FIRST DAY

MONDAY—MORNING SESSION

REPORT ON ALCOHOLIC BEVERAGES

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

The reports of Messrs. Rask (Malt), Rabak (Hops), Laufer (Brewing Sugars, etc.), Rohde (Beer), Munsey (Cereal Adjuncts), Becker (Acidity and *p*H in Beer) consisted of recommendations for some 50 changes in status of methods. These were presented to the sectional meeting on October 14, 1946, and no objections were made to them.

The Associate Referee on carbon dioxide in beer (Mr. Stone) recommended a revision of **14.25** (Carbon Dioxide—Tentative), providing for inclusion of measurement of head-space air, to correspond to similar change to be made by a subcommittee of the American Society of Brewing Chemists. Because of the fact that the wording of the proposed revision will not be available until it is prepared by that subcommittee, it was necessary to postpone action on the recommendation this year.

Mr. Nissen's report on Color and Turbidity in Beer and Wort consisted of a progress report on an investigation of the photoelectric method for the determination of color in those products; and it expressed increased confidence in the Dye Color Reference method previously reported by him (*This Journal*, **29**, 3, 287 (1946)).

Dr. Rohde's report included a statement of the work of the A.S.B.C. on analytical methods.

No reports on the other subjects listed in *This Journal*, **29**, 15 (1946), were submitted except Mr. Valaer's report, which consisted of an evaluation of the method for chromatographic adsorption of wines. It included some suggestions as to future work on methods for wines.

Three subjects, Cereal Adjuncts, Fermentable Extracts in Brewing Sugars and Sirups, and *p*H in Distilled Alcoholic Beverages, were discontinued. One new subject, "Yeast," was initiated and an Associate Referee for it has been designated.

Mr. Laufer, as usual, was of great assistance in correlating the work of the Associate Referees on malt beverages and allied materials with the work of the subcommittees of the A.S.B.C.

The recommendations of the Associate Referees, as they appear in their respective reports, are approved.

Three papers, dealing with analysis of wine, with determination of caramel, and with methods for moisture and for starch and carbohydrates determination by acid hydrolysis, were presented and discussed.

REPORT ON MALT

By CHRISTIAN RASK (Albert Schwill & Co., Chicago, Ill.), *Associate Referee*

It is recommended*—

1. That the following methods (*Methods of Analysis, A.O.A.C.*, 1945) be made official, first action.

14.39, Sampling;
14.40, Preparation of Sample;
14.41, Bushel Weight;
14.45, 1,000 Kernel Weight;
14.52, Moisture in Caramel Malt and Black Malt;
14.61, Diastatic Power.

2. That the following methods (*Methods of Analysis, A.O.A.C.*, 1945) be made official, final action.

14.49, Moisture;
14.50, Preparation of Sample;
14.51, Determination;
14.60, Color of Wort.

For methods **14.39, 14.40, 14.41, 14.45, 14.49, 14.50, 14.51,** and **14.60,** the supporting references to collaborative work are as follows: "Collaborative Malt Analysis Report for 1942," Sub-Committee on Malt Analysis, American Society of Brewing Chemists, W. G. Artis, Chairman; *American Brewer*, **76,** pp. 16, 20, 21, July (1943); *Brewers' Digest, Modern Brewery Age*, July (1943); "Collaborative Malt Analysis for 1944," Sub-Committee on Malt Analysis, American Society of Brewing Chemists, W. G. Artis, Chairman; *American Brewer*, **77,** pp. 41–43, August (1944); *Brewers' Digest, Modern Brewery Age*, August (1944); "Malt—Annual Check Analysis," Sub-Committee on Malt Analysis, American Society of Brewing Chemists, Frank Meindl, Jr., Chairman; *American Brewer*, **78,** p. 46, September (1945); *Brewers' Digest, Modern Brewery Age*, September (1945).

For **14.52,** "Report on Caramel Malt and Black Malt," Christian Rask, Chairman of Sub-Committee of the American Society of Brewing Chemists; *American Brewer*, **76,** pp. 37–38, August (1943); *Brewers' Digest, Modern Brewery Age*, August (1943).

And for **14.61,** see the general references to malt given above and also the following: *Proceedings*, American Society of Brewing Chemists, p. 90, (1941), and p. 124, (1942).

No report on diastatic activity and alpha-amylase of malt was given.

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* For report of Subcommittee D and action by the Association, see *This Journal*, **30,** 53 (1947).

REPORT ON HOPS

By FRANK RABAK[1] (U. S. Department of Agriculture, Washington, D. C.), *Associate Referee*

It is recommended*—

That the following methods (*Methods of Analysis, A.O.A.C.*, 1945) be made official, first action.

14.80, Sampling;
14.81, Physical examination;
14.82, Preparation of sample for chemical analysis;
14.83, Moisture;
14.84, Resins—reagents;
14.85, Soft resins—determination;
14.86, Alpha resins;
14.87, Beta resins;
14.88, Hard or gamma resins;
14.89, Total resins.

The above recommendation is supported by the following references to collaborative work: *Proceedings*, American Society of Brewing Chemists, p. 130 (1941), and p. 116 (1942).

REPORT ON CEREAL ADJUNCTS

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

It is recommended*—

That the following methods (*Methods of Analysis, A.O.A.C.*, 1945) be made official, final action, and that this study be discontinued.

14.65, Sampling;
14.66, Preparation of sample;
14.67, Physical characteristics;
14.68, Moisture—apparatus;
14.69, Moisture—determination;
14.70, Oil or petroleum benzine extract—reagent;
14.71, Oil or petroleum benzine extract—determination;
14.72, Extract—apparatus;
14.73, Extract—standardization;
14.74, Extract—determination;
14.75, Extract—calculation;

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[1] Retired July 31, 1946; formerly at Bureau of Plant Industry, Soils, and Agricultural Engineering, Beltsville, Md. Present address, 2826 39th St., N. W., Washington, D. C.

* For report of Subcommittee D and action by the Association, see *This Journal*, 30, 53 (1947).

14.76, Crude fat or ether extract;
14.77, Protein;
14.78, Ash;
14.79, Crude fiber.

The above recommendation is supported by the following references to collaborative work: *This Journal,* **23,** 174 (1940).

REPORT ON BREWING SUGARS, SIRUPS, WORT, SPENT GRAINS, AND YEAST

By STEPHEN LAUFER (Schwarz Laboratories, Inc., New York 17, N. Y.), *Associate Referee*

It is recommended*—

(1) That the following methods (*Methods of Analysis, A.O.A.C.,* 1945) be made official, first action.

These recommendations are supported by the appended references to collaborative work.

(2) That the following method (*Methods of Analysis, A.O.A.C.,* 1945), **14.92(a),** be made official, final action.

BREWING SUGARS AND SIRUPS

14.90. Extract,

14.91. Non-extract (apparent water):
This Journal, **23,** 174 (1940).

14.92. Fermentable extract:
a) Regular fermentation method,
b) Rapid fermentation method,
This Journal, **28,** 441 (1945), and **29,** 285, (1946); see also references to Wort, **14.105.**

14.93. Protein:
This procedure refers to **14.20** and **37.17,** where both methods are indicated as official.

14.95. Iodine reaction for unconverted starch:
This procedure refers to **14.27(a),** where the method is indicated as official.

14.96. Acidity,

14.97. H-Ion concentration (*p*H):
These procedures refer to Beer, **14.10, 14.11,** and **14.12;** see the references given there.

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* For report of Subcommittee D and action by the Association, see *This Journal,* **30,** 53 (1947).

14.98. Ash:
This procedure refers to **34.9** and **34.10,** where the methods are indicated as official.

14.99. Total reducing sugars:
This procedure refers to **34.39, 34.40, 34.51,** and **34.59,** where the methods are indicated as official. Collaborative work also was carried out as indicated in *This Journal,* **28,** 435 (1945), and **29,** 285 (1946).

WORT

14.103. Specific gravity:
This procedure refers to **14.3,** where the method is indicated as official.

14.104. Original extract of original gravity:
The procedure provides for reading of extract from sugar tables.

14.105. Fermentable extract:
Proceedings of the American Society of Brewing Chemists, 1942, p. 130. "Report of Sub-Committee on Rapid Fermentation Methods" of the American Society of Brewing Chemists, Claude F. Davis, Chairman; *American Brewer,* **76,** December (1943) p. 21; *Brewers' Digest, Modern Brewery Age,* December (1943).

14.106. Iodine reaction:
This procedure refers to **14.27,** where the method is indicated as official.

14.107. Total acidity,

14.108. H-Ion concentration (*p*H):
These procedures refer to **14.10, 14.11,** and **14.12;** see references given there.

14.109. Color:
This procedure refers to **14.60,** where the method is indicated as official.

14.110. Protein:
This procedure refers to **14.20,** where the method is indicated as official.

14.111. Total reducing sugars:
This procedure refers to **14.99, 34.58,** and **34.59,** where the methods are indicated as official.

No report on fermentable extracts in brewing sugars and sirups was given.

REPORT ON BEER

By HUGO W. ROHDE (Jos. Schlitz Brewing Co., Milwaukee 1, Wis.),
Associate Referee

It is most gratifying to note the enlargement of the chapter on "Beer" contained in the 1945, Sixth Edition of the "*Methods of Analysis*," *A.O.A.C.*, as compared with the Fifth Edition published five years earlier. The chapter now covers eight pages, an increase of two and one-half pages and an increase of nine paragraphs.

During the past year no samples have been submitted to former collaborators. As previously reported, the American Society of Brewing Chemists, through its Technical Committee, has undertaken the duties of assigning analytical work to a large number of subcommittees. Dr. Stephen Laufer, former chairman of the Technical Committee, has ably arranged, assigned, and grouped this work under carefully selected chairmanships.

This material is distributed in mimeographed form to the members at the annual conventions, where the reports are presented and discussed and subsequently published in the Proceedings of the Society. Furthermore, the publication of these reports in the trade journals makes them accessible to interested persons.

The Associate Referee respectfully submits the following recommendations:*

(1) That the following methods (*Methods of Analysis, A.O.A.C.*, 1945) be made official, first action.

14.7 Extract of original wort:
"Accuracy of Formula for Calculating Original Gravity," American Society of Brewing Chemists Sub-Committee, Philip P. Gray, Chairman; *American Brewer*, **76**, pp. 20–21, December (1943); *Brewers' Digest, Modern Brewery Age*, December (1943); *This Journal*, **22**, p. 202 (1939).

14.8 Real degree of fermentation or real attenuation,

14.9 Apparent degree of fermentation or apparent attentuation:
This Journal, **22**, p. 202 (1939).

(2) That the following methods (*Methods of Analysis, A.O.A.C.*, 1945) be made official, final action.

14.4 Apparent extract or saccharimetric indication:

14.27a For light beer—Iodine reaction for unconverted starch:
This Journal, **22**, p. 202 (1939).

These recommendations are supported by the appended references to collaborative work.

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* For report of Subcommittee D and action by the Association, see *This Journal*, **30**, 53 (1947).

REPORT ON ACIDITY AND *p*H IN BEER

By KURT BECKER (J. E. Siebel Co., Chicago, Ill.), *Associate Referee*

It is recommended*—

(1) That the following methods (*Methods of Analysis, A.O.A.C.*, 1945) be made official, first action.

14.10. Total acidity—Indicator titration method,

14.11. Total acidity—Potentiometric titration method,

14.12. H-Ion concentration (*p*H)—Electrometric method: *This Journal*, **28**, 448 (1945).

The recommendation is supported by the appended reference to collaborative work.

No report was given on inorganic elements in beer.

REPORT ON COLOR AND TURBIDITY IN BEER AND WORT

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

In last year's report[1] our completed Dye Color Reference equipment and method are pictured and described, consisting of a number of dye solutions representing the color of average American beers. The solutions are arranged in a series from 2° to 5° color in $\frac{1}{4}$° increments and are equivalent to the usual Lovibond scale for beer and wort. These diluted dyes are prepared from a standard stock solution of red, yellow, and blue organic dyes properly blended to produce the required amber color. From several years' experience we have found that such a standard stock solution keeps remarkably well, and as described in the earlier issue of *This Journal*,[1] serves readily for renewal of the diluted colors.

At various intervals within a year's use, photoelectric transmission values have been secured on several of these sets of dye colors. As indicated in Table 1 these colors generally show little, if any, change with a few possible exceptions in the one-year values; but even these changes are less than one-eighth of a degree, which we consider well within experimental error as regards practical possibilities of such visual color comparison methods. The photoelectric transmission values secured for Table 1 were obtained by reading directly through the bottles containing

* For report of Subcommittee D and action of the Association, see *This Journal*, **30**, 53 (1947).

[1] *This Journal*, **29**, 3, 287 (1946).

the dye color solutions. A slight variation in these results is apparent, (as compared by reading the colors in standard cells usually provided with photoelectric instruments) caused, we believe, by lack of optical uniformity of the glass in the bottles.

TABLE 1.—*Stability of dye color reference samples*

Per cent transmission

(Samples Read in Bottles in Cenco-Sheard Sanford Type A Photelometer using Blue (420mμ) Filter)

COLOR	SET NO. 1			SET NO. 2			SET NO. 3		SET NO. 4	
	ORIGINAL READING	AFTER 3 MONTHS	AFTER 6 MONTHS	ORIGINAL READING	AFTER 6 MONTHS	AFTER 1 YEAR	ORIGINAL READING	AFTER 1 YEAR	ORIGINAL READING	AFTER 1 YEAR
2	46.5	46.5	46.5	47.0	46.0	46.0	46.0	45.5	47.0	46.5
2¼	42.5	42.5	42.5	43.0	41.5	41.5	42.0	40.0	43.0	41.0
2½	38.0	37.5	37.0	39.5	39.0	38.5	38.0	37.0	39.0	38.0
2¾	36.0	35.0	35.0	35.5	35.0	35.0	36.0	34.5	35.0	34.0
3	32.0	32.0	32.0	33.0	33.0	32.0	32.0	30.5	33.0	32.0
3¼	30.0	29.5	29.5	30.0	29.5	29.5	30.0	28.5	30.0	29.0
3½	27.0	27.0	26.5	27.5	27.0	26.5	26.0	25.0	28.0	27.0
3¾	25.0	24.5	24.5	25.5	24.5	24.5	25.0	23.5	26.0	25.0
4	23.0	22.0	22.0	23.0	22.5	22.0	23.0	21.5	24.0	24.0
4¼	21.0	20.0	19.5	21.5	21.0	21.0	21.0	19.5	22.0	22.0
4½	18.5	18.5	18.5	20.0	19.0	19.0	19.0	17.5	21.0	20.0
4¾	17.5	17.5	17.5	17.5	18.0	17.5	18.0	16.5	18.0	17.5
5	16.0	16.0	16.0	16.0	16.0	15.5	16.5	15.5	17.0	16.5

During the past year a number of these sets have been sent out to various users, all of whom report very favorable results. In many instances this comparison of solutions seems to be preferred to other methods and some of the main advantages are the economical original cost, the easy periodic renewal of the colors, and the possibility of adjusting the dyes for closer matching in the case of special beers. Also, during the past year, arrangements have been made for the manufacture and distribution of these sets by a well-known equipment manufacturer.[2]

We, therefore, conclude as in earlier reports—but now feel even more confident—that this Dye Color Reference Method is fully satisfactory for beer and wort color estimation and, furthermore, that their stability is very good.

There has been a desire from those who possess photoelectric equipment, to provide a means for beer and wort color estimation photoelectrically. In Table 2 we present some photelometer readings for beer and for Dye Color Reference Solutions having the same visual color value as the

[2] Central Scientific Company, Chicago, Illinois.

beer. For both this is by use of three different filters. It will be seen that by use of the green filter the absorption of the dye colors is greater in the lower range colors, namely $2\frac{1}{2}°$ to 4° Lovibond equivalent, whereas in the darker beers they seem to match quite well both photoelectrically as well as visually. In the case of the blue filter, the absorption of the beer is greater than that of the dye colors. With the blue filter, however, the meter readings do not fall on the scale when using a 5 cm cell. A smaller cell must be used.

TABLE 2.—*Transmission values by Cenco-Sheard Sanford photelometer of beer and dye colors*

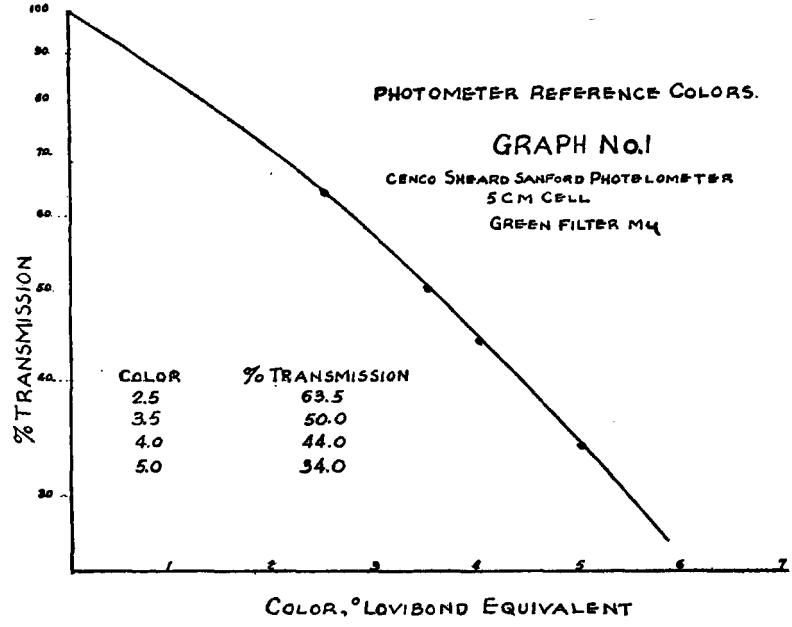
BEER	LOVIBOND COLOR	GREEN FILTER (525 Mμ) % TRANSMISSION		BLUE FILTER (425 Mμ) % TRANSMISSION		WHITE FILTER % TRANSMISSION	
No.	*(Visual)*	*Beer*	*Dye Colors*	*Beer*	*Dye Colors*	*Beer*	*Dye Colors*
A	2.50	63.5	57.0	16.0	21.0	70.0	65.0
B	3.50	50.0	46.0	8.0	12.0	60.0	56.0
C	4.00	44.0	42.0	6.3	9.5	55.0	52.0
D	5.00	34.0	34.0	2.3	5.0	45.0	45.0

The design at first was to prepare these Photometer Reference Colors of the same visual color as the beer and our Dye Color Reference Solutions. It was hoped that a change in the transmission effect of the solution could be accomplished by some means, so that the beer and the dye color would have the same visual color and transmission value. After some experimentation this did not appear to be possible. Accordingly, a separate set of colors was prepared from the stock solution for use as photoelectric checking references, although they do not, at least in the lower range, match visually. From experimentation, as described, it is now possible to prepare these individual colors from the same stock solution used for the visual Dye Color Solutions by pipetting out definite amounts predetermined for each color. This permits accurate and easy preparation for checking of the photoelectric method and equipment.

These Photometer Reference Colors were used on several different types of photoelectric instruments available in our own laboratory and elsewhere at the other collaborators. By connecting the four readings obtained as well as the zero point, smooth curves were obtained which were found to serve very nicely for subsequent color estimation. Graph No. 1 represents a typical curve of this type. To use this curve for beer color estimation, it is only necessary to slightly degas the beer and pour it into the cell of the instrument. By noting where the transmission reading of the sample falls on the curve, the color value of the beer is read directly.

This curve was developed by recording transmission values of a large number of average beers in regular routine laboratory examination, as well as noting their color value obtained by use of the visual Dye Color

Reference Solutions. Table 3 lists a number of such transmission and related dye color values. By means of these values, a mean transmission figure was established for each of the four points selected as the Photom-



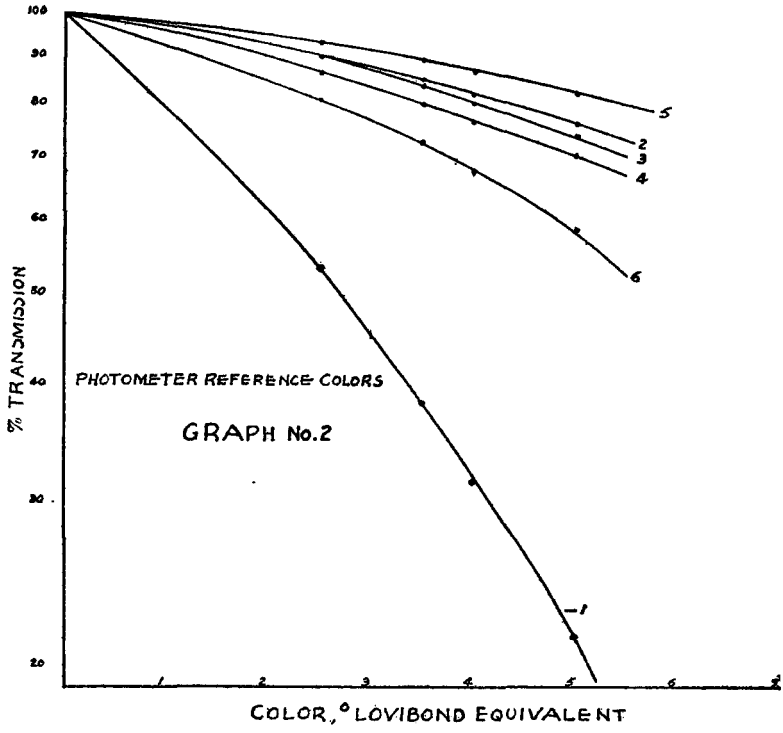
eter Reference Colors. In other words, a mean transmission value is first obtained for the average beer and a Photometer Reference Color Solution adjusted to this same transmission value. When this is once accomplished these same Photometer Reference Colors can be used for establishing

TABLE 3.—*Photometer reference points for development of curves for photoelectric instruments on graph no. 2*

Per cent transmission

PHOTOMETER REFERENCE COLOR	LUMETRON 5 CM. CELL 490 Mμ (GREEN)	FISCHER ELECTRO-PHOTOMETER 11 MM. CELL 525 Mμ (GREEN)	CENCO TYPE B PHOTELOMETER 15 MM. CELL 540 Mμ (GREEN)	COLEMAN MODEL II SPECTRO-PHOTOMETER ½" CELL P.C. 4 525 Mμ (GREEN)	KLETT SUMMERSON PHOTOMETER 12 MM. CELL NO. 54 GREEN 535 Mμ	EVELYN PHOTOELECTRIC COLORIMETER ½" CELL 520 Mμ (GREEN)
2.5°	53.5	90.0	90.0	86.5	93.0	81.0
3.5°	38.5	85.4	84.0	80.0	89.0	73.2
4.0°	31.5	81.7	80.0	76.5	86.5	67.0
5.0°	21.5	76.5	74.0	70.5	82.5	59.1

similar curves for other photoelectric instruments. This has been done, and given herewith in Graph No. 2 are a number of these curves developed on different instruments. In each of these graphs, we believe the curve should be drawn so as to pass through the zero point, since it certainly appears that with zero color one should obtain 100 per cent transmission, if no other light absorbents are present. When using the same type of instrument in different laboratories, under same conditions, very good agreement is obtained. These four Photometer Reference Colors



serve as a suitable method for establishing curves by which the color of beer may be read photoelectrically.

As in the color method, turbidity naturally affects the transmission value and must be carefully avoided. This is particularly the case with wort, and further work is necessary to clarify this point. As will be recalled, a method has previously been given by Nissen and Peterson outlining Color and Turbidity in Beer and Wort.[3] We plan to study further this effect of turbidity by using the proposed present system.

[3] *Proceedings*, American Society of Brewing Chemists, 1942, page 77.

In this work, some experiments were conducted with the inorganic dyes such as iodine, potassium chromate, etc. It appears to be difficult, however, to prepare these inorganic colors so as to visually represent beer colors or match beer in appearance. This is an advantage of the present dye color system. The four Photometer Reference Colors developed herewith have definite visual color values, as follows:

Photometer Reference Color	*Actual Visual Dye Color*
2.5°	2.0°
3.5°	3.0°
4.0°	3.75°
5.0°	5.00°

In other words, these reference colors look like the color of beer and can easily be compared for accuracy and stability.

It is believed that these Photometer Reference Colors will serve as a beginning in the development of a suitable method for determination photoelectrically of color of beer and wort. Further collaborative work, and especially the accumulation of additional results of different beers on different instruments, should be carried out. This we plan to do in the coming months.

The turbidity work was not carried out to any degree during the present year since it was felt more essential to devote time on the Dye Color References and the Photometer Reference Colors. This work should also be furthered during the coming year.

It is recommended*—

That the method prepared by the Associate Referee for color in beer be adopted as tentative, and that the study on photoelectric beer color evaluation, as well as work on beer turbidity methods, be continued.

REPORT ON CARBON DIOXIDE IN BEER

By Irwin Stone (Wallerstein Laboratories, 180 Madison Ave, New York 16, N. Y.), *Associate Referee*

The last detailed report on carbon dioxide in beer by an Associate Referee was submitted in 1939 by Philip P. Gray.[1] This report contained a description of the pressure-air (or manometric) method and some data on reproducibility; and it recommended that this method be adopted as tentative and that it be further studied. Previously, in 1936,[2] the chemical method[3] for determining carbon dioxide in beer had been adopted as tentative.

No further collaborative work on carbon dioxide in beer has been con-

* For report of Subcommittee D and action by the Association, see *This Journal*, **30**, 53 (1947).
[1] P. P. Gray, *This Journal*, **22**, 207 (1939). Report on CO_2 in Beer.
[2] J. H. LeClerc, *Ibid.*, **19**, 177 (1936).
[3] P. P. Gray and I. M. Stone, *Ibid.*, **19**, 162 (1936).

ducted for the A.O.A.C. by the Associate Referees. However, much work has been undertaken by the Subcommittee for Carbon Dioxide in Beer of the American Society of Brewing Chemists, of which subcommittee the present Associate Referee is chairman. The two societies have cooperated very closely. It may, therefore, be well to review our work for the past several years.

Since the report of Gray in 1939, the manometric method has come into constant use for plant and analytical control, completely supplanting the chemical method for this purpose. The chemical method is used only occasionally as a reference method and in laboratories where the manometric apparatus is not available.

The 1941 report[4] of the A.S.B.C. Subcommittee on Carbon Dioxide under the chairmanship of Nissen, indicated satisfactory agreement of average collaborative results by the manometric method. It also showed the need in the manometric method for the employment of a single temperature of measurement and further stressed the necessity of the air-correction. The report also brought out the need for a uniform acceptable pressure-temperature chart for the solubilities of carbon dioxide for use where measurements are made at low temperatures, such as on tank beer.

At that time several charts giving the pressure-temperature-solubility data for carbon dioxide were employed by the brewing industry. The Liquid Carbonic chart based on the solubility of carbon dioxide in water, and the De Markus chart based on the cumulative solubility of carbon dioxide in water and alcohol, tended to give high results. The Wallerstein chart was based on the data of Gray and Stone[1,3] and gave the solubility of carbon dioxide in beer, it having been postulated that the small amounts of alcohol present did not affect the solubility and that the beer-solids acted only as inert diluents. The Wallerstein chart gave overall results which were closer to the value calculated for the A.S.B.C. method conducted at one temperature, 25°C. Other different charts were also available to the analyst.

As part of the chemical control of the brewing process, carbon dioxide is determined on the cold unfinished beer in the tanks in the brewery cellars. These determinations are conducted manometrically in a special pressure sampler and are usually made in the cellar at the low temperatures existing there. It was found that results obtained in these tests were always 0.02 to 0.04 per cent (0.1 to 0.2 volumes) higher than the results on the same beer using the manometric laboratory method at 25°C.

The work of the A.S.B.C. Subcommittee, for the years 1943 to date, under the chairmanship of the author, has been devoted to an attempt to discover the factors causing this discrepancy between the laboratory and the plant results and the effort to eliminate them. One of the first acts of this committee was to prepare a pressure-temperature chart of carbon

[4] Proceedings, A.S.B.C., 95 (1941).

dioxide solubilities in beer from the best available data on hand. This chart is known as the A.S.B.C. Trial Chart No. 1. It was tested by collaborative work and was found to be satisfactory, but the laboratory-plant differences were still encountered. These differences were apparently not due to the chart but were due to other factors in the determinations.

The reports of this subcommittee for 1945 and 1946 detailed the various factors causing the discrepancies and attempted to evaluate their magnitude. Among the factors found to be significant may be mentioned—

(1) The variable head-space transfer of carbon dioxide from the dissolved state in order to maintain carbon dioxide pressure equilibrium during measurement. Differences of 0.02 per cent (0.1 volume) could be accounted for by this factor.

(2) The presence of air in the apparatus used in the plant during the measurement. This tends to make the plant results too high.

(3) The present laboratory method tends to over-correct for the air by using "total air" rather than "head-space air." This over-correction tends to make the laboratory results low. The "head-space air" is considered as that air which comes over in the first 40 ml. of gas entering the absorption buret.

The cumulative effect of these factors produces the differences noted between the laboratory and plant methods. It was found that by correcting for the "head-space air" (instead of "total air") and for head-space transfer (by calculation) in both methods, checks would be obtained. This result would be the "total carbon dioxide" in the container, rather than the customary "dissolved carbon dioxide" as now reported.

From a practical standpoint it was found inadvisable to recommend all these corrections. It was found that their inclusion in the methods would make them too cumbersome and involved and the addition of a new term "total carbon dioxide" would be confusing. The main practical feature of the present manometric method is its simplicity and rapidity. The one change that was suggested for the present A.S.B.C. manometric method which does not detract from its present simplicity is the inclusion of a measurement of "head-space air" in addition to the present "total air." The figure obtained for "head-space air" is substituted for the "total air" in the formula now employed for calculating the carbon dioxide content. The use of "head-space air" for the air-partial-pressure correction has a more logical theoretical basis and gives better values than the previously employed "total air." On containers with less than 3 ml. of air the differences are negligible; with larger amounts of air low results are obtained unless "head-space air" is used. The "total air" figure which is also obtained in the revised method is used only to give an indication of the amount of air present in the container for other control purposes.

The projected work of the A.S.B.C. Subcommittee on Carbon Dioxide

in Beer for this year is to complete the final revision of the methods. When these are acceptable they will be submitted also to the A.O.A.C.

RECOMMENDATIONS*

It is recommended that the tentative manometric method for carbon dioxide in beer (*Methods of Analysis*, **14.25**) be further studied with a view to including a measurement of "head-space air."

No reports were made on soluble starches, distilled spirits, spectrophotometric examination of wines and distilled spirits, or formal titrations.

CHROMATOGRAPHIC ADSORPTION OF WINES

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

Several years ago G. K. Hamill[1] delivered a paper on the above subject before this Association ("Detection of grape wine in blackberry wine"), in which he showed that the presence of grape wine could be detected in blackberry wine by the chromatographic adsorption method. While the method has been in use since that time in the Bureau of Internal Revenue Laboratories and other laboratories as well, no concentrated study equivalent to Mr. Hamill's work has been made. However, it has been found that the method can be simplified in the routine examination of berry wines and it is unnecessary to go through the extensive preparation of the solution. The simplified procedure that is in current use in the Bureau of Internal Revenue Laboratories follows:

CHROMATOGRAPHIC ADSORPTION OF WINE—SIMPLIFIED PROCEDURE

APPARATUS

(a) Adsorption equipment

In the adsorption mixture use white filter-cel only. (The mixture can consist of equal quantities of filter-cel and alumina, or two parts by weight of alumina and one part by weight of filter-cel.)

(b) Ultraviolet equipment.

DETERMINATION

Wet adsorption tube **(a)** with water, dilute 10 ml. wine in 50 ml. glass-stoppered cylinder to 50 ml. mark and pour through adsorption tubes. An 18–20-inch vacuum expedites the operation. Then pass 25 ml. of water through the tube and continue suction of air for 10 minutes. Proceed as in the original method.[1]

It has been found:

(a) That all berry wines exhibit a blue fluorescence.

(b) That apple, peach, raisin, pear, grape, prune, plum, and cherry wines exhibit bright yellow fluorescence.

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* For report of Subcommittee D and action by the Association, see *This Journal*, **30**, 53 (1947).

[1] *This Journal*, **25**, 1, 220 (1942).

(c) That certain substances like elderberry and other very dark red berry wines exhibit unusually deep blue colors.

(d) That the prohibited pokeberry wine, which is sometimes incorporated into wines, exhibits fluorescence that seems characteristic.

(e) That there seems to be a difference in the shades of yellow fluorescence of apple over grapes and smaller differences between those fruits that give yellow fluorescence.

In a letter to the writer, Dr. M. A. Joslyn, Division of Food Technology, University of California, Berkeley, stated:

"With regard to methods of analysis for wines, I feel very strongly that it is now time to introduce chemical methods for the determination of alcohol. We have previously reported upon these, and we have long used the dichromate oxidation techniques for the determination of alcohol in fermented beverages at Berkeley. I also feel that attention should be given to the application of colorimetric techniques for the more rapid determination of the fixed acids of wine. I am not satisfied with the current methods of determining tannin and caramel. Mr. Marsh has done some work on the caramel problem, and I have long been interested in developing more specific methods for the determination of tannin and anthocyanonin pigments in wine."

The writer feels that these suggestions should be given careful consideration.

No reports were made on the following subjects: *p*H in distilled alcoholic beverages; wine; methanol in wines; cordials and liqueurs.

REPORT ON FERTILIZERS

By G. S. FRAPS (Texas Agricultural Experiment Station, College Station, Texas), *Referee*

During the past year, work has been done by the Associate Referees upon potash, phosphoric acid, nitrogen, and boron.

The Associate Referee on Calcium and Sulphur desires to be relieved of the work on calcium but to continue with the work on sulphur. Methods for calcium are sufficient and satisfactory, so it is recommended that the study of the methods for calcium in fertilizer be discontinued and that the title be changed to Associate Referee on Sulphur.

The A.O.A.C. method for potash in fertilizers except for total potassium oxide in organic compounds (**2.41c**, *Methods of Analysis*, 1945) has always been for water-soluble potash as is provided in the laws of almost all the States. The method used for a long time failed in some cases to recover 0.1–0.3 per cent of the water-soluble potash added in the manufacture of mixed fertilizer, which percentage might be as much as 10 per cent of the water-soluble potash added. To obviate this difficulty, about 1903, Carpenter proposed a modification which was not adopted because it provided

for the solution in 5 ml. of concentrated hydrochloric acid to 300 ml. of water and therefore might include some acid-soluble potash. The modification of Kraybill,[1] now official, involves the addition of ammonium oxalate before boiling the solution. The potash determined in mixed fertilizer is still the water-soluble potash. Recently, a proposed uniform fertilizer law required a guarantee of available potash in fertilizers, instead of water-soluble potash. This requirement perhaps was based upon a misunderstanding of what kind of potash is determined by the A.O.A.C. methods. There is no A.O.A.C. definition of available potash so far as fertilizers are concerned, and the use of this term in fertilizer laws would introduce confusion and uncertainty instead of promoting uniformity. In order to avoid further misunderstanding, it is recommended that the heading Potash on page 31, *Methods of Analysis*, 1945, above paragraph **2.40**, be changed to "Water-soluble Potash (official, first reading)."

The Associate Referee on Potash has recommended a method for the recovery of platinum used in potash work, including the preparation of platinum solution, and the method has been subjected to collaborative work. The method as described in *This Journal*, **28**, 782–783, 1945, is recommended for adoption as tentative.

MacIntire, Marshall, and Meyer[2] have proposed an electric air bath for constant agitation of citrate digestions in the determination of citrate-insoluble phosphoric acid. The use of such an apparatus would require several deviations from the present official method. The method for water-soluble phosphoric acid **(2.13)** specifies the use of a 8 cm filter paper, and the mechanical agitation is not vigorous enough to reduce this paper to a pulp as required in the method for citrate-insoluble phosphoric acid **(2.16)**. Filtration on paper pulp or asbestos instead of filter paper might be satisfactory but is not official. The mechanical device involves constant shaking, while the official method requires intermittent shaking. It might be desirable to modify the official method to permit the use of mechanical shaking. It is recommended that a study be made of the use of mechanical shakers in the method for citrate-insoluble phosphoric acid to ascertain whether modifications should be made in the method for water-soluble and citrate-insoluble phosphoric acid to permit the use of mechanical shakers.

According to the work of Jacob, Rader, and Ross,[3] and other papers, the ammonium citrate method gives practically the same results as the citric-acid method for samples of basic slag. Most of the State fertilizer laws require a guarantee of available phosphoric acid, which is determined by means of the ammonium citrate method. The ammonium citrate method, if applicable, comes nearer to meeting the requirements of the State laws than the citric-acid method now used for basic slag. In some States, the

[1] *This Journal*, **18**, 237, 260 (1935).
[2] *Ibid.*, **27**, 272 (1944).
[3] *Ibid.*, **15**, 146 (1932).

phosphoric acid in basic slag is guaranteed as total instead of as citric-acid soluble. It is 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.

The recommendations of the Associate Referee on Phosphoric Acid and other Associate Referees for further work are recommended for adoption.

RECOMMENDATIONS*

It is recommended—

(1) That the study of methods for calcium in fertilizers be discontinued and that the title of the Associate Referee on Calcium and Sulphur be changed to Associate Referee on Sulphur.

(2) That the heading Potash, above par. **2.40,** page 31, *Methods of Analysis,* be changed to Water-soluble Potash (official, first action).

(3) That the method for the recovery of platinum, including the preparation of platinum solution, *This Journal,* **28,** 782–783, 1945, be adopted as tentative.

(4) That a study be made of the use of mechanical shakers in the ammonium citrate method for available phosphoric acid with the object of making modifications in the methods for determining water-soluble and citrate-insoluble phosphoric acid, to permit their use if such changes are found to be desirable.

(5) 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.

(6) That further collaborative work be done on methods of determining nitrogen in ammonium nitrate with special reference to the Devarda method and the use of various connecting bulbs and traps.

(7) That work be continued in accordance with the recommendations of the Associate Referee on phosphoric acid and other Associate Referees.

The report of the Associate Referee on phosphoric acid will be published in a later issue of the *Journal.*

REPORT ON NITROGEN†

By A. L. PRINCE (New Jersey Agricultural Experiment Station, New Brunswick, N. J.), *Associate Referee*

During the past year, Dr. William H. Ross, of the United States Department of Agriculture, called the Associate Referee's attention to the importance of checking up the present methods used for the analysis of

* For report of Subcommittee A and action by the Association, see *This Journal,* **30,** 41 (1947).

† Journal Series paper of the New Jersey Agricultural Experiment Station, Rutgers University, Department of Soils.

total nitrogen in ammonium nitrate. The problem arose from the fact that the Allied War Supplies Corporation, Montreal, Quebec, had received complaints through their U. S. distributors to the effect that Nitroprill Fertilizer Compound does not contain the guaranteed quantity of nitrogen, viz., 33.5 per cent. The mean nitrogen content of 14 shipments, as reported by the producers, was 33.63 per cent, while the mean value as reported by the consumers was 33.10 per cent, or a difference of 0.53 per cent. According to the data submitted by the consumers, all samples analyzed fell below the guaranteed value of 35 per cent.

It has been suggested that the difference in the two sets of determinations may be due to the method used in making the analyses. The method used by the producers is an indirect one. It consists in determining moisture and insoluble impurities in the sample and subtracting the sum of these results from theoretical value for nitrogen in ammonium nitrate to give the total nitrogen. Moisture is determined by weighing 10 grams of the sample on a dry tared 4-inch cover glass and drying for 4 hours at 85°C. Insoluble matter is determined by dissolving 40–50 grams of the sample in 150–200 ml. of water, filtering on an ashless filter, and washing and igniting to constant weight. This procedure is checked periodically against the Devarda method.[1] The consumers' results were obtained by the official A.O.A.C. Kjeldahl method[2] as modified to include the nitrogen of nitrates. The producers are of the opinion that the official Kjeldahl method does not give accurate results as applied to the determination of total nitrogen in ammonium nitrate.

The fertilizer control laws of many of the States specify a limit of tolerance for the plant-food elements. These limits of tolerance differ greatly in the different States. The limit of tolerance for nitrogen differs in different States, but may be as low as 0.1 per cent. The average nitrogen content of the fertilizers consumed in this country amounts to about 5.0 per cent. Inasmuch as Nitroprills contain about six times as much nitrogen as the average fertilizer produced in the United States, it would require more care on the part of different analysts to keep within a tolerance of 0.5 per cent in the analysis of Nitroprills than to agree to within 0.1 per cent in the analysis of the average nitrogen fertilizer. It is apparent, therefore, that if the analysis of Nitroprills is to be kept within the limit of tolerance specified in some of the States it will be necessary to make the analysis with more than ordinary care, or else for the producer to allow for a wider overrun.

In order to insure the closest agreement in the analysis of high analysis materials it is important that the determination be a direct one, and that the same method be used in different laboratories in which the analysis is made.

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[1] *Methods of Analysis*, (1945), **2.31**, 28.
[2] *Ibid.*, **2.24**, 26.

Dr. Ross undertook a study of the procedures followed in the different fertilizer and State control laboratories in carrying out the official Kjeldahl and Devarda Alloy methods. The following report and collaborative study are largely his work, and the Associate Referee is indebted to him for the use of this material in making the present report to the Association.

Through correspondence and the visiting of a number of laboratories in different parts of the country, Dr. Ross found that the procedure followed in the different laboratories varied in three particulars: (a) The type of connecting bulb used in the distillation step; (b) the rate at which the ammonia is distilled; and (c) the manner in which the distilled ammonia is recovered in the receiving flask.

Many types of connecting bulbs are used at present but these may be classified into two groups: (a) Those such as the Davisson scrubber, which permits the accumulation of condensation in the bulb; and (b) those such as the Iowa State Kjeldahl bulb, which do not permit the collection of condensation in the bulb.

The equipment used in different laboratories to recover the distilled ammonia may be considered as being of three types: (a) An adapter of which the lower end dips below the surface of the standard acid in an open receiving flask; (b) an adapter which does not dip below the surface of the acid in the receiving flask, but the flask is closed by means of a Goessman or other type of trap; and (c) the flask is open and no trap is used but the adapter is of such construction that it brings about an automatic dispersion of the ammonia in the standard acid solution during the process of distillation.

A collaborative study was undertaken of the effect of these variable factors on the results obtained in (1) the modified Kjeldahl method; (2) the Devarda method; and (3) a proposed routine laboratory method which consists in distilling the sample with magnesium oxide without prior digestion and doubling the results. The following two samples of Calgary ammonium nitrate were used in making the tests:

Sample 1. Unconditioned granular ammonium nitrate. Moisture content less than 0.1 per cent.

Sample 2. Granular ammonium nitrate conditioned with 2.77 per cent of Kittitas. Moisture content 0.18 per cent.

If Sample 1 had the theoretical nitrogen content of 35.00 per cent, the total nitrogen in Sample 2 should be 33.6 per cent. While there is no way of knowing the true nitrogen content of these two samples, it is thought that the values given are not more than 0.2 per cent too high.

These samples were sent to the following collaborators:

1. Arthur L. Prince, N. J. Agricultural Experiment Station, New Brunswick, N. J., Associate Referee.

2. Oscar I. Struve, Chief Chemist, Eastern States Farmers Exchange, Buffalo, New York.

3. Frank O. Lundstrom, Division of Soils, Fertilizers, and Irrigation, Beltsville, Maryland.

4. H. L. Moxon and A. H. Allen, Virginia-Carolina Chemical Corporation, Richmond, Virginia.

Sample 1 was also sent to:

5. O. W. Ford, Indiana Agricultural Experiment Station, Lafayette, Indiana.

6. W. B. Griem, Wisconsin Agricultural Experiment Station, Madison, Wisconsin.

The instructions sent to the first four collaborators were as follows:

(1) Determine total nitrogen by the Kjeldahl method modified to include the nitrogen of nitrates. In carrying out the distillation step, distill as fast as it is ever likely to be done in any laboratory. Use the distilling apparatus with adapter and receiver as now set up in your laboratory.

(2) Repeat as in (1) but distill as slowly as it is likely to be done in any laboratory.

(3) Determine total nitrogen by the Devarda method.

(4) Determine total nitrogen by the Kjeldahl method modified to include the nitrogen of nitrates. In carrying out the distillation step use a trap as described in *Ind. Eng. Chem., Anal. Ed.*, **18**, 78 (1946). As represented in this paper, the adapter in the distilling apparatus does not dip below the surface of the standard acid solution in the receiver. This device for preventing loss of ammonia in the process of distillation is known as the Goessman trap and was originally designed more than 30 years ago by former Director Goessman of the Mass. Agr. Expt. Station. It is used in the control laboratories of the Massachusetts, Vermont, and Rhode Island Experiment Stations.

(5) Determine total nitrogen in the samples by the simple procedure of distilling without prior digestion and doubling the results.

The instructions sent to the control laboratories of the Indiana and Wisconsin Agricultural Experiment Stations were limited to items (1), (2), and (5).

The results reported by the collaborators are given in the table on the next page.

An Iowa State Kjeldahl connecting bulb was used by Moxon and Allen, and by Ford. Prince used the regular cylindrical type of Kjeldahl connecting bulb, except in one of the Devarda determinations where a baffle type of connecting bulb was used. All other collaborators used a connecting bulb of the Davisson type. The Iowa State connecting bulb acts as a baffle and permits more uniform flow of the ammonia from the distilling flask to the receiver than is possible with the Davisson bulb. When no trap is used in the receiver, a slight loss of ammonia is therefore more likely to occur with bulbs of the Davisson type than with the Iowa State connecting bulb, particularly when the distillation is carried out rapidly. Loss of ammonia may be prevented by distilling slowly with the Iowa State connecting bulb, or by using a Goessman trap in connection with connecting bulbs of the Davisson type.

TABLE 1.—*Results obtained by collaborators in the analysis of the standard ammonium nitrate samples*

Per cent nitrogen

METHOD	TOTAL NITROGEN IN SAMPLE #1						TOTAL NITROGEN IN SAMPLE #2			
	PRINCE (h, i)	STRUVE (a, c)	LUNDSTROM (a, c)	FORD (b, d)	GRIEM (a, c)	MOXON AND ALLEN (b, c)	PRINCE (h, i)	STRUVE (a, c)	LUNDSTROM (a, c)	MOXON AND ALLEN (b, c)
Kjeldahl, rapid distillation without trap	33.96	34.53	—	34.95	34.63	34.70[g]	32.67	33.38	—	33.79[g]
Kjeldahl, medium distillation without trap	—	34.69	—	—	—	34.80	—	33.65	—	33.81
Kjeldahl, slow distillation without trap	33.92	34.77	34.48	34.84	34.76	34.96	32.60	33.73	33.31	33.98
Kjeldahl, rapid distillation with trap	—	34.54	—	—	—	—	—	33.66	—	—
Kjeldahl, slow distillation with trap	33.95	34.66	—	—	—	—	32.48	33.60	—	—
Devarda without trap	34.19	—	—	—	—	—	33.82	—	—	—
Davarda with trap	34.83	—	34.31	—	—	—	33.78	—	—	—
Devarda special connecting bulb and trap	34.92	—	—	—	—	—	33.95	—	—	—
Direct rapid distillation with MgO, without trap	—	34.32	—	34.76[e]	—	—	—	33.48	—	—
Direct slow distillation with MgO, without trap	—	34.40	34.29	34.69[f] 34.41[e]	—	34.92	—	33.30	33.54	33.93
Direct rapid distillation with MgO, with trap	—	34.62	—	34.80[f] —	—	—	—	33.64	—	—
Direct slow distillation with MgO, with trap	35.18	34.72	35.00	—	—	—	34.05	33.76	—	—

[a] Davisson type connecting bulb.
[b] Iowa State type connecting bulb.
[c] Official adapter and receiver.
[d] Bulbed adapter with holes for dispersing ammonia during distillation.
[e] 0.5 gram of sample weighed out directly for analysis.
[f] 10 grams weighed out and diluted to 2 liters from which 100 ml. portions representing 0.5 gram were distilled with MgO.
[g] Slight loss of ammonia detected.
[h] Kjeldahl connecting bulb, cylindrical type.
[i] 5 grams weighed out, and diluted to 500 ml., from which 25 ml. portions representing 0.25 grams were used for analysis. In use of Kjeldahl method, the water was evaporated to dryness in the flask on the steam bath prior to adding salicylic acid.

COMMENTS BY COLLABORATORS TO DR. ROSS

A. L. Prince:

1. The digestion procedures using the Kjeldahl method gave erratic results without the Goessman trap. The results are also low in comparison with the Devarda method. With the Goessman trap more consistent results were obtained but the results were lower than by the Devarda method.

