. for the Fullerton and Hartsells soils, respectively. The curve for the *p*H values of the soils against the calcite additions is given in Figure 2. The maximal *p*H values were 7.4 and 7.5 for the Fullerton and Hartsells soils, respectively. The *p*H of our calcium-saturated soils again fell short of the *p*H 8.3 value that was indicated by the Bradfield and Allison procedure for aerated soil suspensions in the presence of an excess of calcium carbonate.

The conditions of *p*H measurement may account for such differences. Our soils were moistened to a loose paste, stirred and exposed overnight in a room with free interchange with outside air, and again stirred in the morning before *p*H measurements. The Bradfield and Allison's measurements were done usually with 10 g. of soil in 50 or 100 ml. water suspensions followed by a four-hour aeration period. When our calcium-saturated soils were leached with distilled H_2O the *p*H values were raised to 7.9 and 8.1 for the Fullerton and Hartsells soils, respectively. These values are within the *p*H range obtained by Bradfield and Allison on a Miami clay loam after a six-months' contact period with 40 m.e. of calcium carbonate (3, p. 72). There is every indication that the Fullerton and Hartsells soils were in equilibrium with the excess of calcium carbonate after 90 days' contact. The conditions necessary to effect soil-calcium carbonate equilibration in minimal contact period are now under investigation.

CARBONATE DECOMPOSITION VALUES IN RELATION TO OTHER EXPRESSIONS OF BASE-REQUIREMENT

The primary objective of the calcium carbonate-decomposition values obtained from calcium carbonate-soil equilibration was to provide a standard of comparison for any of the laboratory-derived values of exchangeable hydrogen. Of prime concern is the comparison of the barium hydroxide in barium-acetate titration values with those from carbonate decompositions. The graduated increments of calcite additions in our experiment make it possible also to obtain the exchangeable hydrogen, or lime requirement at *p*H 7 which is generally taken as true neutrality, or at *p*H 6.5 which is considered by some (2, 19) as optimal for plant growth. Because ammonium acetate is used so generally to obtain the exchange capacity and the exchangeable bases of soils, the exchangeable hydrogen is often derived from those two values: (Exch. Capacity − Exch. Bases = Exch. Hydrogen). Before a comparison is attempted it is well to consider the known limitations of each of the enumerated methods for exchangeable hydrogen determination in so far as they affect the accuracy of the expressed values.

The "carbonate decomposition" values are based on the carbonate analyses of the soil at the expiration of the experimental period in comparison with the quantity of carbonate applied or those found in the beginning of the experiment. This value can be determined with a high degree of accuracy. Strictly speaking, the carbonate decomposition value cannot be taken as identical with that of "calcium absorption" or "exchangeable hydrogen" replacement at any particular degree of saturation, because an appreciable part of the calcium reacted with the engendered biochemical end-products of sulfuric and nitric acids during the incubation period. The calcium carbonate-equivalence of those end-products may amount to as much as 1000 pounds per 2,000,000 pounds of

soil. This increment may be warranted in the designation of "exchangeable," however, on the grounds that the biochemical activities are an integral part of the soil system, and had it not been neutralized by the applied calcium carbonate, the engendered hydrogen ions would have become a part of the soil complex. When the carbonate decomposition values obtained under natural conditions are compared with any laboratory-determined titration values at equivalent states of neutralization, or *p*H, it should be borne in mind that the last-mentioned procedure will give lower values on account of the attendant experimental conditions that preclude the formation of biochemically engendered hydrogen ions.

The *p*H measurement of soil by means of the glass electrode has become the simplest routine determination in the soil-testing laboratory. On buffered solutions the glass electrode is capable of giving results of the highest precision. On as complex material as a soil, however, the *p*H is the resultant of continually shifting equilibrium between the soil particles, the water solution, and the carbon dioxide of the surrounding atmosphere. In the field, in greenhouse cultures, and in laboratory incubations, soils are affected by variable accumulations of electrolytes. Field soils are particularly affected by seasonal changes in electrolyte content (1, 9, 20, and 25). The electrolyte accumulation in field soils will cause a depression of one-half to one *p*H unit in comparison with measurements of the same soils in which the electrolytes have been removed by leaching. In practice a soil sampled after a prolonged dry and warm spell will give a considerably lower *p*H value than the same soil sampled soon after a heavy rainfall. On the other hand, *p*H readings of soils leached free of their electrolyte content are more readily affected by the carbon dioxide of the atmosphere (1, 23, 27). The significance of such *p*H fluctuations, from either of the above causes, when expressed in terms of calcium carbonate, will vary from a few hundred to several thousand pounds of calcium carbonate per acre, depending upon the buffer properties of the soil in question. The comparison of the various values for exchangeable hydrogen are given in Table 4.