2. The Devarda method without the Goessman trap gave higher results and were somewhat erratic.

3. The Devarda method with the Goessman trap gave in general more consistent and higher values.

4. The Devarda method using a special connecting bulb of the baffle type along with the Goessman trap gave the most uniform as well as the highest results of any of the Devarda procedures.

5. The distillation procedure using MgO and the factor 2 along with the Goessman trap gave very uniform results but the values seem to be slightly high (a little over theoretical on the untreated sample (Sample No. 1).

Oscar I. Struve:

1. The highest results were obtained without prior digestion when the sample was distilled slowly with the Goessman trap but without prior digestion and the result multiplied by two. Traps seemed to be essential in connection with distillation without prior digestion, but the use of a trap seemed to be of no consistent help with the official Kjeldahl method.

Frank C. Lundstrom:

1. The seeming inconsistency in the results reported above by Struve, may be explained by observations made in this laboratory. It has been observed that ammonia may sometimes escape from the receiver when no trap is used in the official Kjeldahl method. The occasional loss of ammonia when no trap is used may be demonstrated by holding red litmus paper over the mouth of the receiver. The extent to which loss of ammonia takes place increases with the rate of the distillation and with the nitrogen in the sample taken for analysis. The effect of the Goessman trap should therefore be more pronounced with the direct distillation method than with the official Kjeldahl procedure when the ammonia distilled is only half as great. Occasional loss of ammonia in the Kjeldahl method has also been observed by Miss K. S. Love of this laboratory.

O. W. Ford:

1. I feel that with material of this nature which is high in nitrogen the most important detail is the accuracy of the weighing of the sample. I would place second the length of time the sample sets in contact with salicylic-sulfuric acid before digestion if one uses this method for the determination of the nitrogen in the sample. With sufficient acid in the receiver, time and speed of distillation has never seemed as important as the speed of the distillation during the first minute or so after the sample has come to the boiling stage during the distillation.

2. In this laboratory we use the gas-heated laboratory construction distillation apparatus which has 13 mm. delivery tubes on the bottom of which are 19 mm. bulbs containing 5 holes for dispersing the ammonia during distillation into the standard acid. The bulbs on these tubes are kept beneath the surface of the standard acid until 150 to 200 ml. of distillate has come over. The receiving flask containing the standard acid is then set on a lower shelf, and the distillation allowed to con-

tinue until the distillation is over. This last step permits the distillation apparatus to be scrubbed free of ammonia so that it can be safely used for the next nitrogen determination.

COMMENTS AND CONCLUSIONS BY DR. W. H. ROSS

1. Ammonium nitrate has more than six times the nitrogen content of the average mixed fertilizer. The fertilizer control laws of certain States specify a limit of tolerance of 0.1 per cent for the nitrogen in fertilizers. While it is possible with care to determine the true nitrogen content of the average fertilizer to within 0.1 per cent, this is not possible in the case of such a high analysis material as ammonium nitrate. It is recommended that the fertilizer control laws be changed so as to permit a higher limit of tolerance for nitrogen in such high analysis materials as ammonium nitrate.

2. Loss of ammonia may sometimes take place when no trap or other equivalent device is used in the receiver of the Kjeldahl apparatus. This occasional loss of ammonia can be demonstrated by holding red litmus paper over the mouth of the receiver. The tendency towards loss of ammonia in this way increases with increase in the rate of distillation and with the nitrogen in the sample taken for analysis.

3. Loss of ammonia in the Kjeldahl or Devarda methods may be prevented by distilling slowly with the Iowa State connecting bulb, or by using a Goessman trap with a connecting bulb of the Davisson type.

4. The procedure of distilling the sample with MgO without prior digestion and doubling the result seems to be a more convenient and accurate method for routine control in ammonium nitrate plants than the indirect one of determining water and impurities in the sample and calculating total nitrogen by subtracting the sum of these results from the theoretical value for nitrogen.

5. The results submitted by the different collaborators differ considerably even when using the same procedure. These differences may be due not only to an occasional loss of ammonia in the distillation step, but to errors in weighing samples that are coated with a conditioning agent, and to variations in the time that the sample sets in contact with the salicylic-sulfuric acid solution before digestion.

6. A careful evaluation of all the results submitted indicates that the nitrogen content of Sample No. 1 is approximately 34.35 per cent. On this basis the calculated content of the nitrogen in Sample No. 2 should be 33.80 per cent. This is in good agreement with the mean of the results obtained under such conditions as to avoid any loss of ammonia.

7. Sample No. 2 was prepared by the same process as those samples which consumers claimed had fallen below guarantee. The results obtained in this study indicate that these samples may not have fallen below guarantee as claimed by the consumer.

COMMENTS BY ASSOCIATE REFEREE

(1) The above data is somewhat disappointing in that very few of the collaborators used the Devarda Alloy method. This is rather surprising since the Devarda Alloy method is strictly the official method for total nitrogen, applicable to the analysis of nitrate salts, an alternate method being the ferrous sulfate-zinc soda method. Furthermore, the Devarda method is also much simpler and more rapid for this purpose than the Kjeldahl method, modified to include nitrates.

(2) Although the Kjeldahl method may be used for this determination

there are certain difficulties which are hard to overcome. For example, the salicylic-sulfuric acid mixture must be applied to the dry material or residue. The error in weighing out 0.5 gram samples of this deliquescent salt for direct analysis may be very appreciable. On the other hand, if an aliquot portion is used from a larger sample, the water must be evaporated to dryness on a steam bath before adding the salicylic-sulfuric acid, otherwise loss of nitric acid will occur on heating.

These two facts, together with variations in the time that the sample sets in contact with the salicylic-sulfuric acid solution before digestion, are, in the opinion of the Associate Referee, more important in causing low and irregular results by the Kjeldahl method than the loss of ammonia in the distillation process. On the other hand, the Goessman trap is a splendid substitute for eliminating the technique of a tube dipping under the acid, as well as a desirable guard against possible loss of ammonia with high analysis materials.

Since writing this report, another problem has come to the attention of your Associate Referee. Mixed fertilizers which are especially high in chlorides and nitrates yield slightly low results for total nitrogen when determined by the modified Kjeldahl Gunning method. This appears to be due to the formation of nitrosyl chloride (NOCl) from the action of HCl on HNO_3 when salicylic-sulfuric acid is added to the fertilizer mixture. In some cases, the total nitrogen may be low by 0.2–0.3 per cent. Shuey[3] has reported work on this problem and proposed a method for correcting this loss. Since a tolerance of only 0.10 per cent from the guaranteed analysis is allowed in some States, it would seem desirable for the Associate Referee to make a study of this problem next year.

RECOMMENDATIONS*

It is recommended—

(1) That further collaborative work be done on methods of determining nitrogen in ammonium nitrate with special reference to the Devarda Alloy method and the use of various connecting bulbs and traps.

(2) That the determination of total nitrogen in mixed fertilizer by the modified Kjeldahl Gunning method be studied in reference to the loss of nitrogen with fertilizers high in chlorides and nitrates.

No report on magnesium and manganese was made.

No report on acid- and base-forming quality was made.

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[3] Shuey, Philip McG, Savannah, Georgia, "Loss of nitrate nitrogen caused by chlorine and a method for determining total nitrogen"; paper presented at Fertilizer Division of the American Chemical Society, Chicago, September 1946.

* For report of Subcommittee A and action by The Association, see *This Journal*, **30**, 41 (1947).

REPORT ON POTASH

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

In accordance with the recommendations of the Association "that work on the details of the method for the determination of potash be continued" (*This Journal*, **28**, 776, 1945), referee work was conducted this year 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 fifteen chemists who found time to do the work and report to the Associate Referee.

Collaborative work on potash in fertilizers was directed to three studies.

(1) Comparison of results obtained by the official method where 1–2 ml of potash-free normal sodium acetate was used in the place of sodium hydroxide to prevent formation of free phosphoric acid during ignition.

(2) Comparison of results obtained by using 80% and 95% alcohol on samples of varying potash content.

(3) Comparison of results obtained by the official method with those obtained by a modification of the official method in which the 80% and 95% acid alcohol[1] was previously saturated with potassium chloroplatinate.

COMMENTS ON RESULTS

The results of the chemists who collaborated on the potash work are reported in Tables 1, 2, 3, and 4. It would appear from the averages of all the results reported that sodium acetate could safely replace sodium hydroxide in the official method (Table 1). Collaborator No. 13 reported an increase of 0.07% potash with sodium acetate on Sample A (0-12-12)[2] and a loss of 0.08% potash with sodium acetate on Sample B (0-20-20). Whereas the difference between the averages was only 0.02% potash, the scope of the study was admittedly somewhat limited, and additional work should be done on a variety of fertilizers before it could be safely recommended to replace sodium hydroxide. Collaborator No. 3 used 80% alcohol by weight (0.8478) formula 30, denatured alcohol. Since the present potash method does not state what alcohol or alcohols are to be used, this collaborator has suggested that it would be of interest to learn the specific gravity of the alcohols being used by other analysts. Several others in reporting indicated dissatisfaction with the present method in this regard. Since many commercial laboratories are not able to obtain ethyl alcohol tax-free, they state that they have gone to the use of other alcohols. The use of other alcohols is questionable, as no data are available on the solubility of potassium chloroplatinate in other alcohols. To clear up this point the Associate Referee recommends to the Association that a survey be made of alcohols being used and that some collaborative solubility studies of these be undertaken. It might be mentioned in this connection that there is a difference of 5.5 per cent at 20/4°C. between 80% ethyl alcohol by volume and 80% ethyl alcohol by weight. The differences in solubility of potassium chloro-

[1] When 80% and 95% acid alcohol is referred to in this paper, it means that acid has been added to 80% and 95% ethyl alcohol. (The Associate Referee assumed that 80% and 95% alcohol meant per cent alcohol by volume instead of by weight.)

[2] Sample A (0-12-12) was part of sample used in A.O.A.C. studies in 1944 and 1945.

platinate in different concentrations of ethyl alcohol have been shown by Allen[3] and Hughes and Ford.[4] It is likely that the compound alcohols being used will show differences that should be investigated.

In Table 2, sixty-six of sixty-eight cases showed higher potash values where 95% alcohol was used in place of 80%. Even collaborator No. 3, who admittedly used alcohol which was stronger than 80% by volume got increases from 0.16% to 0.27% K_2O in three out of four cases and only 0.04% K_2O lower in the fourth

TABLE 1.—*Effect of replacing sodium hydroxide with sodium acetate in the determination of K_2O*

Sample A (0–12–12)[1]

ANALYST NO.	WITH SODIUM HYDROXIDE				WITH SODIUM ACETATE			% K_2O FROM AVERAGE WITH NaOH
	NO. OF ANALYSES	HIGH	LOW	AVERAGE	HIGH	LOW	AVERAGE	
1	6	12.83	12.71	12.76	12.88	12.71	12.79	+.03
2	6	13.20	12.90	13.06	13.20	13.10	13.17	+.11
3[2]	6	13.16	12.83	13.00	13.10	12.81	12.94	−.06
4	6	12.73	12.44	12.56	12.80	12.54	12.65	+.09
5	6	12.89	12.83	12.87	12.91	12.83	12.88	+.01
8	6	13.28	13.10	13.23	13.24	13.06	13.15	−.08
9	6	12.71	12.60	12.67	12.69	12.60	12.66	−.01
10	6	12.47	12.20	12.33	12.55	12.38	12.46	+.13
11	6	13.24	13.17	13.20	13.22	12.95	13.16	−.04
12	6	12.78	12.71	12.75	12.70	12.67	12.66	−.09
13[3]	6	13.19	12.82	13.06	13.23	13.00	13.13	+.07
	6	21.09	20.70	20.96	20.99	20.60	20.88	−.08
14	6	12.86	12.78	12.81	12.82	12.76	12.78	−.03
15	6	12.70	12.55	12.64	12.50	12.40	12.48	−.16
16	6	12.83	12.75	12.77	12.87	12.76	12.81	+.04
17	12	13.32	12.88	13.05	12.96	12.70	12.86	−.19
Average		12.94	12.75	12.85	12.91	12.68	12.84	
Maximum variation		.62	.90	.90	.74	.70	.71	

[1] Sample A used in Tables 1 and 3 was a composite of several fertilizer manufacturers 0-12-12 samples and was part of the same sample used in 1944 and 1945 collaborative work.
[2] Results reported—analyst used 80% alcohol by weight.
[3] Sample B—results reported also for Part 1 *a* & *b* not included in averages for this table.

case when 95% was used. This was on a fertilizer of a relatively low K_2O guarantee where the increase is never as great as on fertilizers of higher K_2O content.

Using 95% alcohol, an average increase of 0.30% K_2O was found with Sample B (0-20-20) while an increase of 0.36% K_2O was obtained with Sample C (0-9-27). The average increase 0.20% and 0.15% K_2O obtained for Samples D and E (8-8-8 and 4-12-8, respectively), were not as great as for Samples B and C but were in line with values obtained in previous collaborative work. The samples selected for this work were composited from samples of the same analysis representing several fertilizer manufacturers. In addition to higher results, considerably better agree-

[3] Allen, H. R., *This Journal*, **22**, 162–167 (1939).
[4] Hughes, C. W., and Ford, O. W., *Ind., Eng. Chem., Anal. Ed.*, **14**, 217 (1942).

ment between individual determinations was reported in most cases in favor of the more concentrated alcohol. In view of this, the Associated Referee *will again* recommend to the Association the use of more concentrated alcohol than 80% by volume for use in the potash method.

In Table 3, eleven of the fifteen collaborators reported higher potash values when the 80% acid alcohol was saturated with K_2PtCl_6 while two reported lower values and two reported no difference. Collaborator No. 2 repeated this part of the work and still reported little if any difference but in submitting his report he remarked that it was difficult in warm weather to hold the temperature of the alcohol down. Hughes and Ford[5] as well as previous collaborators have shown that an increase of temperature of alcohol resulted in an increase of solubility of K_2PtCl_6

TABLE 2.—*Effect of concentration of alcohol on the determination of K_2O*

ANALYST NO.	NO. OF ANALYSES	80% ACID ALCOHOL+ALCOHOL				95% ACID ALCOHOL+ALCOHOL			
		AVERAGE % K_2O				AVERAGE % K_2O			
		B	C	D	E	B	C	D	E
1	6	20.48	26.75	8.25	8.37	20.63	26.95	8.34	8.43
2	6	20.69	26.93	7.98	8.13	21.16	27.48	8.18	8.23
3[1]	6	20.76	27.17	7.90	8.24	20.94	27.44	8.06	8.20
4	6	20.05	26.08	7.72	8.04	20.26	26.51	7.94	8.15
5	6	20.38	25.87	7.98	8.27	20.31	25.88	8.06	8.32
8	6	20.85	26.89	7.92	8.10	20.98	27.23	8.06	8.35
9	6	20.44	26.92	7.90	8.10	20.93	27.29	8.04	8.25
10	6	20.72	26.80	7.55	7.59	21.61	27.42	—	—
11	6	21.27	26.84	8.10	8.24	21.86	27.36	8.39	8.32
12	6	20.94	27.08	8.11	8.28	21.16	27.31	8.22	8.29
13[2]	6	20.83	25.40	7.88	8.08	20.89	26.19	8.02	8.26
	6	12.86	—	—	—	13.06	—	—	—
14	6	20.53	26.53	7.81	8.04	20.70	26.74	8.07	8.22
15	6	20.59	26.42	7.83	7.91	20.89	26.84	8.18	8.26
16	6	20.34	26.99	7.93	8.16	20.77	27.05	8.01	8.21
17	12	20.85	27.30	8.14	8.36	21.04	27.41	8.24	8.48
Average		20.64	26.65	7.93	8.13	20.94	27.01	8.13	8.28
Maximum variation		.93	1.90	.70	.77	1.30	1.60	.45	.33

[1] Results reported—analyst used 80% alcohol by weight.
[2] Sample A—results reported also for Part 2 *a* & *b* not included in averages for this table.

in the alcohol. This can readily be remedied in any laboratory by simply passing the alcohol through a condenser cooled with running water. Collaborators 11 and 12 reported higher values for the 80% alcohol without prior saturation with K_2PtCl_6 than for the 80% alcohol which had been saturated. One of these (No. 11) obtained higher values for the saturated 95% acid alcohol than for the saturated 80% acid alcohol but still slightly lower than for the unsaturated 80% acid alcohol.

[5] Hughes, C. W., and Ford, O. W., *Ibid.*, **13**, 233 (1941).

TABLE 3.—*Effect of saturating acid alcohol with K_2PtCl_6 in the determination of K_2O*

ANALYST NO.	NO. OF ANALYSES	80% ACID ALCOHOL+ALCOHOL						95% ACID ALCOHOL+ALCOHOL					
		SATURATED			NOT SATURATED			SATURATED			NOT SATURATED		
		HIGH	LOW	AVERAGE	HIGH	LOW	AVERAGE	HIGH	LOW	AVERAGE	HIGH	LOW	AVERAGE
1	6	12.94	12.84	12.91	12.83	12.71	12.76	13.10	12.99	13.06			
[1]	3	13.06	12.92	12.97									
2	6	13.10	13.00	13.07	13.20	12.90	13.06	13.30	13.15	13.26			
3[2]	6	13.42	13.25	13.33	13.16	12.83	13.00	13.65	13.38	13.52			
4	6	12.76	12.58	12.67	12.73	12.44	12.56	12.78	12.70	12.75			
5	6	12.91	12.77	12.85	12.89	12.83	12.87	12.97	12.83	12.92			
8	6	13.36	13.18	13.28	13.28	13.10	13.23	13.50	13.24	13.39	13.44	13.16	13.32
9	6	12.83	12.73	12.78	12.71	12.60	12.67	12.91	12.85	12.87			
10	6	12.62	12.27	12.41	12.47	12.20	12.33	12.69	12.47	12.55			
11	6	13.05	12.90	12.98	13.24	13.17	13.20	13.21	13.14	13.18			
12	6	12.83	12.79	12.80	12.78	12.71	12.75	13.02	12.91	12.97			
13	6	13.00	12.88	12.95	13.19	12.82	13.06	13.11	13.00	13.04			
14	6	13.02	12.96	13.00	12.86	12.78	12.81	13.06	13.00	13.03			
15	6	13.35	13.20	13.26	12.70	12.60	12.64	12.90	12.80	12.86			
16	6	12.81	12.69	12.76	12.83	12.74	12.77	12.94	12.89	12.92			
17	6	13.38	13.00	13.22	13.32	12.88	13.05	13.86	13.38	13.52	13.16	13.10	13.13
Average		13.03	12.87	12.95	12.94	12.75	12.85	13.13	12.98	13.06	13.30	13.13	13.23

[1] Not included in averages of this table.
[2] See note at bottom of Table 1.

TABLE 4.—*Kind of alcohol and part of the equipment used in 1946 collaborative potash work*

ANALYST NO.	TYPE OF FILTER	KIND OF ALCOHOL	% ALCOHOL BY VOLUME	% ALCOHOL BY WEIGHT	VOLUME OF ALCOHOL	SP. GR. OF ALCOHOL
1						
2	Sinter Ace B	Formula 30	95		120	.7997 at 25°C.
3	Asbestos Gooch	Formula 30		80	150	.8478
4	Sinter Pyrex M				160	
5	Sinter Pyrex M	Formula 30	80	73.3	150	.8639
8	Sinter Pyrex M	Ethyl U.S.P.	80	73.5	75	.8636 at 15.6°C
9	Sinter Pyrex M	Ethyl	80	72.7	125	.857 at 25°C.
10	Sinter Pyrex M				125	
11	Asbestos Gooch				125	
12	Asbestos Gooch				100	
13	Asbestos Gooch				125	
14	Gooch + Filter paper	Formula 30	80–81 95–96	73–74 93–94	125 80 and 95	.855 at 27° .805 at 27°
15	Gooch + Filter paper	Formula 30	80 95		250	
16	Sinter Pyrex M	Formula 30	80		120	.8590 at 20/4°C.
17	Sinter Pyrex M	Ethyl[1]	80% 95%	73.5 92.4	125	.8593@20/4°C. .8114@20/4°C.

[1] For E. Alcohol 80% by volume =73.5 by wt. and has Sp.Gr. 8593 at 20/4°C.
95% by volume =92.4 by wt. and has Sp.Gr. 8114 at 20/4°C.

The expected results in this comparison would be considerably higher values for the saturated 80% acid alcohol than for the unsaturated 80% alcohol and slightly higher values for the saturated 95% acid alcohol than for the saturated 80% acid alcohol.

To check this, collaborator No. 17 saturated 80% (by volume) and 95% (by volume) acid alcohol by allowing the solutions to remain in contact with the salt for a 60-day period. The samples were shaken once or twice daily to insure complete saturation. At the end of this period 100 ml of each saturated solution was pipetted into carefully tared platinum dishes and evaporated in a vacuum oven at between 45° and 50°C. at 90 mm pressure, dried at 100°C. and weighed. The 100 ml of 80% acid alcohol which was definitely orange in color dissolved 0.1173 gram of K_2PtCl_6 and the 95% only about one-fifth as much. Thus if the 6 ml of 80% acid alcohol became completely saturated in the 15 minute period, it could take up the equivalent of 0.14% K_2O from a quarter gram aliquot sample.

The average difference between the saturated 80% acid alcohol and the unsaturated was 0.10% K_2O for the fifteen collaborators. Two of the collaborators reported no difference while two reported negative values. This is in better agreement than might be expected when one takes into consideration the varying laboratory conditions of the collaborators. About the same increase of 0.10% K_2O was obtained with 95% saturated acid alcohol over the 80% saturated acid alcohol.

Collaborator No. 1 obtained a similar figure with 80% saturated acid alcohol when sodium acetate was used in place of sodium hydroxide. It seems logical to assume that if these trials were made with fertilizers of higher K_2O content more uniform results could be obtained. In the opinion of the Associate Referee this should be considered by the Association either for adoption or as a basis for more intensive collaborative studies.

Collaborators No. 8 and No. 17 compared the saturated 95% acid alcohol with 95% acid alcohol and although in each case the saturated was higher than the unsaturated, not enough work has been done to warrant a statement of what is possible by collaborative work along this line.

Table 4 shows the type of filter and kind of alcohol being used as reported in potash work. There is little doubt on the part of the Associate Referee that the kind of alcohol and the mixtures being used seriously affect the uniformity of potash results. For this reason it will be recommended to the Association that a survey of the alcohols in use be conducted and some solubility studies be made.

LIST OF COLLABORATORS

(1) W. R. Austin and Madalane Buford, Armour Fertilizer Works, Nashville, Tennessee.

(2) C. R. Byers and F. Stewart, Armour Fertilizer Works, Carteret, New Jersey.

(3) R. D. Caldwell, Armour Fertilizer Works (Factory), Atlanta, Georgia.

(4) F. D. McSwiney, Wilson & Toomer Fertilizer Co., Jacksonville, Florida.

(5) H. C. Batton, Swift & Company Fertilizer Works, Norfolk, Virginia.

(6) L. S. Walker, Chemist in Charge Regulatory Service, Vermont Agricultural Experiment Station, Burlington, Vermont.

(7) R. Earle Dickey and H. H. Hanson, State Board of Agriculture, Dover, Delaware.

(8) Katherine W. Ford and Ruth Bishop, Davison Chem. Corp., Baltimore, Maryland.

(9) John Kuzmeski and A. F. Spelman, Massachusetts State College, Agricultural Experiment Station, Amherst, Massachusetts.

(10) William McAllister, Southern State Laboratories, Baltimore, Maryland.

(11) J. F. Fudge and T. L. Ogier, Texas A. and M., College Station, Texas.

(12) W. Chapman, Consolidated Rendering Co., Boston, Massachusetts.

(13) R. M. Smith, Agricultural Department, Chemical Division, Tallahassee, Florida.

(14) H. L. Moxon and R. O. Powell, Virginia-Carolina Chem. Corp., Richmond, Virginia.

(15) S. F. Thornton and A. N. Lineweaver, F. S. Royster Guano Company, Norfolk, Virginia.

(16) R. C. Koch, Swift & Co., Hammond, Indiana.

(17) M. A. Ewan, Purdue University, Agricultural Experiment Station, Lafayette, Indiana.

COMMENTS OF COLLABORATORS

(1) Prefer the use of sodium acetate to sodium hydroxide. Its use gives a more crystalline precipitate of K_2PtCl_6. Would like to see sodium acetate and 95% alcohol considered for the official method.

(2) It is hard to control alcohol temperatures in hot weather.

(3) I used formula 30 denatured alcohol (specific gravity 8478). Industrial firms cannot get ethyl alcohol tax free—have used 80% by weight for over 30 years. Most State laboratories here use 95%. Eighty per cent by volume gives low and erratic results—I assumed that the A.O.A.C. specification must apply to 80% by weight.

(4) Results may be lower than most, as I ground the K_2PtCl_6 rather fine and used small quantities of alcohol in washing.

(8) All samples reground to pass 35 mesh sieve.

(10) I have always had results higher than theoretical by using 95% acid alcohol and 95% alcohol wash. This has been true on all standards used.

(12) In the case of the saturated acid alcohol mixtures it was very noticeable how much K_2PtCl_6 they seemed to dissolve. After their use I had occasion to let them stand around the laboratory and a noticeable amount of the salt disappeared on standing. It has always seemed to me that this soaking period, plus the washing that immediately follow it, have a tendency to dissolve the K_2PtCl_6 salt more than any other period during the analysis.

(15) I used formula 30 alcohol and find some of the platinum has been reduced which is visible in the Gooch crucible when you weigh the K_2PtCl_6 precipitate.

(17) The 80% and 95% acid-alcohols saturated with K_2PtCl_6 were still stable after 3 months of storage at 25–30°C. All of the alcohol used in my work was undenatured ethyl alcohol. I believe that it would be necessary to use considerably more wash alcohol after the ammonium chloride treatment in the case of 95% alcohol than in the case of 80% alcohol to insure complete removal of the ammonium chloride.

RECOMMENDATIONS*

It is recommended—

(1) That the study of substituting sodium acetate for sodium hydroxide in the official method for potash in fertilizers be continued.

(2) In the potash method when referring to alcohol and mixtures of it, that it be definitely specified per cent by volume or per cent by weight.

(3) That the Association approve an increase in the strength of alcohol to not less than 85% *by volume* (official action).

(4) That a survey be made of the alcohols being used at present and a

* For report of Subcommittee A and action by the Association, see *This Journal*, 30, 41 (1947).

collaborative study be made of the solubilities of potassium chloroplatinate in them.

(5) Since one collaborator using formula 30 alcohol obtained reduction of the potassium chloroplatinate upon saturation of the acid alcohol, studies of saturation of the acid alcohol with potassium chloroplatinate should be continued.

(6) That a study be made of the advisability of saturating the wash alcohol with potassium chloroplatinate for use in potash work.

ACKNOWLEDGMENT

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

No report on calcium and sulfur was made.

No report on copper and zinc was made.

A general report on boron—a colorimetric method for determination of boron in soils, which is applicable to plants and to fertilizer materials—is given under "Soils," on p. 308.

REPORT ON CEREAL FOODS

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

The Associate Referees submitted reports in 1945 on the determination of moisture in raisin and white bread.[1] Hydrogen-ion concentration of flour and cereal products by electrometric measurements,[2] proteolytic activity of flour[3] and determination of Fe and Ca in enriched flour and enriched bread,[4] respectively. Since there was no meeting of the Association in 1945, the recommendations will be combined with those for this year.

The recent declaration of phosphorus on the label for cereal products has enlarged the need of a method for the determination of phosphorus in these products in the *Methods of Analysis*. It is recommended that an Associate Referee be appointed to study the application of the present

* W. B. White, *Chief*.
[1] *This Journal*, **29**, 255 (1946).
[2] *Ibid.*, **29**, 49 (1946).
[3] *Ibid.*, **29**, 258 (1946).
[4] *Ibid.*, **29**, 272, 276 (1946).

methods of the Association for the determination of phosphorus in cereal products. Wheat in some granular condition, such as crushed, cracked, or ground, has been subjected to some collaborative study for moisture, protein, ash, crude fiber, and fat, either under cereals or feeds. The methods are the same or else differ only in minor detail for wheat as a feed or as a cereal. On this basis it is recommended that "wheat" appear in the list of other grains in the title preceding sec. **20.70,** page 259. The Associate Referee on methods of analysis of soybean flour is working with the Technical Committee of the National Soybean Processors Association and of the American Oil Chemists Society, although the work has not progressed sufficiently for a report this year.

RECOMMENDATIONS*

It is recommended—

(1) That the dry ashing method for Fe in enriched flour and enriched bread, sec. **20.12,** p. 239, be made official and study discontinued.

(2) That the wet ashing method (*This Journal,* **29,** 272, 1946) for Fe in these products be adopted as official, first action, and study discontinued.

(3) That the dry ashing method for enriched spaghetti, enriched degerminated corn meal, and enriched whole corn meal be made official, first action, and study continued.

(4) That the method for Ca, sec. **20.13,** p. 240, be made official and study discontinued.

(5) That the study of methods for the detection and determination of rye flour in rye bread and mixtures of cereal flours be discontinued.

(6) That the decantation method for determination of H-ion, sec. **20.27,** p. 244, be adopted as official and study discontinued.

(7) That the study on determination of starch be continued.

(8) That further study be given to the determination of acidity of fat in grain, flour, corn meal, and whole wheat and to the correlation of this factor to unsoundness.

(9) That a study of the application of the method for reducing and nonreducing sugars in flour, sec. **20.29,** p. 246, to the determination of sugars in bread and other cereal products be studied and a new Referee appointed.

(10) That the study of the tentative method for the determination of benzoyl peroxide in flour, sec. **20.53,** p. 253, be continued.

(11) That the studies be continued on methods for the determination of available carbon dioxide in self-rising flour containing added calcium carbonate.

(12) That the determination of lactose in bread, *This Journal,* **25,** 630, (1942), be further studied.

* For report of Subcommittee D and action of the Association, see *This Journal,* 30, 53 (1947).

(13) That the determination of milk fat in bread, sec. **20.86**, p. 261, be further studied.

(14) That the semi-autolytic method for proteolytic activity of flour, *This Journal*, **29**, 258, 1946, be discontinued, and that the development of other methods be considered.

(15) That the study of the methods for the determination of moisture, ash, nitrogen, crude fiber, and ether extract in soybean flour and other soybean products be continued.

(16) That studies be made on the detection and determination of soybean flour in cereal products by immunological methods or suitable means of estimation.

(17) That the studies of the determination of added inorganic materials in phosphated and self-rising flour be continued.

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

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

(20) That the methods for the determination of moisture and fat by acid hydrolysis in fig bars and raisin-filled crackers, sec. **20.106** and **20.107**, respectively, be adopted as official.

(21) That the tentative method for the determination of total solids, sec. **20.84(b)**, p. 261, for raisin bread and bread containing raisins and fruits be adopted as official, first action, except that the air-dry sample be dried in an uncovered dish in vacuum oven for about 16 hours at 70°C. under pressure not to exceed 50 mm of Hg and that the study for the determination of moisture, fat, crude fiber, ash, and protein in bakery products be continued.

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

(23) That the study for determination of bromates in flour be continued.

(24) That the determination of apparent viscosity measurements of flour be continued.

REPORT ON IRON IN ENRICHED SPAGHETTI AND ENRICHED CORN MEAL

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

A method for the determination of iron in enriched flour and bread has been published in the Sixth Edition of *Methods of Analysis, A.O.A.C.*,

* W. B. White, *Chief*.

(20.9–20.12 on pages 238–39). The following collaborative study involves the application of the same method to the determination of iron in enriched spaghetti, enriched degerminated corn meal, and enriched whole corn meal.

The enriched spaghetti was submitted in short broken pieces of about 0.5 to 0.75 inch in length. The enriched degerminated meal contained added sodium iron pyrophosphate, and the enriched whole corn meal was fortified with ferrum reductum. The iron compounds in each case were ground and mixed with about 2 per cent of corn starch in a mortar, before addition to the corn meal, in order to insure a better distribution of the enrichment. The corn meal was then mixed in a glass jar containing a few

TABLE 1.—*Iron results on enriched spaghetti*

COLLABORATOR		MG/LB	
1		14.1	
		13.9	Av. 14.0
2		13.5	
		13.5	Av. 13.5
3		13.0	
		13.1	Av. 13.1
4		13.0	
5		13.5	
		13.5	
6		15.80	
		15.80	
7		13.5	
		13.4	Av. 13.5
8		15.2	
		13.8	Av. 14.5
9		13.6	
		13.4	
		13.6	
		13.4	
		13.3	
		15.1	
		14.0	
		13.9	
		14.1	
		13.7	Av. 13.8
10		13.3	Av. 13.3
11		—	
12		14.0	
		13.5	Av. 13.8
	Max. 15.8		
	Min. 13.0		
	Av. 13.8		
	$\delta = 0.75$		
13*		13.5	
		13.7	Av. 13.6

* Received after completion of the table.

large porcelain marbles on the ball mill for 4 hours. Because of the danger of frothing during ashing, apparently caused by the addition of the corn starch, the collaborators were advised to ash very slowly over an open flame or to add 10 drops of oil before ashing.

No attempt was made to determine the theoretical amount of iron present in these samples. The experience gained with this method on these

TABLE 2.—*Iron results on enriched degerminated corn meal and enriched whole corn meal*

COLLABORATOR	ENRICHED DEGERMINATED CORN MEAL		ENRICHED WHOLE CORN MEAL	
	mg/lb		*mg/lb*	
1	14.2		14.2	
	14.6	Av. 14.4	14.6	Av. 14.4
2	15.4		14.5	
	15.1	Av. 15.3	14.9	Av. 14.7
3	14.4		13.9	
	14.3	Av. 14.4	13.7	Av. 13.8
4	13.8	Av. 13.8	13.4	Av. 13.4
5	15.4		14.6	
	15.2	Av. 15.3	14.6	Av. 14.6
6	15.0		14.1	
	15.0	Av. 15.0	13.6	Av. 13.9
7	14.6		15.0	
	14.7	Av. 14.7	14.5	Av. 14.8
8	15.8		15.1	
	15.7	Av. 15.8	14.7	Av. 14.9
9	15.2		14.3	
	15.2		12.6	
	15.1		14.5	
	15.7		14.3	
	15.2		14.5	
	16.0		15.5	
	15.5		15.0	
	16.3		14.4	
	15.5		15.8	
	16.0	Av. 15.6	14.5	Av. 14.5
10	14.5		14.3	
11	16.0		15.1	
	15.7	Av. 15.9	15.1	Av. 15.1
12	14.6		14.3	
	15.0	Av. 14.8	14.0	Av. 14.2
	Max. 15.9		Max. 15.1	
	Min. 13.8		Min. 13.4	
	Av. 15.0		Av. 14.4	
	$\delta = 0.60$		$\delta = 0.50$	
13*	15.1		14.5	
	15.1	Av. 15.1	14.3	Av. 14.4
14*	14.6		13.6	
	15.0	Av. 14.8	13.7	Av. 13.7

* Received after completion of the table.

products and others of essentially known iron content leads to the belief that the average results of analysis on these products represent fairly closely the amount of iron present. On this basis the enriched spaghetti may be considered to contain 13.8 mg per pound, the enriched degerminated corn meal, 15.6, and the enriched whole corn meal, 14.5 mg per pound.

The results obtained from 12 collaborators are given in Table 1 for spaghetti and Table 2 for corn products. No difficulty or adverse comments were received. These results indicate that the method may be satisfastorily applied to these products.

The assistance of the collaborators listed below is gratefully acknowledged.

H. C. Ricks, Mississippi State Board of Health, Jackson, Miss.; L. C. Andrews, Department of Health, New Orleans, La.; J. J. Winston, Jacobs Cereal Products Laboratory, New York, N. Y.; F. H. Luckman, The Best Foods, Inc., Bayonne, N. J.; H. J. Alleman, Kroger Food Foundation, Cincinnati, Ohio; also, Sam P. Fine, H. F. O'Keefe, H. J. Meuron, R. R. Johnson, F. C. Minsker, M. A. McEniry, and D. M. Heller, all of various stations of the Food and Drug Administration.

It is recommended* that this method for the determination of iron in enriched spaghetti and in enriched degerminated corn meal and enriched whole corn meal be adopted as official, first action.

No reports were made on the following subjects: rye flour in rye bread and in flour mixtures; hydrogen-ion concentration; starch in raw and cooked cereals; fat acidity in grain, flour, corn meal, and whole wheat flour; sugar in bread and other cereal foods; benzoyl peroxide in flour; carbon dioxide in self-rising flour.

REPORT ON MILK SOLIDS AND BUTTERFAT IN BREAD

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

Further investigation on the method published in Cereal Laboratory Methods Book, 6th Ed., p. 131, for the determination of lactose and calculation of nonfat milk solids in bread, which has been previously studied collaboratively by the Association, has resulted in some improvement, especially in the length of the determination. It is planned to do further work before reporting it or submitting it to a collaborative study.

It is recommended* that the study be continued.

No reports were made on the following subjects: proteolytic activity of

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* For report of Subcommittee D and action by the Association, see *This Journal*, 30, 53 (1947).
† W. B White, *Chief*.

flour; soybean flour; soybean flour in foods (immunological tests); phosphated flour; noodles.

REPORT ON MOISTURE IN RAISIN, FRUIT, AND WHITE BREAD

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

The determination of moisture in food products is generally considered to be a simple procedure, yet frequently controversies arise because of differences in results obtained on control analyses. These differences are often found to occur because analysts have followed different procedures in determining moisture contents.

Food products which contain fruit, syrups, milk, and other materials that decompose readily on being heated, present the greatest problems. In general, it is advisable to determine the moisture content of such products by drying to an approximately constant weight in a vacuum oven at 70°C. The loss in weight due to decomposition during heating at 70°C. is small and takes place very slowly in practically all food products. At temperatures of 100°C. and 130°C. the loss is appreciable on many food products and varies in proportion to the quantities and types of decomposable substances present.

Tables 1 and 2 list results obtained by investigative work on the determination of the moisture content of various types of bread following procedures which are given as official and tentative in *Methods of Analysis, A.O.A.C.*, Sixth Edition, 1945, and also by other procedures which might be of interest.

Samples were prepared by cutting the loaves in quarters and taking two

TABLE 1.—*Samples dried to completion in oven*

(Results expressed in per cent)

SAMPLE NO.	TYPE	70°C. VACUUM OVEN MIXED WITH SAND	100°C. VACUUM OVEN MIXED WITH SAND	130°C. AIR OVEN MIXED WITH SAND
1	White Bread, Not Enriched*	27.89	27.91	27.99
2	White Bread, Not Enriched*	27.12	27.20	27.28
3	White Bread, Enriched	34.12	34.68	34.96
4	Raisin Whole Wheat Bread	33.22	33.86	34.70
5	Raisin White Bread	34.14	34.56	35.42
6	Cinnamon Fruit Bread	29.81	30.88	31.93
7	Cinnamon Fruit Bread	29.39	30.46	31.23
8	Cinnamon Fruit Bread	31.12	31.81	33.01
9	Cinnamon Fruit Bread	31.48	32.00	33.38

* No sugars and milk solids added.

diagonal quarters for samples. One sample was prepared by passing two quarters twice through a food chopper immediately and placing in a tightly sealed container and allowing to stand several hours before charges were weighed for the determinations. Slices of the other two quarters were spread on paper and allowed to air-dry until sufficiently crisp and brittle to grind. Samples of air-dried raisin and fruit bread were ground twice through a food chopper.

TABLE 2.—*Samples air-dried and oven-dried to completion*

(Results expressed in per cent)

SAMPLE	70°C. VACUUM OVEN		100°C. VACUUM OVEN		130°C. AIR OVEN	
	MIXED WITH SAND	NO DISP. MATERIAL	MIXED WITH SAND	NO DISP. MATERIAL	MIXED WITH SAND	NO DISP. MATERIAL
1	28.00	28.01	28.20	28.18	28.25	28.28
2	27.19	27.21	27.32	27.28	27.36	27.37
3	34.36	34.36	34.76	34.72	34.97	35.00
4	33.28	33.22	33.95	33.80	34.95	35.28
5	34.20	34.11	34.61	34.55	35.51	35.42
6	29.81	29.79	30.94	30.95	31.88	31.90
7	29.50	29.45	30.40	30.50	31.48	31.65
8	31.00	30.80	31.70	31.65	32.75	32.76
9	31.64	31.41	32.25	32.20	33.35	33.15

The determinations were carried out by mixing the undried samples with sand and oven-drying at 70°C.[1] and 100°C.[2] by vacuum methods at a pressure of approximately 50 mm. The drying of the air-dried samples was completed both by dispersing the charge with sand, and by drying without the aid of a dispersing material. Determinations were also carried out by air-oven drying procedure at 130°C.[3] on both undried and air-dried samples.

DISCUSSION

The moisture content of bread can be determined either by the procedure of grinding the sample twice rapidly through a food chopper, weighing charges, mixing with a dispersing material, and oven-drying, or by the procedure of air-drying and oven-drying as given in *Methods of Analysis, A.O.A.C.* Sixth Edition, 1945, **20.84.** The results obtained agree reasonably well. Results can be obtained in shorter time by direct oven-drying but there is too much danger of losing moisture in grinding the sample and weighing out charges, and the procedure is therefore impractical. Air-drying and oven-drying is simpler, and in the opinion of the analyst who conducted this work, it is more reliable.

[1] *Methods of Analysis, A.O.A.C.*, 1945, Sixth Edition, **20.106.**
[2] *Ibid.*, **20.3.**
[3] *Ibid.*, **20.4.**

The results in Tables 1 and 2 show that there are only small differences in moisture contents determined on bread which contained no added sugars and milk solids at the three temperatures. These differences are appreciable, however, when determinations are made under the same conditions on enriched white bread and breads that contain fruit. Because of the variable quantities of decomposable substances contained by bread, especially raisin and fruit breads, it would seem that the most correct moisture content is obtained by completing the drying at 70°C. in the vacuum oven.

Table 2 shows results of moisture determinations made on the samples by air-drying followed by oven-drying, using weighed charges dispersed with sand as directed in *Methods of Analysis, A.O.A.C.* Sixth Edition, 1945, **20.106,** as compared to determinations made without the aid of dispersing material. Results obtained by dispersing the charges with sand are not appreciably greater than those obtained on charges which were weighed directly into the dishes and dried without the aid of a dispersing material. This indicates that after air-drying and grinding, the samples are in such a condition that the moisture is practically all released and satisfactorily removed by oven-drying.

CONCLUSIONS

(1) That the correct procedure for obtaining the most accurate moisture content of breads which contain appreciable amounts of sugars, milk solids, and fruit is air-drying, followed by complete drying at 70°C. under a pressure not greater than 50 mm until the loss in weight is less than 2 mg when trial weights are made at 2-hour intervals.

(2) That it is not necessary to mix air-dried charges of raisin and fruit breads with dispersing materials for oven-drying in order to obtain moisture contents which are correct within reasonable tolerances.

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

No reports were made on moisture in self-rising flour and in pancake, waffle, and doughnut flours, bromates in flour, and apparent viscosity measurements.

REPORT ON BAKING POWDER

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

The Associate Referee[1] submitted a report in 1945 containing collaborative results for total and residual carbon dioxide by the gasometric

* W. B. White, *Chief.*
[1] J. Raymond Chittick, *This Journal*, **29**, 259 (1946).

method (official), and also by the same method, except for the use of hydrochloric acid (1+2) instead of sulfuric acid (1+5), on two baking powders containing added calcium carbonate as a substitute for part of the starch and one baking powder containing no calcium carbonate.

RECOMMENDATIONS†

Since there was no meeting of the Association in 1945, it is now recommended that these gasometric methods sec. **17.4–17.6,** sec. **17.8** and sec. **17.9** be adopted as tentative for baking powder containing added calcium carbonate with the alternate use of hydrochloric acid (1+2) for sulfuric acid (1+5).

The Referee also concurs in the recommendations of the Associate Referee on baking powder—

(1) That additional work be done on various types of baking powders, using the gasometric method for the determination of residual carbon dioxide, with special attention to the time and method of heating in preparation of the residual solution.

(2) That an investigation be made on modifying the present A.O.A.C. gravimetric method by changing from the sulfuric and potassium hydroxide absorption bulbs to the use of "Caroxite" or "Ascarite and Anhydrone."

Furthermore, it is recommended that the study include a comparison of the official gasometric method, sec. **17.6,** p. 209, with a method similar in principle to that given in Quartermaster Corps Tentative Spec. for Baking Powder—C.Q.D. No. 326, May 24, 1946—which in brief involves a correction on the unknown samples determined by analysis of a known sample under the same conditions.

CARBON DIOXIDE IN BAKING POWDERS

By J. Raymond Chittick (Jacques Manufacturing Company, Chicago 4, Ill.), *Associate Referee*

It would seem that the original intent of the authors of the residual carbon dioxide method, as adopted by the A.O.A.C., was to simulate as nearly as possible the reaction occurring during baking conditions. This principle appears to have some merit.

Heating the residual salt solution in excess of the present A.O.A.C. method will increase the amount of carbon dioxide liberated. This is especially noticeable when a neutral salt is added in the test, or the evaporation is carried to dryness.

The retention of carbon dioxide in said solution may be:

(a) Dependent upon the salt concentration.

(b) Upon the alkalinity of the soluble residual salt.

† For report of Subcommittee D and action by the Association, see *This Journal,* **30,** 53 (1947).

(c) Upon the *p*H of the residual solution.

Should the present A.O.A.C. residual method be retained, the method and degree of heating should be more explicitly described.

It is recommended† that additional work be done on various types of baking powders, using the gasometric method for the determination of residual carbon dioxide, with especial attention to the time and method of heating in preparation of the residual solution.

It is further recommended that an investigation be made, modifying the present A.O.A.C. gravimetric method by changing from the sulfuric acid and potassium hydroxide absorption bulbs to the use of "Caroxite" or "Ascarite and Anhydrone."

REPORT ON PLANTS

By E. J. MILLER (Agricultural Experiment Station, Lansing, Mich.), *Referee*

RECOMMENDATIONS*

It is recommended—

(1) That the present Associate Referees continue with their respective problems, except for those studies to be dropped, unless circumstances should make it desirable that they be relieved in individual cases.

(2) That Dr. Benne's recommendations with respect to his problems be carried out: viz.:

(a) That the study of methods for determining carotene in plant tissue be continued.

(b) That the tentative methods for determining chlorophyll in plant tissue be made official, first action, and that the study be dropped.

(c) That the tentative o-phenanthroline colorimetric method for iron be made official, first action, and that the study of methods for determining iron in plants be dropped.

(d) That the study of methods for determining zinc in plants be continued.

No report on sampling (plants) was made.

No report on iodine and boron in plants was made; see report on boron in soils, applicable to plants and to fertilizer materials, page 308.

No report on carbohydrates was made.

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† For report of Subcommittee D and action by the Association, see *This Journal*, **30**, 53 (1947).
* For report of Subcommittee A and action by the Association, see *This Journal*, **30**, 43 (1947).

No separate report was made on zinc; see report on carotene, chlorophyll, iron, and zinc in plants, page 257.

No report on copper and cobalt was made.

REPORT ON CAROTENE, CHLOROPHYLL, IRON, AND ZINC IN PLANTS

By ERWIN J. BENNE, *Associate Referee,* and ELVA L. JONES and GLORIA D. MANALO (Agricultural Experiment Station, East Lansing, Mich.)

There has been but little opportunity for the Associate Referee and his colleagues to devise or investigate new methods for the determination of the above-named constituents of plants since recommendations were made for inclusion of such methods in the 1945 edition of *Methods of Analysis, A.O.A.C.* (1). However, they have kept in touch with the literature since the last reports on these subjects were prepared and there follows a brief review of recent articles, together with recommendations for future study, pertaining to each of these constituents.

CAROTENE

The last report by the Associate Referee on carotene in plant tissue (2) was prepared late in 1944 for presentation at the annual meeting of The Association for that year. A large number of articles published since then show that carotene is still the object of much active research. These recent publications deal with a wide variety of investigations including the effects of processing, drying, and storing on the carotene content of plants used for food and animal feed; the effects of fertilizer treatments on the carotene content of crop plants; the role of carotene in human and animal nutrition; the detection, isolation, and characterization of carotene isomers; and numerous others too diverse in character to fit into general categories. Although the majority of these investigations involved the quantitative determination of carotene, only a few new or modified technics for the determination of this constituent of plant materials have appeared in print during the past two years. Moreover, the majority of changes that have been suggested are minor in nature and do not depart radically from the basic fundamentals of former technics.

Griffith and Jeffrey (3) describe a method with which carotene, total xanthophyll, and chlorophylls *a* and *b* can be determined in a single ether solution. Carotene is isolated chromatographically and evaluated spectrophotometrically. In a later paper these authors (4) describe an apparatus for the rapid determination of carotene in plants. They state that its use together with the method cited above makes it possible to determine beta-carotene and chlorophylls *a* and *b*, and to estimate total xanthophyll,

within an hour from the time a leaf sample is removed from the plant. It is claimed that the apparatus eliminates possible loss from the many transfers involved in the older procedures, decreases the amount of necessary apparatus, reduces the time per determination, is easily constructed, and is self-cleaning.

Kemmerer (5) describes a complete chromatographic method for determining beta-carotene, neo-beta-carotene, "carotinoid X," "impurity A," and alpha-carotene in a variety of plant materials. He also described a technic for determining "pure" carotene which was found to give reliable results in good agreement with those by the A.O.A.C. method. In a later report (6) he presented the results of a collaborative study of both an abridged and a complete chromatographic method for determining the carotenes. Results with the latter were somewhat variable; hence, it was suggested that the method be studied further.

Booth (7) claims to have developed a method for determining carotene in foodstuffs which circumvents the disadvantages of existing methods. Samples are ground in a beaker with a glass rod and quartz sand and extracted with a 3:2 petroleum ether-acetone mixture. Quinol is used to prevent oxidative losses. Other pigments are removed by one of three means, *viz.*: (1) Use of aqueous diacetonol, (2) saponification with alcoholic potassium hydroxide, or (3) chromatographing on a column of aluminum oxide and sodium sulfate. The method has been applied to fresh and preserved materials.

Smith *et al.* (8) determined carotene in tangerines by the method of Moore and Ely (9), modified to permit drying of the extract by filtering through sodium sulfate before reducing the volume and to substitute magnesium oxide for dicalcium phosphate in the adsorption column.

Ham and Tysdal (10) in studying the effects of leafhopper injury on the carotene content of alfalfa modified the method of Moore and Ely (9) to employ an adsorption column consisting of 1 part of magnesium carbonate to 8 parts of sodium carbonate instead of dicalcium phosphate.

Because of the interest that still exists in methods for determining the carotene content of plant materials it is recommended: That the study of methods for this purpose be continued.

CHLOROPHYLL

The last report by the Associate Referee and his colleagues on methods for determining chlorophyll in plant tissue was published in 1944 (11). Only a few methods, or modifications of methods, for this purpose have been published since that time. The articles by Griffith and Jeffry (3, 4) cited above, pertain to chlorophyll as well as to carotene and provide for the determination of total chlorophyll and the individual components, chlorophylls *a* and *b*. Compton and Boynton (12) have published an article under the title of "A rapid method for the determination of chlorophyll in

apple leaves." They point out certain precautions about filtering, sampling, and protecting the extracts from light and discuss the effect of the solvent on the efficiency of extracting the tissue.

It is the opinion of the Associate Referee and his colleagues that the methods for determining total chlorophyll and chlorophylls *a* and *b* now given in *Methods of Analysis, A.O.A.C.* (1) are trustworthy and adequate if used as directed; hence, it is recommended:

(1) That the methods be made official, first action.

(2) That the study be dropped.

IRON

Dr. Hale Cowling served for a time as Associate Referee on methods for determining iron in plants and published a report on this subject in 1942 (13). After his resignation from a position in agricultural chemistry to accept employment with an industrial firm the present Associate Referee was appointed as his successor. Two additional reports on iron (14) have been published since.

Others have also recently investigated methods for determining iron in plant materials. Marcello and Marshall (15) have reported on a comparison of titrimetric and colorimetric methods for determining iron in stock feeds. Results from the thiocyanate colorimetric method were compared with those from the Bradbury-Edwards mercurous nitrate titrimetric method. The results obtained by the two methods were in good agreement; however, the latter method was found to possess several advantages over the former.

Howe (16) has published a report of the 1943–44 committee on the determination of iron in cereal products. Several samples were sent to collaborators to investigate means of bringing iron into solution and methods for evaluating the amount present. Results were obtained from the use of wet-ashing, dry-ashing, and sodium carbonate fusion technics for the former purpose and the o-phenanthroline, the thiocyanate, and several variations of the alpha, alpha'-dipyridyl methods for the latter. It was concluded that the use of dry-ashing and alpha, alpha'-dipyridyl constituted a method as satisfactory for determining iron in cereal products as any other method now available.

The Associate Referee and his colleagues have continued to use the o-phenanthroline colorimetric and the titanous chloride titrimetric methods, as given in the latest edition of *Methods of Analysis, A.O.A.C.* (1), for determining iron in plant materials and have found them to be convenient and reliable. Hence, it is recommended—

(1) That the o-phenanthroline colorimetric method be made official, first action.

(2) That the study be dropped.

ZINC

Dr. Cowling served also as Associate Referee on zinc in plants, and in 1942 published a report on the subject (17). At that time he recommended that a method devised by himself and Miller (18) be accepted as tentative by the Association. This method, which evaluates the zinc photometrically as zinc dithizonate, was submitted for collaborative study under Dr. Cowling's supervision and is included as a tentative method in the latest edition of *Methods of Analysis, A.O.A.C.* Following Dr. Cowling's departure from the field of agricultural chemistry, as mentioned in the preceding section, the present Associate Referee was appointed to succeed him.

Very few articles on methods for determining zinc in plant materials have been published recently. Alexander and Taylor (19) have published a paper on an improved dithizone procedure for determining zinc in food materials. In this method, as in that of Cowling and Miller, zinc is evaluated photometrically in the form of zinc dithizonate; however, interfering ions are removed by different means. These investigators reemphasize the fact that appreciable amounts of zinc may be extracted from pyrex glassware.

Although no other recent articles pertaining to methods expressly designed for determining zinc in plant materials have come to the attention of the authors, it is known to them that numerous investigators concerned with studying the role of zinc in plant nutrition are interested in a highly sensitive and accurate method for this purpose. The authors have used the method of Cowling and Miller and have found it superior to the A.O.A.C. method it replaced; however, to date they have not had sufficient opportunity to make a thorough comparison of it with other available methods. Because of the interest in such methods this should be done as soon as possible; hence, it is recommended that the study be continued.

RECOMMENDATIONS*

It is recommended—

(1) That study of methods for determination of carotene in plant materials be continued.

(2) That methods for determining total chlorophyll and chlorophylls *a* and *b* be made official, first action, and that the study be dropped.

(3) That the o-phenanthroline colorimetric methods for determining iron in plant materials be made official, first action, and that the study be dropped.

(4) That study of the methods for determining zinc in plant materials be continued.

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* For report of Subcommittee A and action by the Association, see *This Journal*, **30**, 43 (1947).

LITERATURE CITED

(1) *Methods of Analysis, A.O.A.C.*, 6th Edition, 1945.
(2) *This Journal*, **28**, 793, 1945.
(3) *Ind. Eng. Chem., Anal. Ed.*, **16**, 438, 1944.
(4) *Ibid.*, **17**, 448, 1945.
(5) *This Journal*, **27**, 542, 1944.
(6) *Ibid.*, **28**, 563, 1945.
(7) *J. Soc. Chem. Ind.*, **64**, 162, 1945.
(8) Arizona Station Mimeographed Report, **70**, **5**, 1945.
(9) *Ind. Eng. Chem., Anal. Ed.*, **13**, 600, 1941.
(10) *J. Amer. Soc. Agron.*, **38**, 68, 1946.
(11) *This Journal*, **27**, 517, 1944.
(12) *Proc. Am. Soc. Hort. Sci.*, **46**, 45, 1945.
(13) *This Journal*, **25**, 555, 1942
(14) *Ibid.*, **27**, 526, 1944, and **28**, 796, 1945.
(15) *Ibid.*, **27**, 161, 1944
(16) *Cereal Chem.*, **21**, 412, 1944.
(17) *This Journal*, **24**, 520, 1941.
(18) *Ind. Eng. Chem., Anal. Ed.*, **13**, 145, 1941.
(19) *This Journal*, **27**, 325, 1944.

MONDAY—AFTERNOON SESSION

REPORT ON FRUITS AND FRUIT PRODUCTS

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

Titratable Acidity:

The Sixth edition of *Methods of Analysis* contains (under **26.28, 26.29**) procedures for the titration of acidity with phenolphthalein indicator, and electrometrically. Comparable results may be expected by either procedure. With highly colored solutions it may be preferable to use the glass electrode, although the dilution procedure, **26.28(b),** has been found suitable. The report of the Associate Referee considers the effect on the titration of the presence of neutral salts and sugars with phosphoric and citric acid mixtures. Additional collaborative work is suggested and the Referee concurs in recommendations of the Associate Referee.

Sampling "Cold Pack" Fruit:

Work by the Associate Referee has shown that it is difficult to obtain a representative sample from a barrel of frozen fruit and sugar. The mixture is heterogeneous, there is a partial separation of juice from the fruit prior to freezing, and there may be a tendency for the fruit to float. The fruit near the sides and bottom of the barrel freezes first. We may expect a concentration of soluble solids near the center when barrels are frozen on their ends. This is indicated in Table 1 of Associate Referee Mills' report. The effect, if any, on the distribution of water-insoluble salts is not apparent. The sample taken should represent the center as well as the outer portions of the barrel. A sample consisting of equal weights of a composite of 3 (or more) outer cores and the center core is proposed. It may not be possible in a heterogeneous system such as this to do other than follow a sampling procedure such as that proposed. Owing to the curvature of the sides of the barrel, it is difficult to devise a sampling procedure when the contents are frozen while the barrel remains in a fixed position on its side. Common factory practice, however, is to rotate the barrel approximately 90° each day during the first several days of freezing and then to stack the barrels on their ends.

The sampling of smaller containers is less difficult. The Associate Referee describes an apparatus and gives a procedure suitable for the purpose. It may be, however, that consideration of the time and effort involved in sampling in this way will suggest the taking of the whole container for the sample. We concur in the recommendation of the Associate Referee that no further work be done on this assignment.

* W. B. White, *Chief.*

Work on the determination of water-insoluble solids of fruits and fruit products has been made the subject of a separate report.

RECOMMENDATIONS†

It is recommended—

(1) That the procedures for sampling frozen pack fruit in barrels and in smaller containers, as described in this year's report of the Associate Referee, be adopted as tentative and that further work on sampling of frozen pack fruit be discontinued.

(2) That studies of methods for the determination of sodium and chlorides be discontinued.

(3) That an Associate Referee be appointed to initiate work in cooperation with the Referee on methods of determining fruit and sugar content of frozen dessert fruits.

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

(5) That further study be made of methods of separating fruit acids for subsequent titration.

(6) That studies of polariscopic methods with particular reference to the possible interference of pectin be discontinued.

(7) That the methods developed by Hartmann for determination of citric, malic, and tartaric acids, published in *This Journal*, **26**, 444 (1943), be studied collaboratively.

(8) That the volumetric procedure for P_2O_5, **26.46, 26.47**, be adopted as official, final action.

(9) That the study of methods for the determination of water-insoluble solids be continued.

REPORT ON WATER-INSOLUBLE SOLIDS OF FRUITS AND FRUIT PRODUCTS

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

The first methods of this Association for water-insoluble solids of fruits and fruit products appear in U. S. Department of Agriculture, Bureau of Chemistry Bulletin No. 65 (1902). Two methods are given: **(a)** Kremla's method modified and **(b)** German official method. In **(a)** 50 grams of sample are macerated in a mortar, transferred with warm water to a muslin filter, and 500 or 1,000 ml of filtrate collected. The insoluble residue is transferred to an evaporating dish, dried, and weighed. In **(b)** water-insoluble solids is determined indirectly by subtracting soluble solids from total solids. These procedures appear in "Official and Tentative Methods

† For report of Subcommittee D and action of the Association, see *This Journal*, **30**, 53 (1947).
* W. B. White, *Chief*.

of Analysis of the Association of Official Agricultural Chemists," 1st edition, XII, 4, 5 (1921).

In 1922 Wichmann[1] published direct methods for the determination of water-insoluble solids with some collaborative data. These procedures were unified and appeared as a tentative method in the second edition of *Methods of Analysis* of this Association, XIV, 7 (1925). The method calls for the use of 25 grams of well mixed fruit sample, boiling with 200 ml of water, filtering and washing the residue on cotton or coarse filter paper previously dried and weighed, and weighing the dried residue in flat-bottomed dishes with covers.

The current tentative procedure for water-insoluble solids (*Methods of Analysis*, 6th edition, 1945) is based in part on the earlier work, with modifications as given by Sale.[2] The Referee has studied their procedure and a report of these studies follows.

FILTERING MEDIA

Three types of filters were investigated: rapid qualitative filter paper, U.S.P. cotton, and milk filter discs. All filters were washed with hot water, dried, and weighed in dishes with tight covers, since they may contain some water-soluble solids and since all readily absorb moisture. Table 1 indicates the amounts of moisture and water soluble material found in the different media before preparation.

TABLE 1.—*Filtering media*

	APPROX. WEIGHT IN GRAMS	APPROX. H_2O BEFORE DRYING	LOSS IN WEIGHT ON WASHING
		mg	*mg*
Cotton (a)	4–5	150	3–5
Filter paper (b)	0.85	35	15–20
Milk filter disc (c)	1.3	45	32

(a) A 5″×5″ square 1/2 the thickness of the roll.
(b) 11 cm circle No. 4 Whatman.
(c) 6″ circle Schwartz Mfg. Co., Two Rivers, Wis.

Table 2 indicates the amount of water-insoluble solids collected on each of the three filtering media taking 50 gram aliquots of a commercial peach jam which was thoroughly mixed in a Waring blendor. From the results it appears that all filters may be suitable. However, the milk filter discs were of flimsy construction, they contain considerable water-soluble material, and they are less suitable.

Some chemists of the Food and Drug Administration prefer cotton for filtering, while others prefer a rapid filter paper. Cotton may permit more rapid filtering and washing of the insoluble solids but filter paper absorbs less water and has the advantage of drying more readily. Cotton expands

[1] H. J. Wichmann, *This Journal*, 6, 37 (1932).
[2] J. W. Sale, *Ibid.*, 21, 502 (1938).

on drying and may be so bulky as to make it difficult to be inclosed in a drying dish. The Referee prefers filter paper to cotton. A circle of paper is used on a Büchner funnel. Circles of cotton on a Büchner funnel are also suitable. With either filtering medium care must be taken by the analyst to prevent the water-insoluble solids from forming a tight mat on the

TABLE 2.—*Water-insoluble solids collected*

Sample of Peach Jam—F. S. 56264-D. Six 1-lb. jars mixed thoroughly in Waring blendor and 50-gram portions taken for analysis

FILTERING MEDIUM	LOSS BY WASHING	WT I S	INSOLUBLE SOLIDS	AVERAGE
	mg	*mg*	*per cent*	*per cent*
(1)	30.2	239.5	0.48	
	30.8	230.4	0.46	0.48
	34.5	242.9	0.49	
(2)	4.3	(Lost)		
	4.4	241.6	0.48	0.48
	2.8	242.3	0.48	
(3)	30.3	249.8	0.50	
	32.7	232.3	0.47	0.47
	31.3	228.7	0.46	
(4)	—	232.7	0.47	
	—	228.3	0.46	0.46
	—	234.9	0.47	
(5)	—	226.0	0.45	
	—	229.0	0.46	0.46
	—	234.1	0.47	

(1) "Perfection" milk filter disc 6"+11 cm Whatman #4 filter paper, with suction on Büchner.
(2) Whatman #4 11 cm, with suction on Büchner.
(3) "Perfection" milk filter disc only with suction on Büchner.
(4) U.S.P. absorbent cotton 9 cm, gravity filtration.
(5) Whatman #4, 15 cm, gravity filtration.

surface of the filter, since this greatly reduces the rate of filtration. This situation may be prevented by regulating the size of the sample, by adding the hot wash water in such a manner that the insoluble solids are lifted from the surface of the filter. A momentary and slight amount of suction may be applied to increase the rate of filtration, but the application of suction requires skill. Only when the washing of the sample is complete should full suction be applied.

VARIATIONS IN PROCEDURE

Table 3 contains the per cent of water-insoluble solids obtained after analyzing seven kinds of commercial preserves with several variations in

procedure; cotton is compared with filter paper, gravity filtration on 60° funnel is compared with filtration on a Büchner, and sample preparation by grinding is compared with sample preparation by comminution using the Waring blendor. There does not appear to be a material difference in the results obtained with cotton and with filter paper. Likewise, results

TABLE 3.—*Water-insoluble solids—variations in procedure**

KIND OF COMMERCIAL PRESERVE	PER CENT WATER-INSOLUBLE SOLIDS							
	FILTERING MEDIUM							
	COTTON				WHATMAN #4 PAPER			
	On 60° funnel		*On Büchner funnel*		*On 60° funnel*		*On Büchner funnel*	
Peach	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)
	—	0.46				0.45		
		0.47				0.46		0.48
		0.47				0.47		0.48
Apricot	0.79	0.78	0.78	0.75	0.79		0.78	
	0.81	0.78	0.80	0.76	0.82		0.80	
Cherry	0.62							
	0.62		0.65		0.59		0.62	
	0.62		0.66		0.59		0.64	
	0.61							
Strawberry	1.07	1.03	1.15		1.06		1.05	
	1.11	1.04						
		1.06	1.15		1.07		1.13	
		1.08						
Raspberry	3.06		3.07		2.97		3.22	
	3.07		3.22		3.12		3.24	
Loganberry	3.26	3.16	3.14	3.19	3.01		3.21	
	3.32	3.30	3.27	3.28	3.15		3.30	
Blackberry	3.31	3.32	3.60	3.46	3.38		3.42	3.37
	3.59	3.35	3.85	3.70	3.54		3.52	3.39

* All 50 g aliquots boiled 30 min. with water Q S 200 ml & 800 ml hot water wash.
(a) Sample comminuted by passing three times through meat type grinder, with mixing.
(b) Sample from (a) further comminuted for two minutes in Waring blendor, with mixing.

obtained by filtering and washing the sample on a 60° funnel are but slightly different from the results obtained by filtering and washing on the Büchner. The results obtained after treatment with the Waring blendor are a little lower than those obtained when the same sample was ground. The water-insoluble solids are in a more finely divided condition. There is a tendency for some finely divided insoluble material to pass through the filter and the water-soluble constituents are more readily removed from

the more finely divided samples. The over-all variations exhibited in this and other tables is due in part to the heterogeneous nature of the samples. The mixture of fruit or fruit products is not uniform and aliquots of the same sample do not contain the same amount of water-insoluble solids. The samples consist of a number of individual fruits of varying size, maturity, and insoluble solids content. This is particularly apparent with fruits having seeds, such as blackberries, red and black raspberries, loganberries, and strawberries. It will be observed from the table that larger variations between duplicates occur with these fruits. It may not be possible to obtain the agreement between aliquots as is obtained for ash or the ash constituents in fruit.

TABLE 4.—*Per cent water-insoluble solids with variation in size of sample*

Commercial Red Raspberry Preserve

TYPE OF INFILTRATION	SIZE OF SAMPLE ALIQUOT		
	50 GRAMS	25 GRAMS	10 GRAMS
Cotton 60° funnel	3.07	3.01	—
	3.06	3.01	—
Cotton on Büchner funnel	3.07	3.08	—
	3.22	3.11	—
Whatman #4, 60° funnel	2.97	2.91	—
	3.12	3.06	—
Whatman #4—Büchner	3.22	2.95	2.82
	3.24	2.98	3.24
Average	3.12	3.01	3.03

VARIATION IN SIZE OF SAMPLE

Table 4 summarizes a study relating to the size of aliquot to be taken for analysis. It appears that a 10-gram aliquot is insufficient and that the values may be a little lower for 25-gram aliquots than with 50-gram aliquots. Either 25 or 50 gram aliquots are generally suitable, the size of aliquot depending on the particular fruit, the ease of filtering and washing, and on the size of filter used. Too large a sample may clog the filter, retard the rate of filtration, and interfere with the removal of the water-soluble ingredients.

Table 5 shows jar-to-jar variations in water-insoluble solids in a case of 12 two-lb. jars of commercial blackberry preserves. The contents of each jar were mixed by passing through a meat grinder three times and stirring after each passage. Three aliquots of 25 grams each were used in the analysis. The data are arranged in increasing order of the water-insoluble

solids contents of the jars. The remaining portions of preserves from six of the jars were further treated separately by mixing for two minutes in a Waring blendor. Again three 25-gram aliquots of each were taken and analyzed for their water-insoluble solids content. Jar No. 3 contained the smallest percentage of water-insoluble solids, 2.46 and 2.48 per cent, while No. 1 jar contained the largest amount, 2.85 and 2.93 per cent, an over-all variation of 0.47 per cent. The grand average of all jars was 2.63

TABLE 5.—*Per cent water-insoluble solids—commercial blackberry preserve*

JAR NO.	EFFECT OF GRINDING					
	% WATER-INSOLUBLE SOLIDS			AVERAGE	VARIATION BETWEEN TRIPLICATES	VARIATION FROM GRAND AVERAGE OF 2.63%
3	2.45	2.45	2.49	2.46	0.04	−0.17
3*	2.38	2.43	2.63	2.48	0.25	−0.15
9	2.34	2.52	2.64	2.50	0.30	−0.13
12	2.46	2.48	2.69	2.54	0.23	−0.09
8	2.35	2.59	2.70	2.55	0.35	−0.08
8*	2.44	2.55	2.59	2.53	0.15	−0 10
5	2.44	2.58	2.74	2.59	0.30	−0.04
6	2.43	2.65	2.69	2.59	0.26	−0.04
2	2.60	2.62	2.63	2.62	0.03	−0.01
2*	2.40	2.63	2.83	2.62	0.43	−0.01
10	2.42	2.66	2.78	2.62	0.36	−0.01
10*	2.48	2.80	2.87	2.72	0.39	+0.09
4	2.59	2.67	2.89	2.72	0.30	+0.09
7	2.62	2.65	2.91	2.73	0.29	+0.10
7*	2.40	2.60	2.68	2.56	0.28	−0.07
11	2.67	2.80	2.81	2.76	0.14	+0.13
1	2.85	2.85	2.85	2.85	0.00	+0.23
1*	2.80	2.98	3.00	2.93	0.20	+0.30

* After analysis of ground sample, remainder was mixed in Waring blendor for 2 minutes.

and the maximum variation between triplicate determinations was 0.43 per cent with Waring blended sample. This is a maximum plus and minus variation from the average of 8 per cent in the three aliquots from the same jar. The data indicate the difficulty in obtaining aliquots of a well mixed jam sample containing the same amount of water-insoluble solids. There is no material difference in the per cent of water-insoluble solids in the ground and the Waring blended aliquots. The jar-to-jar variations are not inordinately large, indicating good mixing of fruit and sugar in the commercially prepared jam.

The Referee recommends* a continuation of studies of methods for the determination of water-insoluble solids of fruit products.

* For report of Subcommittee D and action by the Association, see *This Journal*, **30**, 53 (1947).

No report was made on sodium and chlorides, or on polariscopic methods.

REPORT ON TITRATION OF ACIDS

By H. M. BOLLINGER (Food and Drug Administration, Federal Security Agency, Los Angeles, Calif.), *Associate Referee*

In 1945[1] Subcommittee D recommended the continuation of the study of the titratable acidity of solutions relatively high in phosphate concentration. This recommendation was made because the 1945 collaborative results on synthetic and natural fruit solutions were not quite as precise as had been desired. That year, for each of solutions C, D, and E, four out of six collaborators reported electrometric results within ± 0.2 per cent of the average, but the other two results of each set ranged 0.4 per cent to 1.6 per cent from the average. Furthermore, there seemed to be very little systematic variation by the different laboratories. It therefore appeared that some individuality in the manipulations might be contributing to the variations, although these slight changes were permissible by the directions to collaborators. The most apparent variations in technic revealed by the collaborators' reports were in the size of aliquots taken and the volume of water added before beginning the titration.

Collaborative work in 1942[2] on fruit solutions without added phosphoric acid, and preliminary work by the Associate Referee on solutions approximately .01 *M* in phosphate, had indicated that variability of dilution of the aliquot should have a negligible effect on the calculated acidity at *p*H 8.10 in terms of ml 0.1 *N* NaOH per unit volume of solution. However, variable dilutions for larger concentrations of phosphoric acid, such as have sometimes been found in imitation beverage bases, might readily cause appreciable variation in the calculated acidity at *p*H 8.10.

Another factor which might cause variation is the quantity of saturated potassium chloride solution which is allowed to flow into the titration mixture from the potassium chloride bridge. Potassium chloride is ordinarily considered to be neutral, but large quantities would certainly have some effect on the ionization of fruit acids and phosphoric acid.

Still another factor is the observation that the *p*H of the inflection point of fruit solutions is frequently lower than that which would be expected for corresponding concentrations of fruit acids. Sinclair, Bartholomew, and Ramsey[3] have published curves for orange juice and citric acid which show this same relationship. Phosphate would be expected to raise the *p*H of the inflection point. The effect of the other major soluble fruit constituents, sugars and neutral salts, must therefore have either ap-

[1] *This Journal*, **28**, 69 (1945).
[2] *Ibid.*, **25**, 73, 89 (1942).
[3] Sinclair, W. B., Bartholomew, E. T., and Ramsey, R. C., "Analysis of the Organic Acids of Orange Juice," *Plant Physiol.*, Vol. **20**, 3 (1945).

preciable buffering action or an effect upon the ionization of the fruit acids, or both. Therefore, it seemed desirable to investigate further the effect of neutral salts and sugars on phosphoric and citric acid to determine to what extent these substances would alter the electrometric titratable acidity at *p*H 8.10. This work was combined with a study of the effect of variable sized aliquots and is the basis for this report.

EXPERIMENTAL

Solutions of 0.1 *M* and 0.01 *M* potassium dihydrogen phosphate and citric acid were titrated with the glass electrode (inert atmosphere) with a standard volume of 0.166 *N* and 0.0166 *N* sodium hydroxide with and without added water and saturated potassium chloride solution. The results, shown in Table 1, are averages of triplicate, closely agreeing determinations. It is readily seen that 10-fold dilution of the original aliquot

TABLE 1.—*Acidity (pH 8.10) of 0.1N citric acid and 0.1M KH_2PO_4 solutions under variable conditions of titration*

(ml. 0.1 *N* NaOH per 100 ml. acid solution −27°C.)

VARIATION	ACIDITY
Citric Acid	
(1) 50 ml. 0.1 *N* acid *vs.* 0.1661 *N* NaOH	100.0
(2) 40 ml. H_2O added to (1)	100.0
(3) 5 ml. sat. KCl added to (1)	100.3
(4) 5.00 ml. 0.1 *N* acid+45 ml. H_2O *vs.* 0.01661 *N* NaOH	100.0
(5) 0.5 ml. sat. KCl added to (4)	100.3
Potassium dihydrogen phosphate	
(1) 50 ml. 0.1 *M* acid *vs.* 0.1661 *N* NaOH	94.7
(2) 40 ml. H_2O added to (1)	94.1
(3) 5 ml. sat. KCl added to (1)	97.9
(4) 5.00 ml. 0.1 *M* acid+45 ml. H_2O *vs.* .01661 *N* NaOH	91.2
(5) 5.00 ml. 0.1 *M* acid+85 ml. H_2O *vs.* .01661 *N* NaOH	90.6
(6) 0.5 ml. sat. KCl+4.5 ml. H_2O added to (4)	93.5
(7) 10.00 ml. 0.1 *M* acid+40 ml. H_2O *vs.* .0332 *N* NaOH	92.2

shows no effects with citric acid whereas a 1.8-fold dilution of either a 0.1 *M* or .01 *M* solution of potassium dihydrogen phosphate causes a 0.6–0.7 per cent decrease in titratable acidity. Similarly, a 10-fold dilution of the original aliquot of 0.1 *M* potassium dihydrogen phosphate produces a 3.7 per cent decrease in titratable acidity, and a 5-fold dilution shows a 2.5 per cent decrease. When the ratio of the volume of aliquots after dilution to the volume before dilution is plotted against percentage decrease in acidity, the data fall on a curve, Fig. 1. The use of this curve will be discussed later in this report.

Massive quantities of sat. potassium chloride solution, *e.g.* (1) 5 ml.

added to the titration mixture of 0.1 *M* potassium dihydrogen phosphate or 0.1 *N* citric acid, or (2) 0.5 ml to 0.01 *M* potassium dihydrogen phosphate or 0.01 *N* citric acid has the effect of increasing the titratable acidity. For citric acid, this increase is very slight, only 0.3 per cent; but for potassium dihydrogen phosphate the increase is considerable. It is 3.2 per cent at 0.1 *M* concentration and 2.3 per cent at .01 *M* concentration. For the most precise titrations of potassium dihydrogen phosphate it appears that contaminating potassium chloride should be kept at a minimum. Thus, an allowable error of less than .02 ml. in a titration of potassium dihydrogen phosphate consuming 5 ml. 0.1 *N* NaOH would require that the saturated potassium chloride volume be less than 0.1 ml.

Some information on the acidities of known mixtures is necessary before any calculations on mixtures of fruit acids (citric acid) and phosphoric acid are justified. The following mixtures were titrated with 0.1661 *N* sodium hydroxide, in duplicate, and curves plotted:

(1) 5 ml. 0.1 *M* KH_2PO_4+50 ml. 0.1 *N* Citric Acid+45 ml. H_2O
(2) 50 ml. 0.1 *M* KH_2PO_4+25 ml. 0.1 *N* Citric Acid+15 ml. H_2O

In each case, when the titer for phosphoric acid alone at a given *p*H under nearly identical conditions of dilution was added to the titer for citric acid at the same *p*H, the resultant curve is identical within very small limits with the observed curve over the *p*H range from 7.8 to 9.5. Results for the titration of mixture No. 2 are shown in Table 2. Results for mixture No. 1 are just as satisfactory.

TABLE 2.—*Typical titration data for a mixture of citric acid and* KH_2PO_4

(25 ml. 0.1009 *N* citric acid+50 ml. 0.1000 *M* KH_2PO_4+15 ml. H_2O)

*p*H	TITRATION CIT. ACID ONLY	TITRATION KH_2PO_4 ONLY	SUM OF TITRATIONS	OBSERVED TITRATION OF MIXTURE	DIFFERENCE, ML.
7.80	15.15	26.50	41.65	41.80	.15*
8.00	15.17	27.85	43.02	43.02	.00
8.30	15.20	28.95	44.15	44.17	.02
8.50	15.22	29.36	44.58	44.60	.02
8.75	15.23	29.69	44.92	44.95	.03
9.00	15.23	29.92	45.15	45.18	.03
9.25	15.24	30.09	45.34	45.34	.00
9.50	15.25	30.22	45.47	45.50	.03

* This difference, equivalent to .02 *p*H in highly buffered solution, is within allowable limit of error of *p*H measurement.

It thus appears that in this *p*H range each acid acts in solution as if it were there alone. Assuming that this is true for all possible mixtures, it is possible to determine the organic fruit acids in the presence of phosphoric acid by subtraction of the proper titer correction for the phosphoric acid. The proper corrections for all mixtures of citric acid and potassium dihydrogen phosphate will be found in the section headed "Discussion."

Of course, the phosphate must be determined by an independent accurate method after which the equivalent quantity of phosphoric acid or potassium dihydrogen phosphate is calculated.

Since it appeared that sugars probably have some effect in altering the shape of the titration curve and also that massive quantities might change the calculated titratable acidity, the following experiments were performed. A 15 g portion of a commercial preparation of powdered dextrose was titrated alone. It showed a trace of acidity and increasingly strong buffer effect above *p*H 8.5. The same experiment was made with N.B.S. No. 41 dextrose, which showed no free acidity but exhibited the same buffer action. Similarly, N.B.S. No. 17 sucrose was a much weaker buffer even above *p*H 9. Selected *p*H values, which well define the electrometric curves for each of these substances, together with the corresponding sodium hydroxide consumption, are listed in Table 3.

TABLE 3.—*Electrometric titration of sugar solutions*

(15 g. sugar in 100 ml. water vs. 0.166 *N* NaOH)

*p*H	TITRATION COM. POWDERED DEXTROSE	TITRATION NBS NO. 41 DEXTROSE	TITRATION NBS NO. 17 SUCROSE
	ml.	*ml.*	*ml.*
4.95	0	—	—
6.53	—	—	0
6.59	0.05	0	—
8.00	0.10	0.02	0.01
8.50	0.15	0.07	0.02
8.80	0.23	0.20	0.04
9.00	0.33	0.33	0.06
9.50	1.00	1.15	0.20

When the above titers for 15 g sugar were subtracted from the titrations of citric acid +15 g sugar, the resultant curves were identical within experimental error with that of pure citric acid up to *p*H 8.8. However, when the same procedure was followed with potassium dihydrogen phosphate data, the resulting curve was quite different throughout the entire range of *p*H from 7.8 to 9.5, except that the inflection point of the corrected curve came at the same titer as did pure potassium dihydrogen phosphate. The resultant effect of adding 15 g of dextrose to the titration mixture containing 50 ml. of 0.1 *M* potassium dihydrogen phosphate is to lower the *p*H at all points and therefore to increase the acidity at *p*H 8.10 by 2.3 per cent. Corrected titration data for potassium dihydrogen phosphate solutions with and without N.B.S. No. 41 dextrose are listed in Table 4.

DISCUSSION

When solutions of partially neutralized citric acid (.02 *M*) of *p*H less than 7–7.5 are diluted 10-fold, the *p*H of the solution is raised considerably,

TABLE 4.—*Corrected titrations of KH_2PO_4 with and without added dextrose*

(50 ml. 0.1 *M* KH_2PO_4 in 100 ml. water)

TITRATION 0.166 *N* NaOH	NO ADDED DEXTROSE	15 G. ADDED DEXTROSE	DIFFERENCE
ml	*p*H	*p*H	*p*H
26.00	7.78	7.62	0.16
28.00	8.10	7.95	0.15
29.00	8.40	8.23	0.17
29.50	8.65	8.48	0.17
29.90	9.01	8.76	0.25
30.10	9.35	8.95	0.40

approximately 0.25 to 0.35 *p*H units. However, when the *p*H is between 7.90 and 8.20, the change in *p*H on 10-fold dilution is less than 0.1. Above 8.20 there is an increasingly sharp lowering of *p*H on 10-fold dilution, *e.g.*, at 8.30 the change is −0.2, at 8.50 it is −0.4, at 9.00 it is −0.5. Unfortunately, the *p*H at which partially neutralized potassium dihydrogen phosphate (.06 *M*) shows no change on 10-fold dilution is 8.7 instead of 8.1. The change in *p*H at 8.10 for the same degree of dilution is +0.17, but at this *p*H phosphoric acid is a strong buffer so that a substantial quantity of sodium hydroxide is consumed in changing the *p*H only slightly.

The acidities of a few mixtures of citric acid and potassium dihydrogen phosphate have been shown to be equal to the sum of the acidities of the individual acids. If it is assumed that this condition holds for all mixtures, then certain general conclusions may be drawn from the experiments reported herein. Thus, the rate of change of the percentage correction for the titratable acidity of potassium dihydrogen phosphate decreases with increasing dilution (see Fig. 1), and the change in titratable acidity of citric acid is negligible. Therefore, it is evident that the largest variations in titratable acidity of mixtures will result when the titration is small and most of the acidity is due to potassium dihydrogen phosphate. This variation in titratable acidity could not normally exceed 3.7 per cent, which corresponds to the difference in acidity per unit weight of potassium dihydrogen phosphate when it is titrated at .06 *M* and .006 *M* end point concentrations.

Again using the curve (Fig. 1) and making the same assumption (the sum of the partial acidities of the individual acids is always equal to the total acidity) it is possible to calculate the correction to be applied to the titration of any mixture of citric acid and potassium dihydrogen phosphate that titrates approximately 50 ml. 0.1 *N* sodium hydroxide and is contained in an initial volume of 50 ml. If the initial volume is greater, the calculated correction will be smaller. The required correction for any mixture may be calculated as follows, and the corrections are listed in Table 5:

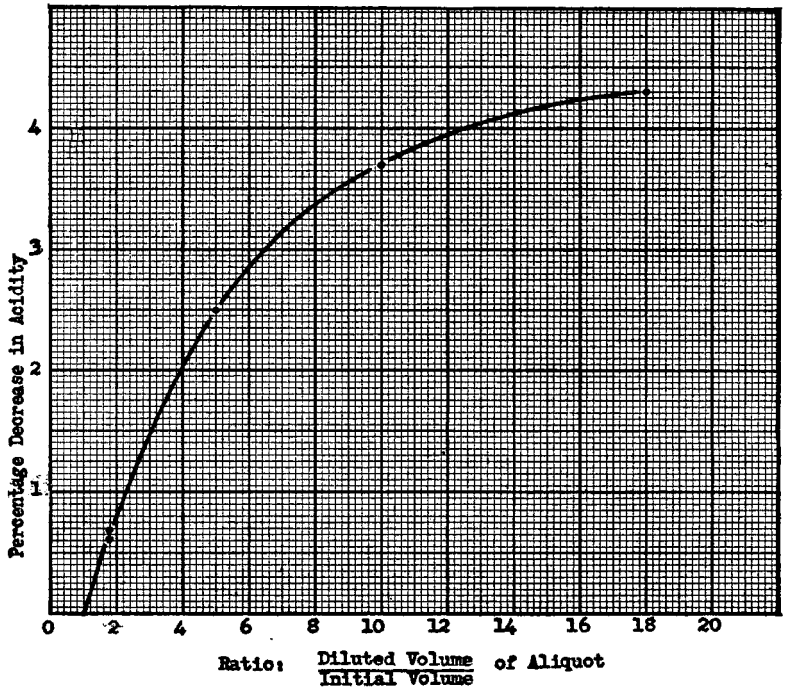


FIG. 1.—Decrease in Titratable Acidity (*p*H 8.10) of 0.1 *M* KH_2PO_4 with Increased Dilution.

Let V_1 = ml. 0.1 *N* citric acid
V_2 = ml. 0.1 *M* KH_2PO_4
$V_1 + V_2 = 50$ ml.

P = percentage decrease in acidity (Fig. 1) at ratio: $\frac{V_1+V_2}{V_2}$

0.947 = percentage of 1st H of 0.1 *M* KH_2PO_4 neutralized (no initial dilution), see Table 1.

Then $T = V_1 + .947V_2$ = Calculated titration without correction for decrease in acidity of KH_2PO_4 on dilution (ml.)

$T_c = V_1 + \frac{.947V_2(100-P)}{100}$ = Calculated titration corrected for decrease in acidity of KH_2PO_4 (ml.)

$C = T - T_c = \frac{.947V_2P}{100}$ = Difference between titrations (ml.)

As shown in Table 5, the correction to be applied rises very sharply until the potassium dihydrogen phosphate content is equal to 10–12 ml. of 0.1 *M* solution. It never exceeds 0.24 ml. Thus, the percentage error in deter-

mining citric acid by difference will not exceed 0.8 per cent of the citric acid present, even when no correction is applied.

The above discussion has been concerned entirely with pure solutions of citric acid and potassium dihydrogen phosphate; however, fruit product solutions generally contain natural or added sugars. Added sucrose and dextrose (15 g) were shown to have no effect on the titratable acidity of citric acid, but these substances do increase the acidity of potassium dihydrogen phosphate and thus tend to cancel out the effects of dilution. Sufficient data have not yet been obtained to permit more than a rough

TABLE 5.—*Titration corrections for mixtures of citric acid and KH_2PO_4*

(Percentage error in citric acid by difference if correction is not applied)

V_1*	V_2	$\frac{V_1+V_2}{V_2}$	P	T	T_c	C	Error in Citric Acid, by Diff.
0	50	1.00	0	47.35	47.35	0.00	0.0
5	45	1.11	0.1	47.61	47.57	0.04	0.8
10	40	1.25	0.2	47.88	47.80	0.08	0.8
20	30	1.67	0.5	48.41	48.27	0.14	0.7
30	20	2.50	1.1	48.94	48.73	0.21	0.7
35	15	3.33	1.6	49.21	48.98	0.23	0.7
38	12	4.16	2.1	49.36	49.12	0.24	0.6
40	10	5.00	2.5	49.47	49.23	0.24	0.6
45	5	10.0	3.7	49.74	49.57	0.17	0.4
47	3	16.7	4.3	49.84	49.72	0.12	0.3
50	0	—	—	50.00	50.00	0.00	0.0

* For definitions of symbols see paragraph immediately preceding.

estimate of the effect of these substances in phosphoric-citric mixtures. However, it may be safely concluded that no acidity increase greater than 2.3 per cent would result upon addition of 15 g dextrose to citric acid-potassium dihydrogen phosphate mixtures which consume ca 50 ml. 0.1 *N* NaOH in titration to *p*H 8.10.

All the comparisons in this report are based on determinations with a single glass electrode system, but it seems reasonable to expect that potential differences in other systems, *e.g.*, solution junction potentials, changes in asymmetry potential of glass electrodes, etc., would have a negligible effect, since the magnitudes of the potentials themselves are relatively small.

SUMMARY

The following facts concerning citric acid, potassium dihydrogen phosphate solutions and their mixtures have been elucidated by the use of a single glass electrode system:

(a) The titratable acidity at *p*H 8.10 of citric acid is not changed significantly

by 10-fold dilution, presence of neutral salts (5 ml. sat. KCl in 100 ml.), or 15 g. of dextrose or sucrose per 100 ml.

(b) The titratable acidity of 0.1 *M* KH_2PO_4 at *p*H 8.10 is influenced appreciably by 10-fold dilution (−3.7 per cent), presence of 5 ml. sat. KCl (+3.2 per cent), presence of 15 g. dextrose per 100 ml. (+2.3 per cent).

(c) The titratable acidity of mixtures of citric acid and KH_2PO_4 is equal to the sum of the acidities of the individual acids when measured at the same level of dilution.

(d) The titration of all mixtures of KH_2PO_4 and citric acid titrating ca 50 ml. is never more than 0.24 ml. 0.1 *N* NaOH below the titration that is calculated when the effect of dilution on the KH_2PO_4 (0.1 *M*) is neglected. The above difference rises sharply with increase in KH_2PO_4 concentration and reaches a maximum (0.24 ml.) at 10–12 ml. 0.1 *M* KH_2PO_4 in presence of 40–38 ml. 0.1 *N* citric acid. Therefore, citric acid in mixtures which titrate approximately 50 ml. 0.1 *N* NaOH may be calculated by difference as follows:

$$\text{ml. 0.1 } N \text{ citric acid} = \text{ml. 0.1 } N \text{ NaOH} - 0.947\ (\text{ml. 0.1 } M\ KH_2PO_4)$$

The error in the calculated citric acid due to variation in the titratable acidity of the KH_2PO_4 constituent will not exceed 0.8 per cent and will be even smaller when the citric acid contributes more than 20 per cent of the acidity.

The above-mentioned data on mixtures of citric acid and potassium dihydrogen phosphate indicates very strongly that the variations in electrometric titratable acidity reported for the collaborative solutions C, D, and E in 1945 were not due to the different small quantities of water added to the samples before titration. Also, the relatively small concentration of potassium dihydrogen phosphate (.01 *M*) could not in any way contribute significantly to the variations in titratable acidity by indicator methods when very large dilutions were sometimes employed. Thus, the variations between collaborators are still not explained.

This year's work by the Associate Referee has demonstrated conclusively that the determination of individual fruit acids or phosphoric acid mixtures by mathematical solution of equations expressing the shape of the individual electrometric curves is extremely impracticable if not impossible. This is true because the parameters of the titration curves are extremely sensitive to variations in dilution, salt concentration, neutral soluble solids, etc., at all *p*H values except in the vicinity of *p*H 8. Thus it appears that the usefulness of the glass electrode applied to mixed fruit acids is limited to the measurement of the titratable acidity.

RECOMMENDATIONS*

It is recommended—

(1) That a new series of solutions be sent to collaborators, so that a better conclusion can be drawn concerning the precision that may be expected in the determination of acidity by titration to *p*H 8.10 with various commercial glass electrode instruments. The composition and plan of analysis of some of these solutions should be arranged so that the

* For report of Subcommittee D and action of the Association, see *This Journal*, 30, 53 (1947).

assumptions and tentative conclusions of this year's work by the Associate Referee are more thoroughly tested.

(2) That further work leading toward the analysis of mixtures for the individual fruit acids by mathematical operations on the glass electrode titration curves be abandoned.

(3) That further study be made of methods of separating fruit acids, so that they may be titrated individually or in smaller groups that have similar chemical properties.

No reports were made on fruit acids, phosphoric acids, or potassium.

REPORT ON SAMPLING COLD PACK FRUIT

By PAUL A. MILLS (Food and Drug Administration, Federal Security Agency, Seattle, Wash.), *Associate Referee*

Earlier work by the Associate Referee has demonstrated analytically that in the sampling of barrels of cold pack or frozen fruit diagonal cores do not provide representative samples. Likewise, cores taken vertically midway between the side of the barrel and the center are not representative.

In 1945 the following fruits and containers were obtained: Three cans packed with 25 lbs. of strawberries, taking at the same time, portionwise, a 1 quart jar of fruit to represent each can. Three cans were packed with 30 pounds of a mixture of 4 parts strawberries and 1 part sugar. Packing was done in a commercial packing plant by their workmen, and consisted of adding fruit and sugar alternately until the can was full and contained the desired quantity of material. Approximately ½ lb. of the sugar was spread on top of the fruit. While each can was being filled a quart jar was also filled, portionwise, and the exact required quantity of sugar was added. Cans and jars were placed in storage 0°F., where they remained until sampled.

Later in the season 2 barrels were packed each containing a net weight of 375 lbs. of blackberries. One contained 375 lbs. of washed and sorted berries only, and a No. 10 can full of the same fruit was collected, portionwise, while the barrel was being filled. The other barrel was filled with 337½ lbs of fruit and 37½ lbs. of water to represent 10 per cent of water. While this barrel was being filled, 63.74 oz. of berries was collected portionwise, in a No. 10 can and 7.08 oz. of water was added. The barrels were both frozen on their sides at 0°F. for 48 hours and then stored top up at 10–15°F. The cans were stored with the barrels.

Sampling:

During the winter each barrel was sampled using a hollow tubular

trier approximately 1 inch in diameter and 40 inches long. Eight vertical cores were taken from bottom to top and through the length of each barrel.

Four cores were taken at the edge or chime, spaced evenly around the circumference.

Three cores were taken spaced evenly around the barrel midway between the edge and center and

One core was taken at the center.

It was found that the use of a towel dipped in warm water, wrung dry and wrapped around the trier, greatly facilitated the removal of a core. Care must be taken to avoid dripping water into the sample container. The trier, powered with a $\frac{3}{8}''$ electric drill motor, could be inserted about one-third the length of the barrel before the core froze in and had to be removed. Boring was continued in the same hole. Each core was kept separate.

The 30 lb. tin cans of strawberries could be sampled only from the top. These cans are approximately 12″ in diameter and 16″ high. An attempt to use the 2″ hollow trier equipped with a stop to prevent boring through the bottom was made on one can of straight berries and one can of 4+1 pack, but it was found impossible to remove the bottom inch or so of the core, which consisted largely of frozen juice or sugar solution, and this method was abandoned.

A large polished wood auger $1\frac{1}{2}''$ in diameter and sufficiently long was obtained. The lead screw was filed off to a button so it would not puncture the can. The angle of the cutting edges, which was approx. 175° with no leading cutters, was expected not to cut through the tin bottom. This large auger was powered with a $\frac{3}{8}''$ electric drill motor and operated through a special container which was used to collect the drillings. The cans were drilled vertically until the auger was stopped by the tin bottom. The drillings were carefully collected in the special container and transferred to a pint jar. Any drillings which fell into the hole were picked up by inserting the auger and turning carefully by hand a few times and lifting them out. If care was taken to drill vertically the bottom of the cans was not punctured; if however, the auger was held at an angle the cutting edges would pierce the bottom. Five borings were made, four within $\frac{1}{2}''$ of the side and evenly spaced around the circumference, and one at the center. Each subsample was analyzed separately. (Can Number 3 was bored 4 times, 3 around the edge and 1 at the center.) Each boring extended completely to the bottom of the can.

ANALYSIS

Soluble solids by refractometer at 20°C., uncorrected for insoluble solids, and insoluble solids were determined on the samples collected.

Results of the analysis are shown in Table 1.