$CaCO_3$ EQUILIBRATION VS. TITRATION TO *p*H 7 IN Ba-ACETATE

In the comparisons of Table 4, the soil-calcium carbonate equilibration results, Method A, were taken as standard, following Bradfield and Allison's definition of a calcium-saturated soil, although our results are based on actual soil-calcite contact, whereas theirs are identified with their laboratory procedure, using successively calcium hydroxide, carbon dioxide, and equilibration with carbon dioxide of the atmosphere. The titrations with barium hydroxide in .5 *M* barium-acetate, Method B, show a deviation from Method A to the extent of about 1.1 m.e. per 100 g. Since Method B was carried out under conditions which precluded the formation of biochemically engendered acids, it may be presumed in view of previous discussion, that this method accomplishes as extensive

a hydrogen replacement of the soil as does the extended contact with calcium carbonate, except for the increment resultant of nitrification and sulfofication during the incubation. This difference should not be disregarded if Method A is to be taken as standard. Furthermore, it is expected that its value would vary considerably with the type of soil and organic matter content. In order to establish a comparison of these values on a broader basis it will be necessary to extend this study on a greater number and greater variety of soils. The titration method, as

TABLE 4.—*$CaCo_3$ equilibration values in comparison with other methods of determination of exchangeable hydrogen of Fullerton and Hartsells soils*

METHOD OF DETERMINATION	EXCHANGEABLE HYDROGEN (m.e. PER 100 g)			
	AS DETERMINED		DERIVATIONS FROM METHOD A	
	FULLERTON	HARTSELLS	FULLERTON	HARTSELLS
A. Soil-$CaCO_3$ Equilibration	7.4	8.0		
B. By titration with $Ba(OH)_2$ in 0.5 *M* Ba-acetate-24-hrs. contact period	6.2	7.0	1.2	1.0
C. By *p*H vs. Ca-absorption curve (fig. 3) —unleached soils				
at *p*H 7.0	6.5	6.5	0.9	1.5
at *p*H 6.5	5.2	5.2	2.2	2.8
D. By same as C, but on leached soils				
at *p*H 7.0	5.3	5.4	2.1	2.6
at *p*H 6.5	4.0	4.2	3.4	3.8
E. By Ammonium Acetate	2.3	3.5	5.1	4.5

previously outlined (21) may well qualify as a quick procedure for determination of lime requirement. With the use of simple laboratory equipment, one person can complete 40 to 50 determinations per day.

$CaCO_3$ EQUILIBRATION VS. LIMING *p*H 7

Methods C and D are not independent methods. They represent a part of the 90 day soil-calcite incubations, similar to A in Table 4, but involve fractional calcite additions, so that varying levels of base saturation and corresponding *p*H values could be obtained. The *p*H values for C and D, in Table 4, were taken from the "Absorption Curve" of Figure 3. This curve differs from that in Figure 2, in that the *p*H values are plotted, not against the *calcite additions*, as in Figure 2, but against the *calcium absorptions*, as obtained from Table 3. The *titration curves*, that is, the plotting of *p*H values against calcium carbonate additions, are not indicative of any real relationship between the expressed variables, except where the complete decomposition of the added calcite may be presumed. This point is being emphasized because it is common to find reports on the effect of varying applications of ground limestone upon soil

*p*H values where the extent of calcium absorption, or carbonate decomposition, is given no consideration (2, 6, 26).

The exchangeable hydrogen indications by Method C, in Table 4, show that had the soils been limed to *p*H 7, and *p*H determined without leaching, the lime requirement would have been 6.5 m.e. for each soil. This quantity is close to that obtained by the laboratory titrations to *p*H 7 by barium hydroxide in .5 *M* barium-acetate. If, on the other hand,

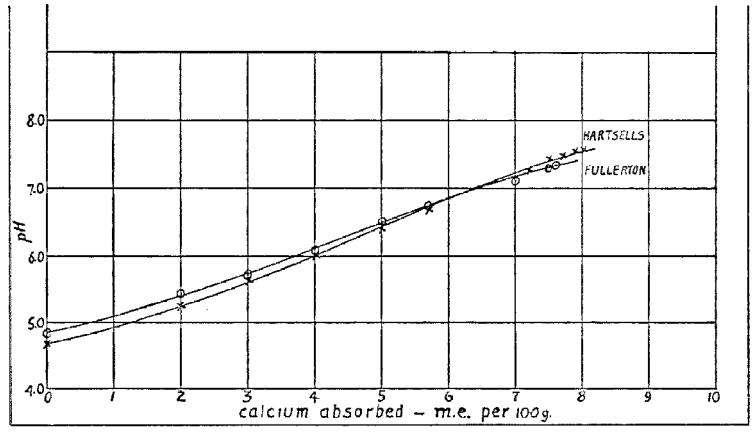

FIG. 3.—Effect of increasing calcium absorptions upon *p*H values of Fullerton Silt Loam and Hartsells Fine Sandy Loam.