TABLE 1.—*Results in soluble and insoluble solids*

Per Cent

	1	2	3	4	5	6
	AVE. OF OUTER CORES (4)	AVE. OF MIDDLE CORES (3)	CENTER CORE	AVE. OF OUTER CORES AND CENTER CORE	SAMPLE COLLECTED WHEN CONTAINER WAS PACKED	VARIANCE OF COL. 4 FROM COL. 5
						Dif. *Per cent*
			Bbl. of Straight Blackberries			
Sol. Solids	9.09	9.32	9.70	9.40	9.61	−0.21 or 2.2
Insol. Solids	5.44	6.31	6.50	5.97	5.84	+0.13 or 2.2
			Bbl. of Watered Blackberries			
Sol. Solids	8.38	8.47	8.36	8.37	8.30	+0.07 or 0.8
Insol. Solids	4.85	5.49	5.30	5.08	4.87	+0.21 or 4.3
			Can of Straight Strawberries			
Sol. Solids	9.08	—	8.95	9.01	8.89	+0.12 or 1.3
Insol. Solids	2.44	—	2.23	2.34	2.45	−0.11 or 4.5
			Can of Straight Strawberries			
Sol. Solids	9.22	—	8.58	8.90	8.52	+0.38 or 4.4
Insol. Solids	2.45	—	2.16	2.31	2.45	−1.14 or 5.7
			Can of 4+1 Strawberries			
Sol. Solids	21.24	—	26.15	23.70	24.61	−0.91 or 3.7
Insol. Solids	2.24	—	2.24	2.24	2.06	+0.18 or 8.7
			Can of 4+1 Strawberries			
Sol. Solids	26.08	—	29.65	27.87	27.85	+0.02 or 0.1
Insol. Solids	1.99	—	2.54	2.27	2.03	+0.24 or 11.8

DISCUSSION

Each barrel was sampled from the bottom through its entire length. Sampling from the bottom insures the inclusion of all the juice which always collects at the bottom and is always present.

The composition varies from the edge to the center due to freezing. Freezing progresses from the outside and is faster at the bottom than at the top because of the dead air space at the top left there to take care of the expansion. The water in the fruit freezes first and tends to increase the soluble solids toward the center. At the same time an inverted cone of frozen material probably is formed which tends to funnel juice from the unfrozen portion toward the center. As the outer layers solidify they expand and tend to produce pressure toward the center and pack the unfrozen berries closer together. This pressure is demonstrated by the mounding observed at the top of containers of frozen fruits. These phenomena are shown by the analyses in Table 1.

In sampling, then, the center and the outer edge, as the two extremes, should be represented. Table 1 shows that such sampling gives a reasonably close representation of the contents.

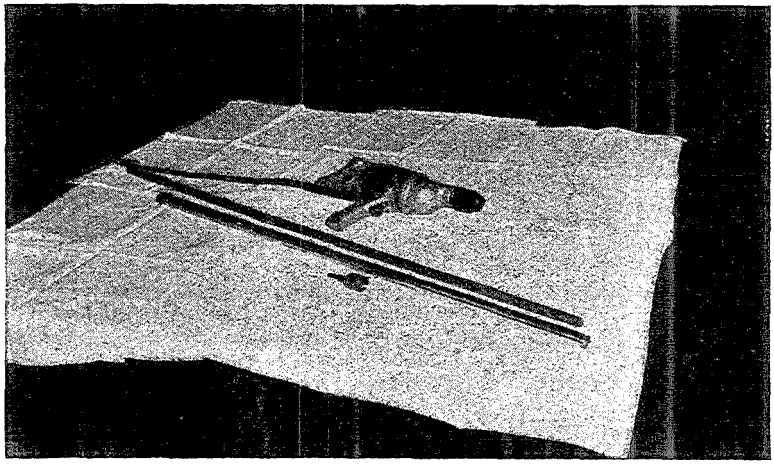


FIG. 1.—Trier for sampling barrels.

The following tentative procedure and equipment for sampling of frozen fruit for chemical analysis are recommended:

For barrels:

Use a stainless steel or corrosion resistant tube approximately 1¼″ in diameter and 36″ long, one end of which is serrated and set to run freely, the other end of which has a removable cap and connection for use of electrical energy in drilling. To assist in removal of core samples use a wooden ram smaller in diameter but longer than the tube.

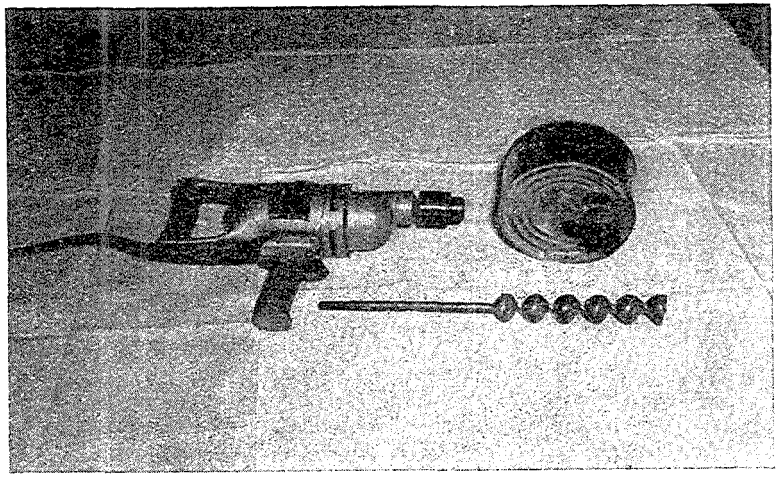
Remove the bottom of the barrel and take three cores evenly spaced around the circumference near the chime, parallel to the length of the barrel and through its entire length. Take a fourth core at the approximate center of the barrel. Composite the three outer cores and take equal weights of the center core and outer cores for analysis.

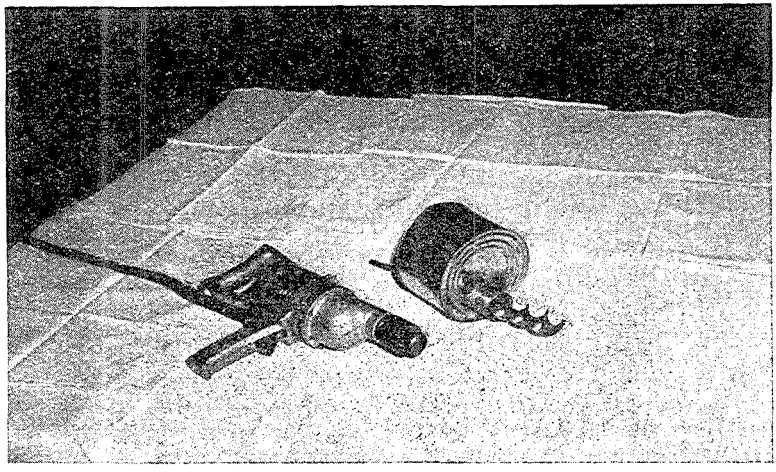
For smaller containers:

For sampling use a modified corrosion resistant auger approximately 1 to 1½″ in diameter and 19″ long which can be operated with electrical energy. The auger should have no lead screw or cutters and the angle of the face should be approximately 170–175°. In conjunction with the auger and for collection of the borings, use a corrosion resistant sampling can open at one end, approximately 6″ in diameter and 4″ high, with an outlet at the other end about 1″ long near the circumference and slightly larger than the diameter of the auger.

Take three vertical borings evenly spaced about the circumference approximately ½″ from the edge of the container, and take one boring at or near the center. Use the sampling can in conjunction with the auger and remove both simultaneously to prevent borings from falling through delivery outlet. Composite the borings about the circumference and take as the sample for analysis an equal weight of the center and outer borings.

The cooperation of the National Fruit Canning Co., Seattle, Wash. for the use of its facilities in furnishing the material sampled is gratefully acknowledged. H. L. Halladay and C. P. Marshall, Food and Drug Administration, Seattle, Wash. assisted with the sampling, and I. B. Berch, U. S. Food and Drug Administration, Seattle, Wash., collaborated in the photography.





FIGS. 2 and 3.—Auger and funnel for sampling cans.
(Funnel made from No. 10 tin. Should be of stainless steel.)

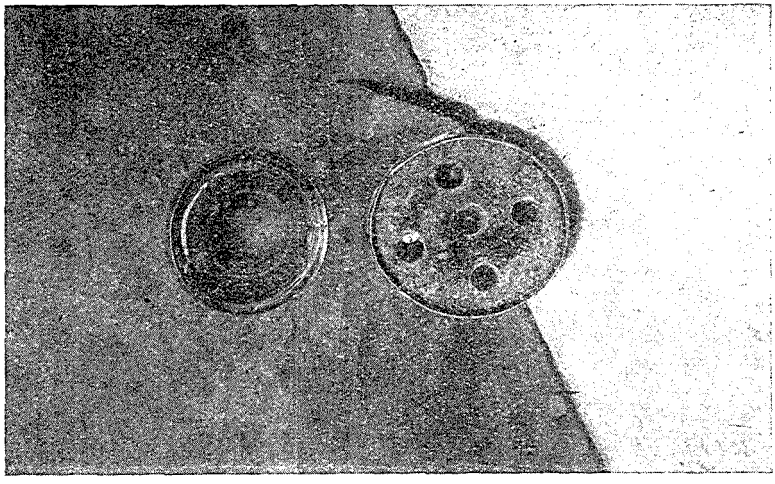


FIG. 4.—Can of frozen strawberries after sampling.

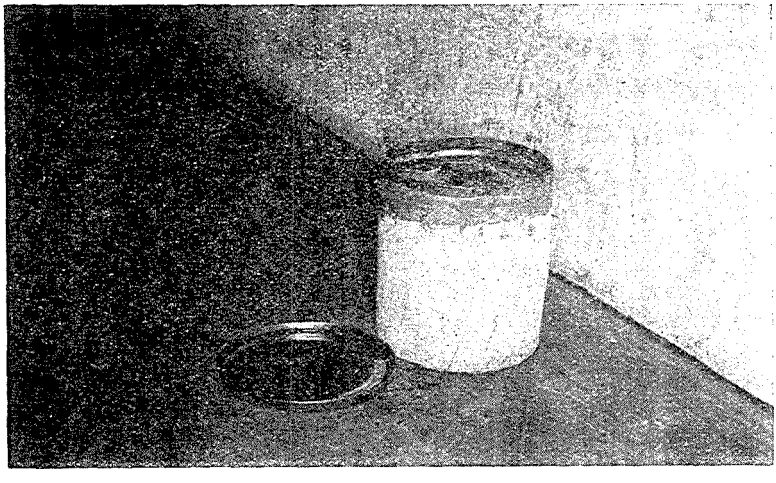


FIG. 5.—Can of frozen strawberries showing mounding.

It is recommended* that further work on the sampling of cold pack fruit be discontinued.

* For report of Subcommittee D and action of the Association, see *This Journal*, **30**, 53 (1947).

REPORT ON CACAO PRODUCTS

By W. O. WINKLER (Food and Drug Administration, Federal Security Agency, Washington 25, D. C.), *Referee*

The work on cacao products this year has progressed, but none of the projects started has been completed. Only one Associate Referee's report was received this year, that on the determination of lecithin. The work on other projects has not stood still, however, and a considerable amount of work has been done on the determination of maltose and lactose in the presence of other reducing sugars.

Maltose.—Mr. E. W. Meyers, the Associate Referee on Maltose, has assembled and studied a number of methods of arriving at the maltose content of mixed sugar samples such as occur in cacao products. The work has not reached the stage where the Associate Referee is ready to recommend study of a particular method, but he anticipates that this will be done during the coming year.

Lactose.—Because of the use of sweetening ingredients other than sucrose in cacoa products, there is need for a method of determining lactose in the presence of other reducing sugars. The Referee has studied some methods, particularly fermentation procedures, and has devised a fairly rapid one which has given good results in the hands of one chemist. It is the intention of the Referee to submit the procedure to an Associate Referee during the coming year for study and comparison with other suitable methods.

Pectic Acid.—Determinations of pectic acid by the tentative method have shown that the final precipitate (pectic acid) is often of a colloidal character if the first saponification is made at temperatures around 25°C. This makes filtration very difficult. To overcome this it is necessary to reduce the temperature to 20°C. or lower in the first saponification. It is therefore recommended that this change be made in the tentative method for determination of pectic acid.

Lecithin.—The Associate Referee on Lecithin has submitted a report in which a collaborative study was made of samples before and after addition of known amounts of lecithin. Inasmuch as recoveries were somewhat low on the samples with added lecithin, the Referee concurs in the recommendation that the work be continued.

RECOMMENDATIONS*

It is recommended—

(1) That the work on lecithin be continued.

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

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* For report of Subcommittee D and action by the Association, see *This Journal*, 30, 53 (1947).

(3) That methods for the determination of cacao ingredient be studied further.

(4) That work on the determination of fat in beverage bases and other refractory material be continued.

(5) That line 7 of paragraph 2, page 227, under section **19.16,** reading "Cool the bottle and contents to about 20° (below 25°)" be changed to read "cool the bottle and contents to 20° or lower," for the reasons given in this year's report of the Referee.

(6) That further collaborative study be made of the tentative method for pectic acid.

REPORT ON LECITHIN IN CACAO PRODUCTS

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

In his Report on Cacao Products, 1945, the Referee suggested that the method for lecithin reported in 1941 (*This Journal,* **25,** 717, 1942) should be studied collaboratively.

Two samples of sweet chocolate were sent to collaborators. Both samples were made of the same chocolate, but Sample B contained nearly 0.30 per cent of added lecithin. Collaborators were requested to determine lecithin by the method cited above, but to take a 5 gram sample and make up to 200 ml. This slight change was made to facilitate the withdrawal of a 100 ml aliquot and to bring the phosphoric acid well within the range of the molybdenum blue method. Collaborative results are given in the following table:

TABLE 1.—*Collaborative results*

COLLABORATOR NO.	SAMPLE A		SAMPLE B		
	DET. NO.	LECITHIN	DET. NO.	LECITHIN	ADDED LECITHIN FOUND (B—A)
		per cent		*per cent*	*per cent*
1	1	0.232	1	0.528	0.296
	2	0.227	2	0.466	0.239
	3	0.218	3	0.453	0.235
	4	0.223	4	0.476	0.253
2	1	0.262	1	0.460	0.198
	2	0.255	2	0.460	0.205
3	1	0.214	1	0.450	0.236
	2	0.216	2	0.459	0.243
4	1	0.223	1	0.446	0.223
	2	0.221	2	0.450	0.229

COMMENTS OF COLLABORATORS

William Horwitz—Minneapolis Station:

Since in Sample B, determinations 1 and 2 did not check, both samples were repeated on July 9, including a new standard curve which was identical with the original (July 3). A Klett-Summerson photoelectric colorimeter with test tube cells and filter 64 was used. It gave a straight line standard curve. After standing overnight, four samples and the standard curve were completed in seven hours.

We have no criticisms to make regarding the method although the aliquots were slightly cloudy.

Halver C. Van Dame—Cincinnati Station:

It seems that it might be a good idea to filter the 100 ml aliquot directly into the Kjeldahl flask, as there seems to be some chocolate residue left after evaporation, no matter how carefully the aliquot is drawn off.

There were no directions for calculating phosphoric acid on the sample so I assumed no correction was made for the volume occupied by the insoluble part of the sample. While this is not very much it would amount to about 2 or 3 ml and possibly should be considered. I did not make any corrections in my calculations.

Harry W. Conroy—Kansas City Station:

The device used for measuring 100 ml. of the solvent solution of sample is a slight modification of one shown in the Journal of the A.O.A.C., 1932 (p. 594).

The use of filter paper on the end of the pipet was found to be unhandy and the resulting solution was somewhat cloudy.

The 200 ml sample and solvent is poured into a bottle having a stopper which permits entry of a Knorr tube and pressure bulb. A 1-inch length of 19 mm extraction tube thimble is slipped over the lower end of the Knorr tube and pressure applied. The resulting solution is clear and no air is forced through if the thimble is kept under the solvent.

Matthew L. Dow, Frederick M. Garfield—St. Louis Station:

The results obtained on the collaborative samples sent to St. Louis are shown in Table 2.

TABLE 2.—*Collaborative results*

SUB NO.		A1		A2		B1		B2	
		DOW	GARFIELD	DOW	GARFIELD	DOW	GARFIELD	DOW	GARFIELD
Size of Sample	Gm	5.0000	5.0015	5.0000	5.0060	5.0000	5.0017	5.0000	5.0017
Ave. Photometer reading	Cm	3.68	3.40	(*)	3.63	6.36	6.24	6.27	6.32
P_2O_5 (Graph)	Mg	0.100	0.09	—	0.10	0.197	0.20	0.199	0.202
P_2O_5 in Sample	Gm	0.020	0.018	—	0.020	0.039	0.040	0.040	0.040
Lecithin ($P_2O_5 \times 11.37$)	%	0.227	0.205	—	0.227	0.443	0.455	0.455	0.455

The curve was prepared from fresh standards and reagents. Every point fell on the line. Each analyst prepared and ran his own samples. The aliquots were pipetted out of the flasks using gentle suction. One of the determinations (marked *) ap-

peared to be invalid because the suction was accidentally broken during pipetting and some of the settled material was drawn up into the pipette. From our experience with this series of samples, it appears that if any of the sediment on the bottom of the flask gets into the sample, high P_2O_5 results are obtained. Perhaps a caution statement should be inserted into the method to warn against errors of this type. It is possible that the unusually high preliminary results obtained were due to this very thing.

DISCUSSION

It is recognized that the volume of the insoluble material causes the results to be slightly too high; however, this error is less than 2 per cent and does not appear important.

A slight turbidity in the filtered aliquot does not appear to be seriously objectionable, as it is due chiefly to sugar and cacao starch, which do not contain P_2O_5.

The close agreement of most of the analytical results is gratifying. The failure in all cases but one to show the true amount of added lecithin is disappointing. The reason for this is not clear, but it may be due to the age of the lecithin used. This lecithin was six years old; however, it was purified before use by the method used by Winkler and Sale (*This Journal*, **14**, 537, 1931).

It may be that there was progressive decomposition during the warming and mixing of the sample.

It is recommended* that work on lecithin in cacao products be continued.

No reports were given on theobromine, malt solids, pectic acid, chocolate constituents, lactose, and fat.

REPORT ON SUGAR AND SUGAR PRODUCTS

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

It is recommended*—

That the study of unfermented reducing substances in molasses (*This Journal*. **29**, 242, 1946) be continued.

That the study for the determination of moisture be continued.

That tables of density of sucrose solutions at various temperatures be calculated.

That the Zerban and Martin values for refractive indices of dextrose and invert sugar solutions (*This Journal*, **27**, 295, 1944) be adopted as tentative. This table was subsequently expanded to show intervals of 0.1

* For report of Subcommittee D and action by the Association, see *This Journal*, 30, 53 (1947).

per cent; it is published under "Changes in Methods of Analysis," *This Journal*, **30**, page 77.

That the study of the applicability of electrodeposition to the direct quantitative determination of dextrin in honey and honeydew honey (*Ind. Eng. Chem. Anal. Ed.*, **16**, 23–25) be continued.

That a study of the characteristic properties of the dextrins of honey and honeydew honey be undertaken with reference to their application to methods of analysis of honey.

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

That the method for the determination of resinous glaze in confectionery as described in this year's report of the Associate Referee be subjected to collaborative study.

That further study be made on the determination of dextrose, maltose, and dextrins, by copper reduction methods in pure sugar mixtures.

That the tentative methods, **34.133-34.155,** inclusive, be subjected to collaborative study.

That the procedures in N.B.S. Circular C440, pp. 324–334, for measurement of transmission of solutions of commercial sugar products be considered with a view to their future adoption as tentative methods. (*See also,* Browne and Zerban, Sugar Analysis, pp. 584–607 (1941).)

No reports were made on the following subjects: unfermented reducing substances in molasses; drying methods; densimetric and refractometric methods.

REPORT ON HONEY AND HONEYDEW HONEY

By GEORGE P. WALTON (Production and Marketing Administration, United States Department of Agriculture, Washington 25, D. C.), *Associate Referee*

In accordance with the 1944 recommendation for work under this subject, efforts have been made to develop a method of separating dextrin from the other constituents of honey by means of electrodeposition of the dextrin.

Written suggestions for exploratory work for the development of such a method, based upon the Williams and Johnson method for the determination of pectins by electrodeposition,[1] were sent to several prospective

[1] Ind. Eng. Chem., Anal. Ed., 16, 23–25 (1944).

collaborators in December 1944 and January 1945. Suitable samples of true honey and honeydew honey accompanied the procedure outline.

The method outlined comprised precipitating the crude dextrin by the addition of ethyl alcohol, as in the A.O.A.C. tentative method;[2] dissolving the precipitated dextrin in hot water; diluting with distilled water to an estimated dextrine concentration of around 150 milligrams per 100 ml; and depositing or reprecipitating the dextrin in pure form by suitable electrolysis of an alcoholic solution of the dextrin.

Since it had been shown that honey colloid particles usually carry charges of positive electricity, it was assumed that the honey dextrin would accumulate at the cathode of the electrolysis cell. This assumption seemed to rule out use of the simple type of cell in which the cathode is a layer of mercury. Accordingly, a description, with drawings, of two alternative cell designs was drawn up, and sent to the prospective collaborators.

It was taken for granted, from Williams and Johnson's work on the electrodeposition of pectins, that a source of 220-volt DC electricity would be required to effect the electrolysis. It transpired that such electric current was not available in the other laboratories to which samples had been sent. Only Carl F. Snyder, our General Referee for Sugar and Sugar Products was in a position to collaborate with the Associate Referee in the exploratory work on the proposed method.

Samples of Western Cedar honeydew honey, and of Buckwheat honey, prepared for the collaborative work, were used in the experiments. The present A.O.A.C. method was followed to the completion of the operation of dissolving the crude dextrin in hot distilled water and passing the hot solution through the filter. An electrolysis cell of the second design shown in the description and drawings appended to this report, was made available by the General Referee, and was used in all of the trials.

Concentrations of crude dextrin varying from 65 to 154 milligrams (35 to 83 milligrams of "corrected" dextrin) per 100 ml of the solution electrolyzed, were used in the trials. In every case ethyl alcohol was added, a little at a time, to the aqueous solution of the dextrin, with continuous mixing, until incipient precipitation of the dextrin occurred, as shown by faint cloudiness of the mixture. The total volume of the mixture in the electrolysis vessel was then brought to 100 ml with alcohol of the same strength. In the several trials, the alcohol content of the mixture varied from 45 to 67½ per cent (by volume).

The first few trials showed too great electrical conductance of the solutions, in spite of the low electrolyte (ash) content of the honeys. Although the electrolysis vessel was kept immersed in ice water, current flow, and the temperature of the dextrin solutions, increased rapidly in every case,

[2] *Methods of Analysis*, 6th edition (1945), 34.98, 583.

and the current had to be interrupted in order to cool the solutions. The solutions carried too much current in all of these trials. For example, using 220-volt DC for electrolyzing a solution of approximately 140 milligrams of crude dextrin, from Buckwheat honey, contained in 100 ml of 67½ per cent (by volume) alcohol, the flow of current rose from less than 250 to 450 milliamperes, in 20 minutes. Upon turning off the current, cooling the solution, and again electrolyzing, the current flow was 150 milliamperes, but soon rose to 0.5 ampere. In this experiment a considerable quantity of reddish-brown flocculate was observed. This had settled to the bottom of the cell, and was therefore resting on the anode. It appeared to be an irreversible colloid, did not redissolve in hot water, and probably was chiefly protein material.

A small quantity of each of the two ion-exchange resins (I R #100 and I R #4) mentioned by Williams and Johnson (*loc. cit.*) was obtained, for the purpose of reducing the electrolyte content of the dextrin solutions Three experimental runs were made, in which the aqueous dextrin solutions were successively passed through columns several centimeters thick of each ion-exchange resin. Although in one trial the initial current flow was reduced to as low as 50 milliamperes, and it was possible to continue the electrolysis for several hours, no appreciable deposit of material was observed on either electrode, in this experiment.

Although our experimental attempts to separate the dextrin of honey by electrolysis, or electrodeposition, so far have been unsuccessful, a number of features of the procedure have not yet been explored. It is our opinion that additional work should be done with particular attention given to removal of interfering electrolytes, and to adjustment of the voltage of the electric current to the requirements of the method.

I wish to gratefully acknowledge the help and facilities afforded by Mr. Snyder, General Referee, in the greater part of the experimental work.

RECOMMENDATIONS*

In addition to again calling attention to the considerations, brought out in the 1944 report, that cause the present tentative method for determining dextrin in honey to be recognized as an empirical one, it is recommended—

(1) That the study of the applicability of electrodeposition to the direct quantitative determination of dextrin in honey and honeydew honey be continued;

(2) That a fundamental study of the characteristic properties of the

* For report of Subcommittee D and action by the Association, see *This Journal*, 30, 53 (1947).

dextrins of honey and honeydew honey be undertaken as opportunity permits;

(3) That with regard to the official method for the determination of free acid in honey, collaborative work be undertaken with a view to making the determination more definite with respect to the end point of the titration with 0.1 *N* sodium hydroxide, by establishing the length of time during which the faint pink color of the phenolphthalein indicator should persist.

Suggestions for a Simple Electrolysis Cell for Use in Determining the Dextrin in Honey by Electrodeposition

In the Williams and Johnson method (*loc. cit.*) for the determination of soluble pectin and pectic acid by electrodeposition, the pectic substances are deposited upon the anode; hence an easily prepared and conveniently operated cell with cathode of mercury as bottom layer in a beaker fitted with side tube, was employed by the authors of that method.

When the substance to be determined is deposited upon the cathode, however, mercury cannot be used. As the cathode, it does not provide an electrode surface suited to the drying and weighing of the deposited substance, and its use as anode is undesirable. It appears advisable, therefore, to have both electrodes of platinum, in determining the dextrin content of honey by electrodeposition.

Williams and Johnson have pointed out the advantage in having the layer of solution that is subje cted to electrolysis completely contained between transverse, stationary electrodes, the area of each of which is practically the same as that of a cross-section of the liquid layer. No stirring is required, it is stated, and deposition is completed in five hours.

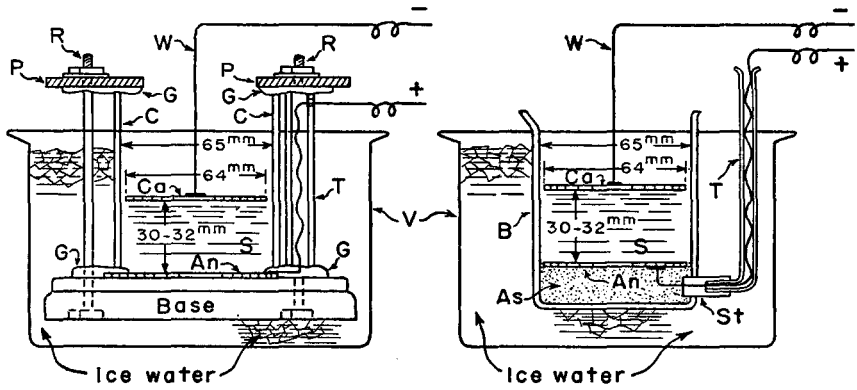
The voltage and current density recommended, in fact all of the quantitative data included in the suggested method are based upon use of a cell of 65 mm. internal diameter and a solution volume of 100 ml., forming a solution layer approximately 31 mm. thick.

Two alternative arrangements of the parts of such an electrolytic cell, more particularly of the anode fittings, are shown in the accompanying drawings. The first cell consists of a glass tube, or bottomless glass cylinder, furnished with a leak-proof, platinum bottom (which serves as the anode) which is provided by tightly clamping the cylinder to a disc of non-perforated sheet platinum, after adjusting a thin gasket of gum rubber between cylinder end and platinum disc. The second cell is made by drilling a hole through the wall, near the bottom of a 250 ml. Griffin-type beaker (to provide for the anode connection); fitting this hole with rubber stopper through which the anode lead is passed; flowing in warm petroleum asphalt, of the grade used on automobile storage batteries, to a level just above the stopper; then inlaying the platinum-disc anode in the upper surface of the asphalt, and making sure the anode is clean and bright after the asphalt has hardened.

Since provision for cooling the cell in ice and water should be made, either cell should have a water-tight glass-tube conduit for protecting the anode lead from short-circuiting.

Although less readily constructed, the first cell can be more quickly and easily prepared for use, or disassembled for cleaning. The same readily demountable electrode of platinum gauze, described in the article by Williams and Johnson, may be used as cathode in either of the cells described.

SKETCH OF ELECTROLYSIS CELL
(ALTERNATIVE ANODE FITTINGS)



An = Anode
Ca = Cathode
S = Solution to be electrolyzed
B = Beaker, or
C = Bottomless cylinder of glass
G = Rubber gaskets
R = Tie-rods between base and plate-ring
P-P = Plate-ring for clamping C tightly to G and platinum anode-bottom of cell
T = Glass tube to shield anode connection
St = Rubber stopper
As = Asphalt of grade used to cap storage batteries
W = Platinum wire △
V = Outer cooling vessel, for ice and water

△ *Electrodes are thin platinum discs, the cathode can be made of fine-mesh Pt. gauze with stem, W, of fairly rigid Pt wire.*

U. S. DEPARTMENT of AGRICULTURE
Production and Marketing Administration

Negative 781

REPORT ON CONFECTIONERY

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

In consideration of solvents for removal of lac from glazed candies as recommended last year,[1] it appears that a mixture of absolute alcohol and benzol will remove the glaze. Extraneous material such as sugar, acids, and fat included in the extract can be removed by suitable washing of the dried residue after evaporating the solvent. After some trials a suitable method was worked out as follows:

PROPOSED METHOD FOR ESTIMATION OF LAC IN CANDIES

Place 50 grams of candy in a 400 ml beaker. Add 50 ml of a mixture of benzol and absolute alcohol and cover with a water glass. Place on a steam bath, heat to boiling and simmer for a few minutes, stirring occasionally. Decant the liquid into a tared round glass dish of about 100 ml. capacity having a flat bottom about 2¾ inches in diameter. Repeat, using a similar mixture, and finally rinse twice with two 25 ml portions of absolute alcohol, simmering and stirring each rinsing liquid.

Add each liquid to the glass dish previously placed over the steam to evaporate the alcohol-benzol mixture. Allow to remain on bath until alcohol is all removed, rotating the dish, as it goes to dryness, to spread the extract uniformly over the bottom surface. If fat appears to be present wash with 50 ml. petroleum benzene, stirring and warming. Decant and add 20 ml. of water, digest on bath, stirring and rubbing well. Decant, break up and dissolve lumps with 95% alcohol, evaporate alcohol, and repeat washing and alcohol treatment to constant weight; usually about 5 such treatments are required. Dry in 100° oven and weigh.

Results on known lac added to various candies are shown in Table 1. Authentic bleached candy shellacs were used excepting Sample C-3 which

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

SAMPLE	TYPE OF CANDY	LAC ADDED	LAC FOUND
		Per cent	*Per cent*
A & R I	Sugar coated almonds	0.54	0.53
A & R I	Sugar coated almonds	0.27	0.26
A & R I	Hard sugar candy	0.54	0.51
S-1	Hard sugar candy	0.34	0.32
M.L.C.-2	Small sugar coated lozenges	0.53	0.53
M.L.C.-3	Hard sugar candy—aged one month after adding glaze	0.34	0.36
W.Z.-1	Small sugar coated lozenges	0.72	0.67
R.P.-1	Small sugar coated lozenges	1.06	1.06

was unbleached. Each number represents lac obtained from a different concern or a different lot. The lac was added in alcoholic solution and allowed to dry well before assaying.

The above results indicate reasonably good recoveries can be obtained

[1] *This Journal*, **28**, 535 (1945).

considering the type of materials involved. Table 2 illustrates recovery of added glaze in a commercial sample of glazed, chocolate-coated peanuts.

TABLE 2.—*Recovery of glaze added to commercially glazed candy*

SAMPLE NO.	TYPE OF CANDY	AMOUNT FOUND BY ANALYSIS	AMOUNT ADDED	RECOVERY OF ADDED LAC
		Per cent	*Per cent*	*Per cent*
M.L.C.-3	Choc. coated peanuts	0.21	0.34	0.32

It is recommended* that the proposed method be subjected to collaborative study.

No report on reducing sugars was made.

No report on corn sirup and corn sugar was made.

REPORT ON COLOR AND TURBIDITY IN SUGAR PRODUCTS

THE MEASUREMENT OF COLOR IN SOLUTIONS OF SUGAR PRODUCTS

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

INTRODUCTION

The word "color" in the present report has the customary meaning as it is used by the sugar chemist who "as a rule wants information on the quantity and nature of the coloring matter present in sugar products."[1] What is actually measured is the extent of light transmission (or absorption) by a solution of the sugar product under known conditions. The quality of absorption may be ascertained by the use of appropriate instruments and computations, and the color defined in the sense of the word color as used by the physicist.

The following description of procedure is taken from the chapter on colorimetry in National Bureau of Standards Circular C440, "Polarimetry, Saccharimetry, and the Sugars," pages 300–334. The method is applicable to the colorimetry of cane and beet sugars, sirups, and molasses, and to corn and maple sugars and sirups. A spectrophotometer is required or an instrument designed for abridged spectrophotometry capable of yielding results at some few wave lengths, one of which should be λ 560 mμ. See also, Browne and Zerban, Sugar Analysis, pp. 574–609.[1]

* For report of Subcommittee D and action by the Association, see *This Journal*, **30**, 53 (1947).

[1] C. A. Browne and F. W. Zerban, "Physical and Chemical Methods of Sugar Analysis," page 574. (John Wiley & Sons, Inc., New York, N. Y., 1941.)

DEFINITIONS AND SYMBOLS

Brix = grams of sucrose in 100 g of solution. In impure solutions, Brix is taken to represent dry substance, d.s., in 100 g of solution as determined densimetrically, refractometrically, or by drying.

c = g of d.s. in 1 ml = Brix × true density/100.

b = thickness (or length) in cm of the light-transmitting layer of solution.

T = transmittance = $T_{solution}/T_{solvent}$.

t = specific transmissivity = transmittancy reduced to unit conditions as regards thickness and concentration.

$t = \sqrt[cb]{T}$ or $-\log t = 1/cb\ (-\log T)$.

$-\log t$, the specific absorptive index, is a measure of the absorbing power of the unknown amount of sugar color associated with 1 g of dry substance.

λ = wave length expressed in millimicrons, mμ.

PREPARATION OF SOLUTIONS

For transmission readings the concentration of sugar solids should correspond to about 60 Brix. Solutions of white sugars require long cells particularly if readings are made at the longer wave lengths where b may be 20 cm. On the other hand, darker products such as raw or soft sugar and molasses may require dilution with colorless sugar or syrup to render them readable and shorter cells may be used (b = 1 to 2 cm depending upon wave length).

(1) *Where Color Dilution is Unnecessary*

Weigh 60 g of the sugar ± 1 cg in a tared Erlenmeyer flask or beaker. Add 40 g of hot water in small quantities at a time, shaking or stirring until all is dissolved and the solution has become thoroughly mixed. Filter through asbestos as described below.

(2) *Dark Products Requiring Dilution of Color*

A high grade granulated or tablet sugar (the latter coarsely pulverized) selected for absence of color is recommended as a color diluent. If the dark sugar product is moist or a liquid such as molasses, its dry substance content is determined beforehand and recorded.

PROCEDURE

Weigh 50 to 59 g ± 1 g of the diluent sugar in a tared beaker recording the weight. Add 1 to 10 g of the dark product (dry basis), again recording the weight. Dissolve and dilute to 60 Brix with hot water as in (1). The weights given above may be varied in accordance with the darkness of the colored product. After dilution and mixing, filter the solution through asbestos as described below.

PREPARATION OF ASBESTOS

Treat each 25 g of long fibered Powminco asbestos (A grade or longer) with 250 ml of a 40 percent solution (sp.g. 1.43) of sodium hydroxide in Pyrex flask, mix, cover the flask loosely, and boil 30 minutes. Dilute with water and filter with suction in a Büchner (without paper). Wash to remove alkali, drain and transfer the asbestos to a beaker or flask and add a mixture of 250 ml hydrochloric acid 1.20 sp.g. and 25 ml nitric acid sp.g. 1.42. Mix and heat on a steam bath 30 minutes. Dilute with wa-

ter and filter as above. Wash the asbestos until Cl can no longer be detected. Dry at 100–110°C and store.

Filtration of Syrup

For filtration, glass filters with fritted disc such as the Büchner type Pyrex, 60 ml, fineness C, are preferable. Porcelain Gooch crucibles fitted with discs of filter paper and adapters may be used. 250 ml glass suction flasks serve as receivers.

PROCEDURE

Mix prepared asbestos with water and pour it into the filter, starting the suction. Drain and tamp the asbestos tightly with a large, blunt stirring rod to form a pad about 0.5 cm thick. Wash the pad a few times with water and drain.

Add a few grams of dry asbestos to the prepared syrup and thoroughly mix. Pour a few ml of this mixture in the filter, apply suction until water in the filter has been replaced and leaving the mat still covered with syrup. Stop the filtration, replace the receiving flask with a clean, dry one and proceed to filter the remaining syrup. The asbestos is not allowed to become uncovered with syrup during or at the end of filtration. A second filtration is frequently necessary to insure a suitable filtrate. Determine and record the refractometric Brix of the well mixed filtered syrup.

MEASUREMENT OF TRANSMITTANCY

The technique of transmittancy measurement varies with the type of instrument being used and it may be assumed that this is familiar to the operator. With spectrophotometers, the sugar solution is usually compared with distilled water each contained in cells with plane parallel end faces and with equal length of light path. The length of cell to be chosen is discussed above. Highest transmittancy is found in the red part of the spectrum and lowest in the blue and it is sometimes advantageous to vary the cell length for different spectral regions. Customarily 3 to 5 readings are taken at each wave length. The positions of the cells with reference to each other and to the entrance pupils of the photometer are reversed and an equal number of readings recorded. The mean of these readings is recorded as the value of T. With some instruments scale readings are in angular degrees from which T may be calculated. With photoelectric instruments, two check readings may be sufficient. In any event the value of T or $-\log T$ is sought, and one of the wave lengths should be 560 mμ.

CALCULATION OF RESULTS

(1) When dilution of color was unnecessary there have been recorded Brix, thickness, b, and T_λ. From Brix calculate c as described above or by reference to an appropriate table. From T find $-\log T$ and substitute these values in the equation

$$-\log t_\lambda = 1/cb(-\log T_\lambda)$$

at each wave length at which observations were made. From readings at every 10 or 20 mμ a curve may be plotted, $-\log t$ against wave length, to provide a picture of the absorbent properties of the solution.

(2) When color has been diluted with white sugar a correction is to be made for any absorbency of the diluent. A 60 Brix solution of the diluent sugar is photometered and $-\log t$ for each wave length is applied as a correction to the results obtained for the diluted mixture. Following the directions for dilution of color given under (*2*) above, there are available—

(*1*) The weight and d.s. content (100%) of the diluent sugar.
(*2*) The weight and Brix or d.s. of the dark product.
(*3*) The Brix and c of the filtered solution.
(*4*) $-\log T_\lambda$ and $-\log t_\lambda$ of the diluent.
(*5*) $-\log T_\lambda$ and $-\log t_\lambda$ of the filtered solution.

From the weights of d.s. (*1*) and (*2*) obtain the per cent of colored d.s. and the per cent of diluent d.s. in the mixture. Since it may be assumed that the original proportions of diluent and of colored d.s. have not changed in the filtrate, we find the per cent of c (*3*), attributable to the diluent sugar, calling it c_D and the per cent due to the dark product, c.

In the mixture it is obvious that the absorbency to be designated as $-\log T_M$ is made up of two parts, the $-\log T$ of the colored product and $-\log T_D$ of the diluent, that is,

(a) $$-\log T_\lambda = (-\log T_{M\lambda}) - (-\log T_{D\lambda}).$$

Calculate the value of $-\log T_{D\lambda}$ from c_D of the diluent d.s. in the mixed solution and its specific absorptive index, $-\log t_{D\lambda}$ for each wave length as follows:

(b) $-\log T_{D\lambda} = c_D b(-\log t_{D\lambda})$ to obtain the corrected values for $-\log T_\lambda$ in the mixture and recalculate the values of $-\log t_\lambda$.

Provided the diluent sugar is so pale as to affect results for $-\log t$ only in the third decimal the above correction may be omitted in many cases, particularly when the sugar product is very dark.

It is recommended*—

(1) That the procedure for the measurement of color in sugar products as outlined above and as described in detail in Circular C440 of the National Bureau of Standards (already cited) be adopted as a tentative method.

(2) That the method receive further study by this Association, particularly with regard to (a) instruments for measuring transmittancy, and (b) the filter aid to be used in preparing solutions for transmittancy measurement.

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* For report of Subcommittee D and action by the Association, see *This Journal*, 30, 53 (1947).

REPORT ON SOILS AND LIMING MATERIALS

By W. H. MacIntire (The University of Tennessee Agricultural Experiment Station, Knoxville), *Referee*

Because of the problem of inadequacy of analytical personnel in laboratories from which collaboration might have come, the work done since the last annual meeting of this Association has been chiefly the results of studies conducted within the laboratories of Associate Referees. The Referee has functioned solely in a suggestive capacity.

The Associate Referee on Zinc in Soils has pursued studies upon a combination of dithizone-spectrographic method that is outlined in his report. A requested contribution, "The Polarographic Determination of Zinc in Soil" from the Hawaiian Agricultural Experiment Station was referred to the Associate Referee and was offered as a contributive paper during the 1946 meeting.[1]

Work done in the laboratory of the Associate Referee on Boron indicates the feasibility of the adaptation of carmin as an indicator for determination of boron in aqueous extracts of soils.

The studies of the Associate Referee on H-ion concentration have dealt with seven aspects relative to *p*H of soils of the arid and semi-arid regions and have indicated the feasibility, if not desirability, of utilizing virtually air-dry soils for the determination of *p*H on samples from arid and semi-arid regions.

In recognition of the importance of the large increase in the use of slags as liming materials and, in particular, recognition of their distinctive chemical characteristics resultant from type and source, the Associate Referee for Liming Materials has concentrated his efforts upon specifically appropriate analytical procedures to differentiate between potential neutralization capacity and actual neutralization effectiveness. A new aspect of his comprehensive studies was inclusion of blast furnace slags and recognition of the vitiative factor of sulfide content; and his procedure is given in detail in the following report, p. 295.

RECOMMENDATIONS*

It is recommended—

Soils:

(1) That the "combination dithizone-spectrographic method" and the polarographic procedure for the determination of zinc in soils be studied further.

(2) That the determination of copper in soils be studied.

(3) That the utilization of carmin as an indicator in the determination of boron content of soils be studied further.

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[1] *This Journal*, 30, 182 (1947).

* For report of Subcommittee A and action by the Association, see *This Journal*, 30, 41 (1947).

(4) That further studies of *p*H in soils of the arid and semi-arid regions be based upon soil systems of moisture content representative of air-dry soil.

(5) That the analytical technique previously proposed for ignition of charge and distillation of fluorine therefrom be studied collaboratively.

(6) That the "2-point" titration procedure for the determination of exchangeable H in soils be studied further, in relation to liming practice.

(7) That a survey and comparisons be made of methods for the determination of phosphorus, (a) that fraction in "available" state, and (b) the proportions of organic-inorganic forms therein.

(8) That a survey and comparisons be made of methods for the determination of "exchangeable" K in soils.

Liming materials:

(9) That the direct titration against bromcresol green (Method 2) be adopted as optional for the determination of the neutralization value of blast furnace slags.

(10) That the tentative procedures be annotated by the statement, "without correction for sulfide content."

(11) That correction for sulfide sulfur content be studied further.

REPORT ON LIMING MATERIALS

By W. M. SHAW (University of Tennessee Agricultural Experiment Station, Knoxville, Tenn.), *Associate Referee*

The present report constitutes a chronological record of the efforts exerted and collaborative results obtained during the past two years on the subject of determination of the potential neutralization value of blast furnace slags by a simplified procedure.

STATUS OF METHODS FOR THE DETERMINATION OF NEUTRALIZATION VALUE OF SLAGS

(A) MEMORANDUM TO DR. W. H. MACINTIRE, REFEREE ON SOILS AND LIMING MATERIALS, 1945

In correspondence with K. D. Jacob, Mr. Williams of the National Slag Association, and ourselves, Mr. C. J. Schollenberger of the Ohio Station advanced two points of criticism of the recently adopted A.O.A.C. method (*This Journal*, **27**: 532, 1944) for the determination of the neutralization value of slags. He stated that the potentiometric titration is not always feasible or convenient as a routine procedure and that the method does not prescribe correction for the sulfide sulfur content of the slag. He proposed a 2-stage titration procedure and the use of phenolphthalein in the final titration, the oxidation of the sulfide being effected by hydrogen peroxide during the dissolution of the slag by the dissolvent acid.

These points had been accorded consideration in the course of our investigation on the titrative methods for the determination of the neutralization value of slags, and were embodied in a paper presented at the 1944 meeting of the Association (*This Journal*, **28**: 310–335, 1945). Under the heading of "Utility of Indicators . . . ," we demonstrated that neither methyl orange nor phenolphthalein is appropriate in the titration of the acid solution of slags, and concluded, "Nevertheless, when a *p*H meter is not at hand, values within 2 per cent of true results can be obtained by titration to *p*H 5.2 against Bromcresol green." We had not given the indicator titration in the A.O.A.C. procedure in 1944, since we had found the *p*H meter satisfactory and considered it a usual accessory in chemical laboratories. However, should titration against an indicator prove more feasible in some laboratories, we proposed an optional titration procedure for blast furnace slags for our report at the 1946 meeting of A.O.A.C.

We believe that the direct titrative technique against either Bromcresol green or methyl red to *p*H 5.2 is feasible and simpler than the 2-stage titration procedure suggested by Schollenberger. Moreover, the proposed separation of phases in the titration system at some point before the final titration is not deemed in keeping with physico-chemical principles.

We do not have sufficient evidence as to the completeness of the oxidation of the sulfide sulfur when hydrogen peroxide is introduced into the system prior to the dissolution of the slag with solvent acid. Apparently, an appreciable fraction of generated hydrogen sulfide is volatilized during the dissolution of the slag, even in the presence of hydrogen peroxide. Since the addition of hydrogen peroxide appears to afford only a partial and inconstant correction, it would seem expedient to grant an allowance of 3.5 per cent as medial for the ever present sulfide (in the range of from 2.5 to 5.0 per cent calcium carbonate-equivalence) in the making of routine analyses. Some data seem to warrant the proposed allowance, but further analytical work, especially as to the sulfate sulfur content of the slag solution obtained with and without use of hydrogen peroxide, should be done before definite answer to admissibility of the proposal.

It should be remembered that in the appraising of the liming value of a slag, a probable error of 1 or 2 per cent in the chemical evaluation would not be of economic significance when other factors are given consideration. The disintegration and effectiveness of the slag after incorporation into the soil is governed by mechanical make-up, R_2O_3 components and combinations, the effects of quenching, and other factors not yet well known.

Hence, the following titration technique is proposed for study as an optional procedure for the analytical evaluation of *blast furnace slags*.

Titration against indicators. Effect dissolution of slag as under **(a)** [*This Journal*, **27**, 532, 1944] until "cool to room temperature"; then dilute to 150 ml. with CO_2-

free H_2O and add either 2 drops of a 1 per cent solution of Bromcresol green or 4 drops of a 0.2 per cent solution of methyl red. Back titrate with 0.5 *N* NaOH by quick introduction of 15 ml. NaOH with constant swirling and continue the titration dropwise until the color matches or oversteps that of a buffer solution of *p*H 5.2 with same indicator and identical concentration after agitation for only 2–3 seconds. The net ml. acid consumption times 5 expresses neutralization value in terms of $CaCO_3$.

It was suggested that 6 or 8 additional samples of slag be obtained from Mr. Williams for use, in a prescribed collaborative study with Mr. K. D. Jacob, Mr. C. J. Schollenberger, Mr. H. T. Williams of the Standard Slag Company of Youngstown, Ohio, and by the Referee's laboratory, on the titration procedures submitted by us and the one suggested by Mr. Schollenberger. It was hoped that the results could be presented at the 1946 meeting of the A.O.A.C. Upon approval by the Referee, the following instructions were sent out.

(B) INSTRUCTIONS FOR COLLABORATIVE WORK ON THE DETERMINATION OF NEUTRALIZATION VALUE OF BLAST FURNACE SLAGS (Aug. 25, 1945)

"The objective of the present collaborative work is to evolve a simplified titrative technique as an alternative to the present tentative potentiometric procedure of titration to *p*H 4.8. The identical portions of the eleven referee samples are representative of blast furnace slags produced in the United States. Upon receipt, the samples are to be subjected to no processing. Correction for the presence of sulfide sulfur is by-passed for the present, since choice of titrative technique and indicator will not be governed by such correction."