the soil had been leached prior to *p*H determination, as in D, the apparent lime requirement would have been 5.3 and 5.4 m.e. for the two soils, respectively. This difference gives emphasis to the influence of electrolytes on *p*H value of the same soil with same degree of saturation. If 6.5 had been the desired *p*H, the soils should have been built up with either 5.2 m.e. or 4.1 m.e., depending upon whether the *p*H was determined in presence or in absence of electrolytes native to the soil after its contact with limestone. Although these facts are well known, there is no agreement among soil investigators as to how to dispose of the disturbing factor of electrolyte content of field and greenhouse soils, in making *p*H measurements.

The results for exchangeable hydrogen by ammonium acetate, Method E, are much lower than those obtained through the use of the other methods.

CHOICE OF LABORATORY PROCEDURE FOR EXCHANGEABLE HYDROGEN

In a search of the literature one is bewildered in finding so many related and unrelated methods for the determination of exchangeable hydrogen and lime requirement of soils. Methods range from extremes of funda-

mentals to mere changes of technique or to variance in concentration of reagents. For example, the Jones (11) calcium-acetate procedure is similar to the Brown ammonium acetate method (4) in that both are based on single contact with salts of a weak acid. The Jones method, however, prescribes use of only 1/30 of the equivalent salt concentration that is generally used with replacement methods. The serial calcium hydroxide titration method of Dunn is similar in principle to the Veitch method except for the longer contact period and the use of *p*H instead of phenolphthalein for measurement of the resultant soil reaction. The present investigation was based upon two principles: (A) that the standard for a base-saturated soil must be the actual equilibration in contact with calcite under proper temperature and moisture conditions and in contact with normal atmosphere and (B) that, for routine determination, the procedure should be simple, adapted for quantity output of easily reproducible results that would approach those obtained by calcium carbonate equilibration.

The simplified barium acetate-barium hydroxide titration procedure is deemed to meet the above-stipulated qualifications, especially since the results come nearest to the values indicated by the calcium carbonate equilibration, as shown in Table 4. Additional experimental data should be obtained concerning the relation of this titration procedure to the calcium carbonate-equilibration values. Except for the poorly buffered sandy soils, it appears improbable that the unduly high indications of liming needs would be obtained through the use of the barium acetate-barium hydroxide method, since in most instances, the laboratory titration values have been found to need "liming factors" to raise them to the higher lime requirements found in actual field trials (2, 5, 6, 19).

SUMMARY

Exchangeable hydrogen has come to be recognized as the underlying cause of soil acidity and lime requirement of soils. It is to be regretted that the *p*H determination which is being widely employed in soil-testing laboratories has only limited application for ascertaining the *quantity* of lime required to raise a soil to some desired saturation level. The exchangeable hydrogen is an indefinite quantity that varies with the method employed for its determination.

In recognizing the need of a standard for "base saturation" of soils various authorities have come to the conclusion that a "base saturated" soil is one that will not further react with an excess of calcium carbonate when exposed to ordinary atmospheric conditions. The carbonate decomposition effected by a soil in the attainment of such equilibrium is considered as equivalent to its exchangeable hydrogen content.

The present contribution deals with an attempt to supply a simple routine method for the determination of exchangeable hydrogen of soils

and to evaluate such procedure in the light of results obtained by actual contact of soils with a standard carbonate material, namely calcite. A procedure based on titration of soils with calcium hydroxide in 0.5 *M* calcium-acetate was presented in 1941 and that work has been extended to include the barium hydroxide titration in 0.5 *M* barium acetate and determinations by ammonium acetate on 13 soils. There was little difference in the values obtained by calcium hydroxide and barium hydroxide titrations, whereas ammonium acetate gave much lower results, particularly on soils of moderate to high organic matter content.

Carbonate decompositions were obtained after 90 days contact on 8 soils treated with moderate excesses of 100-mesh calcite, and on 2 soils which received treatments in greater excess and in more graduated increments. The *p*H values were determined and plotted against calcite additions as well as against calcium absorptions. The soils that were in equilibrium with calcium carbonate had a *p*H of 7.4 to 7.5, whereas the same soils leached registered a *p*H 8.0. At the calcium-absorption levels equivalent to the indicated base requirement by the simplified barium hydroxide-barium acetate titration, the incubated soils had a *p*H about 7.1. It is believed that the proposed "2-point" titration procedure for exchangeable hydrogen of soils is more in line with the base exchange principle, and gives results nearest to those obtained by soil-calcium carbonate equilibration. It is proposed that the comparison between the calcium carbonate equilibration method and the proposed titration procedure be extended to a greater number and greater variety of soil types.