METHODS TO BE USED

"I. *Potentiometric Titration.*

Weigh a .5-gram charge of slag that has been ground to pass a No. 80 sieve and transfer into a 250-ml. Erlenmeyer flask. Wash down with a minimum of water and introduce 35 ml. of 0.5 *N* HCl, while swirling the contents.[1] Heat over a Bunsen burner to gentle boiling, *agitate frequently* to prevent caking until the bulk of the sample has dissolved, and boil gently 5 minutes. Cool to room temperature; transfer to 150 ml. beaker, dilute with CO_2-free water to about 80 ml., and dip the glass electrode assembly into the solution. Back-titrate with 0.5 *N* NaOH, adding the first 15 ml. rapidly and continuing the titration dropwise with vigorous stirring, until the *p*H meter indicates *p*H 4.8 for one minute. The net acid volume used times 5 represents the neutralization value of the slag in per cent $CaCO_3$-equivalence."

"II. *Titration to p*H *5.2 by Bromcresol Green.*

Effect dissolution of the slag as under (a) (*This Journal*, 27, 532) until "cool to room temperature"; then dilute with CO_2-free H_2O to about 150 ml. and add 1 ml. of 30 per cent H_2O_2[2] and 3 drops of a 1 per cent solution of Bromcresol green.[3] Back titrate with .5 *N* NaOH, adding the first 15 ml. rapidly and continue titration drop-

[1] Where a shaking machine of the Ross-Kershaw type is available, it will be found convenient to agitate the slag suspensions for 10 minutes on the machine before application of heat.

[2] As per suggestion of Mr. C. J. Schollenberger.

[3] Methyl red indicator, 4 drops of a 2 per cent solution, may be substituted for Bromcresol green with some advantage in visibility of end point. This indicator is very unstable, however, and requires renewal of buffer solution every few days.

wise, contents of stoppered flask being agitated vigorously after each addition until the indicator tint matches or slightly oversteps that of a *p*H 5.2 phthalate buffer solution of like volume and indicator concentration after agitation for 2–3 seconds."

"III. *Two-stage Titration, with Intervening Filtration.* [Directions by C. J. Schollenberger]

Transfer a 2-gram charge to a 250 ml. volumetric flask, preferably a Pyrex phosphate flask. Wash down with a little water and add 60 ml. 1 *N* HCl while swirling to prevent caking. Boil gently for 5 minutes, with sufficient agitation during heating to keep undissolved particles in suspension. At the end of this time, test the escaping steam with a strip of moistened lead acetate paper; if no reaction for sulfide is obtained, decomposition of the sample may be considered complete. Cool and dilute to about 200 ml. Add 1 ml. 30 per cent hydrogen peroxide to oxidize iron. Start the back titration by introducing 15 ml. 1 *N* NaOH, stopper the flask and shake thoroly; test by dropping in a bit of methyl orange paper.[4] If the test paper, *floating on the solution*, turns pink or orange at once and does not become yellow within one minute, insufficient alkali has been added. In this case, add more, 1 ml. at a time, until a repetition of the test with a fresh bit of the test paper causes the latter, floating on the solution, to become yellow within one minute. Record the total volume of 1 *N* NaOH added in the first stage of the titration (X).

"Fill the flask to the 250 ml. mark, shake well at once and several times during 10 minutes, releasing pressure from decomposing peroxide each time. Filter on a dry fluted paper. Transfer 200 ml. of the clear filtrate to a flask, add 1 ml. hydrogen peroxide and 10 drops of phenolphthalein solution and start the second stage of the titration, dropwise, until the mixture becomes distinctly pink and retains that color after shaking. If there is much manganese in the solution, the dark color of the precipitated higher hydrated oxide may conceal the indicator color; in this case, let the mixture stand until settled sufficiently for a portion of the clear solution to show color, or filter. If the solution shows no indicator color after filtration, add a drop or two of peroxide before completing the titration. The end point is a faint but unmistakable pink not disappearing after thorough shaking. The volume of 1 *N* NaOH used in titrating the 200/250 aliquot of the original mixture, the second stage, is designated (X^1).

"The neutralization value of the slag is calculated by the formula

$$2.5\ (60 - X - 1.25\ X^1) = \text{per cent calcium carbonate equivalence.}"$$

PRESENTATION AND EVALUATION OF RESULTS

"The calcium carbonate equivalence of each sample is to be determined in triplicate by each procedure, and the respective mean values are to be tabulated and compared with the computed calcium-magnesium equivalences of the furnished analysis, *without* correction for sulfur, and the deviations between the analytical means and these Ca+Mg values are to be recorded. The total Ca+Mg equivalences are used merely as a convenient yardstick rather than as absolute values. A more nearly correct basis of comparison would be the Ca+Mg found as having been dissolved by the standard acid that was used in the titrative procedure, with

[4] Thin filter paper saturated with 0.1 per cent methyl orange solution and dried. Methyl orange is quickly yellowed and finally bleached by hydrogen peroxide in acid solution, hence is unreliable when used in solution for the foregoing test, or after the test paper has been in the solution for some time; but the peroxide is largely or entirely decomposed by passing through the paper, so that with the technique described good indications are obtained.

correction for sulfate content of the solution. This extra analytical work may be done if time admits.

"Comments as to the relative feasibility and expeditiousness of the several procedures are sought in order to facilitate and expedite decision as to the admissibility of any procedure that is included in the outline."

COLLABORATIVE RESULTS

1945–1946

The results of the collaborative work during part of 1945 and part of 1946 are given in Tables 1–4, and summarized in Table 5. Mr. C. J. Schollenberger failed to report any work with the potentiometric or the Bromcresol green titration procedures, and his 2-stage titration results were obtained through his own version of oxidation by hydrogen peroxide, rather than the technique prescribed in the formulated outline. Although they are not strictly comparable with the other findings, his results are included to indicate Mr. Schollenberger's interest in some method for the determination of the neutralizing value of slags.

DISCUSSION OF RESULTS

The primal objective was to compare the two titrative procedures proposed for the determination of the neutralization value of blast furnace slags upon basis of accuracy, precision, and expeditiousness. It was suggested that degree of accuracy by these procedures be judged from the extent of which the results are in agreement with those found by analysis of Ca+Mg. Precision may be judged from reproducibility of results by each analyst and by the concordance with results of the several collaborators.

The collaborative results with 3 titrative procedures are given in comparison with the analyzed Ca+Mg values in Table 5. The potentiometric titration to *p*H 4.8 shows agreement within 1 per cent of the Ca+Mg results in the hands of two analysts, whereas the results of the third analyst show a mean error of 1.9 per cent. Since the bracketed results show deviations several times as large as the mean deviations and were probably accidental, they were not included in the over-all averages. The titrations to *p*H 5.2 with Bromcresol green gave results concordant with those by potentiometric titrations in one laboratory, fair agreement in another, and poor agreement in the third. The results by the 2-stage titration procedure gave good agreement with the Ca+Mg in one laboratory, fair agreement in two laboratories, and very poor in the fourth laboratory. A cursory inspection of these results might suggest rejection of all of the titrative procedures as lacking in precision and accuracy. Upon closer examination of the data, however, it appears probable that many of the results were obtained under conditions of strain from overwork, insufficient time for observation of simple details, and lack of time

TABLE 1.—*Results by E. J. Fox, U. S. Department of Agriculture, submitted Sept. 28, 1945*

SLAG	PER CENT $CaCO_3$-EQUIVALENCE BY METHODS—														
	A. POTENTIOMETRIC pH=4.8				B. METHYL RED				B. BROMCRESOL GREEN			C. PHENOLPHTHALEIN			
	(1)	(2)	(3)	Avg.	(1)	(2)	(3)	Avg.	(1)	(2)	Avg.	(1)	(2)	(3)	Avg.
A	97.5	94.0	95.0	95.5	89.4	91.8	92.5	91.2	93.8	92.5	93.2	84.9	84.0	83.9	84.3
B	91.5	91.3	88.8	90.5	83.2	85.9	85.9	85.0	86.5	86.0	86.3	79.7	78.7	79.0	79.1
C	92.5	92.0	91.8	92.1	87.2	89.8	89.0	88.7	91.5	90.5	91.0	82.5	80.6	80.2	81.1
D	86.7	84.9	84.8	85.5	81.6	82.4	81.3	81.7	86.8	84.3	85.6	77.3	74.4	73.3	75.0
E	97.0	96.5	96.1	96.5	91.5	91.0	93.8	92.1	95.4	95.5	95.5	90.0	86.8	88.7	88.5
F	89.5	91.4	91.4	90.8	89.4	88.2	87.5	88.4	88.0	80.5	84.3	83.5	81.5	80.8	81.9
G	90.0	90.0	89.6	89.9	85.9	83.6	87.3	85.6	86.5	89.8	88.3	79.4	76.9	81.8	79.3
H	87.0	85.2	84.5	85.6	80.2	82.0	76.6	79.6	84.5	83.5	84.0	73.2	74.0	72.0	73.1
I	81.1	78.8	78.1	79.3	74.4	74.6	74.6	74.5	83.0	78.5	80.8	67.5	68.4	68.9	68.3
J	86.3	84.2	84.7	85.1	81.3	81.3	79.2	80.6	88.0	84.0	86.0	74.5	73.1	73.2	73.6
K	89.8	89.3	89.1	89.4	85.2	86.1	85.6	85.6	90.8	87.5	89.2	79.6	78.6	79.0	97.1

COMMENT: Too much H_2O_2 renders indicators unstable. Better results obtained by cutting indicated quantities in half.
Potentiometric method employs too low *p*H value, giving high results.
Analyses of samples not available for comparison with above results.

or desire to correct gross and apparently accidental errors in the results. In one instance, for example, the results by the 2-stage titration technique showed a constant error of minus 9 per cent. The Associate Referee had advised the collaborator that such deviations were discordant with results that were obtained with only ordinary care. The response was an expression of regret of inability to do further work then on the proposed methods. In another instance, triplicate determinations exhibit good precision, yet results show obvious errors of plus 5 and 6 per cent calcium

TABLE 2.—*Results by C. J. Schollenberger, Ohio Agricultural Experiment Station, submitted May 8, 1946*

SLAG	PER CENT $CaCO_3$—EQUIVALENCE BY METHODS—					
	A. POTENTIOMETRIC	B. BROMCRESOL GREEN	C. PHENOLPHTHALEIN*			
			(1)	(2)	(3)	Avg.
A			87.2	86.6	88.8	87.5
B			82.3	81.6	85.2	83.1
C			87.2	85.4	89.7	87.8
D			80.0	79.7	82.2	80.6
E			94.8	—	93.3	94.1
F	Reported "not done"	Reported "not done"	81.5	—	85.8	83.7
G			84.8	—	84.3	84.6
H			81.3	—	81.0	81.2
I			74.4	—	75.8	75.1
J			79.2	—	83.7	81.5
K			86.7	87.0	83.3	85.7

* With oxidation of sulfide by H_2O_2.

carbonate-equivalence. This titrative procedure has been followed in numerous determinations, but in no instance did we encounter errors much beyond 1 or 2 per cent. Our own findings and comments were communicated to the analyst, but we were unable to obtain any further work or any explanation for such extraordinary results.

ADDITIONAL WORK

In addition to the numerous determinations by the several titrative procedures, the Associate Referee determined the calcium and magnesium dissolved by the 0.5 *N* hydrochloric acid of the titrative evaluations, and compared the results with those obtained when the slags were dissolved in hydrochloric acid of higher concentration in the laboratories of the Standard Slag Company. The sulfate sulfur content of each solution also was determined and the total Ca+Mg values were corrected for this increment by subtraction of the calcium carbonate-equivalence. As an over-all determination of the neutralization value of the eleven slags,

TABLE 3.—*Results from Laboratory of The Standard Slag Company, Youngstown, Ohio, submitted Nov. 26, 1945*

SLAG	PER CENT $CaCO_3$-EQUIVALENCE BY METHODS—											
	A. POTENTIOMETRIC				B. BROMCRESOL GREEN				C. PHENOLPHTHALEIN			
	(1)	(2)	(3)	Avg.	(1)	(2)	(3)	Avg.	(1)	(2)	(3)	Avg.
A	92.50	92.25	92.75	92.50	94.00	95.50	94.50	94.67	91.25	90.63	91.25	91.04
B	91.40	91.50	91.25	91.40	90.25	90.25	89.50	90.00	87.35	87.97	87.65	87.66
C	89.75	90.25	90.00	90.00	91.25	90.00	90.25	90.50	91.25	90.95	90.95	91.05
D	84.00	84.50	84.00	84.20	86.75	86.25	86.75	86.58	83.13	83.43	83.43	83.33
E	97.50	97.00	96.00	96.80	97.50	97.50	98.00	97.67	96.57	96.25	96.40	96.41
F	90.50	91.00	90.50	90.70	95.00	95.50	94.75	95.08	91.57	92.02	91.87	91.82
G	89.25	89.25	89.25	89.30	92.50	92.00	92.25	92.25	88.90	88.90	88.90	88.90
H	83.75	83.75	83.50	83.70	92.50	92.25	92.50	92.42	84.68	84.05	84.38	84.37
I	79.00	78.50	78.75	78.80	96.00	96.50	95.00	95.83	76.88	76.73	76.73	76.78
J	83.50	83.50	83.50	83.50	88.75	87.75	87.75	88.08	83.75	83.43	83.43	83.54
K	88.75	88.75	88.50	88.70	90.00	91.00	90.50	90.50	87.80	87.65	87.65	87.50

COMMENTS: In comments on Methods B and C "our chemist reports that he was able to observe the end point with phenolphthalein more readily than with bromcresol green, but a considerable amount of time was involved in test Method C because of the intervening filtration. The gelatinous precipitate which accompanied the first addition of sodium hydroxide filtered very slowly. A coarser filter paper proved undesirable, as the filtrate was not clear."

TABLE 4.—*Results by W. M. Shaw, Associate Referee, Tennessee Agricultural Experiment Station, August 1945*

SLAG	PERCENTAGE $CaCO_3$-EQUIVALENCE BY METHODS—											
	A				B				C			
	(1)	(2)	(3)	Avg.	(1)	(2)	(3)	Avg.	(1)	(2)	(3)	Avg.
A	92.3	92.3	—	92.3	92.8	92.8	92.8	92.8	93.1	93.1	93.8	93.3
B	87.0	87.3	—	87.2	87.5	87.8	87.5	87.6	86.6	87.5	87.5	87.2
C	89.0	88.5	89.5	89.0	90.0	90.3	90.0	90.1	89.7	90.3	90.0	90.0
D	82.8	83.0	—	82.9	83.5	83.5	83.5	83.5	82.8	83.8	83.1	83.2
E	95.8	96.0	96.3	96.0	96.8	96.8	97.0	96.9	96.3	96.9	97.2	96.8
F	90.5	90.0	—	90.3	90.8	90.5	90.8	90.7	90.0	90.9	89.4	90.1
G	89.0	88.8	—	88.9	88.8	89.0	89.0	88.9	88.4	90.0	87.5	88.6
H	83.0	83.5	—	83.3	82.8	82.8	83.0	82.9	83.1	83.8	82.5	83.1
I	76.8	76.8	—	76.8	77.0	77.0	77.0	77.0	76.3	76.9	77.5	76.9
J	83.5	82.8	82.5	82.9	83.0	83.5	83.5	83.3	82.5	84.1	82.8	83.1
K	87.0	87.5	—	87.3	88.0	88.5	88.0	88.2	86.9	86.9	88.1	87.3

A. Potentiometric titration to *p*H 4.8 by means of glass electrode.
B. Titration to *p*H 5.2 against bromcresol green and phthalate buffer, as reference.
C. Two-stage titration, with intervening filtration, as suggested by C. J. Schollenberger.

TABLE 5.—*Summary of collaborative results by titrative procedures with Ca plus Mg analyses—in per cent of $CaCO_3$-equivalence*

SLAG	Ca+Mg	POTENTIOMETRICALLY						BY BROMCRESOL GREEN						2-STAGE TITRATION							
		E.J.F.		ST'D.		W.M.S.		E.J.F.		ST'D.		W.M.S.		E.J.F.		ST'D.		W.M.S.		C.J.S.	
		Per cent	*Dev.*	*Per cent*	*Dev.*	*Per cent*	*Dev.*	*Per cent*	*Dev.*	*Per cent*	*Dev.*	*Per cent*	*Dev.*	*Per cent*	*Dev.*	*Per cent*	*Dev.*	*Per cent*	*Dev.*	*Per cent*	*Dev.*
A	93.1	95.5	+2.4	92.5	−0.6	92.3	−0.8	93.2	+0.1	94.7	+1.6	92.8	−0.3	84.3	− 8.8	91.0	−2.1	93.3	+0.2	91.7	−1.4
B	88.0	90.5	+2.5	91.4	(+3.4)	87.2	−0.8	86.3	−1.7	90.0	+2.0	87.6	−0.4	79.1	− 8.9	87.7	−0.3	87.2	−0.8	87.8	−0.2
C	89.3	92.1	+2.8	90.0	+0.7	89.0	−0.3	91.0	+1.7	90.5	+1.2	90.1	+0.8	81.1	− 8.2	91.1	+1.8	90.0	+0.7	90.7	+1.4
D	83.0	85.5	+2.5	84.2	+1.2	82.9	−0.1	85.6	+2.6	86.6	+3.6	83.5	+0.5	75.0	− 8.0	83.3	+0.3	83.2	+0.2	84.0	+1.0
E	96.0	96.5	+0.5	96.8	+0.8	96.0	0.0	95.5	−0.5	97.7	+1.7	96.9	+0.9	88.5	− 7.5	96.4	+0.4	96.8	+0.8	97.2	+1.2
F	89.8	90.8	+1.0	90.7	+0.9	90.3	+0.5	84.3	(−5.5)	95.1	+5.3	90.7	+0.9	81.9	− 7.9	91.8	+2.0	90.1	+0.3	88.2	−1.6
G	87.8	89.9	+2.1	89.3	+1.5	88.9	+1.1	88.3	+0.5	92.3	+2.4	88.9	+1.1	79.3	− 8.5	88.9	+1.1	88.6	+0.8	88.0	+0.2
H	82.3	85.6	+3.3	83.7	+1.5	83.3	+1.0	84.0	+1.7	92.4	+6.8	82.9	+0.6	73.1	− 9.2	84.4	+2.1	83.1	+0.8	83.7	+1.4
I	79.8	79.3	−0.5	78.8	−1.0	76.8	(−3.0)	80.8	+1.0	95.8	+6.5	77.0	(−2.8)	68.3	−11.5	76.8	−3.0	76.9	(−2.9)	78.4	−1.4
J	83.9	85.1	+1.2	83.5	−0.4	82.9	−1.0	86.0	+2.1	88.1	+3.0	83.3	−0.6	73.6	−10.3	83.5	−0.4	83.1	−0.8	86.7	+2.8
K	87.7	89.4	+1.7	88.7	+1.0	87.3	−0.4	89.2	+1.5	90.5	+1.1	80.2	+0.5	79.1	− 8.6	87.7	0.0	87.3	−0.4	85.7	−2.0
Mean	—	—	1.9	—	0.9	—	0.6	—	1.3	—	3.2	—	0.7	—	8.9	—	1.2	—	0.6	—	1.3

NOTE. Initials represent, respectively, E. J. Fox, (St'd.) Standard Slag, W. M. Shaw, and C. J. Schollenberger.

they were subjected to dissolution in 0.5 *N* hydrochloric acid, sequential removal of SiO_2 and R_2O_3 from the solution, conversion of chlorides to oxalates, which were ignited to carbonate and titrated (*This Journal*, **28**, 330, 1945. The results of these additional analyses have been assembled in Table 6. Comparisons of columns I and II of Table 6 demonstrate that the 0.5 *N* hydrochloric acid was as effective as the more concentrated hydrochloric acid in the dissolution of the calcium and magnesium from the eleven slags. In most instances the sum of the bases dissolved by 0.5 *N* hydrochloric acid in the Referee's laboratory was

TABLE 6.—*Neutralization value of blast furnace slags, by analyses of Ca plus Mg and by basicity determinations*

SLAG	ACID-SOLUBLE			0.5 *N* ACID-SOLUBLE					BASICITY†		
	Ca	Mg	I* / Ca+Mg	Ca	Mg	SO_4	II / Ca+Mg	II_c / Ca+Mg −SO_4	1	2	III / AVG.
A	87.1	6.0	93.1	86.0	6.4	.08	92.4	92.3	92.3	92.3	92.3
B	81.4	6.6	88.0	81.1	7.1	.18	88.2	88.0	87.5	87.8	87.7
C	71.9	17.4	89.3	71.4	17.8	.08	89.2	89.1	89.5	89.3	89.4
D	78.3	4.7	83.0	77.5	4.8	.08	81.9	81.8	82.8	83.3	83.1
E	72.6	23.4	96.0	71.6	26.1	.55	97.7	97.1	97.5	97.3	97.4
F	67.3	22.5	89.8	65.8	25.3	.78	91.1	90.3	89.5	89.8	89.7
G	74.3	13.5	87.8	73.2	14.7	.26	87.9	87.6	87.5	87.0	87.3
H	70.6	11.7	82.3	70.6	13.2	.42	83.8	83.4	83.0	82.8	82.9
I	72.2	7.6	79.8	72.0	4.5	.20	76.5	76.3	77.0	77.3	77.2
J	73.7	10.2	83.9	72.6	11.5	2.36	84.1	81.7	82.0	81.5	81.8
K	74.1	13.6	87.7	72.7	16.1	1.08	88.8	87.7	87.5	87.3	87.4

* Analyses by Standard Slag Company laboratories.
† By dissolution in 0.5 *N* HCl, removal of SiO_2 and R_2O_3, evaporation of filtrate, conversion to oxalate, ignition, and titration of carbonate residue (See *This Journal*, 28, 330, 1945).

slightly greater than the sum obtained by means of the more concentrated acid in the laboratory of the Standard Slag Company. In general, the magnesium determinations in the Referee's laboratory were higher than those made in the Standard laboratories, which may be ascribed to difference in analytical technique. The single exception was the 3.1 per cent lower value obtained by our analysis of Slag I. This value was foreshadowed by the consistent results of the earlier titrative determinations given in Table 4. That slag was the only one that has shown a consistent minus value of nearly 3 per cent by each of the 3 titrative procedures as compared with the over-all analytical data of column I, Table 6.

The sulfate sulfur was determined after dissolution of the slag and expulsion of the generated hydrogen sulfide by boiling, as prescribed in the titrative determinations. The results show that not all of the sulfur in the slag is present solely as sulfides and that correction for the equivalence of

total sulfur content might impart a minus error of 1 to 2 per cent in the values indicated by titrative procedures.

The analytical data given under "basicity" in Table 6 were designed to replace the acid-soluble Ca+Mg as reference values in a titrative determination, since this determination obviates the separate determinations of Ca and Mg and also supplies an automatic correction for the sulfate sulfur content of a slag. These basicity determinations show an agreement with the corrected 0.5 *N* hydrochloric acid soluble Ca+Mg within a mean deviation of ±0.4 per cent. The agreement with acid soluble Ca+Mg is equally good, save the two cases in which the values differ by over 2 per cent. It has been shown that in one instance this difference is due to the high value of the Mg determination and in the other, due to presence of sulfate sulfur.

Compared with the "basicity" determinations, the 3 titration procedures gave fairly satisfactory results, mean deviation of only 0.6 to 0.7 per cent and in only 3 instances of the eleven samples were the deviations in the range of 1 to 1.6 per cent. The two colorimetric titration procedures give like results but, because of its rapid and easy manipulation, the single titration to *p*H 5.2 against Bromcresol green is deemed the preferable procedure.

THE SULFIDE SULFUR FACTOR

The eleven slag samples studied carry from 0.81 to 1.66 per cent total sulfur, chiefly, if not entirely, as sulfide. Since that form would be oxidized to sulfuric acid in the soil, it has been contended that the sulfide would neutralize its equivalence of the liming material, although some of the sulfide sulfur may be volatilized as hydrogen sulfide when the acidic soil is slagged. Nevertheless, the question of sulfide correction for sulfide content has been considered. The suggested oxidation of the sulfide by means of hydrogen peroxide in the dissolvent acid has not proved admissible as a means of automatic correction. A simple method for the evaluation of the sulfide content is being tested. If the eleven samples are representative of the output of blast furnace slags, the calcium carbonate correction for sulfide would be from 2.5 to 5.0 per cent with an average of 3.5 per cent calcium carbonate-equivalence.

It is recommended*—

(1) That the direct titration against bromcresol green (Method 2) be adopted as optional for the determination of the neutralization value of blast furnace slags.

(2) That the tentative procedures be annotated by the statement, "without correction for sulfide content."

(3) That correction for sulfide sulfur content be studied further.

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* For report of Subcommittee A and action of the Association, see *This Journal*, 30, 41 (1947).

REPORT ON HYDROGEN-ION CONCENTRATION OF SOILS OF SEMI-ARID REGIONS

By W. T. McGEORGE (Agricultural Experiment Station, Tucson, Ariz.), *Associate Referee*

Research work on the *p*H of semi-arid soils during the past few years has been along the following lines:

1. Salinity—*p*H relationships
2. Base—exchange *p*H relationships
3. Sesquihydrate—*p*H relationships
4. *p*H of soil separates
5. Comparison of *p*H by soil paste method with isohydric *p*H values and the *p*H of exchange neutrality.
6. Effect of variation in absolute weight of soil used for the *p*H determination at high soil:water ratios
7. *p*H at low moisture content.

The results of these studies are given briefly in this report and are given in detail in Tech. Bul. 104, Arizona Agricultural Experiment Station.

(1) The *p*H of soil increases with increase in concentration of CO_3 ions, HCO_3 ions, and decreases with increase in concentration of chloride, sulfate, and calcium ions. There is no relation between total salinity and *p*H because of the opposite effect of the several ions.

(2) The *p*H value of the soil increases with an increase in the percent age of replacable sodium in the exchange complex and the ratio of sodium to the total exchange capacity. All sodium saturated soils, in the absence of neutral salts, have *p*H values approximating *p*H 10.2 when the *p*H value is determined at 1:10 soil:water ratio. When the determination is made on the soil paste the *p*H decreases with increase in exchange capacity of the soil. The absorbing capacity of the soil is increased by leaching the soil with salt solutions of high *p*H values but not by salt solutions of *p*H 8.5 or lower.

(3) Sesquihydrates exhibit base exchange properties and high *p*H values which, like the semi-arid soils, are affected by salinity and dilution.

(4) All the soil separates possess an absorption capacity for cations and exhibit high *p*H values. The sand particles usually have a higher *p*H than the silt and clay particles.

(5) The *p*H value of the soil as determined on the soil paste agrees very closely with the isohydric *p*H and the *p*H of exchange neutrality.

(6) Variation in the absolute weight of soil with a constant soil:water ratio materially affects the *p*H value of the soil suspension. For *p*H determinations on soils at 1:10 soil:water ratio nothing less than 20 grams of soil in 200 ml. of water should be used.

(7) For the determination of soil *p*H at low moisture content the use of alcohol has been studied. The *p*H values obtained with 50 per cent

ethanol or 50 per cent methanol at 1:1 soil:alcohol ratio agree very closely with the *p*H obtained on the soil paste.

Future studies on the *p*H determination for semi-arid soils should concern the *p*H of the soil at moisture contents below that representing a soil paste, approximately the moisture equivalent, and that representing an air-dry soil. There is evidence that equilibrium values may be obtained with the glass electrode for air-dry soils. There is some difference of opinion as to whether these equilibrium values represent true *p*H values, the *p*H of a thin film in equilibrium with the carbon dioxide content of the air and glass, or the effect of increased resistance on the product of the grid current and resistance. Then again it is known that in preparing a soil paste for the *p*H determination the soil is severely puddled and as much as 90 per cent of the water may be bound during the preparation of the paste. Thus the active water content of the prepared soil paste may be closely that of an air-dry soil.

REPORT ON BORON IN SOILS

By C. A. Evans* and J. S. McHargue,† Agricultural Experiment Station, Lexington, Ky.

This report is limited to notes on a colorimetric method for the determination of boron in soils, which is also of applicability to plants and to fertilizer materials. Several methods for the colorimetric determination of boron have been used with varying degrees of success. On the whole, the Berger-Truog method has been the most satisfactory. A disadvantage of this method is the care with which the concentration of sulfuric acid must be maintained at a given percentage. In a search for another reagent, a water extract of cochineal and later, carmin, the aluminum salt of cochineal, showed promise. The color change induced by carmin is from red to a purple, as governed by the incidence of boron. Repeated trials demonstrated that the material obeys Beer's law. The initial difficulty was overcome by the use of a boron-free Corning florence type flask in which the color was developed.

The method is extremely simple. Sufficient carmin is dissolved in C.P. sulfuric acid to give an intensity of approximately 60 per cent transmittance against air at 600 mμ wave length on the Coleman Spectrophotometer. This amount can be varied, provided a new curve is run for each amount of reagent made up.

The standard curve was constructed by making up a solution of either boric acid or sodium borate in concentrations of from 1 to 12 p.p.m. of boron in a graduated series. One ml. of the standard was taken to give

* Resigned September 15, 1946.
† Associate Referee on Boron in Soils.

concentrations of 1 p.p.m., 2 p.p.m., etc., of boron and put in Corning florence flask, and 10 ml. of the reagent (sulfuric acid and carmin) was added from an automatic burette. The reagent dispensing bottle is so constructed as to prevent entrance of moisture and, hence, the reagent concentration is constant. The color develops at once and is read in the spectrophotometer at 600 mμ. A blank of 1 ml. of H_2O to 10 ml. of the

Standard Curves

p.p.m.	% TRANS.	% TRANS.	% TRANS.	% TRANS.
1	95	92	94	91
3	84.5	77.5	83	80
5	75	6.5	73	69.5
7	65	57.5	68	60.5
9	55	48	62	54
11	49	41.5	57	47
13	42.5	34.5		

FERTILIZER	TRANS. FOUND	DET. BY A.O.A.C.
Sample 3	49.5= .435#/100	.43 #/100
Sample 6	48 = .45 #/100	.445#/100
PLANT MATERIAL		
No boron	80 =2.6 p.p.m.	
×boron added	55.5=7.1 p.p.m.	
2.5×boron added and diluted 2.5 times	55 =7.3 p.p.m.	
5×boron added and diluted 5 times	57 =6.9 p.p.m.	

reagent is used as the reference and the spectrophotometer set at 100 when the reference is in place.

Samples of boric acid in various salt solutions were tried for the interference of other ions. Concentrations of various salts gave little if any indication of interference with the exception of the nitrates, which must be absent.

Soil extracts, plant material, and fertilizers were tested. Soils were extracted with hot H_2O and the extract was evaporated to a small volume and a 1 ml. aliquot taken. The plant material was ashed and the ash taken up with 1–25 sulfuric acid, made to volume, a 1 ml. aliquot taken. Fertilizer was extracted with hot water. The extract was evaporated to "dryness" with sulfuric acid to destroy nitrates, the residue taken up with 1–25 ml. sulfuric acid, made to volume, and a 1 ml. aliquot taken.

Results from recovery of boron added to plant material were highly satisfactory. Four 5-gram samples of mustard plants were taken and boron was added to three of the samples of three concentrations. Boron recovery was between 95 and 97 per cent.

When two fertilizer samples were analyzed by the A.O.A.C. method, 90 per cent recovery of boron was obtained.

The curves may vary from time to time, but whenever a curve is run from standards, simultaneous runs of unknowns afford checks on boron recovery. This suggests a factor of either time or temperature.

Because of the resignation of the senior author, the present notes admittedly are based on insufficient data, but they are presented with the recommendation* that further work be done upon a promising method for the colorimetric determination of boron, utilizing carmin as an indicator.

REPORT ON ZINC IN SOILS

By LEWIS H. ROGERS (Department of Soils, Florida Agricultural Experiment Station, Gainesville, Fla.), *Associate Referee*

Several methods for the determination of total or extractable zinc in soils are currently employed in various laboratories. Colorimetric dithizone procedures have been described by Holmes,[1] Sandell,[2] and others. A polarographic procedure is described by Takazawa and Sherman.[3] Spectrographic procedures have been used by Scott and Mitchell,[4] and others.

In the author's laboratory, a combination dithizone-spectrographic method is being employed. For zinc, the extracted soil solution is evaporated to dryness, organic matter destroyed with hydrogen peroxide, the residue taken up in dilute hydrochloric acid, the *p*H adjusted to 8.5, and the solution extracted with several portions of dithizone in carbon tetrachloride. The combined carbon tetrachloride extracts are evaporated to dryness, taken up in a minimum of chloroform, an internal standard solution added, and aliquots dried on graphite electrodes. These are then analyzed spectrographically using conventional plate calibration and intensity ratio procedures.

It is recommended* that the study be continued.

No report was made on exchangeable calcium and magnesium, or on exchangeable hydrogen.

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* For report of Subcommittee A and action by the Association, see *This Journal*, 30, 41 (1947).

[1] *Soil Sci.*, 59, 77–84 (1945).

[2] "Colorimetric Determination of Traces of Metals," Interscience Publishers, p. 458, 1944.

[3] *This Journal*, 30, 182 (1947).

[4] *J. Soc. Chem. Ind.*, 62, 4 (1943).

REPORT ON WATERS, BRINE, AND SALT

By ANNA E. MIX (U. S. Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Referee*

RECOMMENDATIONS*

It is recommended—

(1) That methods for the determination of fluorine in salt be studied.

(2) That the tentative procedure for preparation of sample: salt (37.108, p. 653) and the tentative method for sulfate: salt (37.113, p. 654) be further studied.

(3) That studies on methods for boron in waters be continued.

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

REPORT ON INSECTICIDES AND FUNGICIDES

By J. J. T. GRAHAM (Production and Marketing Administration, Livestock Branch, Insecticide Division, Beltsville, Md.), *Referee*

The General Referee wishes to express his appreciation to the Associate Referees for their assistance in this work. He has examined their reports and concurs in their respective recommendations.

In the application of sec. **6.110**, page 74, of *Methods of Analysis*, sixth edition, to samples of derris and cubé powder of low rotenone content, it is required that before the crystallization from carbon tetrachloride is carried out, a sufficient quantity of pure rotenone must be added to insure that the weight of the precipitate, expressed as rotenone, will be at least one gram.

Because of the fact that in many cases the added rotenone is an appreciable part of the precipitate, it is very important that it be as pure as it is possible to obtain. It has been the experience of the chemists in the Referee's laboratory that rotenone purchased under the designation "C.P" was not of sufficient purity to avoid errors in this determination. It has therefore been our practice to repurify such material before using it in the determination of rotenone according to **6.110.**

The following procedure has been found to yield rotenone that is satisfactory for use as a reagent, and it is recommended† that it be added to sec. **6.110.**

PREPARATION OF PURE ROTENONE REAGENT

From "C.P. Rotenone."—Dissolve the rotenone in hot carbon tetrachloride, cool in a refrigerator or ice-bath until precipitation of the rotenone-carbon tetrachloride

* For report of Subcommittee D and action by the Association, see *This Journal*, **30**, 59 (1947).
† For report of Subcommittee A and action by the Association, see *This Journal*, **30**, 41 (1947).

solvate has ceased. Filter thru a Büchner funnel and wash once or twice with ice-cold carbon tetrachloride. Concentrate filtrate, crystallize, and filter as above described. Transfer the crystalline residues to a beaker, add ca twice their volume of alcohol, and heat nearly to boiling. It is not necessary that the crystals dissolve completely in the alcohol. Cool to room temperature, filter thru a Büchner funnel, and draw air thru the crystalline residue until most of the alcohol is removed. Remove the rotenone from the funnel, dry in air, and finally heat for 1 hour at 105°C. Mother liquors may be concentrated and the rotenone-carbon tetrachloride solvate allowed to crystallize. The crystalline material may be used for further purification, or it may be kept for preparation of wash solutions or for seeding to induce crystallization in the analytical procedure.

REPORT ON FLUORINE COMPOUNDS

By C. G. DONOVAN (Production and Marketing Administration, Livestock Branch, Insecticide Division, Beltsville, Md.)
Associate Referee

The fluorine methods as previously adopted and published in the Sixth Edition of the "Official and Tentative Methods of Analysis of the A.O.A.C." have proved satisfactory and no changes in any of the procedures are proposed at this time.

It is recommended*—

That the lead chlorofluoride method, *Methods of Analysis*, 6th Ed., par. **6.18** and **6.19**, for the determination of fluorine, be adopted as official, final action.

REPORT ON RODENTICIDES

By JOHN W. ELMORE (Bureau of Chemistry, State of California Department of Agriculture, Sacramento 14, California),
Associate Referee

During the year 1945 the first collaborative work on rodenticides by the Association was undertaken. At that time zinc phosphide was used in considerable quantities for rodenticidal purposes, and it was therefore decided to use this material for the collaborative study. Three laboratories in addition to that of the Associate Referee collaborated in the work.

INSTRUCTIONS TO COLLABORATORS

Samples of zinc phosphide and oat groats plus spreader were sent to each of the collaborators. The following letter enclosing the directions for the determination of the zinc phosphide was also sent to them:

"Dear XXXX:

"The writer, who is an Associate Referee on Rodenticides, A.O.A.C. (Association of Official Agricultural Chemists), has been referred to you as willing to do collabo-

* For report of Subcommittee A and action by the Association, see *This Journal*, 30, 41 (1947).

rative work on methods of analysis. The subject selected for investigation this year is the determination of phosphide phosphorus in zinc phosphide and in zinc phosphide-poisoned grain.

"An account of previous work on this subject was published as a contributed article in the *Journal of the A.O.A.C.*, Vol. 26, (1943), page 559, reprint of which is enclosed. However, some changes in procedure have been made by this laboratory and these have been incorporated in the enclosed copy of 'A.O.A.C. collaborative method for determination of phosphide phosphorus.'

"For work on determination of phosphide phosphorus in poisoned grain it was thought best to avoid the uncertainties of composition of prepared poison baits by supplying the oat groats plus spreader and the zinc phosphide separately. A sample of the active poisonous ingredient can thus be weighed out for each determination and added to a weighed quantity of the inert ingredients (oat groats plus spreader).

Collaborative Results on Phosphide Phosphorus

Results in percentages of Zn_3P_2 found

ANALYST	WITHOUT GROATS	WITH GROATS	DIFFERENCES
Oscar I. Struve, Eastern States Cooperative Milling Corp. Buffalo, N. Y.	95.62	93.26	2.36
Abraham Alter, Health Department, Washington, D. C.	94.10	90.59	
	94.32	90.64	
		90.78	
	Av. 94.21	90.67	3.54
A. B. Heagy and J. E. Schueler, Inspection & Regulatory Service, College Park, Md.	A 98.04	88.34	
	98.46	88.39	
	Av. 98.25	Av. 88.36	9.89
	B 99.24	88.21	
	98.97	88.28	
	Av. 99.10	Av. 88.25	10.85
John W. Elmore, Bureau of Chemistry, California State Dept. of Agriculture	96.00	92.75	
	96.10	92.55	3.40
	Av. 96.05	Av. 92.65	

Analytical determination can then be made of the active ingredient and one has the definitely known added amount against which to check.

"In your work kindly follow the enclosed 'A.O.A.C. collaborative method for determination of phosphide phosphorus' in detail. It will be appreciated if your report includes at least two results of each analysis and be in my hands as soon as practicable but not later than August 1, 1945."

1. DETERMINATION OF PHOSPHIDE PHOSPHORUS IN PRESENCE OF OAT GROATS

"Place three of the gas wash bottles "B" Fig. 1, in the absorption train. These should be of the type designated, each containing 200 ml 1.5% $KMnO_4$ solution. Place 25 grams "oat groats plus spreader" in flask "A" and set in water bath heated to 50°C. Weigh 0.20–0.25 gram of the "zinc phosphide" in a small *glass* thimble and drop into "A." Connect the apparatus as shown and start the suction. Add 75 ml of HCl (1+3) *previously heated to* 50°C to flask "A" through the funnel. With the re-

action flask heated to 50°C, draw air through the apparatus, about six bubbles per second, for *two* hours. Continue as directed in the method at "Disconnect and pass SO_2. . . ." Report results of analysis as per cent Zn_3P_2 in the "zinc phosphide" weighed."

2. DETERMINATION IN COMMERCIAL ZINC PHOSPHIDE

"Proceed as directed in "1" but omit the addition of the 25 grams "oat groats plus spreader."

"Report results of analysis as per cent Zn_3P_2 in the 'zinc phosphide' weighed."

COMMENT OF COLLABORATORS

Abraham Alter.—"Lacking a mechanical stirrer I followed A.O.A.C. method for P_2O_5 under page 22 and 23, 12(b). . . . Shuman's ammonium molybdate stabilized with tartaric acid was used for the precipitations. . . . On emptying the generator flask when oat groats and spreader was used a distinct 'phosphorous' odor prevailed. This does not happen when these are absent. . . . While there is no P_2O_5 ppt. in the contents from the third gas absorber when the spreader is used, a slight ppt. does appear on the straight PH_3 generation of the zinc phosphide. . . . Wouldn't it be advisable to place the 100 cc. aliquots in one beaker, then boil down to desired volume for P_2O_5 precipitation?"

A. B. Heagy and J. E. Schueler.—"We are of the opinion that it would be more economical of time and labor to combine the decolorized solutions into a 1 liter volumetric flask and use a 200 ml aliquot for analysis, rather than take three separate aliquots. . . . In one determination, on boiling the decolorized solution, we encountered a brown precipitate, probably manganese dioxide. Subsequently we allowed the sulfur dioxide to pass through the solution a few minutes after clearing and had no further difficulty in the boiling. . . . The presence of the oat groats made it necessary to use manual agitation to provide effective stirring. . . . The appearance of a dark green color on the addition of alkali to the yellow phospho-molybdate precipitate made the end point difficult to read. We wonder if it would be advisable to recommend the gravimetric procedure for completing the analysis. Time did not permit our making an investigation of this phase."

DISCUSSION

Three sets of results are in fair agreement, those of one laboratory being somewhat out of line. All indicate that some loss of phosphide phosphorus occurs in the presence of groats. A similar loss was noted by the writer when analyzing zinc phosphide by this method and weighing the sample in a gelatin capsule as suggested in the "Procedure" in the original article on this subject (*This Journal,* **XXVI,** page 563). Probably the organic matter interferes with conversion to PH_3. The apparent loss of Zn_3P_2 is about 4 per cent of the amount present which would amount to 0.02 per cent with a poison bait containing 0.60 per cent zinc phosphide. The correct result would involve the use of a factor which possibly would be justified if further collaborative results should establish the approximate constancy of the difference.

The actual per cent of Zn_3P_2 in the commercial products is uncertain. The material used in this investigation was analyzed by the writer by fusion in a peroxide bomb. There was found zinc 73.0 per cent, and phos-

phorus 24.2 per cent, making a total of 97.2 per cent. Zinc phosphide, calculated from total phosphorus gives 101.0 per cent and from the total zinc 96.1 per cent.

RECOMMENDATIONS*

It is recommended—

(1) That the method for phosphide phosphorus in rodenticides be further studied.

(2) That methods for the analysis of rodenticides containing "1080" (sodium fluoroacetate), and "Antu" (alpha-naphthylthiourea) be studied.

REPORT ON NICOTINE AND NORNICOTINE ANALYSIS

By C. VERNE BOWEN (U. S. Department of Agriculture, Bureau of Entomology and Plant Quarantine, Division of Insecticide Investigations, Beltsville, Md.), *Associate Referee*

The 1944 report on nicotine (*1*) recommended that work on the determination of nicotine and nornicotine be continued. In that year some collaborative work had been done on this subject, but the results were not entirely satisfactory. In the method based on the formation of nitrosonornicotine (*2*) and subsequent steam distillation of the nicotine from the solution made just basic with sodium hydroxide, the amount of base added is critical. The addition of an excess of sodium hydroxide over that needed to make just basic to phenolphthalein permits steam distillation of the nornicotine compound, giving high results for nicotine and consequently low results for nornicotine. Unless the alkalinity is carefully controlled, there is difficulty in obtaining results that check (*3*).

With this difficulty in mind it was decided to try various basic materials to alkalize the solution containing the nicotine and the nitrosonornicotine prior to steam distillation. Nornicotine solutions were treated with acetic acid and sodium nitrite as described in the method of analysis which follows, and then made alkaline with magnesium oxide, barium hydroxide, or calcium oxide and steam-distilled. Precipitates were obtained in the steam distillates from barium hydroxide and calcium oxide on the addition of silicotungstic acid. The distillate from magnesium oxide gave no precipitate, and it was decided to use this material in the current study. The presence of sodium chloride with the magnesium oxide does allow the steam distillation of some of the nitrosonornicotine; consequently a large excess of hydrochloric acid that will require neutralization with sodium hydroxide should be avoided. Since nornicotine is volatile with steam from solutions made alkaline with all of these bases,

* For report of Subcommittee A and action of the Association, see *This Journal*, **30**, 41 (1947).

sufficient time must be allowed for the complete formation of the nitroso compounds.

In the analytical investigation the official method (*4*) was followed for the steam distillation. The distillate which had been made acid with hydrochloric acid was concentrated by heating until the volume was less than 250 ml., and it was then transferred to a 250-ml. volumetric flask. The washings were added to the flask and the liquid was brought to the mark. A 100-ml. aliquant was taken, and the total steam-volatile alkaloids (nicotine and nornicotine) were precipitated with silicotungstic acid solution (12 per cent), filtered, and ignited according to the A.O.A.C. procedure for nicotine (*4*), giving residue A. Another 100-ml. aliquant was placed in the steam-distillation flask, boiling stones were added, and the solution was concentrated by boiling to a small volume (about 10–15 ml.), after which the sides of the flask were washed down with a small amount of water, the solution was made neutral to phenolphthalein, and 2 ml. of acetic acid (30 per cent) and 0.5 gram of solid sodium nitrite were added. After standing at room temperature for 20 minutes, the solution was made alkaline by adding an excess of a freshly prepared magnesium oxide-water paste and steam-distilled into 3 ml. of hydrochloric acid (1+4) as above. The distillate contained the nicotine, which was precipitated, filtered, and ignited as for the first aliquant, giving residue B.

The nicotine was calculated from residue B and the nornicotine from the difference between residues A and B.

$$\text{Weight of B} \times 0.1140 = \text{weight of nicotine in aliquant}$$

$$(\text{Weight of A} - \text{weight of B}) \times 0.1042 = \text{weight of nornicotine in aliquant}$$

$$\frac{\text{Weight of alkaloid}}{\text{Weight of sample in aliquant}} \times 100 = \% \text{ alkaloid}$$

In both cases the distillation was continued until a few drops of fresh distillate failed to give an opalescence when tested with silicotungstic acid solution (12 per cent). Warming and cooling the distillate after addition of the silicotungstic acid makes the test more delicate (*5*).

Magnesium oxide should be an ideal base to use for this determination, since an excess always results in solutions of the same alkalinity.

COLLABORATORS

The following collaborators took part in the study:

C. R. Bigelow, New York State Agricultural Experiment Station, Geneva, N. Y.

J. J. Campodonico, Tobacco By-Products and Chemical Corporation, Louisville, Ky.

R. R. Chesson, The American Tobacco Company, Richmond, Va.

J. P. Clingman, R. J. Reynolds Tobacco Company, Winston-Salem, N. C.

A. J. Cox, California Department of Agriculture, Sacramento, Calif.

A. B. Heagy, State of Maryland Department of Chemistry, College Park, Md.

R. Jinkins, Insecticide Division, Livestock Branch, Production and Marketing

TABLE 1.—*Collaborative results on nicotine and nornicotine*

CHEMIST[1]	SAMPLE NO. 1			SAMPLE NO. 2					
	MgO			MgO			NaOH		
	NICOTINE	NOR-NICOTINE	TOTAL	NICOTINE	NOR-NICOTINE	TOTAL	NICOTINE	NOR-NICOTINE	TOTAL
a	0.54	0.04	0.58	38.74	1.29	40.03			
	0.52	0.04	0.56	38.08	1.95	40.03			
b	0.51	0.05	0.55	37.84	1.64	39.48	36.74	3.07	39.81
	0.51	0.04	0.55	38.28	1.18	39.46	36.50	3.31	39.81
c	0.51	0.03	0.54	38.85	1.19	40.04	39.36	0.66	40.02
	0.46	0.04	0.55	38.46	1.78	40.24	39.05	0.80	39.85
	0.49	0.05	0.54	38.80	1.32	40.12			
	0.47	0.07	0.56	38.37	1.62	39.99			
d and e	0.47	0.04	0.51	38.84	2.37	41.21			
	0.46	0.06	0.52	38.55	2.83	41.38			
	0.46	0.06	0.52	38.54	1.91	40.45			
	0.48	0.04	0.52	38.69	1.79	40.48			
f	0.54	0.03	0.57	38.79	1.31	40.10			
	0.53	0.05	0.58	38.51	1.30	39.81			
g	0.49	0.06	0.55	36.95	2.11	39.06	38.69	1.43	40.12
	0.49	0.06	0.55	36.61	2.25	38.86	38.81	1.43	40.24
h	0.54	0.03	0.57	38.23	1.21	39.44	39.76	0.44	40.20
	0.54	0.02	0.56	38.17	1.40	39.57	39.68	0.50	40.18
	0.53	0.03	0.56	38.97	0.91	39.88	39.86	0.37	40.23
i	0.53	0.03	0.56	39.33	0.81	40.14			
	0.51	0.03	0.54	38.97	0.73	40.22			
j							37.23	3.29	40.52
k							39.19	1.02	40.21
l[2]				35.24	4.82	40.06			
						39.98			
m	0.51	0.05	0.56						
	0.47	0.07	0.54						
n[2]	0.48	0.05	0.53						
	0.48	0.06	0.54						40.54
	0.48	0.06	0.54						40.51
Ave.	0.500	0.046	0.548	38.29	1.71	39.96	38.62	1.48	40.17
Range	0.46–0.54	0.02–0.07	0.51–0.58	35.24–39.49	0.73–4.82	38.86–41.38	36.50–39.86	0.50–3.31	39.81–40.54

[1] The designation of the chemist does not conform with the order of the collaborators as given.
[2] Entire sample used in each case, no aliquants taken. New sample taken for nornicotine determination.

Administration, U. S. Department of Agriculture, Chicago, Ill.

W. T. Mathis, Connecticut Agricultural Experiment Station, New Haven, Conn.

W. Ralston, Tobacco By-Products and Chemical Corporation, Richmond, Va.

K. E. Rapp, Kentucky Agricultural Experiment Station, Lexington, Ky.

F. J. Roth, California Department of Agriculture, Sacramento, Calif.

J. E. Schueler, Jr., State of Maryland Department of Chemistry, College Park, Md.

F. A. Spurr, Insecticide Division, Livestock Branch, Production and Marketing Administration, Agricultural Research Center, Beltsville, Md.

RESULTS

Sample 1, a powdered low-nicotine tobacco from Kentucky, said to contain about 0.33 per cent of nicotine.

Sample 2, a commercial nicotine sulfate solution.

The results of analyses reported by the collaborators are presented in Table 1. The agreement in the case of Sample 1 is very good for the total alkaloids. For Sample 2 the total-alkaloid reports of individual chemists show good agreement, but a comparison of results from the different analysts suggests a variation in methods for either weighing samples or measuring aliquants. On comparison of the results for Sample 2 using magnesium oxide with those using sodium hydroxide, it is seen that in most cases the percentage of nornicotine is larger in the magnesium oxide method.

In order to eliminate the possibility of error due to improperly calibrated apparatus for the aliquant measurement, two chemists used the entire sample for each alkaloid determination.

It has been suggested that, rather than evaporate the steam distillate before taking the aliquant, the liquid be diluted suitably to a definite volume and an aliquot taken. This should be equally satisfactory.

The possibility of loss of alkaloid during the process of concentration was presented by one of the collaborators. This has been carefully investigated by condensing the liquid removed by boiling, adding silicotungstic acid solution and hydrochloric acid, as in the usual nicotine precipitation, and allowing to stand overnight. No precipitate formed; thus it is indicated that the loss of alkaloids on concentration was negligible.

The Associate Referee appreciates the cooperation of the collaborators.

RECOMMENDATIONS*

It is recommended that the work on the determination of nicotine and nornicotine be continued with attention being centered on the apparatus.

LITERATURE CITED

(1) BOWEN, C. V., *This Journal*, **28**(3), 578–585 (1945).

(2) MARKWOOD, L. N., *Ibid.*, **26**, 283–289 (1943).

* For report of Subcommittee A and action of the Association, see *This Journal*, **30**, 41 (1947).

(3) BOWEN, C. V., and BARTHEL, W. F., *Ind. Eng. Chem., Anal. Ed.*, **15**, 740–741 (1943).
(4) *Official and Tentative Methods of Analysis, A.O.A.C.*, 6th Ed., p. 74 (1945).
(5) BOWEN, C. V., and BARTHEL, W. F., *This Journal*, **27**, 224 (1944).

REPORT ON METHODS FOR ANALYSIS OF DDT AND INSECTICIDAL PREPARATIONS CONTAINING DDT

By ELMER E. FLECK (Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, U. S. Department of Agriculture, Beltsville, Md.), *Associate Referee*

Since the last report on DDT (*1*), the development of methods has proceeded along three lines: (a) Methods for the determination of small amounts of DDT, chiefly colorimetric and spectrographic (*2*)–(*7*); (b) methods for the determination of the p,p′-isomer content of DDT (*8*)–(*14*); and (c) the determination of DDT by the hydrolyzable-chlorine and total-chlorine methods (*15*)–(*20*).

It is toward the third line that this year's collaborative work has been directed. The hydrolyzable-chlorine method (*15, 16, 17*) has been subjected to collaborative study and has been adopted by the Association as a tentative method for the p,p′-isomer of DDT. As pointed out in the earlier report (*1*), the method gives high and somewhat erratic results when applied to technical-grade DDT. Soloway, Schechter, and Jones (*10*) have shown that under reflux conditions the o,p′-DDT gives results that are high by 12.6 per cent. They have also shown that 2-trichloro-1-*o*-chlorophenyl ethanol (the half-condensation product) gives results high by 176 per cent. Forrest, Stephenson, and Waters (*18*) have found that this half condensation product neutralizes nearly four equivalents of alkali, liberating three equivalents of chloride per mol under reflux conditions.

Technical DDT contains from 20 to 25 per cent of o-p′-DDT (*8, 9*) and about 0.2 per cent of the half condensation product (*19*). The latter compound is of minor importance as long as the DDT conforms to joint Army-Navy specifications (JAN-D-56A); but substandard DDT is usually characterized by a rapid rise in content of this impurity. The over-all result on analyses of technical DDT meeting specifications is a hydrolyzable chlorine value too high by about 5 per cent.

These factors have led to the increased use of total-chlorine methods for determination of technical-grade DDT. While the requirements for JAN-D-56A technical-grade of DDT allow a total-chlorine content of between 48 and 51 per cent, the usual finding for technical DDT which meets the other requirements of this grade is close to the theoretical 50 per cent.

Carter and Hubanks (*20*) and Donovan (*21*) have described modifica-

tions of the Umhoefer (*22*) method, using sodium and isopropanol, which are satisfactory for rapid determination of DDT both as spray residues and in insecticidal preparations. These methods have been used for a great number and variety of DDT determinations in the laboratories of both the Bureau of Entomology and Plant Quarantine and the Production and Marketing Administration, U.S.D.A.

In this determination it is essential that isopropanol of boiling point 82.5°C. be used. The azeotrope containing 12.1 per cent of water and boiling at 80.4°C. *cannot* be used. It has been shown that the conversion of organic chlorine to sodium chloride by sodium and isopropanol is complete at the end of 15 minutes' refluxing.* The time of the determination has therefore been shortened. The procedure also has been modified by using 50 per cent aqueous isopropanol to eliminate the residual metallic sodium.** The time may be shortened further by using electrometric titration methods for the determination of chloride ion, because this makes possible the elimination of the use of decolorizing carbon and of the extraction of colored bodies by the isoamyl alcohol-ethyl ether procedure.

Samples prepared from technical-grade DDT were submitted for collaborative analysis by the following procedures. The results are shown in Table 1.

DDT BY TOTAL CHLORINE

METHOD 1

(1) Weigh a quantity of sample containing about 1.00 g. of DDT, transfer to a 250-ml. volumetric flask, and make to volume with chlorine- and thiophene-free benzene. Shake until the DDT is dissolved and the solution well mixed. Transfer a 25 ml. aliquot to a 250–500 ml. standard tapered Erlenmeyer flask.† Evaporate on a steam bath until most of the benzene is removed. It is not desirable to evaporate to dryness, as DDT may decompose with loss of hydrochloric acid. Add 25 ml. of 99 percent isopropanol and 2.5 g. of metallic sodium in the form of ribbon or cut in small pieces, and shake the flask to mix the sample with the alcohol. Connect to a reflux condenser and boil gently for at least ½ hour. Shake the flask occasionally. Eliminate excess metallic sodium by cautiously adding 10 ml. of 50 per cent isopropanol through the condenser at the rate of 1–2 drops per second. Boil for an additional 10 minutes, and then add 60 ml. of water, cool the flask, and transfer the contents to a 250-ml beaker. Add 2–3 drops of phenolphthalein solution, neutralize by adding nitric acid (1+1), and then add 10 ml. in excess. Add a slight excess of N/10 silver nitrate and coagulate the precipitated silver chloride by digesting on a steam bath for ½ hour with frequent stirring. Cool, filter through a fast qualitative paper, and wash thoroly with distilled water. Add 5 ml. of saturated ferric alum solution, and determine the excess silver nitrate in the filtrate by titration with N/10 potassium thiocyanate. Subtract the quantity of silver nitrate found in the filtrate from that originally added. The difference will be that required to combine with the chlorine in the DDT. One ml. of N/10 silver nitrate is equivalent to 0.0035457 g. of

* Unpublished results from Hercules Powder Co., Analytical Laboratory

** Unpublished results from R. H. Carter.

† The method sent out to the collaborators called for a 0.75 g. sample made up to 100 ml. with a 10-ml. aliquot being used for a determination. These changes were made subsequently in order to minimize sampling errors. Direct weighing of the sample may be substituted for the aliquoting, provided the weighing does not introduce an error of more than 0.1 per cent in the sample weighed.

TABLE 1.—*Collaborative results on technical DDT and DDT-containing insecticides*

ANALYST	TECHNICAL DDT	COMMERCIAL 10% DDT DUST	10% DDT AND 1% ROTENONE DUST	5% DDT OIL SOLUTION	5% DDT, 5% ORGANIC THIOCYANATES, AND 0.1% PYRETHRIN
J. B. LaClair, California Bureau of Chemistry	97.68 97.88	10.88 10.86	9.92 10.12	4.82 4.84	4.60 4.68
Average	97.78	10.87	10.02	4.83	4.64
D. E. Gordanier, P. J. McLaughlin, Hercules Powder Co.	100.50 100.38 100.52	11.14 11.11 11.01	10.37 10.42	4.97 4.96	4.87 4.86
Average	100.47	11.09	10.40	4.97	4.87
C. G. Donovan, Production and Marketing Administration, U.S.D.A.	98.30 98.30 97.82 97.82				
Average	98.03				
Marcia Klein, Bureau of Entomology and Plant Quarantine, U.S.D.A.	98.56 98.56	10.78 10.78	9.93 9.93	4.80 5.00	4.77
Average	98.56	10.78	9.93	4.90	4.77
Elmer E. Fleck, Bureau of Entomology and Plant Quarantine, U.S.D.A.	99.81 99.94	10.92 10.95	10.14 10.18	4.98 4.99	4.93 4.91
Average	99.88	10.93	10.16	4.99	4.92
P. E. Hubanks, Bureau of Entomology and Plant Quarantine, U.S.D.A.	100.00 98.86	11.06 11.20	10.32 10.16	4.93 5.11	4.82
Average	99.43	11.13	10.24	5.02	4.82
General Average	99.00	10.97	10.15	4.92	4.80
15 ml. aliquot used	99.28 99.52 99.76 99.52				
Average	99.52				

chlorine. To obtain the percentage of DDT multiply the chlorine value by 2.

When electrometric titration is used proceed as directed under (1), through "neutralize by adding nitric acid (1+1)," then add 6 ml. excess. Cool the flask to room temperature and then transfer the contents to a 400 ml. beaker. The volume should be 200–250 ml. Titrate the Cl with N/10 silver nitrate using Ag-AgCl electrodes on an electrometric titrimeter (Fisher titrimeter or the equivalent). Calculate the percentage DDT as above.

NOTE: When the electrometric procedure is used the decolorizing carbon step in method (3) and the isoamyl alcohol-ethyl ether extraction methods (3), (4), and (5) may be omitted.

METHODS FOR THE DETERMINATION OF DDT IN VARIOUS INSECTICIDAL MIXTURES BY TOTAL-CHLORINE PROCEDURES—APPLICABLE IN ABSENCE OF OTHER CHLORINATED COMPOUNDS

(2) METHOD FOR THE DETERMINATION OF TOTAL CHLORINE IN DUSTING MIXTURES CONTAINING DDT IN ABSENCE OF ORGANIC MATTER

Weigh a quantity of sample containing about 0.75 g. of DDT, transfer to a 100–200 ml. volumetric flask, and add exactly 100 ml. of chlorine- and thiophene-free benzene. Shake well until the DDT is dissolved and the solution is well mixed. Allow to settle and transfer a 10-ml. aliquot to a 250–500 ml. standard tapered Erlenmeyer flask. From this point proceed as directed in method (1) beginning "Evaporate on a steam bath. . . . "

NOTE: If free sulfur is present use the hydrogen peroxide procedure outlined in method (6).

(3) METHOD FOR THE DETERMINATION OF TOTAL CHLORINE IN DUSTING MIXTURES CONTAINING DDT IN PRESENCE OF ORGANIC MATTER SUCH AS COLORING MATTER, PLANT RESINS, ETC.

Weigh a quantity of sample containing about 0.75 g. of DDT and transfer to a 100–200 ml. volumetric flask. Add a small amount of chlorine-free decolorizing carbon (0.5–1.0 g.) and exactly 100 ml. of chlorine- and thiophene-free benzene. Shake until the DDT is dissolved and the solution well mixed. Filter into a narrow-necked flask through a fast qualitative paper without suction, keeping the funnel covered with a watch glass to avoid loss from evaporation. Transfer a 10-ml. aliquot to a 250–500 ml. standard tapered Erlenmeyer flask. Proceed as in method (1) beginning with "Evaporate on steam bath . . . " through "then add 60 ml. of water." Cool, add 2 or 3 drops of phenolphthalein solution, neutralize by adding nitric acid (1+1) dropwise, and then add 10 ml. in excess. Cool, if necessary, to room temperature, transfer contents of flask and aqueous washings to a small separatory funnel, and shake with 15 ml. of a mixture of equal volumes of isoamyl alcohol and ethyl ether. Draw off the aqueous layer into a second separatory funnel and extract again with 15 ml. of the isoamyl alcohol-ethyl mixture. Draw off the aqueous layer into a 250-ml. beaker. Wash the two extracts successively with 10 ml. of water, and repeat with a second washing with another 10 ml. of water. Combine the aqueous wash solutions with the aqueous solution in the beaker. From this point proceed as directed in method (1) beginning "Add a slight excess of N/10 silver nitrate. . . . "

NOTE: Test the decolorizing carbon for the presence of chlorides by heating with dilute nitric acid (1+4), filtering, and adding silver nitrate solution to the filtrate. If chloride is present, wash with warm dilute nitric acid until washings no longer give a positive test.

(4) METHOD FOR THE DETERMINATION OF TOTAL CHLORINE IN MINERAL OIL SPRAYS CONTAINING DDT AND IN THE ABSENCE OF ORGANIC MATTER (PLANT EXTRACTIVE MATERIAL, ORGANIC THIOCYANATES)

Transfer a quantity of sample containing 0.065–0.075 g. of DDT to a 250–500 ml.

standard tapered Erlenmeyer flask. From this point proceed as directed under method (1) beginning "Add 25 ml. of 99 per cent isopropanol. . . ."

NOTE: If DDT content is less than 2 per cent, use the isoamyl alcohol-ethyl ether extraction in method (3) to remove excess oil.

(5) METHOD FOR THE DETERMINATION OF TOTAL CHLORINE IN MINERAL OIL SPRAYS CONTAINING DDT AND ORGANIC MATTER SUCH AS PLANT EXTRACTIVE MATERIAL FROM PYRETHRUM OR DERRIS AND/OR CUBÉ

Use method (4) with the isoamyl alcohol-ethyl ether extraction of method (3).

(6) METHOD FOR THE DETERMINATION OF TOTAL CHLORINE IN MINERAL OIL SPRAYS CONTAINING DDT IN THE PRESENCE OF ORGANIC THIOCYANATES WITH OR WITHOUT PLANT EXTRACTIVE MATERIAL

Transfer a quantity of sample containing 0.065–0.075 g. of DDT to a 250–500 ml. standard tapered Erlenmeyer flask. Add 25 ml. of 99% isopropanol and 2.5 g. of metallic sodium in the form of ribbon or cut in small pieces, and shake the flask to mix the sample with the alcohol. Connect to a reflux condenser and boil gently for at least ½ hour. Shake the 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 minutes and then add 60 ml. of water. Add 5 ml. of 30% hydrogen peroxide, a few drops at a time, thru the top of the condenser. Heat the mixture in the flask to boiling and boil for 15 minutes. Add 5 ml. more of 30% hydrogen peroxide and boil again for 15 minutes. Cool, add 2 or 3 drops of phenolphthalein solution, neutralize by adding nitric acid (1+1) dropwise, and then add 10 ml. in excess. Cool, if necessary, to room temperature, transfer contents of flask and aqueous washings to a small separatory funnel, and shake with 15 ml. of a mixture of equal volumes of isoamyl alcohol and ethyl ether. Draw off the aqueous layer into a second separatory funnel and extract again with 15 ml. of the isoamyl alcohol-ethyl ether mixture. Draw off the aqueous layer into a 250-ml. beaker. Wash the two extracts successively with 10 ml. of water, and repeat with a second washing with another 10 ml. of water. Combine the aqueous wash solutions with the aqueous solution in the beaker. From this point proceed as directed in method (1) beginning "Add a slight excess of N/10 silver nitrate . . . " or electrometric titration as in method (1) may be run directly on this solution.

NOTE: Use the hydrogen peroxide and isoamyl alcohol-ether extraction procedure on dispersible powders or sprays that contain surface-active agents or other ingredients that react with silver nitrate.

A blank should be run on reagents used in these procedures. A.O.A.C. Methods **6.152** or **12.42** may be substituted in the above procedures for the titration of chloride ion.

DISCUSSION

The results shown in Table 1 indicate that the methods are adequate in all cases except for technical-grade DDT. A study of method (1), as submitted for collaborative testing, for possible sources of the variation of results, indicates that the size of the sample and the method used in taking it may be at fault. As the method originally stood, one drop of the silver-nitrate solution corresponded to approximately a half of 1 per cent of DDT. The evaporation of benzene during sampling is another possible source of error. It is felt that a 25-ml. sample of a solution of 1.00 g. of DDT made up to 250 ml. with benzene would reduce this possible source of trouble. This is indicated by the results of P. E. Hubanks, who included several determinations of technical DDT using a 15-ml. aliquot of the

same benzene solution used in the original method (1). A larger sample is also indicated by the occasional presence of small blanks and the difficulty in accurately correcting this possible source of error.

It is recommended*—

That methods (2) through (6) be adopted as tentative methods for the determination of DDT in the absence of other organic chlorine-containing compounds.

That method (1), with the revised sampling procedure, be adopted as a tentative method and that this method be subjected to further collaborative study and compared with samples of pure p,p'-DDT.

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No reports were given on the general subjects, disinfectants, or leathers and tanning materials.

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* For report of Subcommittee A and action by the Association, see *This Journal* **30**, 41 (1947).

APPOINTMENTS

Lannes E. Davis, Division of Soils, California Agricultural Experiment Station, Davis, Calif., has been appointed Associate Referee on Hydrogen-ion Concentration of Soils, as successor to W. T. McGeorge of Tucson, Ariz.

Howard Bennett, Food and Drug Administration, Kansas City, Mo., has been appointed Associate Referee on Formaldehyde as a Preservative.

CORRECTION—FEBRUARY JOURNAL

In the paper on "The Polarographic Determination of Zinc in Soil," by Futoshi Takazawa and G. Donald Sherman, published in the preceding number of this *Journal*, (**30**, 185, 1947), 4th line under heading "Recovery of Zinc," the figure "74.7," should read "74.4."

CONTRIBUTED PAPERS

PLANT CONTROL METHODS FOR DETERMINING MOISTURE AND REDUCING SUGARS AFTER ACID HYDROLYSIS, IN GRAINS AND OTHER STARCHY MATERIALS*

By N. LACKTMAN (Chief Control Chemist, Publicker Industries, Inc., Philadelphia, Pa.)

It is well known that the various methods used by the fermentation industry for determining starch in grains, potatoes, etc., will give rather widely varying results. Furthermore, that industry is not interested in starch alone, but also in the soluble carbohydrates capable of being fermented into alcohol. The A.O.A.C. method **27.33** for grains and stock feeds, since it determines starch only, is not a suitable basis for evaluating fermentation efficiency in a single plant, nor for making comparisons between different plants. Of still less value for this purpose is the Alcohol Tax Unit method of reporting proof gallons of alcohol per bushel of grain, since the industry uses a wide variety of grains, etc., each with its own fairly wide range of moisture content. But the writer has found, in his laboratory, that the following method for total reducing sugars figured as starch gives a very satisfactory approximation of the total fermentable carbohydrates. It is, of course, recognized that some of the reducing sugars produced by acid hydrolysis of grains are not fermentable, but this source of error is not excessive in ordinary plant routine.

It is, of course, necessary to also know the moisture content, in order to convert the "starch" result to a water-free, or a uniform moisture, basis. The moisture method is somewhat similar to the A.O.A.C. method for Malt (**14.49**, *Methods of Analysis*). In the writer's laboratory the determination is made on a representative sample, ground in a laboratory mill. The moisture losses in grinding have been found to be never over 0.5 per cent. A single heating period is used, both for grains and potatoes, without an additional reheating to obtain "constant weight." Potatoes are ground in a meat chopper, and the charge is mixed with sand and heated four hours.

The methods follow:

STARCH AND OTHER FERMENTABLE CARBOHYDRATES

Prepare sample as indicated under A.O.A.C. methods **27.1** and **27.2.** Introduce a weighed quantity of sample representing 2 to 2.5 grams of the material on the dry basis (this would represent about 10–15 grams of ground white potatoes) into a 250 ml Kohlrausch flask, or any 250 ml volumetric flask which can be attached to a reflux condenser. Add about 150 ml of water and 20 ml of HCl (Sp. gr. 1.125), heat the mixture under a reflux condenser for 2 hours, cool and nearly neutralize with NaOH. Complete the volume to 250 ml, filter and determine the reducing sugars as dextrose in 25 ml of the filtrate using the Munsen-Walker general method described in Par. **24.39** of *Methods of Analysis*, sixth ed. Weight of reducing sugars as dextrose $\times 0.90 =$ weight of total carbohydrates figured as starch.

* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., Oct. 14, 15, and 16, 1946.

MOISTURE

Weigh 5 grams of the ground sample into a covered aluminum dish at least 50 mm in diameter and not exceeding 40 mm in height. Place the dish with weighed sample of grain in a hot air oven, with the temperature being kept at 100° to 105°C., for four hours. At the end of the four-hour period, place the dish in a desiccator, cool, and weigh. Do not put back in the oven for constant weight. Report the loss in weight as moisture. For ground, white potatoes or any other starchy materials high in moisture, take a sample of the material representing about 1.5 grams of dried solids for the moisture determination. Use a 125 ml flat bottom porcelain dish containing a layer of washed and ignited sand for this type of moisture determination.

METHODS OF ANALYSIS OF WINE

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

Before going into specific details concerning the various methods of analysis of wines, the writer believes that this time is as good as any to take stock of our wine methods, and to consider where they originated and how much we can further improve them by eliminating those that are clearly unnecessary, and those that are not being used because they are expensive of time and equipment, substituting in their place those that will be used and hence be of value.

In the interest of unification, simplification, and ease of interpretation, it is desirable that all wines be analyzed in about the same manner, so that the results of one chemist will agree as nearly as possible with those of another on the same class of products.

The most universally used set of methods are the official and tentative methods of analysis of the Association of Official Agricultural Chemists (to be referred to hereinafter as the A.O.A.C.).

The first comprehensive compilation of A.O.A.C. methods, including the analysis of wine, appeared in Bulletin 107, of the U. S. Bureau of Chemistry, revised in 1908. The methods of wine analysis covered 6½ pages out of 260 pages.

The current volume of "A.O.A.C. Methods" (6th Edition) now has 8½ pages covering wine analysis. It is to the everlasting credit of the early wine chemists, including Bigelow, Tolman, Munson, Burd, Trescot, Alvord, Eoff, Ingle, and Hartmann, and others who had tested well the soundness of the early methods, that these methods stood the test of time so well that few changes have appeared necessary during the 40 odd ensuing years.

The earliest, and at the same time one of the most comprehensive, study on wines ever made is recorded in Bulletin 59, Bureau of Chemistry, Department of Agriculture, 1900, prepared under the direction of W. D.

* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 14–16, 1946.

Bigelow and entitled "The Composition of American Wines." The following determinations were made on 845 samples of all varieties obtained from all wine areas of the United States:

Specific gravity; alcohol by volume; glycerol; glycerol-alcohol ratio; extract; ash; ash-extract ratio; total, volatile and fixed acids; volatile acid = total acid ratio; "extract-rest";† polarization; reducing sugar; NaCl; K_2SO_4; H_2SO_3; protein; tannin and other coloring matter.

In this Bulletin Dr. Bigelow discusses and interprets the results of these analyses. A few of his observations are that: " . . . these analyses are not to be accepted in any way as expressing the quality of American wine"; . . . "The average extract of wine 6 months old is 2.9 grams per 100 ml for red wine and 2 grams per 100 ml for white wine. When over 2 years of age the average extract content is 2.65 for red and 1.75 grams per 100 ml. for white. A red wine is regarded with suspicion which contains less than 2.4 or more than 3.25 grams per 100 ml, and a white wine which contains less than 1.5 grams per 100 ml of extract."

About that time (1903) Dr. H. W. Wiley authorized the analysis of American wines made by wine-makers who had received awards at Paris in 1900. This work was published as Bureau of Chemistry-Bulletin No. 72, by H. W. Wiley, entitled "American Wines at the Paris Exposition of 1900, Their Composition and Character." The determinations made were about the same as in Bulletin 59, but they also included "total and free tartaric acid," "phosphoric acid," "total sulphurous acid," and "undetermined extract."

The third, and most extensive, survey was made by Alvord, Ingle, and Hartmann. While the results were compiled during the years 1914–1916, much of the actual analytical work was performed several years before that time. This work was widely distributed in mimeographed form, but as far as the writer knows, it was never published. The following 16 determinations were made:

Alcohol and glycerol; non-sugar solids; reducing sugar; polarizations; ash; alkalinity of ash; fixed acids; volatile acids; tartaric acid, free tartaric acid, and cream of tartar; chlorine; nitrogen; pentosans; color; condition of sample; neutralizing test; per cent of P_2O_5 in ash.

All of these methods were listed in old Bulletin 107, Revised, with the exception of alkalinity of the ash, nitrates, pentosans, and the neutralizing test. "The Neutralizing Test" is of decided advantage (it is claimed) in judging white wines for pomace origin. It is conducted as follows:

"Neutralize 50 ml of wine in a porcelain dish with NaOH and allow the mixture to stand 2 hours. If the wine turns dark and a sediment forms on the bottom, this may be indicative of pomace origin. A straight wine gives a faint rose color. The test made on red wines is of little use for pomace detection, red wines giving a greenish

† Obtained by deducting from the total extract the sum of sugar (in excess of 0.1 gram), glycerol ash and fixed acids.

precipitate. The determination of nitrates as an indication of watering would be serviceable only as indicating water added after fermentation, since a yeast would deplete the nitrate content during fermentation."

Rarely do the commercial winery laboratories, no matter how well they are equipped, go into such detailed analyses as were made in the three important wine papers mentioned above, and by the author as shown elsewhere,[1] and in his more recent analyses of wines.

The principal determinations made in the wineries, and these are made by practically all of them except the very smallest, are: alcohol by volume (more often by Ebulliometer), total acids, volatile acids, and sulphur dioxide. The better equipped winery laboratories will make other tests as well, usually color, *p*H, and microscopic tests for the purity of their yeast or for bacterial contamination of the wine. However, Experiment Stations, State and city laboratories, may employ all the A.O.A.C. methods and other special methods as well.

The methods regarded as routine, for either standard wine or one that is under suspicion, by the Internal Revenue laboratory are: (1) alcohol by volume; (2) total solids (dried for 24 hours at 100° under forced draft); (3) ash; (4) tannin; (5) total acids; (6) volatile acids; (7) natural acids; (8) nitrogen as protein; (9) phosphates as P_2O_5; (10) alkalinity of water soluble ash; (11) total alkalinity of ash; (12) specific gravity at 60°.F; (13) color "as is" in $\frac{1}{8}$ inch cell of Lovibond tintometer; (14) weight of one gallon of the wine at 60°F.; (15) tests for artificial color (caramel, coal tar, and other artificial colors). On certain samples it may also be necessary to make a sulphur dioxide determination, to determine the various mineral constituents of the ash, or to perform several other special tests as the situation may require.

While practically all of the methods of analysis used by the author on the many wines analyzed during the past few years are essentially those of the A.O.A.C., some have been modified or abbreviated in order to save time and encourage their use. It is obvious that as long as chemical methods for routine work are short in detail, quick in results, and reasonably accurate, they will be used; long methods requiring much time and complicated equipment will be seldom used except for research work The details of the modified and abbreviated procedures are given in the key which follows.

KEY TO METHODS USED BY BUREAU OF INTERNAL REVENUE

(1) *Alcohol by Volume.*—A.O.A.C. *Methods of Analysis*, sec. **15.4.**

(2) *Total Solids.*—10 ml of the wine are evaporated to apparent dryness on a steam bath and dried approximately 24 hours (overnight) in a current of air at 100° C. These solids will be lower than solids in solution determined by numerous other

[1] "Apple Juice and Apple Wine," "Blackberry and Other Berry and Fruit Wines," and "Raisins and Raisin Wine," by Peter Valaer, have been published in mimeographed form and are obtainable from the Alcohol Tax Unit, Bureau of Internal Revenue, Treasury Department, Washington 25, D. C.

methods but have the advantage of being quite dry and are easily burned off without loss of ash.

(3) *Ash.*—Using residue from (2) treat as in A.O.A.C. sec. **15.16.**

(4) *Tannin.*—Folin-Denis Reagent **(a)** To 750 ml of distilled water add 100 grams of sodium tungstate, 20 grams phosphomolybdate acid and 50 ml. of 85% phosphoric acid. Reflux for 2 hours, cool, dilute to 1 liter. **(b)** Saturate distilled water with C.P. anhydrous Na_2CO_3. Allow to stand overnight, shake thoroughly, and filter. **(c)** Standard tannic acid solution. Dissolve exactly 100 mg tannic acid (c.p.) in a liter of distilled water. This solution must be freshly prepared for each determination.

Determination.—Place 0.1 ml of wine in a Nessler tube containing about 90 ml of distilled water. Add 1 ml of Folin-Denis Reagent and make up to 100 ml mark with water. Then add 5 ml of saturated Na_2CO_3 solution and shake well. After standing one hour, begin to transfer the material from the large tubes to small Nessler tubes (about $\frac{5}{8}$ inch inside diameter and 6 inches long, capacity 25 to 30 ml). The small tubes are filled to the same depth and compared with standards prepared in the following manner:

Into Nessler tubes containing about 90 ml of water add the standard tannic acid solution in quantities beginning at 0.1 ml and continue at 0.1 intervals until up to 1 ml then continue at 0.2 intervals until 2 ml are added to one tube, then prepare tubes cont. 2.0, 2.5, 3.0, 4.0, 5.0 ml of standard tannic soln. At the end of one hour these standards are filled into small Nessler tubes as described above. The small Nessler tubes containing the wine and the Nessler tubes containing the standard tannic acid solution are compared and the amount of tannins or tannic acid indicated in the wine reported as grams per 100 ml.

(5) *Total Acids.*—A.O.A.C., sec. **15.22(a).**

(6) *Volatile Acids.*—A.O.A.C., sec. **15.23.**

(7) *Natural Acids.*—Calculate volatile acid and total acid in terms of the acid (citric, malic, or tartaric) naturally present in the fruit used to make the wine and subtract the former from the latter.

(8) *Nitrogen as Protein.*—A.O.A.C. sec. **15.34.**

(9) *Phosphate as P_2O_5: Phosphates.*—Introduce 100 ml sample of wine into a casserole of about 250 ml. capacity and add 20 ml. magnesium nitrate solution and evaporate the solution to apparent dryness on a steam bath and place the casserole in an oven at about 100° C. until the contents are quite dry (best overnight). Ignite carefully in a muffle furnace at a dull red heat (about 550–600°C.). By careful manipulation, avoid splattering, decrepitation or loss of material due to rapid ignition. To the cool, well ashed mass in the casserole add 50 ml of 10% nitric acid and heat on steam bath, cool. Transfer the solution with rinsing into a glass-stoppered flask convenient for shaking. To the solution add ammonium hydroxide until it is distinctly alkaline to litmus, then neutralize with concentrated nitric acid adding 2 or 3 drops in excess.

Add 25 ml. of freshly filtered ammonium molybdate solution and shake continuously for at least 30 minutes. A shaking machine is convenient, otherwise shake thoroughly by hand. Filter promptly through a Gooch crucible or Büchner funnel, using a filter mat of well-washed paper pulp. Wash the precipitate with water until neutral to phenolphthalein, testing with N/3 NaOH (such as is to be used for final titration).

Add an excess of N/3 NaOH and titrate the excess with N/3 HNO_3, using phenolphthalein as an indicator. (Add N/3 NaOH until no yellow precipitate shows and add over 5 ml of the N/3 NaOH solution in excess.)

Solutions Required.—Magnesium nitrate solution—15 grams of magnesium nitrate in 200 ml. distilled water.

Ammonium molybdate solution—See A.O.A.C. sec. **2.7.**

Standard acid and alkali N/3—Each ml equivalent to .00103 grams of P_2O_5.

Example.—30 ml N/3 NaOH was used to dissolve the precipitate and furnish the excess.

23 ml of N/3 nitric acid was used to neutralize the excess alkali.

30-23 = 7 ml N/3 NaOH was used up by the precipitate.

$7 \times 10 = 70 \times .00103 = .0721$ grams of P_2O_5 in 100 ml of samples used for analyses.

This method is essentially the A.O.A.C. Method for the determination of phosphates in fertilizers, merely modified slightly to suit the material.

(10) *Alkalinity of Water-Soluble Ash.*—A.O.A.C. sec. **15.17.**

(11) *Total Alkalinity of Ash.*—To the total ash from 10 ml of wine obtained as described above add 10 ml. of N/10 HCl or N/10 H_2SO_4 and heat to incipient boiling, wash into a 250 ml beaker and titrate with N/10 NaOH using phenolphthalein as an indicator. The ash may be taken up with excess standard acid in the dish itself and titrated. Express the total alkalinity of the ash in terms of number of ml of N/10 acid required for the neutralization of the ash of 100 ml of wine.

(12) *Specific Gravity at 60°F.*—A.O.A.C. sec. **16.3.**

(13) *Depth of Color.*—Determined in a $\frac{1}{2}$ inch standard cell by the use of the Lovibond tintometer, using red glass slides (200 N.T.) and the brown slides (brewer's scale, series 52). These are usually the only slides required, but some dark red Eastern wines may require a bit of blue, and some unusual fruit wines may require some yellow color slides for exact matching of color. As a rule it is best to confine the readings as near as possible to red and brown.

(14) *Weight of one gallon of wine at 60°F.*—Multiply the weight of one gallon of water at 60°F. (8.33722 lbs.) by the specific gravity at 60°F.

(15) *Caramel.*—A.O.A.C. sec. **15.38, 15.39.**

(16) *Hamil-Simonds Test for Foreign Fruit Material.*—Blackberries and other berries impart mauve to purple shades, while some unknowns show "off" shades, others approach the clear purples of control tubes (*This Journal*, **25**, 220, 1942).

(17) *Coal-Tar Colors.*—A.O.A.C., *Methods of Analysis*, Chapter 21.

A REVIEW OF THE METHODS FOR THE DETECTION OF CARAMEL COLORING IN WINE AND OTHER ALCOHOLIC LIQUORS

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

An examination of commercial alcoholic liquors for caramel coloring has always been an important step in their analysis, and some very good methods have been proposed for this purpose.

There has been further progress since the publication of the 6th Edition (1945) of the Official and Tentative Methods of Analysis of the A.O.A.C. in which for the first time there appeared in the chapter on wines a chemical test for caramel—the Milos Test and its confirmation (**15.38–9**). If the chemist follows carefully the details of Milos' method and, with

* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 14–16, 1946.

any colored residue he might obtain, pursues the confirmatory procedure, he will determine positively the presence of caramel and not report caramel unless it is actually present.

Chemistry in the United States moves so swiftly that even during the time the collaborative work on the Milos Test was going on (1944), Mallory and Love of the San Francisco Laboratory, Alcohol Tax Unit, Bureau of Internal Revenue, were working on a caramel test which was intended to be, and is generally rated, approximately quantitative. At the same time, A. P. Mathers, of the Bureau of Internal Revenue, was busy in the East on another method which had as its principal objective speed, as well as accuracy. The principal purpose here is to discuss these two recent methods and to compare them and coordinate them with other methods of caramel detection that are in use, and also to make them all available to those who may have use for them.

CARAMEL IN WINES

When caramel is added to wine or liquors as commercial caramel, the determination is very simple indeed, and the proof quite positive. The only time when the decision becomes at all difficult or uncertain is when partially cooked and partially caramelized material has been added. Caramel is a substance that is not only black itself, but it also has a high coloring or tinctorial power, and thus a very small amount of it will color brown a very much larger volume of wine or other fluid. An example of such a partly cooked and partly caramelized substance is a normal grape concentrate which has been further heated or cooked by a free flame. Such a product is not pure, condensed must, since it has been reheated at a high temperature (during which it has lost all or most of its original characteristics) for the express purpose of producing a coloring agent. It thus has practically no value except for coloring. This is not the case, however, with even the darkest (almost black) imported raw sugar, with the darkest and oldest commercial black strap molasses, with old fruit wines made from elderberry, plums, or dried fruit (which often get almost black with age), or with any normal grape or other fruit juice concentrates which are very old and have thus become quite dark. Furthermore, we must exclude sherry wine made by any approved and accepted process in the United States or abroad, even the darkest Amoroso or Oloroso sherries which have been fortified in Spain with condensed must. None of these are classed as artificially colored nor have they ever been officially found to contain, or reported as containing, caramel. Ordinary grape or fruit juice concentrates made by the usual vacuum process, or by the steam jacketed kettle process (very rarely used at the present time), do not give positive caramel tests by the methods we will discuss. When, however, the owner of such concentrates boils his product over a free flame, caramel is almost certain to be formed. Such concen-

trates are not rated as pure condensed must, and their addition to wines constitutes rectification.

Caramel, and such caramelized coloring products as have been described, are used by those who wish to make their grossly over-ameliorated, stretched, substandard, spurious, and imitation wines look as if they were standard articles. Coal-tar dyes have also been resorted to, but as a rule they are too bright and obvious, and also very simple to detect.

The two new caramel methods that are to be discussed are alike in that certain precipitation agents are used which drag down with them any caramel present, when these agents are added to suspected wine. The Mallory-Love procedure is the longest method on record, with long descriptive details of its operations. Because this method has recently been published and is readily accessible, only the barest details will be given here. It was published in the Analytical Edition of Industrial and Engineering Chemistry (Vol. 17, p. 631, October 15, 1945), where the method and its discussion covers 6½ pages of small print. Very briefly, it is as follows:

To 25 ml of wine in a graduated cylinder add separately 3 carefully weighed dry reagents, add a specially prepared precipitating reagent, and allow the cylinder containing the mixture to stand overnight. Then filter the liquid through a paper-padded Gooch crucible and wash with 4 different liquids. Transfer the residue† to a beaker with a .5 *N* NaOH solution and water, and boil the fluid and filter into a 50 ml graduate cylinder. When acidified and made up to volume as prescribed, read the color in a 0.5 inch cell of the Lovibond tintometer and record the depth of color of the brown slides. The reading is then corrected for a manipulation loss of 12.5% and calculated to per cent of original color by dividing the corrected reading by the color reading of the original wine and multiplying by 100.

The simplest and shortest method so far devised for the detection of caramel is that of Alex P. Mathers which, because it has not so far been published, is given here in all necessary details.

Add to 10 ml of the filtered wine, in a Babcock cream bottle or ordinary 50–100 ml centrifuge bottle, 1 ml of pectin solution (1.0 gram pectin dissolved in 100 ml of 25% alcohol), 3 drops conc. HCl and 50 ml of ethyl alcohol, mix thoroughly, centrifuge, and decant the supernatant liquid. Take up the residue in 5 ml water, add 3 drops conc. HCl, add 50 ml alcohol, and again mix, centrifuge, and decant. Repeat this step (adding only acid and alcohol) until the supernatant liquid is colorless. Take up the residue in 10 ml water; a brown solution indicates caramel. Confirm by adding to this solution, in the Babcock bottle or other special bottle or test tube, 1 ml of 2,4-dinitrophenylhydrazine reagent (1 g 2,4-dinitrophenylhydrazine, 7½ ml of H_2SO_4, 67½ ml of ethyl alcohol 95%). Place the bottles in a beaker of boiling water for 30 minutes. Set aside to cool 30 minutes. If caramel is present, a precipitate should form by the time the tube is heated 15 minutes. If a precipitate has not formed by the end of the cooling period, caramel is absent. Disregard any precipitate formed thereafter.

† The residue at this stage may be washed, according to Mallory-Love, to remove any color not caramel, by disintegrating the paper retaining any residue on the filter pad and washing it into another Gooch, with acetone-HCl reagent, and then finishing the test as described.

An interesting item follows, from the minutes of the meeting of the Technical Advisory Committee of the Wine Institute, December, 1945. Dr. Marsh (University of California) after testing wine by the quantitative method of Mallory-Love is thus reported:

"Results indicated the test may prove to be the most satisfactory method yet developed for determining caramel. He stated he did not believe the method is specific for caramel but is perhaps better than any test yet devised. A disadvantage is that substances other than caramel are precipitated—for example, the substances responsible for the blackening of apricots—and there is evidence that the material thrown out is not caramel, but this point had not been proven. (Marsh ran the precipitate through three precipitations and the nitrogen content increased.) Since the substances responsible for the blackening reactions are universal to all products, the tawny color left in any baked or old wine would show as caramel."

Such material referred to by Dr. Marsh, of course, is not caramel and such substances have never been, and never would be, reported as caramel by Milos' test, properly followed by the confirmatory test as outlined in the 6th Edition of *Methods of Analysis;* or by the newly devised Mathers test as given above.

During the latter two procedures in making the tests for caramel, it is readily seen whether the final residue is due to the various kinds of commercial caramel or to saccharine matter that has been heated to a very high temperature, transforming the sugar molecule into quite a different substance with a high tinctorial power which we loosely call caramel.

Tom Scott, in a recent article—"Some Notes on Caramel" in "Vines and Wines,"—expressed an apprehension, after experimenting with the Mallory-Love procedure, that even a mere trace of caramel might give a positive test and thus get them into legal trouble. Mr. Scott, and all others so concerned, can be assured that, as far as this Bureau is concerned, substances that look like caramel, or under some circumstances behave like caramel, will not be reported as caramel. While it is believed that faithful adherence to Milos' test with its confirmatory test will show the presence of caramel, and will not show a positive test when commercial caramel is not there, such results from the Milos or the Mallory-Love procedure are carefully checked in this laboratory by the Mathers procedure above described. This process will give a negative test on natural substances such as old wines which have turned quite black; elderberry, plum, dried fruit, old pokeberry, or other berry material which once may have been indescribably red, but which has turned quite black with age; very old grape or other fruit concentrates that have turned dark; any old, darkened fruit material; old wood extracts from oak chips or from old whiskey; the very darkest raw or brown sugar; or new or very old blackstrap molasses. None of these substances show a positive test with Mathers' method for caramel, or by any other method mentioned above if the suggestions given herein are followed.

An interesting experiment, involving all of the accepted methods for caramel detection, was made with one representative each of the most troublesome classes of products encountered during extended laboratory experience in analyzing wine for caramel, particularly those wines suspected of being substandard, spurious, rectified, or of being imitations. (Not many of the products described below are actually found on sale.) These difficult samples fall into the following classes:

(1) Very old elderberry wine, old plum wine, dried fruit wine, or similar material which, because of age, exposure, light, air, oxidation, and perhaps some heating and other factors, has turned very dark.

(2) Heated carbohydrate material which may have been cooked, in liquid form, or dry, in a number of ways, in some instances to about 150°C. (302°F.). Such a product could be produced by heating dextrose sugar with 4 per cent ammonium sulphate in the manner of manufacturing commercial caramel, but not hot enough to reach the true caramel stage. Dextrose and sucrose may be heated still higher without producing a product responding positively to the above caramel test.

(3) Solutions of the darkest raw Cuban sugar, and of other forms of commercial brown sugar which are exceedingly dark but which have not been heated above the temperature used in the normal production of commercial cane sugar. (This class, of course, does not include any commercial brown sugar to which caramel coloring has been actually added, if indeed there is any such product on the market.)

(4) All forms of commercial molasses (blackstrap) such as is used for the production of industrial alcohol, even molasses that has become very black from age and exposure but which has received no deliberate heating, aside from that which is necessary during the commercial production of cane sugar.

It was found that wines which consist in whole or in part of one or more members of any of the four classes above mentioned, but which do not contain any caramel coloring whatever, may give colored solutions in the Milos procedure as outlined in the *Methods of Analysis*, 6th Edition, when the final residues, obtained by the process, are dissolved in water. While these solutions do not look exactly like caramel there may be some persons unfamiliar with this type of chemical work who would be deceived at this stage. However, this is not the end of the Milos test. This final solution, if colored, is not submitted to the "confirmatory test—tentative," and this will eliminate the color if it is not due to caramel. Should this colored aqueous liquid obtained by the first part of Milos' procedure be submitted to the Mathers test as described above the result will also be negative at the end of the test if the color is not due to caramel.

The Mallory-Love test[1] will sometimes give quite dark solutions at the

[1] Mallory, G. E., and Love, R. F., Ind. Eng. Chem., Anal. ed., 17, 633 (1945).

end of this procedure, and this color may be eliminated only in part by the additional procedure described on page 634 of the same Mallory-Love article. Any final color in the aqueous liquid which is about to be read in the Lovibond tintometer, whether obtained by the method described on page 633, or by the acetone-hydrochloric washing process described on page 634, should be promptly submitted to the "confirmatory test—tentative" above mentioned or to the Mathers test described above. In the interest of certainty both could be easily and quickly applied. The Mallory-Love procedure requires 25 ml of wine, and the final solution which is to be read in the tintometer as caramel is made up to 25 ml. Only 10 ml of this solution would be required for each confirmatory test, and the 5 ml remaining (after diluting to 10 ml) could be used directly for the di-nitrophenylhydrazine test already described.

The final aqueous liquids from the A.O.A.C. confirmatory procedure and from the Mathers test are also quite satisfactory to use and are used for the di-nitrophenylhydrazine test.

CARAMEL IN SPIRITS

It sometimes occurs, in testing for caramel in spirituous liquors with Marsh reagent, and with cyclohexanol reagent, that positive indications will be obtained with both reagents when there is no caramel present. Cyclohexanol was made an alternate solvent in the official method because this reagent indicated the absence of caramel when the spirit being analyzed had been stored a long time (8 years or more in charred wood), or when certain coal-tar dyes were present, or in beverage spirits that had been quick-aged with raw, uncharred, or untoasted white oak chips, and sometimes even with toasted chips. This was gone into fully in the Associate Referee's published report for 1944 (*This Journal,* **28,** 467, 1945).

There have been other occasions, however, when both reagents gave indications of caramel coloring in the lower layers of the test when there was present in the liquor: an infusion of peat-dried barley malt, as in making certain Scotch-flavored liqueurs, or an unusually heavy infusion of both toasted and untoasted white oak chips, or very dark colored wine solids or certain dyes, but no caramel coloring.

If it is necessary to actually prove the presence of caramel in the spirits, the sample should be analyzed by the "confirmatory test—tentative" under wine (sec. **15.39**), followed by the Mathers test. All of the substances which have been described above that give a colored liquid are quickly eliminated by these two tests, the final solution being usually water white or practically colorless. Should the final aqueous solutions be brown either at the end of the Mallory-Love, Mathers, or A.O.A.C. "confirmatory-tentative," they should be further confirmed by the di-nitro-phenyl-hydrazine reagent, in the manner already described.

If the special Mallory-Love method for spirits (*loc. cit.,* page 635)

is used on spirits which are heavily colored with uncharred and untoasted white oak chips, or with charred or toasted chips, there may be a substantial amount of color in the solution that may be read as caramel coloring. However, no caramel color is indicated if these final aqueous solutions are submitted to the confirmatory test (sec. **15.39**), and to Mather's procedure above, further confirmed by 2-4-di-nitro-phenyl-hydrazine reagent test described above.