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NOTE ON DETERMINATION OF METHANOL

By GEORGE F. BEYER (Bureau of Internal Revenue, Washington 25, D. C.)

A note on the preparation of the modified Schiff's reagent used in connection with the determination of Methanol or wherever decolorized basic Fuchsin solution is used:

It has come to the attention of the Laboratory of the Bureau of Internal Revenue that it was very difficult, if not impossible, to obtain Basic Fuchsin or Rosaniline HCl that could be decolorized with sodium sulfite and hydrochloric acid. Therefore, this Bureau made an effort to obtain some of this dye from manufacturers in the United States. It also contacted the U. S. Consulate in Switzerland in the hope of getting some of Kahlbaum's Rosaniline HCl which can be decolorized.

The samples received in the United States could not be decolorized and none was obtainable in Switzerland.

In view of this situation certain experiments were made which resulted in the following treatment. In the preparation of 250 ml of the modified Schiff's reagent, dissolve the Fuchsin or Rosaniline hydrochloric acid in about 5 ml 95% ethyl alcohol plus a little water and heat, then add the remainder of the water, sodium sulfite, and hydrochloric acid. Allow to stand at least 24 hours before using. If the solution has a brownish or amber color, add about 0.5 gram of Darco, shake well, and in about 10 minutes filter. The filtrate should be water-white.

Spectrophotometric determinations showed that the decolorized Fuchsin solution using Darco (an activated carbon) gave the same results as Kahlbaum's Rosaniline hydrochloric acid which needed no activated carbon treatment for decolorization.

BOOK REVIEWS

Les Isotopes, Rapportes et Discussions, Published by the secretaries of the council under the auspices of the Scientific Commission of the Solvay Institute, Brussels R. STOOPS, Editor—411 pages.

This book consists of a collection of papers on isotopes given at the 7th meeting of the International Solvay Institute at Brussels in 1947. There are nine reports, 7 in English and 2 in French. The authors and the subject matters discussed are as follows:

1. F. JOLIOT.—Modes de formation, constitution et filiation des isotopes artificiels.
2. K. T. BAINBRIDGE.—Some results of mass-spectrum analysis.
3. C. K. INGOLD.—Isotopes in the Spectroscopy of Polyatomic Molecules with special reference to the Benzene Molecule.
4. M. DE HEMPTINNE.—Les isotopes comme moyen d'investigation de spectres de bandes.
5. F. A. PANETH.—The preparation of radioactive Tracers.
6. A. LANGSETH.—The preparation of organic deuterium Compounds.
7. C. DE HEVESY.—Application of labeled Phosphorus.
8. M. CALVIN.—Radiocarbon and its Application in Chemistry and Biology.
9. D. RITTENBERG.—The use of N^{15} and D for the Study of chemical Processes in the living Cell.

The first 4 papers are concerned chiefly with the theoretical aspects of isotope chemistry, while the last 5 deal with practical applications. The papers by Hevesy, Calvin, and Rittenberg should prove particularly useful to the biologist and biochemist. Good bibliographies and discussions are appended to each report.

E. P. LAUG

Carl Alsberg, Scientist at Large. Edited by JOSEPH S. DAVIS. Stanford University Press, Stanford, California, 182 pages. Price $2.00.

Older members of the Association of Official Agricultural Chemists will find this volume on the life and work of Carl L. Alsberg of especial interest. The book was planned soon after his untimely death in 1940, but the War delayed its earlier publication. Separate chapters by five of his former close associates record the growth and development of this remarkable man through the various phases of his extraordinarily productive career. The volume is edited by Joseph S. Davis, who collaborated with Alsberg at the Food Research Institute, Stanford University, and who contributes an impressive Foreword.

Readers of this Journal will doubtless find most interest in the chapter by Fred B. Linton covering Alsberg's career as Chief of the Bureau of Chemistry. This was the period during which he was active in the work of the Association of Official Agricultural Chemists and the Association of American Dairy, Food, and Drug Officials. The four remaining biographical chapters are, however, equally interesting. Their content is indicated by their titles: "The Making of the Man," "Work in the Natural Sciences," "University Professor and Administrator," and "Social Scientist Beyond the University." They demonstrate the accuracy of Dr. Davis's statement in the Foreword that "Other natural scientists and social scientists have achieved greater fame, but few have left so deep and constructive an impression on a circle so far-reaching in intellectual interests and in geographical distribution."

The book closes with three of Alsberg's own papers, which are especially well chosen, and a classified bibliography of his publications. These three papers exemplify his delightfully simple style, the broad field of his interests, and the innate modesty of this very lovable man. P. B. DUNBAR

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