DETERMINATION OF DDT, PARTICULARLY IN MILK AND FATS, BY THE SCHECHTER PROCEDURE

By P. A. CLIFFORD (U. S. Food and Drug Administration, Federal Security Agency, Washington 25, D. C.*)

The colorimetric procedure of Schecter *et al.* (1) has been applied successfully to the determination of DDT as spray residue (2). Advantages of the method are its specificity and ability to distinguish between the two main isomers of DDT. Simple tests can assure the absence of off-shades in the final photometric color. Since the latter publication appeared, the writer has tried to utilize more fully the remarkable sensitivity of the Schechter method, especially when it is applied to milk and fats.

There are two main and related sources of difficulty with the procedure: first, the quantity of inert organic material included with the DDT sample may be excessive and the nitration cannot be controlled; this results in "burning up" of the DDT; second, extraneous yellow colors may result from incomplete removal of partially oxidized organic material when it is present in excess. The spray residue application specifies removal of surface DDT from a large sample of fresh fruit by means of a simple benzene wash. Except for small amounts of wax and coloring matter, little organic matter is included, and aliquots of the wash solution representing several hundred grams of fruit can be handled without undue difficulty.

There is, of course, a limit to the quantity of residue, remaining after evaporation of the benzene wash aliquot, that can be handled in the nitration. Thus the following results were obtained when 1.0 milligram quantities of DDT were nitrated in the presence of increasing quantities of "apple wax" and "spinach wax." The DDT was a 75–25 per cent mixture, respectively, of the p,p'- and o,p'-isomers, and the waxes were the materials remaining after evaporation of the benzene strip solutions of unsprayed apples and of dried, fresh spinach. A modification of the procedure was the use of a 1+4 mixture of ethyl-petroleum ether, instead of straight ethyl ether, in the extraction of the nitration mixture. The mixed ethers extract much less interfering yellow color from the digest, and such interference is much easier to remove in the subsequent alkali washings. The results are given in Table 1.

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TABLE 1.—*Recoveries of DDT from fruit and vegetable "wax"*
(.75 mg p,p'-DDT and .25 mg o,p'-DDT added in all cases)

	APPLE WAX (MG)				SPINACH WAX (MG)			
	100	200	300	400	100	200	300	400
p,p'-DDT found (mg)	.765	.750	.678	.650	.718	.709	.721	.689
o,p'-DDT found (mg)	.230	.204	.156	.142	.248	.219	.133	.112
Per cent recovery	99.5	95.4	83.4	79.2	96.6	92.8	85.4	80.1
Indicated p,p'-isomer in DDT found (per cent)	76.9	78.6	81.3	82.1	74.3	76.4	84.4	86.0

Significant losses, especially of the o,p'-isomer, are noted with 200 mg. of either wax, and this quantity might be taken as an upper residue limit for successful application of the method. In ordinary work waxy residues should be much less. It is fortunate in spray residue work that the quantity of this extraneous material can so simply be limited.

With many products, notably fats, this is not the case. Accordingly, more sensitive colorimetric ranges were investigated in order to allow a determination upon workable quantities of sample material. The full sensitivity of the method is not utilized when spray residue is determined but, knowing the optical densities of the DDT colors when developed by this technic, it is possible to calculate a practical sensitivity limit, working with longer cells and smaller volumes, for the Schechter procedure. A 10 cm cell containing slightly less than 9.0 ml. was available for use with the neutral wedge photometer. By dissolving the nitrated residues in only 3.0 ml. of benzene and developing the colors by adding 6.0 ml. of standard methylate (less than a two-fold volume will give turbid solutions) it was reasoned that nearly the full photometric scale could be covered with DDT colors ranging up to only 20 micrograms. This was confirmed by the photometric readings of the colors produced by mixing 6.0 ml. of standard methylate and equivalent micro-quantities of pure tetra-nitro p,p'-DDT dissolved in 3.0 ml. of benzene (30.1 mmg. tetra-nitro-DDT =20.0 mmg. DDT).

Difficulties arose, however, when attempts were made to derive standard curves by actually nitrating and extracting 0–20 mmg. quantities, in increments of 4.0 mmg., of both pure isomers. (Nitrations were carried out in un-etched 50 ml. Erlenmeyer flasks.) Density readings were erratic and usually fell far below the theoretical values of equivalent quantities of the pure tetra-nitro derivatives. Destruction of DDT was indicated, since microgram quantities of the nitro-derivatives could be carried through the extraction and alkali washing procedures without loss. Gentle nitration for 15 minutes in all oil-bath at 70°C. gave color densities of about 92 per cent of theory, but if the nitration period was extended to one hour, recoveries were only about 78 per cent. Nitrations at other

temperatures and for varying periods of time gave no better results. However, it was found that if these microgram quantities of DDT were nitrated along with 10 milligrams of oleic acid, close to theoretical color densities were obtained with the usual spray residue nitration technic (*i.e.*, mixture brought slowly from ice-bath temperature to about 85°, then placed on the steam bath at 95–100° for one-half hour). Why the use of an inert "buffer" material should be necessary with microgram quantities of DDT and unnecessary with the spray residue range is unexplained. It appears that the nitration always destroys a little DDT, especially in the absence of some protective organic material, and the proportion becomes large when microgram quantities of DDT are dealt with.

Besides use of the oleic acid, other modifications of technic were necessary before satisfactory standard curves, covering a 0–20 microgram range in steps of 4 mmg., were obtained for both isomers. Use of the 10 cm. cell, and of the total nitrated residue in the color development, magnified the interference of yellow off-shades and turbidity, and the following precautions are all designed to eliminate these effects.

(1) All solvents were carefully redistilled from all-glass apparatus.

(2) Straight petroleum ether (B.P. 30–65°) was used for extraction of the diluted nitration mixture as it included much less interference, in the form of oxidation by-products, than ethyl ether or mixtures of ethyl ether and petroleum ethers. It should be pointed out, however, that the nitration products of DDT are very sparingly soluble in petroleum ether and losses may occur in the extraction in those cases where more than a few tenths milligram of DDT has been nitrated. Ethyl-petroleum ethers, 1+4, are recommended for the spray residue range.

(3) *Thorough* washing of the petroleum ether extracts was found to be essential. With only the 10 mg. of oleic acid present as protective material in the nitration, it was still necessary to wash three times with 10 ml. portions of 10 per cent W/V potassium hydroxide, shaking a full two minutes each time. (As many as six extractions may be necessary with actual samples.) No DDT is lost in the washing process.

(4) After the final wash with saturated sodium chloride solution, the petroleum ether extract was filtered through carefully washed and dried fine glass wool. Cotton tended to introduce yellow color, and asbestos partially adsorbed the nitro-DDT derivatives.

(5) It was discovered that heating the flasks, in which the washed ether extracts had been evaporated, for 1 hour at 100°C., tended materially to reduce off-shades and turbidity, and this practice was adopted as routine. Nitro-derivatives of DDT are not lost, and interfering materials, especially when actual samples are run, tend to be volatilized.

(6) It was necessary to pay particular attention to the elimination of turbidities in the final color. The standard methylate solution could not

be used until it had settled perfectly clear. Methylate solutions made from clean metallic sodium were more satisfactory than those made from commercial sodium methylate. The lower tube of the automatic buret from which the methylate solution was dispensed was tightly packed with alkali washed asbestos and the solution was thus re-filtered immediately before use.

(7) All glassware was rinsed with strong alcoholic potassium hydroxide solution before cleansing by means of the usual cleaning solution—soap and water routine.

With these precautions, satisfactory standard curves were obtained for 0–20 mmg. quantities of both main isomers. The precision attainable is illustrated by the following recoveries with various mixtures of pure p,p′- and o,p′-DDT. Twenty mmg. of total DDT was taken in all cases, and the proportion of p,p′-DDT ranged from 75 to 95 per cent in steps of 5 per cent. The color mixtures were resolved as previously outlined in the spray residue procedure (2).

The precision attainable with the pure isomers and the 0–20 mmg. range is remarkable, but the writer found this micro-range hardly practicable with actual samples. Traces of unremoved yellow color were apt to interfere, and good resolution of isomeric mixtures was usually not obtained.

This interference was reduced by half by extending the range to 0–50 mmg. in steps of 10 mmg. and by reading the colors in a 5.0 cm. cell. (Ten milligrams of oleic acid was again used for the nitrations.) Such a range has ample sensitivity for the detection of significant quantities of DDT in foods and can be used in conjunction with the spray residue procedure. Both ranges can be varied according to the manner in which the benzene solution of the DDT nitro-products is handled; if, with the spray residue range, the nitro-products are taken up with only 5 ml. of benzene and the color developed by mixing with 10 ml. of methylate, the range covered is 0–0.2 mg. The 0–50 microgram range can be expanded by taking up the nitro-products in an appropriate volume of benzene and developing a 3 ml. aliquot with the usual 6 ml. of methylate.

The Schechter procedure has been applied successfully to the determination of DDT in fats, especially the body fats of experimentally fed animals (3.) In most cases the DDT content has been so high that limitation of sample size was not a factor and direct nitrations were feasible. It was soon found that the quantity of sample that could be handled directly must be strictly limited. The following tests, wherein 50 microgram and 1.0 milligram quantities of DDT were nitrated in the presence of increasing amounts of pure butterfat, illustrate this point. The DDT was again a 75–25 per cent mixture of the pure p,p′- and o,p′-isomers. Nitrations were done in the usual manner with 5 ml. of the nitrating

acids, *i.e.*, the mixtures were brought slowly from ice-bath temperature to 85°C., then held for one-half hour on the steam-bath. Recoveries are given in Table 2.

It is noted that losses become serious if more than 100 mg. of fat is present in the nitration step, and this quantity might be set as an upper limit for the amount of fat sample that can be handled directly. More will cause destruction of DDT, and quantities of fat above 0.5 gram are apt to oxidize in a semi-explosive manner.

In several cases the progress of this oxidation reaction was checked as follows: One-half gram of butterfat was placed in a test-tube immersed in an ice-cold water bath, then 10 ml. of the ice-cold nitrating acid was

TABLE 2.—*Determination of pure p,p'- and o,p'-DDT with a 0–20 mmg range*

(Total DDT added = 20.0 mmg)

p,p'-DDT ADDED	o,p'-DDT ADDED	p,p'-DDT ADDED	p,p'-DDT FOUND	o,p'-DDT FOUND	TOTAL DDT FOUND	INDICATED p,p'-ISOMER IN DDT FOUND
mmg	*mmg*	*per cent*	*mmg*	*mmg*	*mmg*	*per cent*
19	1	95	18.7	1.2	19.9	94
18	2	90	17.7	2.0	19.7	90
17	3	85	16.8	2.9	19.7	85
16	4	80	16.0	3.9	19.9	80
15	5	75	14.5	5.2	19.7	74

added. A thin-walled thermometer was placed in the test-tube to serve as a stirrer. The bath temperature was slowly raised, and its temperature compared with that of the reaction mixture. Usually, at about 50°C., the temperature of the mixture would rise abruptly 10° to 15° above bath temperature and would remain above it until both would again coincide at about 70°. Due to lag, the temperature indicated by the inner thermometer at this "flash point" is probably not the true temperature of the reaction mixture, and local temperatures may exceed it by many degrees. Hence, as 50° appeared to be a critical point in the nitration step, recovery tests were made in which the nitration was conducted in careful stages. Fifty micrograms of DDT and 200 milligrams of butterfat were used; the mixtures were brought slowly from ice-bath temperature to 40° and held for 15 minutes, then brought to 50° and held 15 minutes longer, then held for 15 minutes at 10° intervals up to 90°, then held on the steam-bath for 15 minutes to complete the reaction. (It took about 7 minutes to go from a certain bath temperature to one 10° higher.) As a rule, recoveries were no better than those listed in Table 3. Nitrations with 1+1 mixtures of conc. sulfuric acid and ordinary conc. nitric acid did not bring up the full DDT colors and if, more than a few milligrams of

fat was present, nitrations with dry potassium nitrate and sulfuric acid totally destroyed the DDT. Apparently there was no point in changing the simpler nitration technic used with spray residue samples. However, it was established that better recoveries were obtained when larger volumes of nitrating acids were used (at least 5 ml.). This must be because excessive local temperatures in the nitration are more quickly dissipated by the larger volume and heat capacity of the reaction mixture.

If, in the case of fats, sample size must be limited to 100 milligrams for the direct nitration, a precision in the determination of one microgram must be achieved in order to determine DDT content within 10 p.p.m. This would appear nearly impossible. And recent developments

TABLE 3.—*Recoveries of DDT added to butterfat*
(Direct nitration)

FAT ADDED (mg)	0–50 MICROGRAM RANGE				0–1.0 MILLIGRAM RANGE			
	37.5 mmg p,p′- AND 12.5 mmg o,p′- ADDED				.75 mg p,p′- AND .25 mg o,p′- ADDED			
	p,p′	o,p′	RECOVERY	INDICATED p,p′	p,p′	o,p′	RECOVERY	INDICATED p,p′
			per cent	*per cent*			*per cent*	*per cent*
None	35.8	13.5	99	73	.740	.230	97.0	76.3
50	34.9	13.3	96	72	.753	.239	99.2	75.9
100	33.9	12.6	93	73	.700	.222	92.2	75.9
200	31.7	9.4	82	77	.726	.188	91.4	79.4
300	—	—	—	—	.547	.103	65.0	84.2

in the entomological field point up the need for the accurate determination of traces of DDT smaller than 10 p.p.m. in food fats, cheeses, and milk (4). A suitable method of isolation of DDT from the fat sample would solve the difficulty, but, because of the solubility of DDT in fats, and their mutual solubility in most organic solvents, a simple method of separation was not immediately apparent.

A first attempt to isolate DDT from larger quantities of fats is presented for whatever interest it may possess. An alkaline saponification was held to be infeasible because it would destroy DDT, but it was found that one-half gram quantities of fats could be saponified with nitric acid without losing it. The technic was as follows: 0.5 gram of sample was treated with 5 ml. of fuming nitric acid brought slowly from ice-bath temperature at 100° and held at 100° for 1 hour. The mixture was then diluted with 25 ml. of ice-water and titrated to alkalinity (phenolphthalein) with 10 per cent sodium hydroxide. Prolonged shaking was necessary in establishing an end point. The solution was poured into a 250 ml. glass-stoppered cylinder and diluted to 75 ml. with rinsings of the flask; then 75 ml. of 95 per cent alcohol was added and the solution was

extracted four times with 50 ml. portions of petroleum ether. The extracts were siphoned off and combined in a separatory funnel, washed twice with small portions of 10 per cent alcohol and evaporated to dryness in a 125 ml. Erlenmeyer flask. The small amount of residue was then nitrated and carried through the procedure as usual. Results with the 0–20 microgram range on added amounts of DDT ranging from 2 to 25 p.p.m. (0.5 gram sample) were quite good (within 1 p.p.m.) but sensitivity, even of this micro-range, was taxed, and the advantage gained by this procedure with respect to a workable sample size was thought not sufficient to warrant its adoption. More effective means of separation were sought and a method based on the extraction of DDT from oils or liquid fats with anhydrous methanol showed promise. However, it was not developed, as interfering quantities of fat were usually included in the methanolic extract.

A procedure involving hydrolysis of larger samples of fat by means of pancreatic lipase was more successful. First trials were made with two different samples of commercial pancreatin and 5.0 gram quantities of butterfat; various amounts of the dry commercial products were mixed with the fat in glass-stoppered Erlenmeyer flasks and the whole shaken into emulsions after the addition of 25 ml. of sodium biphosphate-sodium hydroxide buffer solutions of various *p*H. The mixtures were allowed to remain in a hot-air cabinet at 40–42°C. for different periods of time and were shaken at intervals; they were then removed, diluted with 50 ml. of 95 per cent alcohol and titrated to the pink color of phenol red (*p*H ca 7.5–8.0). The solutions were then rinsed into 250 ml. glass-stoppered graduated cylinders, diluted to the 150 ml. mark with water and extracted four times with 50 ml. portions of petroleum ether.* The combined petroleum ether extracts were washed twice with 20 ml. portions of 50 per cent alcohol, evaporated to dryness in tared flasks and the progress of hydrolysis noted from the weight of unsaponified residue.

Hydrolysis was rapid at the start but became progressively slower. With 5.0 grams of butterfat, 48 hours hydrolysis time usually was necessary to reduce unsaponified residues to 5 per cent of original sample weight (250 mg.). This is seen to be still too much for a successful nitration.

A more active lipase preparation was made from fresh hog pancreas gland as detailed later under "Reagents." At first, a glycerol solution of the active principles was prepared by stirring overnight a portion of the de-fatted, dried, and ground gland with a 17-fold weight of 87 per cent glycerol (5). Five ml. of the strained extract gave much better hydrolysis (with butterfat, usually less than 100 mg. residue after 48 hours), but

* The partition of DDT between this mixture of 2 volumes water with 1 of 95 per cent alcohol, and petroleum ether, strongly favors the ether; the coefficient expressed as concentration (alcohol) divided by concentration (petroleum ether) equals 0.0278. Thus two extractions as above should remove more than 99 per cent of the DDT from the solution of soaps. Because mechanical separation of the two layers by means of a siphon arrangement is not entirely complete, two more precautionary extractions were made.

even better results were obtained by direct addition of 200 mg. of the ground gland itself. Use of the solid gland caused some sludge to appear at the liquid interface during the petroleum ether extraction of the unsaponifiables, but this was not troublesome. Portionwise addition of the enzyme during the course of hydrolysis appeared to offer no great advantage. Methods for the preparation of more active lipolitic extracts (6) were considered, but not investigated. Possibly certain commercial enzyme preparations may be as efficient as the product employed in this investigation.

Good emulsification during hydrolysis was found necessary and the addition of small amounts of both dry sodium oleate and dry bile salts helped in this regard. To maintain the emulsion, constant shaking of the flasks, after the addition of glass beads, was adopted. Because the available hot-air cabinet was too small to accommodate a shaking machine, operations were transferred to a 37.5°C. incubation room where a "swirling" shaker of the Ross-Kershaw type (phosphoric acid determination) could be used. Better emulsification, due to shaking, more than compensated for this somewhat less than optimum temperature, even though the action of this type of shaker is too gentle and sometimes tends to agglomerate or "churn" the liberated fatty acids. No doubt more vigorous shaking would yield a more rapid hydrolysis.

The sodium biphosphate-sodium hydroxide buffer solution finally adopted is described below under "Reagents." The solution is adjusted to *p*H 8.0 and during hydrolysis the *p*H of the fat-buffer mixture falls rapidly from this initial value to about 6.2. The enzyme preparation employed was found to work satisfactorily at a slightly acid *p*H; no advantage was noted when attempt was made to hold the hydrolysis mixture at *p*H 7.5 (phenol red) by the occasional addition of *N* sodium hydroxide during the course of the hydrolysis. A very high concentration of buffer salts would be necessary automatically to hold the mixture alkaline, because of the relatively large mass of liberated fatty acids.

No difference was noted in the rate of hydrolysis of 5 gram samples of butterfat, beef fat, or corn-oil, and little difference in the quantity of unsaponified residue. The amount of unsaponifiable material may be a limiting factor with some fats; with butterfat it may approach 0.5 per cent, or 25 milligrams on a 5 gram sample basis. Most residues, remaining after hydrolysis and extraction, had a sterol-like odor and appearance.

The lipase hydrolysis is noted to be slow. With a 5 gram sample, about 48 hours hydrolysis time was found necessary to reduce the quantity of unsaponified residue below the workable limit of 100 milligrams. After an overnight hydrolysis (18–20 hours) about 250 mg. of residue was recovered from 5 gram samples of butterfat—too much to nitrate directly. However, if an early result was desired, it was found possible to dissolve such residues in a measured volume of benzene, and evaporate down and

nitrate an aliquot calculated to contain not more than 100 milligrams of residue. It was found convenient, in routine work, to hydrolyze samples over the week-end, or longer, if time was no immediate object. Early experiments with both main isomers showed no significant loss of DDT during a prolonged hydrolysis (up to two weeks time).

Schechter *et al.* have recently outlined a method (4) in which the DDT-containing fat is dissolved in chloroform and the fat removed by repeated extraction with sulfuric acid and sulfuric acid-fuming sulfuric acid mixtures. Details of this procedure were available through personal communication during the course of most of the work reported here, in particular their method for the isolation of DDT (along with the fat) from milk samples. However, the lipolitic separation of DDT from fats was further studied in order to provide an alternate method. It will be found slower but perhaps more convenient. Details of procedure are given below and the presentation is meant to follow that of the spray residue method (2). General principles, and most of the reagents used, are the same, with new reagents as indicated. Preparation of samples by means of the lipase hydrolysis is given in detail. Preparation of standard curves, and the calculations, remain about the same for the 0–1.0 milligram (spray residue) range with slight differences in technic as noted; and directions for setting up a micro-range (0–50 micrograms) are given.

REAGENTS

With the following exceptions and additions, reagents are as listed on pp. 196–197 of the spray residue method above cited.† Instead of reagent **(b)**, use either a 1+4 by volume mixture of *redistilled* ethyl-petroleum ethers (0–1.0 mg. range), or straight redistilled petroleum ether (0–50 mmg. range). Instead of reagent **(c)** use a 10 per cent W/V solution of reagent grade KOH. Use the same methylate solution of the strength specified under **(e)** for both ranges. Prepare only from clean metallic sodium and preserve in a suitable dispensing system with all outlets closed off or trapped against CO_2 and moisture. An automatic buret filled by means of pressure from a hand bulb is convenient; if this is used, pack the inner delivery tube to buret with dry, alkali washed asbestos, to filter the solution immediately before use. In addition to the above include:

(g) *Bile Salts.*—("Difco-Bacto"; Difco Laboratories, Detroit, Michigan).

(h) *Sodium Oleate.*—(Neutral powder; J. T. Baker & Co.).

(i) *Buffer Solution.*—Weigh 50 grams reagent grade $NaH_2PO_4 \cdot H_2O$ into a 1 liter volumetric flask and dissolve in ca 600 ml. H_2O. Add a few drops phenolphthalein indicator and about 325 ml. of *N* NaOH solution. Cool the flask to room temperature and add from a buret more NaOH solution until, with the solution nearly at volume, a faint pink color persists. (About 350 ml. *N* NaOH, in all, is required.) Make to mark and check with a *p*H meter, if desired. The *p*H should be 8.0–8.2. (The solution is susceptible to mold growth, but this can be suppressed by occasional heating on the steam bath.)

(j) *Lipase Preparation.*—Grind fresh hog pancreas glands through a food chopper and soak overnight in acetone. Pour the mixture into large centrifuge bottles, centrifuge, and decant the acetone. Repeat the acetone wash, shaking up the material

† *This Journal*, **29**, 196 (1946).

thoroughly, then wash twice with a 1+1 acetone-ether mixture. Finally, wash three times with ether, or until all fat is removed. Allow the extracted material to air-dry, then grind to 20-mesh size in a Wiley mill. Preserve the dry powder at all times in a refrigerator.

PREPARATION OF SAMPLE

A—*Butter, Pure Fats, and Oils.*—Weigh 5.00 grams of sample into a glass-stoppered 125 ml. Erlenmeyer flask, add several dozen glass beads and 200 mg. each of bile salts and sodium oleate (g) and (h). Add 25 ml. of buffer solution (i) and shake the mixture into an emulsion. (With some fats it may be necessary to warm slightly.) Make sure the temperature is not above 45°, then add 200 mg of the ground gland (j) and shake vigorously for several minutes longer. Place in an incubator at 37.5° C., or better, in a cabinet or hot-air oven at 40–42°. Constant shaking during the hydrolysis is highly desirable; if a mechanical shaker is not used, remove the flasks at frequent intervals, especially at the start, and shake vigorously to re-establish the emulsion. Allow hydrolysis to proceed for at least 24 hours (48 hours or longer if time permits).

Remove the flasks and add 50 ml. of 95% alcohol, rinsing down the stoppers carefully. Shake vigorously to dissolve the fatty acids, add 5 drops of .04% phenol red indicator and titrate with *N* NaOH solution, slowly and with constant shaking, to a salmon-pink end-point (*p*H = ca 8.0). (With 5.0 gram samples of most fats, about 20–22 ml. of *N* alkali is required; much less may indicate incomplete hydrolysis.) Make sure the end point persists after vigorous shaking, then pour the solution into a 250 ml. glass-stoppered graduated cylinder, including the beads, and rinse to the 150 ml. mark with water. Cool under the tap, then add 50 ml. of redistilled petroleum ether and shake vigorously for 1 minute. The layers should separate cleanly in 3–5 minutes. Blow off the upper ether layer into a 500 ml. separatory funnel by means of a siphon arrangement similar in principle to that of a wash bottle. (The lower end of the delivery tube is sharply bent to a "U" shape, with the open end, about ½ inch long, directed upwards and slightly flared. In operation, the delivery tube is slipped down through its stopper until the open limb is only slightly above the level of the liquid interface and the upper liquid is removed by applying pressure at the mouthpiece.) Extract three more times with 50 ml. portions of the petroleum ether, rinsing out the hydrolysis flask with each successive portion, then wash the combined extracts by shaking vigorously with two 20 ml. portions of a 1+1 by volume mixture of water and 95% alcohol. (Emulsions here are indicative of incomplete hydrolysis. Any sludge-like solid material included with the ether extracts usually disperses into the alcohol layers and can be eliminated when these are drawn off.) Evaporate the washed petroleum ether extracts, portionwise, to dryness in a tared 125 ml. Erlenmeyer flask, rinsing the funnel with 1–2 small portions of petroleum ether. Remove traces of solvent by means of a gentle current of air, cool and determine the weight of residue. If the quantity of residue is under 100 milligrams, it can be nitrated directly; if not, dissolve the residue in a measured volume of benzene and evaporate to dryness an aliquot calculated to contain not more than 100 mg.

B—*Liquid Milk.*—(Extraction procedure of Schechter *et al* (4)). Weigh 100 gram samples of well-mixed fresh or formaldehyde preserved milk into large centrifuge bottles and add 100 ml. of 95% alcohol. Mix, then add 50 ml. of redistilled petroleum ether, stopper bottles with clean rubber stoppers and shake vigorously for 1 minute. Centrifuge to break emulsions and blow off the upper layers into separate 125 ml. glass-stoppered Erlenmeyers with a siphon arrangement as described above. If emulsions do not break readily, add 1–2 drops conc. HCl, shake vigorously, and re-centrifuge. Extract in the same manner with three additional 50 ml. portions of

petroleum ether, combining extracts in the proper flask by evaporating off the ether from an extraction during the time subsequent extracts are being prepared. Finish the evaporation with the aid of a gentle current of air.

Proceed with the lipase hydrolysis as outlined under (A). (One hundred grams of cow's milk will yield roughly from 3 to 7 grams of fat; the quantity can be gauged by visual inspection. If a high-fat milk is encountered, the hydrolysis should be allowed to proceed for as long a time as is practicable.)

C—*Cheese; Ether Extracts of Animal Tissues.*—Work a quantity of cheese, calculated to yield about 5.0 g. of fat, into a suspension with about 100 ml. water, add 100 ml. 95% alcohol and extract with petroleum ether as in (B). Proceed with the lipase hydrolysis as in (A). Handle up to 5.0 g. quantities of the ether extractives of animal tissues (mostly fat), directly as in (A). (Small amounts of protein material do not interfere with the lipolitic hydrolysis.)

DETERMINATION

A. *Preparation of Standards.—For the 0–1.0 milligram range,* proceed as given on pp. 197–8 (2), but use the 1+4 mixture of redistilled ethyl-petroleum ethers, instead of straight ethyl ether, in the extractions; and the 10% KOH solution, instead of 5% NaOH solution, in the alkali wash of the extract. Instead of using cotton, filter the washed ether extract through a ½ inch layer of washed and dried fine glass wool, previously wetted with the ether mixture, and held in a filter tube of appropriate size. Evaporate the ether extract to dryness, and heat flasks for one hour at 100°C. Use 25 ml. redistilled benzene to dissolve the residue. Photometric readings and calculations are as given (*loc. cit.*, pp. 198–200).

For the 0–50 microgram range, add 10, 20, 30, 40, and 50 mmg. of the pure isomers, in benzene solution, to a series of 50 ml. Erlenmeyer flasks, each containing 10 milligrams of U.S.P. oleic acid in benzene solution, and evaporate to dryness. (Small lead rings are convenient for weighting the flasks.) Nitrate as before and extract with straight redistilled petroleum ether. Wash the extract *three times* with the 10 per cent KOH solution, shaking a full two minutes each time. Heat final washed residues for 1 hour at 100°C., take up in 3.00 ml. redistilled benzene and develop the color with 6.00 ml. of the standard methylate solution, **(e)**. Read in a 5 cm. cell. (Standard curves for both isomers should be as linear and reproducible as those of the higher range.) Calculate factors as given above, but express results for both isomers in micrograms (instead of milligrams) of DDT.

If shorter cells with photoelectric instruments that cover a shorter density range are used, particular attention must be paid to matching of cells, to their cleanliness, and to the complete removal of extraneous yellow color and turbidity.

B—*Determination of DDT in Residues Remaining after Hydrolysis.*—Carefully nitrate not over 100 milligrams of the dry unsaponified residue, obtained under "Preparation of Sample" and contained in a 125 ml. Erlenmeyer flask, with 5–7 ml. of the nitration mixture **(a)**. Depending on the range to be employed in the estimation, extract with either straight petroleum ether, or with the 1+4 ether mixture. To insure thorough removal of interfering oxidation by-products, wash *six times* with 10 ml. portions of the 10% KOH solution. Heat final residues for 1 hour at 100°C. as usual. (In most work, residues will be invisible after this treatment.) If the quantity of DDT is unknown, use the 1+4 ether mixture in the extraction, dissolve the final residue in 10 ml. of redistilled benzene, and develop an exploratory color with a 5 ml. aliquot and 10 ml. of methylate. Estimate from the 0–1.0 milligram data after taking into account this aliquot factor. A check may sometimes be made with 3 ml. of the remaining benzene solution, 6 ml. of methylate and the 0–50 microgram range. If the latter color is too weak for an accurate result, work up an-

other sample, dissolve the total final residue in 3 ml. benzene, and develop with 6 ml. of methylate for estimation with a normal 0–50 microgram range.

Subtract the proper intercepts from photometric density readings, calculate the quantities of both isomers, and refer to the weight of sample taken for a final result. Always check for the presence of interfering yellow color by means of auxiliary readings in the blue at about 450 millimicrons.

The performance of the method is illustrated by the following recovery experiments in which 5.0 gram samples of pure butterfat and 100 gram samples of milk were carried through the method after the addition of various quantities of DDT in the form of the mixed pure isomers (Table 4). DDT was added to the fat samples by evaporating to dryness, in the hydrolysis flasks, a benzene solution of the proper amount; the fat was then added and warmed slightly to dissolve it. The DDT was added to the milk samples in the alcohol with which the milk is diluted before the first petroleum ether extraction. Neither the butterfat nor the milk (a composite of six different samples) gave any test for DDT, or appreciable interfering color.

TABLE 4.—*Recovery of DDT from butterfat and milk*

(DDT added as a 75–25% mixture of p,p'- and o,p'- isomers)

BUTTERFAT (5 GRAM SAMPLE)					MILK (100 GRAM SAMPLE)				
DDT ADDED	DDT FOUND	RECOVERY	INDICATED p,p'	RANGE USED	DDT ADDED	DDT FOUND	RECOVERY	INDICATED p,p'	RANGE USED
p.p.m.	*p.p.m.*	*per cent*	*per cent*		*p.p.m.*	*p.p.m.*	*per cent*	*per cent*	
2.0	2.0	100	68	0–50 mmg.	.05	.06	120	80	0–50 mmg.
5.0	4.3	86	78	"	.10	.10	100	77	"
10.0	9.0	90	79	"	.20	.18	90	72	"
25.0	24.8	99	76	"	.50	.46	92	75	"
100	96.0	96	77	0–1.0 mg.	2.00	1.93	97	74	0–1.0 mg.
500	483	97	76	"	10.0	9.54	95	77	"

Recoveries, on the whole, are seen to be good. Small losses, partly mechanical, and possibly due in part to slight decomposition of DDT, cause them usually to be a little low. This loss appears proportional to the quantity of DDT present and with the lower, more sensitive, range microgram amounts may be determined with small error.

SUMMARY

The nitration step in the Schechter colorimetric procedure for the determination of DDT has been more closely investigated in order to

determine how much organic material of various kinds may be present before serious losses of DDT occur. Precautions in the establishing of more sensitive colorimetric ranges are described. A method in which DDT is isolated from fatty samples by means of a lipolitic hydrolysis is presented. The sensitivity and accuracy of this procedure is illustrated by results on butterfat and milk.

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RATIO OF LABILE CHLORINE TO TOTAL CHLORINE IN DDT SPRAY-RESIDUE DEPOSITS IN SOUTHERN INDIANA APPLE ORCHARDS

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Pyrethrum, rotenone, and nicotine are subject to rapid decomposition or volatilization under conditions of orchard practices for codling moth control. Gunther (*1*) and other experimenters have reported a rapid loss of toxicity in DDT spray-residue deposits in orchards. These facts promote the supposition that DDT in insecticidal spray residues may be decomposed by sunlight and that this degradation of DDT may interfere in the analysis of DDT spray residues.

Three colorimetric methods for analysis of DDT spray residues have been developed—the nitration technique of Schechter and Haller (*2*), the xanthydrol-pyridine method of Stiff and Castillo (*3*), and the dinitrophenylhydrazine method of Wichmann, *et al.* (*4*). All these methods are complicated and require equipment not usually available in small laboratories. The DDT molecule contains 5 chlorine atoms, one of which is comparatively labile and can be converted to the inorganic halide by refluxing with alcoholic potash. By refluxing with metallic sodium in the presence of isopropyl alcohol all 5 atoms of chlorine can be converted to the inorganic halide. The analysis for total chlorine or labile chlorine is relatively simple and readily adapted to routine studies of spray residues. However, any degradation products of DDT present in the spray residue will contain organic chlorine compounds, which can be expected to interfere with the total-chlorine or labile-chlorine analysis as a measure of the quantity of DDT present in the residue.

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In their study of the decomposition and volatility of DDT and some of its derivatives, Wichmann, *et al.* (*5*) have demonstrated that DDT may be decomposed by irradiation with ultraviolet light. They have further shown that the resulting degradation products are more volatile than the *p,p'*-isomer of DDT. In their study of a simple spray residue deposited by a spray of Deenate (25 per cent DDT wettable powder) with lime and Wyoming bentonite, they showed that there was no accumulation of DDT degradation products after 42 days' weathering. They conclude that, although the DDT present in spray residues may be decomposed by ultraviolet irradiation, the degradation products are volatilized under orchard conditions and do not contaminate the DDT spray residue.

It is the purpose of this paper to present data accumulated in two years' study of DDT spray-residue deposits, which show that the findings of Wichmann, *et al.* (*5*) are applicable to a wide variety of spray schedules and to orchard conditions in southern Indiana.[1]

METHODS OF STUDY

The spray residues on samples of 30 fruits each were analyzed in 1945, and on samples of 600 leaves each in 1946. The fruit was picked at random over the entire tree, from two or more trees in each plot. The foliage samples were picked from ground level on three trees of each plot. Total-chlorine and labile-chlorine determinations were made on aliquots of a benzene solution of the residue obtained by stripping the fruit or foliage in a known quantity of benzene.

Total chlorine was determined by refluxing the benzene solution of the spray residue with metallic sodium in the presence of isopropyl alcohol, separating the inorganic halide by extraction, and titrating by the Volhard technique. Labile chlorine was determined in the same manner except that alcoholic potash was employed in the refluxing instead of sodium and isopropyl alcohol. These analytical methods are described in detail by Wichmann *et al.* (*4*).

The results of these studies are reported as the ratio of labile chlorine to total chlorine in the residue deposits. Determinations on technical DDT and various DDT formulations have shown this ratio to agree substantially with the theoretical ratio of 1 to 5, or 0.20.

1945 EXPERIMENTS

In 1945 the DDT spray residues on apples from 8 spray treatments were studied. The spray treatments included three wettable-powder formulations, used alone and with mineral oil, and two formulations in which technical DDT was dissolved in organic solvents and applied in the form of aqueous emulsions. Each plot received eight DDT spray applications during the growing season, four at a dosage of $\frac{3}{4}$ pound and four at

[1] Workers of the Division of Fruit Insect Investigations of the Bureau of Entomology and Plant Quarantine applied these sprays.

½ pound of DDT to 100 gallons. The interval between applications ranged from 8 to 21 days. Samples of fruit for chemical analysis were taken before and after the sixth, seventh, and eighth sprays and at harvest.

The ratios of labile to total chlorine in the spray residues on these samples are shown in Table 1. The ratios for the spray residues ranged from 0.17 to 0.24, with a maximum difference of 0.04 from the theoretical

TABLE 1.—*Ratio of labile chlorine to total chlorine in DDT spray residues on apples, 1945*[1]

TREATMENT (QUANTITIES PER 100 GAL.)	RANGE FOR OBSERVATIONS ON DIFFERENT DATES	AVERAGE
Wettable powders:		
Deenate (25% DDT); wettable sulfur 3 lb. in 3rd and 4th cover sprays.	0.18–0.22	0.20
DDT-kaolin (33% DDT)	.19– .22	.20
DDT-Pyrophyllite (Pyrax ABB) (50% DDT)	.17– .23	.20
Wettable powders with oil:		
Deenate wettable sulfur 3 lb. in 3rd and 4th cover sprays; mineral oil 1 qt. in 2nd, 5th, 6th, 7th, and 8th cover sprays; Fermate[2] 1 lb. in 5th and 6th cover sprays.	.17– .20	.19
DDT-kaolin (33% DDT); wettable sulfur 3 lb. in 3rd and 4th cover sprays; mineral oil 1 qt. in 2nd, 4th, 5th, 6th, 7th, and 8th cover sprays.	.18– .24	.20
DDT-Pyrophyllite (Pyrax ABB); mineral oil 1 qt., Wyoming bentonite 0.25 lb. in all but 1st cover spray.	.18– .20	.19
Emulsions:		
DDT in benzene; emulsified with B-1956[3] in 1st 3 cover sprays; added to 1 qt. of emulsive mineral oil in last 5 sprays; Wyoming bentonite added in last 3 sprays.	.17– .20	.19
DDT in xylene; emulsified with B-1956	.18– .20	.20

[1] The ratio for the insecticides before application was 0.19, except for DDT-Pyrophyllite (Pyrax ABB), where it was 0.20.
[2] Ferric dimethyl dithiocarbamate.
[3] A glycerol phthalic alkyl resin emulsifier.

ratio. Of the 56 samples tested, 22 gave the theoretical ratio of 0.20, 16 showed a difference of 0.01, and 12 a difference of 0.02 from the theoretical. Thus, in 50 of the 56 samples studied the ratio of labile to total chlorine in the spray residue was found to be within 10 per cent of the theoretical ratio.

1946 EXPERIMENTS

In 1946 the determinations were made of DDT residues on foliage at harvest following six cover sprays during the season. A total of 22 different treatments were applied in 2 orchards. The spray treatments include 8

wettable-powder formulations, 4 formulations with oil adhesive or wetting agent, and 3 DDT-nicotine bentonite (nicotine sulfate plus Mississippi bentonite) spray schedules.

The ratios of labile chlorine to total chlorine in the original insecticide and in the spray residues for each treatment are shown in Tables 2 and 3. The ratio in the original insecticide varied less than 0.01 from the theoretical. In residue deposits on foliage it ranged from 0.19 to 0.23. Eight of the

TABLE 2.—*Ratio of labile chlorine to total chlorine in DDT spray residues on Grimes Golden foliage, 1946*

(All sprays contained 0.5 lb. of DDT per 100 gal. unless otherwise stated. Six sprays were applied and samples taken 36 days after final spray)

TREATMENT	IN INSECTICIDE	IN RESIDUE
Wettable powders:		
DDT-Pyrophyllite (Pyrax ABB) (50% DDT)	0.20	0.22
DDT-Pyrophyllite (Pyrax ABB) (50% DDT) plus B-1956	.20	.20
DDT-Silene[1]-Wyoming bentonite (50% DDT)	.21	.22
DDT (aerosol grade) wet with B-1956	.19	.23
DDT on tobacco dust (25% DDT)	.20	.20
Emulsions:		
DDT in xylene:		
Emulsified with B-1956	.21	.23
Emulsified with Wyoming bentonite	.21	.20
DDT in Velsicol AR-50[2] emulsified with B-1956	.21	.21
DDT-nicotine bentonite-mineral oil combinations[3]:		
Deenate (50% DDT)	.19	.19
DDT-Panther Creek bentonite (20% DDT)	.20	.23

[1] A commercial calcium silicate.
[2] An alkylated naphthalene.
[3] 0.25 lb. of DDT per 100 gal.

samples gave the theoretical ratio of 0.20, 7 showed a difference of only 0.01, 4 a difference of 0.02, and only 3 as much as 0.03 from the theoretical ratio. Thus in 19 of the 22 observations the ratio was found to be within 10 per cent of the theoretical.

SUMMARY AND CONCLUSIONS

The combined data for both years show that 37 per cent of the residues contained labile and total chlorine at the theoretical ratio, that an additional 31 per cent were within 5 per cent, 21 per cent were within 10 per cent, and the remaining 11 per cent were within 20 per cent of the theoretical ratio. Colorimetric analyses of two solutions of DDT from foliage residues analyzed by Carter were found to agree within 7.5 per cent with

the total-chlorine DDT analysis.[2] These studies include 78 observations covering 9 wettable DDT powders, 4 formulations of technical DDT in organic solvents, 3 wettable powder DDT formulations with mineral oil, 3 spray schedules employing nicotine-bentonite with DDT formulations, and 2 tests of DDT formulations with wetting agents.

The colorimetric methods for determining DDT are not easily adapted to routine analysis of DDT spray residues. The total chlorine or the labile chlorine method could be readily adapted, with equipment generally

TABLE 3.—*Ratio of labile chlorine to total chlorine in DDT spray residues on Rome Beauty foliage, 1946*

(All sprays contained 0.75 lb. of DDT per 100 gal. unless otherwise stated. Six sprays applied and samples taken 41 days after final spray)

TREATMENT	IN INSECTICIDE	IN RESIDUE
Wettable powders (DDT 50%):		
Deenate[1,3]	0.19	0.22
DDT-Pyrophyllite (Pyrax ABB)[1,3]	.20	.20
Niatox[2]	.21	.22
DDT-Bancroft clay[2,3]	.21	.20
DDT (aerosol grade)-Bancroft clay	.19	.20
DDT-china clay[2,3]	.21	.19
DDT-Silene-Wyoming bentonite[3]	.21	.20
Wettable powders (DDT 50%) with mineral oil in last 4 sprays:[1]		
Deenate	.19	.21
DDT-Pyrophyllite (Pyrax ABB)	.20	.21
Emulsion: DDT in xylene emulsified with B-1956	.21	.21
Solution: DDT in kerosene-xylene (3:1)	.21	.21
N-nicotine bentonite-oil plus Deenate 0.5 lb. per 100 gal.	.19	.19

[1] Kolofog (sulfur-bentonite dust) 3 lb. per 100 gal. added to 1st two sprays.
[2] Kolofog 3 lb. per 100 gal. added to 1st spray.
[3] DN-111 (dicyclohexylamine salt of dinitro-o-cyclohexylphenol) 0.75 lb. per 100 gal. added to final spray.

available, but these methods would be subject to interference by degradation products of DDT and by other chlorinated insecticides, or auxiliary spray materials. The studies of Wichmann, *et al.* (*5*) on the decomposition of DDT by ultraviolet irradiation have demonstrated the volatility of DDT degradation products. The fact that their analyses show a very close agreement between DDT residue loads calculated from total chlorine content, and those obtained by the colorimetric method of Schechter and Haller (*2*), suggests that total chlorine and/or labile chlorine analysis would be a satisfactory basis for the estimation of DDT in spray residues on fruit of known history. Additional data here presented show that this is the case with Indiana apples.

[2] Analyses by R. H. Carter of the Bureau of Entomology and Plant Quarantine.

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UREASE ACTIVITY AND OTHER CHEMICAL CRITERIA AS INDICATORS OF INADEQUATE HEATING OF SOYBEAN OIL MEAL

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The change in status of soybean oil meal from a relatively minor member of the group of high-protein feedstuffs to the principal member of the group, serving in some animal diets as the sole representative of this class of feedstuffs, has been so rapid that new problems have inevitably appeared. Information that was adequate when soybean oil meal was commonly fed as 5 per cent of the diet may not be adequate when it makes up 20 or 25 per cent of the diet, and differences among different lots of meal which are unimportant at the 5 per cent level of feeding become important at the higher levels. Bird and Burkhardt (1) reported that the feeding of commercial soybean meals as principal source of protein, in a practical starting mash for chicks, revealed considerable variability among meals. Investigations of factors affecting the nutritive value of soybean oil meals have been concerned for the most part with the heat-treatment applied to the meals. This work has been reviewed recently by Barnes and Maack (2).

It is known that both under-heated and over-heated meals are of inferior nutritive value, but little is known of the fundamental nature of the changes brought about by heating except that the availability of the sulphur-containing amino acids is affected. Recently it has been reported by Bowman (3) and by Ham and Sandsted (4) that unheated soybeans and soybean oil meal contain material which retards or inhibits the action of proteolytic enzymes. The practical importance of this finding has not yet been evaluated. Presumably a thorough understanding of the changes

* In undertaking this work, the authors acted as a subcommittee of the Committee on Animal Nutrition of the National Research Council. This report is published with the approval of the Committee.

brought about by heating would permit development of a chemical test that would distinguish under-heated and over-heated meals from those heated properly. In the absence of such a test, it was pointed out by Caskey and Knapp (5) that the intensity of heat-treatment required for development of high nutritive value appeared to be the same as that required for destruction of the enzyme urease, and that inadequately heated soybean oil meals could be detected by measuring their urease content. These investigators described a simple test for urease activity, and it showed that in a series of 16 samples of soybean products the 8 samples that were inadequately heated according to chick-growth tests had a measurable urease content, whereas the adequately heated meals did not. Since this relationship could have been fortuitous, testing of a larger number of samples seemed desirable. Furthermore, questions were raised as to the possibilities that results might be seriously affected by variation in incubation temperature, and that some meals might be sufficiently alkaline to give a misleading result. Obviously the test is not applicable to the detection of over-heated meals.

The purpose of this study was to investigate the validity of the urease test, and any other promising chemical tests, as means of distinguishing inadequately heated soybean oil meals.

PROCEDURE

Two series of samples of soybean oil meals and other soybean products were used in these studies. Series A consisted of 18 samples, all but one of which had been subjected to chick-growth tests. Samples 4, 5, and 9 were extracted, 6, 16, and 18 were hydraulic, and 7, 13, 14, 15, and 17 were expeller meals. All of the foregoing were commercial meals. Samples 1, 3, and 11 were extracted meals which were not subjected to accessory cooking. Samples 10 and 12 were experimentally processed extracted meals, the former of which was subjected to more and the latter to less than the usual heat treatment. Sample 2 consisted of soybean grits, and sample 8, of unprocessed soybeans. Series B consisted of 9 samples of known history. Sample 1 of Series B consisted of unprocessed beans representative of the material from which samples 3, 5, 6, and 7 were prepared. Samples 2 and 3 were extracted and not subjected to accessory cooking; the former was air-dried and the latter was dried in a commercial drier. Samples 5, 6, and 8 were extracted meals, and 7, 9, and 10 were expeller meals, all prepared according to standard commercial practice.

These samples were submitted to the members of the subcommittee and to each of seven collaborators.†

† The authors gratefully acknowledge the collaboration of Dr. John S. Andrews, General Mills, Inc., Minneapolis, Minn.; Mr. C. O. Gourley, Beacon Milling Co., Inc., Cayuga, N. Y.; Mr. F. H. Hessel, Cooperative G. L. F. Mills, Inc., Buffalo, N. Y.; Mr. R. C. Holder, Central Soya Co., Inc., Decatur, Ind.; Mr. H. C. Schaefer, Ralston-Purina Co., St. Louis, Mo.; Mr. Carl H. Schroeder, Larro Research Farm, Detroit, Mich.; Dr. Oscar I. Struve, Eastern States Cooperative Milling Corporation, Buffalo, N. Y.

Each of the subcommittee members and collaborators tested the samples for urease according to the following method.

TEST FOR UREASE ACTIVITY IN SOYBEAN OIL MEAL

REAGENTS

0.1% *phenol red solution.*—Dissolve 0.1 gram phenol red in 15 ml. of 0.02 *N* NaOH. Dilute with recently boiled H_2O to 100 ml.

0.05 *molar phosphate buffer solution.*—Dissolve 3.402 grams KH_2PO_4 in approx. 100 ml. H_2O. Dissolve 4.355 grams K_2HPO_4 in approx. 100 ml. H_2O. Combine the two solutions, add 10 ml. 0.1% phenol red solution and dilute to 1000 ml. Use recently boiled distilled water throughout.

Buffered urea solution.—Dissolve 15 grams of urea in 500 ml. 0.05 molar phosphate buffer solution (containing phenol red). Keep in refrigerator under toluol.

PROCEDURE

For each sample of soybean oil meal to be tested, prepare 2 test tubes containing 10 ml. of 0.05 molar phosphate buffer solution, and two test tubes containing 10 ml. of buffered urea solution. Add 0.200 gram of the soybean oil meal to each test tube. Place one tube containing urea and one without urea in a water bath at 25°C., and the remaining two tubes in a bath at 30°C. (Two additional tubes containing buffered urea solution, but no soybean oil meal, should be prepared and one of these should be held at each of the two temperatures mentioned above.) Let stand 30 minutes, shaking at 5 minute intervals. After 30 minutes record color change (or lack of change) for all tubes and determine *p*H electrometrically

This method is based on the procedure of Caskey and Knapp (5), but specifies the quantity of soybean oil meal, the frequency of shaking, and the temperature of incubation more exactly. Two incubation temperatures are specified in order to see if results are unduly influenced by small differences in temperature; and the blanks containing soybean oil meal but no urea are introduced as means of detecting soybean oil meals which would give an alkaline reaction in the absence of urea.

The suggestion was made to the collaborators that they also determine the thiamin and water-insoluble nitrogen content of the samples and grade them according to appearance and taste if they regularly used these procedures in their laboratories. Each of these variables is known to be influenced by extent of heating. The procedure recommended for water-insoluble nitrogen was the one described in the Methods of Analysis of the Association of Official Agricultural Chemists, Sixth Edition (6).

Large enough samples of 10 of the meals of Series A were submitted to three of the collaborating laboratories for chick-growth experiments, and similar samples of the 9 meals comprising Series B were submitted to four laboratories for the same purpose.

FEEDING TRIALS WITH SOYBEAN OIL MEAL

Groups of 30 chicks (Rhode Island Reds, or Barred or White Plymouth Rocks) were fed the following diet from hatching to five weeks of age:

	Per cent
Ground yellow corn	35*
Pulverized heavy oats	10
Wheat bran	10
Wheat flour middlings	10
Alfalfa meal	5
Soybean oil meal	25
Butyl fermentation solubles	1.5*
Vitamin A & D oil (400 D, at least 1000 A)	0.25
Limestone or oystershell flour	1.75
Bone meal or defluorinated superphosphate	1
Salt	0.5
$MnSO_4 4H_2O$	0.012

Each lot of soybean oil meal to be tested was supplied to one group of chicks as a constituent of this diet. Butyl fermentation solubles and alfalfa meal were each furnished from a common source. Other ingredients were furnished by the individual collaborators. Sufficient feed for the 5 weeks test (50 lbs. for each group) was mixed at the beginning of the test and a one-half pound sample of each lot of mixed feed was removed for analysis.

The chicks were weighed at the beginning of the tests and at weekly intervals thereafter. Feed consumption was recorded at weekly intervals, and records of mortality were kept.

RESULTS AND DISCUSSION

The means of the results obtained in the chemical tests by all collaborators, are summarized in Table 1, with their standard errors. The results of the urease test show excellent agreement among the collaborators as to which samples were sufficiently heated to destroy urease and which were not. Samples 1, 2, 3, 6, 8, 11, and 16 in Series A, and samples 1, 2, and 3 in Series B contained sufficient urease to "cause an increase in the *p*H of the solution of one unit," the criterion established by Caskey and Knapp (5). Of the samples other than those mentioned above, none even approached a positive test, the greatest increase in *p*H reported by any collaborator being 0.12. Thus the urease test divided the meals very sharply into two classes, with no truly intermediate samples. Sample 16, Series A, was closer to the intermediate range than any other sample, giving a mean increase in *p*H of 1.42 ± 0.12, at 25°C., the standard error in this case being the largest in the series.

Comparison of the values obtained at 25° and 30°C. reveals a slightly greater change in *p*H at the higher temperature in practically every case, but in no case did the difference in temperature lead to different conclusions regarding efficacy of heat-treatment. Furthermore, many of the differences due to temperature were actually less than the standard errors

* These percentages of corn and soybean oil meal were so varied as to equalize the protein content of all diets at approximately 20 to 21 per cent.

of the means. From the standpoint of conclusions to be drawn, the specification of Caskey and Knapp that the temperature be held between 25° and 30°C. would seem to be justified. However, differences in temperature within this range did cause appreciable differences in response of the urease-positive meals in the tests of some collaborators, and the use of a water-bath to maintain a definitely specified temperature is probably desirable. The greatest difference reported for any sample, due to the 5°

TABLE 1.—*Urease activity and thiamine and water-insoluble nitrogen content of soybean oil meals*

SERIES	SAMPLE NO.	UREASE ACTIVITY, CHANGE IN *p*H		THIAMIN, MICROGRAMS PER GRAM	WATER-INSOLUBLE *N*, PER CENT OF TOTAL *N*
		AT 25°C.	AT 30°C.		
A	1	1.78±0.05	1.79±0.07	12.76±1.13	42.70±1.95
	2	1.93±0.06	1.99±0.04	12.81±1.00	47.64±3.05
	3	1.86±0.06	1.96±0.06	11.64±0.62	58.97±3.70
	4	0.01±0.02	0.01±0.02	3.87±0.26	93.35±0.68
	5	0.00±0.02	−0.02±0.02	1.29±0.12	93.89±0.52
	6	1.84±0.04	1.88±0.09	9.13±0.61	63.07±1.75
	7	0.00±0.02	−0.02±0.01	1.40±0.09	92.33±0.64
	8	1.89±0.06	1.95±0.06	9.67±0.60	56.98±4.44
	9	−0.01±0.02	−0.04±0.01	3.04±0.25	93.08±0.56
	10	−0.05±0.01	0.00±0.01	1.16±0.06	94.10±0.45
	11	1.89±0.09	2.01±0.04	10.86±0.93	49.01±3.86
	12	−0.04±0.02	0.01±0.01	3.59±0.20	92.59±1.08
	13	−0.06±0.02	−0.04±0.01	0.50±0.16	90.96±0.79
	14	−0.06±0.03	−0.05±0.01	0.73±0.18	92.45±0.59
	15	−0.07±0.02	−0.07±0.01	0.51±0.12	92.93±0.56
	16	1.42±0.12	1.52±0.10	4.03±0.23	82.53±2.45
	17	−0.02±0.03	−0.02±0.01	1.35±0.12	92.26±0.77
B	1	1.84±0.06	1.94±0.05		
	2	1.98±0.07	2.02±0.06		
	3	1.83±0.07	1.88±0.07		
	5	−0.01±0.01	0.00±0.01		
	6	−0.02±0.02	0.01±0.01		
	7	−0.03±0.01	0.01±0.01		
	8	−0.02±0.02	0.00±0.02		
	9	−0.03±0.01	0.01±0.01		
	10	−0.04±0.01	0.03±0.01		

temperature change, was 0.65. One collaborator reported that there was greater loss of ammonia at the higher temperature, from tubes in which alkalinity developed, and that therefore 25°C. was preferable to 30°C. as the incubation temperature. He stated further that when a high degree of alkalinity developed, the *p*H had by no means reached its maximum at the end of ½ hour and that therefore care should be taken to make the *p*H reading after an exact interval of incubation.

The results obtained with tubes containing soybean products but no urea, and with those containing urea but no soybean products, showed very clearly that none of the 26 samples was capable of producing an alkaline reaction in the absence of urea, under the conditions of this test. The largest increase in *p*H was 0.30. Since these results were wholly negative, no detailed summary is presented here.

Of the various questions these investigations were designed to answer, the most important was the one concerning the relationship between

TABLE 2.—*Results of chick growth tests, series A. Average weights, 5 weeks, as % of best group in each test*

COLLABORATOR NO.		11	12	13	U.M.*
Sample No.	1	83	81	84	86
	3	78	80	77	66
	4	97	93	92	91
	5				95
	6	65	72	70	76
	7	96	87	100	91
	8				55
	9				79
	10	92	94	85	84
	11	75	75	75	71
	12	100	100	99	78
	13	94	86	93	95–100
	14				96
	15				82
	16				84
	17				94
	18	94	76	84	
Smallest difference for significance (19:1 odds)		11		15	
Average of best group (gms.)		265	306	214	

* Results obtained at University of Maryland in different experiments in each of which there was a positive control group for comparison.

nutritive value of soybean oil meal and urease activity, or other chemically determinable properties. In Tables 2 and 3 are summarized the results of the chick growth tests with the meals of Series A and B, respectively. The average weights are given as percentage of the best group in each test, to permit easier comparison. The tables also show the average weight in grams of the best group in each test so that the average weight in grams of any group may be calculated if desired. The figures in the last column of Table 2 were obtained, not all in the same test, but in several tests at the University of Maryland; however, in each test there was at least one positive control group on a good diet with which the other groups could be

compared. These results are included here because some of the samples of Series A were not subjected to collaborative growth tests. They agree rather well with the other results in most cases where comparisons can be made, the chief exception being sample 12.

Of special interest are the growth responses of chicks fed the urease positive meals; namely, samples 1, 2, 3, 6, 8, 11, and 16 of Series A, and 1, 2, and 3 of Series B. Sample 18 of Series A should also be included in this group. It was not included in the collaborative urease tests, but gave a strong positive reaction for urease. Sample 2 of Series A was not used in a chick growth experiment. The urease positive meals were fed to a total

TABLE 3.—*Results of chick growth test, series B. Average weights, 5 weeks, as % of best group in each test*

COLLABORATOR NO.	1	2	3	6
Sample No. 1	60	71	55	56
2	67	64	56	69
3	89	84	85	86
5	98	81	98	98
6	100	92	100	100
7	91	81	95	97
8	93	72	97	94
9	94	100	99	89
10	94	98	95	88
Smallest difference for significance (19:1 odds)		14		
Average weight of best group (gms.)	343	232	377	390

of 33 lots of chicks, and in only 2 lots did the average weight at 5 weeks exceed 86 per cent of the weight of the "best group," or positive control. One of these two lots was fed sample 18 of Series A by Collaborator No. 11, and the result in this case was in sharp disagreement with the results reported by the other two collaborators for the same meal. The other lot which attained an average weight exceeding 86 per cent of the "best group" was the lot fed sample 3 of Series B by Collaborator No. 1. This result, though slightly higher than those reported by the other three collaborators, was in approximate agreement with them.

As indicated in Tables 2 and 3, any average weight, in order to be significantly poorer than that of the best group, would need to be at most 86 per cent of the "best group" in the case of Collaborator No. 2, 89 per cent in the case of No. 11, and 85 per cent in the case of No. 13.

Of the lots fed urease-negative samples, all but 9 attained average weights that were more than 86 per cent of the "best group," and of these

9, five were lots which in each case gave a result that disagreed with those of all the other collaborators (see samples 12 and 13, Series A, and samples 5, 7, and 8, Series B). Of the remaining 4 groups that made relatively poor growth with urease-negative meals, two were fed sample 10 of Series A, a meal that had been exposed purposely to heat treatment believed to be excessive.

Although lack of agreement among the collaborators in the growth tests has been pointed out in several cases, it appears that in general there was rather good agreement among the different laboratories, especially in view of the number of ingredients in the diet, and of the fact that only the alfalfa meal, fermentation solubles, and soybean oil meal were uniform for all collaborators. The results reported by Collaborator No. 2 deviated most widely from the general trend. This investigator agreed with the others in obtaining poor growth with samples 1, 2, and 3, the urease-containing meals; but he also found samples, 5, 7, and 8 to be relatively poor, and these samples supported good growth in the other laboratories.

Collaborator No. 1 reported mortality ranging from 3 to 23 per cent for individual groups. Mortalities reported by other collaborators were 3 to 13 per cent by No. 2, 0 to 3 per cent by No. 3, 0 to 3 per cent by No. 6, 0 to 17 per cent by No. 11, 0 to 5 per cent by No. 12, and 7 to 37 per cent by No. 13. It does not appear that the mortality was related to the nutritive value of the soybean oil meals. The high mortality of 13 per cent reported by Collaborator No. 2 occurred in the group fed sample 2 of Series B, a relatively poor meal, but the highest mortality figures reported by Collaborators No. 11 and 13 occurred in the groups fed samples 10 and 12, respectively, of Series A, and these meals supported good growth in the tests involved, as well as in other tests. It is also worthy of note that the data on growth obtained in tests where mortality was high agree rather well with data obtained in tests where it was low. Collaborator No. 11 reported the occurrence of "pasted" vents in all groups during the first week of the experiment, and also some difficulty due to accumulation of feed in the mandibles of the chicks fed samples 3 and 6.

These results would seem to support the acceptibility of the urease test as a means of detecting inadequately heated meals. Of the 27 samples involved in these tests, 11 gave a positive reaction for urease; and, judged by the feeding trials, there were no meals of high nutritive value among these 11. Of the 16 urease-negative meals, three (samples 9, 10, and 15, Series A) did not produce uniformly good results in feeding trials. Sample 10 was supposedly overheated, but the reasons for the poor performance of the other two are unknown. As stated previously, the urease test cannot be expected to detect low nutritive value that is due to causes other than inadequate heating.

These findings give no basis for fears that the urease test might discriminate unjustly against some meals that had been subjected to mild but

adequate heat treatment. Samples 1, 11, 12, 16, and 18 of Series A, and sample 3 of Series B were believed to be on the "border-line" with respect to adequacy of heating, but the urease test classified all these samples except 12 as inadequately heated, and the growth tests supported this classification. In any large random selection of commercial soybean oil meals the proportion of meals subjected to "border-line" heat treatment would certainly be smaller than in these two series, and the proportion of inadequately heated meals would be very much smaller.

Three of the collaborators made thiamin determinations on the samples of Series A. The results are shown in Table 1. A uniform method was not specified for this determination, the methods currently in use in the various laboratories being employed. It is therefore not surprising that the collaborators reported divergent results for some samples. The three collaborators were in good agreement in dividing the samples into two groups, one of which had a high, and the other a low, thiamin content. The lowest figure for the "high" group was 9.13 for sample 6, and the highest figure for the "low" group was 4.03 for sample 16. The conclusions derived from the thiamin assays agree with those based on urease except in the case of sample 16. This sample was sufficiently heated to destroy most of the thiamin but not enough to destroy the urease. The results of chick growth tests at the University of Maryland support the conclusions of the urease test rather than those of the thiamin determination.

The results of determinations of water-insoluble nitrogen, expressed as per cent of total nitrogen, are also given in Table 1. As in the case of the thiamin determinations, the agreement among the actual figures reported by the different investigators was not very good, but all the results were in agreement in indicating that samples 1, 2, 3, 6, 8, and 11 were not heated sufficiently to bring about the desired change in protein solubility. Sample 16 which had been heated sufficiently to destroy the thiamin, but not the urease, was on the border-line with respect to its content of water-insoluble nitrogen.

After the completion of these studies Evans and St. John (7) reported that the nutritive value of soybean meals was related to the so-called "glutelin fraction" of the protein. Since this method is based on solubility of protein, it would seem that it also might give misleading results for such a meal as sample 16; however, it would have the advantage of detecting overheated as well as underheated meals.

The results of classification of the meals of Series A according to appearance and taste are summarized in Table 4. On the basis of appearance, samples 1, 2, 3, 8, and 11 could be classified correctly as inadequately heated; but sample 6 which clearly belonged in this class on the basis of other tests, was not so classified because any color change due to heating was obscured by the presence of black beans. Sample 16 was also omitted from the inadequately heated group.

TABLE 4.—*Appearance and taste of soybean meals, series A*

SAMPLE NO.	COLLABORATOR NO. 5	COLLABORATOR NO. 7	COLLABORATOR NO. 8	COLLABORATOR NO. 9
		Appearance		
1	Cream	Straw	Light yellow	Raw
2	Cream	Straw	Light yellow	Raw
3	Cream	Straw	Light yellow	Raw
4	Light brown	Light toasted	Brown	Cooked
5	Brown	Dark	Brown	Over-cooked
6	Green-gray	Black bean	Gray	—
7	Brown	Medium dark	Brown	Cooked
8	Cream	Yellowbean	Yellow & brown	Raw
9	Brown	Light toasted	Brown	Cooked
10	Brown	Medium dark	Brown	Cooked
11	Light brown	Straw	Yellow	Raw
12	Tan	Medium light	Brown	Cooked
13	Medium brown	Dark	Red-brown	Cooked
14	Light brown	Medium dark	Brown	Cooked
15	Dark brown	Dark	Brown	Cooked
16	Tan	Medium light	Light brown	Cooked
17	Brown	Medium dark	Brown	Cooked
		Taste		
1	Bland	Neutral	Raw	Raw
2	Bland	Neutral	Raw	Raw
3	Bland	Neutral	Raw	Raw
4	Bland	Neutral	Toasted	Cooked
5	Nutty	Sandy	Toasted	Over-cooked
6	Bitter	Raw	Raw	Very raw
7	Nutty	Neutral	Toasted	Cooked
8	Bitter	Raw	Raw	Very raw
9	Bland off-flavor	Neutral	Toasted	Cooked
10	Slight toasted	Sandy	Toasted	Cooked
11	Slight bitter	Neutral	Raw	Raw
12	Bitter	Slight raw	Toasted	Cooked
13	Nutty	Roasted	Toasted	Cooked
14	Nutty	Stale	Toasted	Cooked
15	Nutty	Bitter	Toasted	Cooked
16	Bitter	Flat	Partly toasted	Cooked
17	Bland	Slight roasted	Toasted slightly bitter	Cooked

On the basis of taste, only samples 6 and 8 were unanimously classified as inadequately heated. Furthermore, Collaborators No. 5 and 7 found sample 12, which was adequately heated according to all other tests, to be "bitter" or "slightly raw"; whereas they found samples 1, 2, and 3, which clearly deserved to be classed as raw, to be "bland" or "neutral." On the basis of these results, appearance and taste would seem to be undependable criteria for general use, although they probably could be very helpful

to one who had had considerable practice in using them for this purpose.

CONCLUSIONS

The urease test of Caskey and Knapp, applied to soybean meals, yields valuable information regarding the heat-treatment to which they have been exposed. Any meal which gives a positive test for urease by this method (an increase in *p*H of 1.0 or more) should be suspected of low nutritive value due to inadequate heating.

When this test is used, it is important that observations of *p*H be made after approximately ½ hour of incubation, and that they be made after identical periods of incubation in the case of samples that are to be compared with each other. Useful results can be obtained merely by holding the incubation temperature between 25° and 30°C., but more reproducible results can be obtained by using a water bath to maintain a definitely specified temperature.

Determinations of thiamin and of water-insoluble nitrogen lead, in most cases, to conclusions regarding efficacy of heat treatment that are identical with the conclusions drawn from the urease test. In the one exception to this agreement which occurred in these studies, the results of chick-growth tests supported the conclusions drawn from the urease test rather than those drawn from determinations of thiamin and water-insoluble nitrogen.

Appearance and taste are not dependable criteria for general use in the detection of inadequately heated soybean meals.

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IMPROVEMENT OF THE STEENBOCK RACHITOGENIC DIET BY A SUPPLEMENT OF LYSINE

By Philip S. Francis* (National Dairy Research Laboratories, Baltimore, Maryland)

In the experience of this laboratory the growth rate of rats on the Steenbock rickets-producing diet is very slow, considerably less than one

* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 14–16, 1946.

gram per day over a 23 to 25 day depletion period on the average, with some individual rats growing as little as 0.25 grams per day. The animals have low resistance to disease, and a few frequently die during the depletion period. Some of the individual animals do not develop satisfactory rickets because of insufficient growth during depletion. As rickets can develop only while there is bone growth, it would seem logical that increased growth should result in a greater degree of rickets. It was felt that correction of any deficiency in the diet other than that necessary to produce rickets would result in a more rapid growth rate of the rats being depleted.

Because of their importance to growth of young animals, the first nutritional factors of the diet to be investigated were the essential amino acids. Generally accepted figures on the amino acid contents of corn and wheat gluten were used in compiling Table 1. The rat requirements are those determined by Rose in 1937 (3).

TABLE 1.—*Per cent essential amino acids supplied in the Steenbock rachitogenic diet**

Data compiled from references (1) and (2)

AMINO ACID	76% CORN	20% WHEAT GLUTEN	TOTAL	REQUIREMENT (3)	DEFICIT
Methionine	—	0.46	0.46	0.6	none
Cystine	0.07	0.42	0.49		
Lysine	0.10	0.36	0.46	1.0	0.54
Tryptophane	0.10	0.14	0.24	0.2	none
Threonine	0.38	0.54	0.92	0.6	none
Leucine	1.34	1.86	3.20	0.9	none
Isoleucine	0.15	0.72	0.87	0.5	none
Phenylalanine	0.18	0.68	0.86	0.7	none
Tyrosine	0.44	0.92	1.36		
Histidine	0.15	0.40	0.55	0.4	none
Valine	0.43	0.72	1.15	0.7	none
Arginine	1.08	0.80	1.88	0.2	none

* Yellow corn, 76%; wheat gluten, 20%; calcium carbonate, 3%; sodium chloride, 1%.

The data presented in Table 1 indicate that the diet provides ample quantities of all the amino acids save lysine, which is present in only one-half the amount needed by the rat. Microbiological assay showed the lysine content of the diet to be approximately 0.6 per cent. A preliminary experiment demonstrated that an addition of 0.5% lysine to the Steenbock rachitogenic diet nearly doubled the growth rate of rats during depletion. The animals receiving the lysine supplement showed satisfactory rickets after a 16 day depletion period.

The normal depletion period for the strain of rats used in this labora-

tory is 23 to 25 days on the Steenbock rickets-producing diet. The following experiment showed definitely that this depletion time can be materially shortened by a 0.5% lysine supplement to the diet.

Four groups of 10 rats each, one rat in each group from each of 10 different litters, were placed on the Steenbock rachitogenic diet. Groups I and II received a supplement of 0.5% lysine incorporated in the diet, while groups III and IV received the diet unsupplemented. Group I was depleted for 13 days, group II for 16 days, group III for 20 days, and group IV for 23 days. The animals were weighed at the beginning and the end of the depletion period, and their daily growth was calculated. Two rats from group II and one from group III died of respiratory infections during the depletion period. Each group was started on an assay period when its depletion period had ended. The rats were fed 5.7082 units of reference oil (diluted in corn oil) in 3 doses of 0.1 ml. each, on the first, third, and fifth days of the assay period. On the eighth day, the animals were killed and their bones (radii) examined. Experimental data are shown in Table 2.

TABLE 2.—*Growth during depletion periods on rachitogenic diets*

GROUP	DAYS DEPLETION	GRAMS BODY WEIGHT		GRAMS GAIN	
		BEGINNING	END	TOTAL	PER DAY
I. Steenbock diet +0.5% lysine	13	47.2	61.5	14.3	1.10
II. Steenbock diet +0.5% lysine	16	46.6	62.9	16.3	1.02
III. Steenbock diet unsupplemented	20	47.7	58.8	11.1	0.56
IV. Steenbock diet unsupplemented	23	49.6	66.4	16.8	0.73

The rats of group I grew at a rate of 1.10 grams per day during the 13 day depletion period, while those of group II showed a growth rate of 1.02 grams per day for 16 days. Group III, receiving no lysine supplement, grew 0.56 grams per day, and group IV gained 0.73 grams per day. Thus, the lysine supplement produced a growth rate increase of nearly 39 per cent.

Photographs of the bones are shown in Figure 1.

The radii of the rats depleted for 13 days with the lysine supplement show clearly defined healing in all but one case. The bones of the animals in group II, depleted for 16 days with a lysine supplement, have such a wide rachitic metaphysis that the healing is perfectly defined throughout.

The fourth bone in the group shows no healing, but the metaphysis is well-developed. Group III, depleted for 20 days on the unsupplemented diet showed less well defined healing than group I, with 3 bones showing doubtful healing because of very narrow metaphyses. The bones of group IV are comparable to those of group II.

This experiment shows that a 13 to 16 day depletion period with lysine supplemented diet produces the same degree of rickets obtained in 20 to

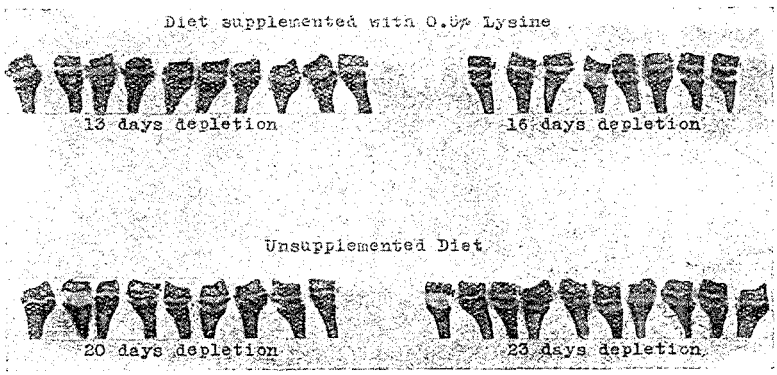

FIG. 1.—Healing in Radii.

23 days with the unsupplemented diet. Thus, with the strain of rats used in the above work, the depletion period is shortened by 7 days.

It would seem that any good source of lysine, if sufficiently low in phosphorus, could be used to raise the lysine level to meet the rat requirement. Blood fibrin,[1] showing by analysis about 8.8 per cent lysine (microbiological assay) and 0.09 per cent total phosphorus, was thought to be satisfastory as a source of lysine. Jones in 1939 (5) obtained good rickets with alcohol-ether extracted blood fibrin as the protein in purified rachitogenic diets. The fibrin used in the following work was a highly refined product, and no alcohol-ether extraction such as that used by Jones in 1938 (4) was made.

The Steenbock rachitogenic diet, the diet supplemented with lysine, and the diet modified with blood fibrin, were fed to rats in order to compare the development of rickets and the clarity of the healing line. The following experiment was accordingly made.

Three groups of 12 rats, litter mates and evenly matched for body weight, were depleted for 16 days. This depletion period was selected because it was shown in the first experiment to be optimum for the lysine-supplemented diet. Group I received the Steenbock rickets-producing

[1] Wilson Laboratories, Chicago, Ill.

diet, group II received the diet plus 0.5% lysine, and group III received the diet modified to contain 5.7% blood fibrin. The fibrin was incorporated at a level sufficient to supply 0.5% lysine, the percentages of corn and wheat gluten being altered proportionately. The Steenbock diet contains 76% corn, 20% wheat gluten, 3% calcium carbonate, and 1% sodium chloride. The fibrin-modified diet was composed of 71.8% corn, 18.5% wheat gluten, 5.7% blood fibrin, 3.0% calcium carbonate, and 1.0% sodium chloride. The rats were weighed on the sixth, eleventh, and sixteenth days of the depletion period. At the end of the depletion 3 negative controls from each group were killed. The remaining rats were fed the reference oil as in the first experiment.

The lysine and fibrine supplements produced about 33 per cent more growth during the depletion period than the unsupplemented diet, as shown in Table 3.

TABLE 3.—*Growth during 16 days depletion on rachitogenic diets*

GROUP	GRAMS BODY WEIGHT		GRAMS GAIN	
	BEGINNING	END	TOTAL	PER DAY
I. Steenbock diet unsupplemented	45.8	60.7	14.9	0.94
II. Steenbock diet+0.5% lysine	45.9	68.4	22.5	1.41
III. Steenbock diet+5.7% fibrin	44.2	66.2	22.0	1.38

The negative control bones from each group show some widening of the cartilage band, but the lysine-supplemented group was the only one in which the metaphysis had developed. The bones of the fibrin supplemented group presented an atypical appearance, with an irregular metaphyses and spotty calcium deposits along the epiphyses.

Photographs of the bones are shown in Figure 2.

The bones of group I show in general the development of such a narrow metaphysis as to make accurate scoring impossible. Only 2 bones from the group show well defined healing. The healing of the bones from the lysine-supplemented group II is clear and well defined in every case. The fibrin-supplemented group showed only one bone which could be scored for healing.

The foregoing work should be of interest to laboratories performing routine vitamin D assays. The more rapid growth rate of rats on depletion is very desirable because the animals develop rickets in a shorter period of time, this shorter depletion period allowing less time for the animals to develop respiratory and other infections. The cost of assays would be reduced if an inexpensive lysine source were used. Pure lysine at the present time is too expensive to be used in routine vitamin D assays. Further experiments will be directed toward finding a natural source of

lysine that will prove as satisfactory as the pure lysine in giving improved growth and the more rapid development of rickets.

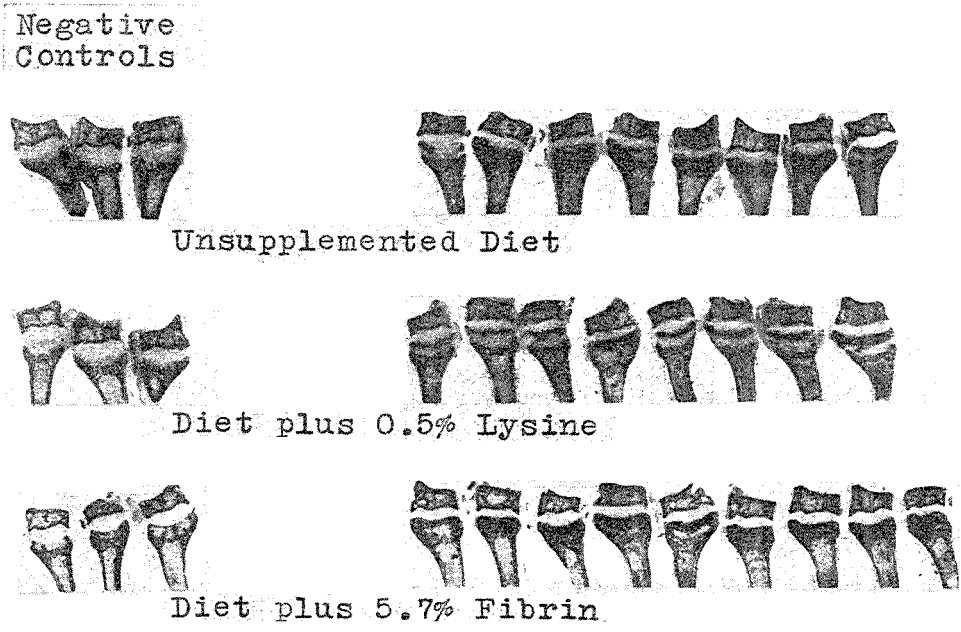

FIG. 2.—Healing in Radii after 16 Day Depletion Period.

SUMMARY

Experiments were made to determine the effect of a lysine supplement on the Steenbock rachitogenic diet. The results of these experiments definitely indicate that rickets can be produced in a shorter period of time when the diet is supplemented with lysine, as the improved growth rate of the rats results in a more rapid development of rickets.

Blood fibrin was found to be unsatisfactory as a source of lysine in the rickets-producing diet. Although it promotes growth on a par with pure lysine, it inhibits the development of a satisfactory rachitic metaphysis.

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THE DETERMINATION OF CHLORIDES IN COMMERCIAL FEEDING STUFFS

By G. S. FRAPS and E. E. BROWN (Texas Agricultural Experiment Station, College Station, Texas)

Determinations of chlorides in samples of commercial mixed feeds collected by the Texas Feed Control Service have been made for a number of years and published annually. The many deviations found from the guaranteed content of salt have amply justified such work. Chlorides are of natural occurrence in certain feeds, averages for which are given in Table 1. Correction needs to be made for the chlorine derived from such feeds in estimating the added sodium chloride content of a mixture.

Several different methods have been used for the purpose of determining chlorides (1), including purification of the extract with carbon black or lead acetate (2) and extraction with a solution of picric acid (3). Both of

TABLE 1. *Average percentage of chloride expressed as sodium chloride in feeds high in chloride*

	PER CENT
Alfalfa (Western)	.98
Beet pulp, dried	.87
Blood, dried	.75
Buttermilk, dried	2.38
Coconut oil meal or cake	1.11
Fish meal	.57
Meat meal	2.77
Meat scraps	1.31
Meat and bone scraps	1.54
Molasses	1.12
Tankage	1.53

these methods gave satisfactory results on most of the mixtures tested, but there were some mixtures on which the results were not entirely satisfactory. Our latest method involves boiling with nitric acid to destroy the interfering substances, as is done in the A.O.A.C. method for chlorides in cheese (4). It has been found satisfactory with all samples except one. This sample contained oxide of iron which was not dissolved by the nitric acid, could not be filtered out, and the color interfered with the titration.

The method has been compared with the A.O.A.C. method for soluble chlorine in grain and stock feed **27.61** (5) with satisfactory results. Some of the many comparisons are given in Table 2. However, the nitric acid method is much more rapid than the A.O.A.C. method, chiefly because filtration is not needed. For this reason it is worthy of consideration. The

method follows. The procedure given for salt in mineral feeds has been used on a number of samples.

TABLE 2.—*Salt content by A.O.A.C. method and nitric acid method*

	A.O.A.C.	NITRIC ACID		A.O.A.C.	NITRIC ACID
	Per cent	*Per cent*		*Per cent*	*Per cent*
Turkey mash	.78	.75	Starting mash	.45	.39
Dairy feed	.29	.21	Dairy feed	1.11	.93
Turkey mash	1.02	.99	Sweet feed	1.35	1.31
Dairy ration	2.05	2.10	Dairy feed	1.05	1.07
Sweet feed	1.68	1.62	Dairy feed	1.95	1.98
Laying mash	1.02	.96	Horse and mule feed	.27	.23
Egg mash	.51	.56	Concentrate for hogs	1.23	1.22
Meat & bone scraps	1.02	1.08	Complete hog ration	1.26	1.16
Growing mash	1.41	1.29	Hogs concentrate for		
Laying mash	1.17	1.11	meat & bone scraps	2.58	2.58
Hay supplement	.48	.45	Meat & bone scraps	1.41	1.46
Chick starter	.66	.72	Dairy feed	1.23	1.05
Dairy feed	1.32	1.35	Growing mash	.81	.69
Dairy feed	.96	.93	Meat & bone scraps	1.44	1.50
Turkey starter	1.74	1.74	Egg mash pellets	1.23	1.16
Laying mash	.45	.51			

CHLORIDES IN FEEDS

REAGENTS

(1) *Standard silver nitrate.*—(A little stronger than 0.1 *N* to simplify calculations.) Weigh out 35.6 grams of pure crystallized silver nitrate. Dissolve and make up to 2000 ml. Determine exact strength of solution by titrating against 10 ml. of exactly 0.2 *N* hydrochloric acid, which has been neutralized with pure powdered calcium carbonate, using potassium chromate as indicator (Reagent 5). Adjust so that 20 ml. of the silver nitrate solution is equal to 10.26 ml. of the acid.

(2) *Standard ammonium or potassium sulfocyanate (thiocyanate).*—Dissolve about 18 grams of the ammonium salt, or 20 grams of the potassium salt, in 2000 ml. water. Measure out 25 ml. of the standard silver nitrate, add 2 ml. ferric indicator (Reagent 3) and 10 ml. of the nitric acid (Reagent 4) and run in the sulfocyanate solution from a buret, slowly and with stirring, until a light brown color appears which does not disappear on stirring. Readjust if necessary to exact equivalence with the silver nitrate solution.

Cross-check the silver nitrate solution for the Volhard titration as follows: Add 25 ml. of the silver nitrate solution (Reagent 1) to 10 ml. of 0.2 *N* hydrochloric acid, coagulate the precipitate of silver chloride, filter on quantitative filter paper and wash the precipitate 8 times with cold water. Add to the filtrate 2 ml. ferric indicator (Reagent 3) and 10 ml. nitric acid free from lower oxides of nitrogen (Reagent 4), and titrate the excess of silver nitrate in the filtrate with the sulfocyanate solution (Reagent 2). Subtract the volume of the sulfocyanate from the total volume of silver nitrate added. The difference is the volume of silver nitrate equivalent to the 10 ml. of 0.2 *N* hydrochloric acid. 20 ml. of the silver nitrate solution should again be

equivalent to 10.26 ml. of the acid. If it is not, adjust both silver nitrate and sulfocyanate solutions to this value. Restandardize the adjusted silver nitrate according to the Mohr titration and use the proper factor.

(3) *Ferric indicator.*—Use a solution of 30 grams of ferric potassium sulphate in 300 ml. of water; or take 100 grams ferrous sulphate (copperas), add about 300 ml. water, 50 ml. concentrated nitric acid, and 5 ml. sulphuric acid, adding the latter slowly, while stirring. Evaporate to a paste, to drive off nitrous fumes, and dissolve in water to a solution of about 10%.

(4) *Nitric acid, free from lower oxides of nitrogen.*—Add 400 ml. of water to 1200 ml. pure concentrated nitric acid in a large flask, containing a few small pieces of broken quartz or porcelain. Boil until colorless. Keep in dark place.

(5) *Potassium chromate.*—Dissolve 25 grams in 500 ml. of distilled water. Use 2 ml. for each titration.

DETERMINATION

Weigh 2 grams of feed into a 300 ml. Erlenmeyer flask; add about 20 ml. of water and 10.00 ml. of standard silver nitrate solution. Shake until the sample is completely moistened.

Add about 20 ml. of conc. nitric acid, and next add about 10 ml. of 6% potassium permanganate and shake. Heat until the liquid is clear and remove from the flame as soon as brown fumes again appear. Cool, and rinse down the inside of the flask thoroly with water. Add 10 ml. of 5% urea to destroy the nitrous oxides and shake again. Rinse down the inside of the flask with water to remove last traces of nitrous oxides. Let stand for about five minutes. Add 10 ml. of acetone and 2 ml. saturated ferric potassium sulphate solution. Titrate the excess of silver nitrate with standard ammonium thiocyanate to a permanent brownish-red color.

If nearly all the silver nitrate is used up by the feed, repeat the work, using 20 ml. or more of silver nitrate solution.

The volume of silver nitrate used up (in ml.) multiplied by 0.3 gives the percentage of chlorides expressed as common salt (NaCl).

DETERMINATION OF SALT IN MINERAL FEED HIGH IN SALT

Transfer 2.000 grams to a 200 ml. volumetric flask and dissolve the soluble part in water. Make up to mark and filter, discarding the first 10 ml. To 20 ml. of the clear solution, add about 0.5 gm. pure calcium carbonate suspended in 40 ml. water to neutralize any acid, and 1 ml. of potassium chromate. Titrate with standard silver nitrate to a permanent pink color. The volume of standard silver nitrate (in ml.) multiplied by 3.0 gives the percentage of chlorides expressed as NaCl.

If less than 4 ml. of silver nitrate is used, repeat using 50 ml. of the solution; then ml. of silver nitrate times 1.2 equals per cent sodium chloride.

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NOTES

A Portable Apparatus for Determining Sediments in Cream

By FRANK H. COLLINS (U. S. Food and Drug Administration, 501 Post Office Building, Cincinnati 2, Ohio.)

The apparatus described in this paper can be used in creameries and other places where suction and other laboratory conveniences are not available, or in connection with a water aspirator it will serve as a convenient work table.

This apparatus, weighing about 100 pounds, collapses to a self-contained chest measuring 28″×15″×19″ high (Fig. 2). It is convenient to carry and may be transported in an auto trunk or inside a coach or sedan. When expanded, the removable front forms a rigid work table 28″×17¾″, 38″ high (Fig. 3) providing ample space for sediment testing. It is made rigid by means of wire cable braces, tightened with a turnbuckle.

The cabinet as illustrated dimensionally in Fig. 1 houses, in addition to the pump and motor, two end supports for the table. One of these carries a coiled extension cord and electrical fixtures. Provision has been made beneath the table top for a storage tray for prepared sediment pads. Unused pads may be conveniently stored in a small swing-out box as shown in Fig. 4. The cabinet has been shortened slightly so that it no longer accommodates the multiple storage trays in the base which are shown in Fig. 4.

This apparatus has given satisfactory service since its construction in May, 1945. It uses a rotary (liquid) pump instead of the conventional compressor-vacuum pump of the oil seal, ring piston type. With the former type there is no troublesome oil dilution when used with hot solutions. By means of a system of dual tanks, it develops positive action on liquid and maintains a vacuum of 25 to 27 inches.

The system is initially filled with water. The flow of liquid through the apparatus is as follows:

(1) From the funnel sediment pad holder through the pad into the top of a tank holding about one quart; (2) then out at the bottom of this tank and through the pump into an elevated tank of about a gallon capacity; (3) it enters this tank through a tubulation in the bottom, then overflows through a length of garden hose.

The smaller tank maintains a head of liquid at the intake port of the pump, preventing the pumping of air with consequent loss of vacuum. Should all of the liquid be replaced by air leakage around the filter pad or otherwise, the lower tank and the entire system between the pump and filter pad may be quickly refilled with liquid from the elevated tank by means of a toe-operated by-pass valve. Incidentally, this back flow of liquid forces air back through any filter pad still in place, often assisting in the filtering of a refractory sample.

The following parts and materials, exclusive of lumber, were used in the construction of this apparatus.

	Cost
1. Worthington Rotary Pump, Type GI, Size ½″, Reference No. HH5492	$12.00
2. Motor—¼ H.P., 1725 RPM	6.90
3. Flexible Coupling, Boston FGN 12, ½″	2.50
4. All rubber electric cord, 35 ft. 14-2	5.52
5. Plug for cord,	

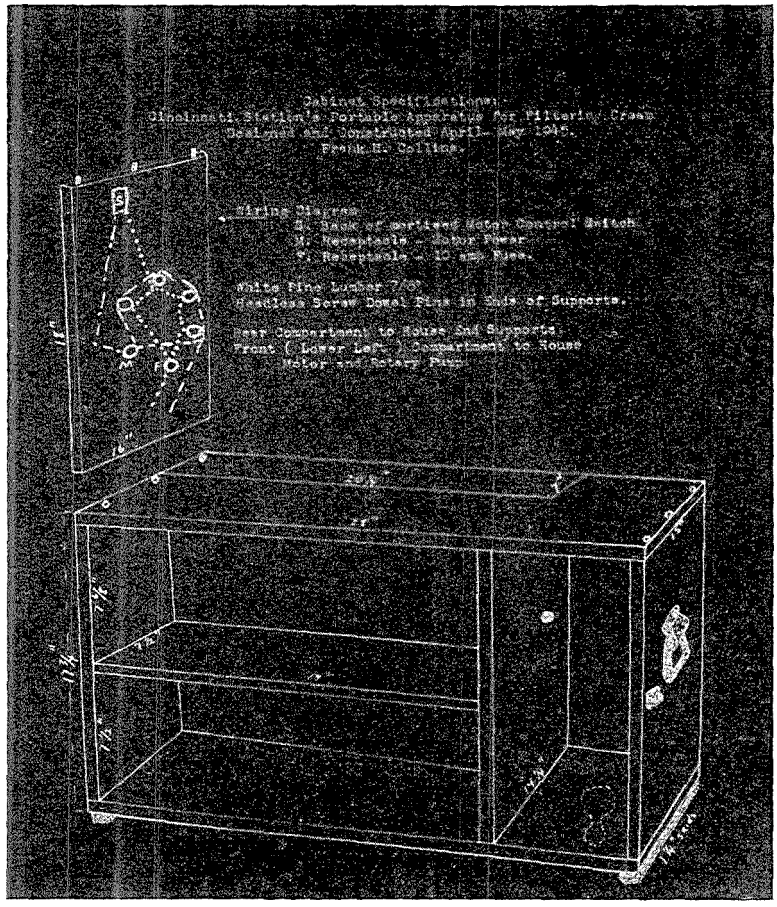


FIG. 1

6. Sediment pad holder and funnel
7. Tank, approximately 1 quart capacity, from old Grunow refrigerator (to be adapted) 1.00
8. Tank, approximately 1 gallon capacity—vacuum tank from old auto (to be adapted) No Cost
9. Electrical Fixtures:

   1 toggle switch (wall type) with plate
   6 receptacles—open wiring
   5 screw-in plug bases
   10 amp. fuses.

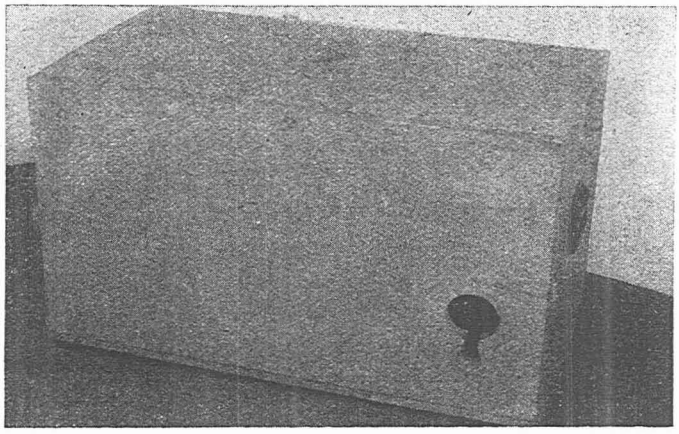


FIG. 2

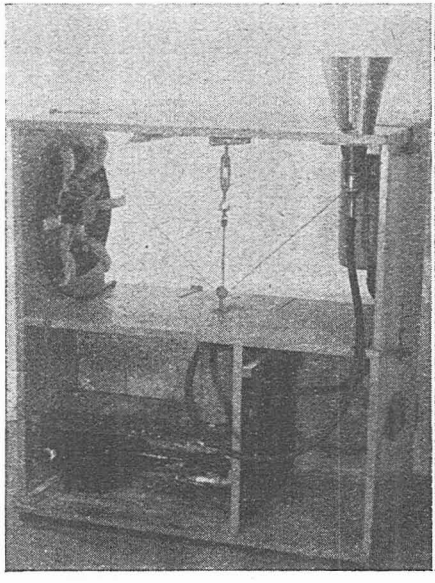


FIG. 3

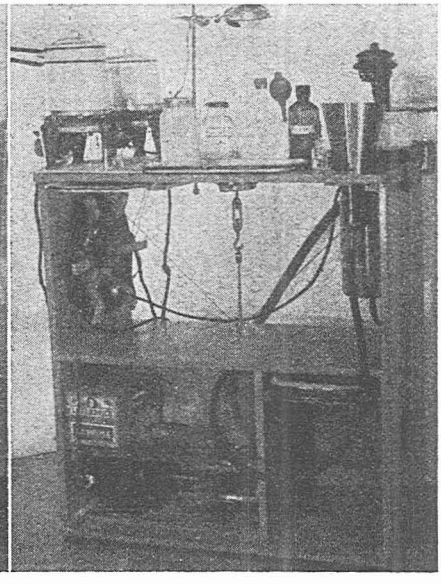


FIG. 4

10. Miscellaneous Hardware and Equipment:

2 chest handles (5″)
5 hasps (8″)
9 hasp staples
1 single pulley (small)
1 double pulley (small)
steel cable
1 turnbuckle
suction hose
garden hose
pipe fittings:
2—½″ nipples,
3—reducing T's (½ to ¼″)
3—¼″ hose nipples
1—¼″ shut-off valve with nipple.

A Note on the Determination of Starch

By O. S. Rask (U. S. Industrial Chemicals, Inc., Baltimore, Md.)

In connection with certain fermentation studies which will be reported elsewhere, the writer has made extensive use of the A.O.A.C. tentative method* for determining starch by hydrochloric acid dispersion and subsequent alcohol precipitation. In so using this method the writer (1) changed somewhat the preliminary washings of the sample, (2) introduced a new mechanical technique for dispersing the previously washed sample in the hydrochloric acid reagent, and (3) replaced the Gooch crucibles by sintered glass filtering crucibles fitted with asbestos pads. These three changes seem to be sufficient improvements in the method to warrant this note.

1. The preliminary washings as conducted are as follows:

Wash the weighed sample on a 9.0 cm. paper filter successively with three portions of ether, two of alcohol, five of water, two of alcohol and finally three of ether, preferably anhydrous. Before introducing the washed sample into the dispersing acid, dry it at room temperature or in an oven at a temperature not higher than 50°C. until substantially all of the ether has evaporated off.

The advantages of such a series of washings are that they keep down enzymatic actions and leave the washed sample in a preserved condition so that the dispersing acid need not be applied immediately afterward, as is required when the last washing is with water. Accordingly, the analyst may wash any reasonable number of samples simultaneously and then from that point he may, any time at his convenience, resume manipulation of each sample individually without danger of any diastatic actions which are likely to occur when water is the final washing medium.

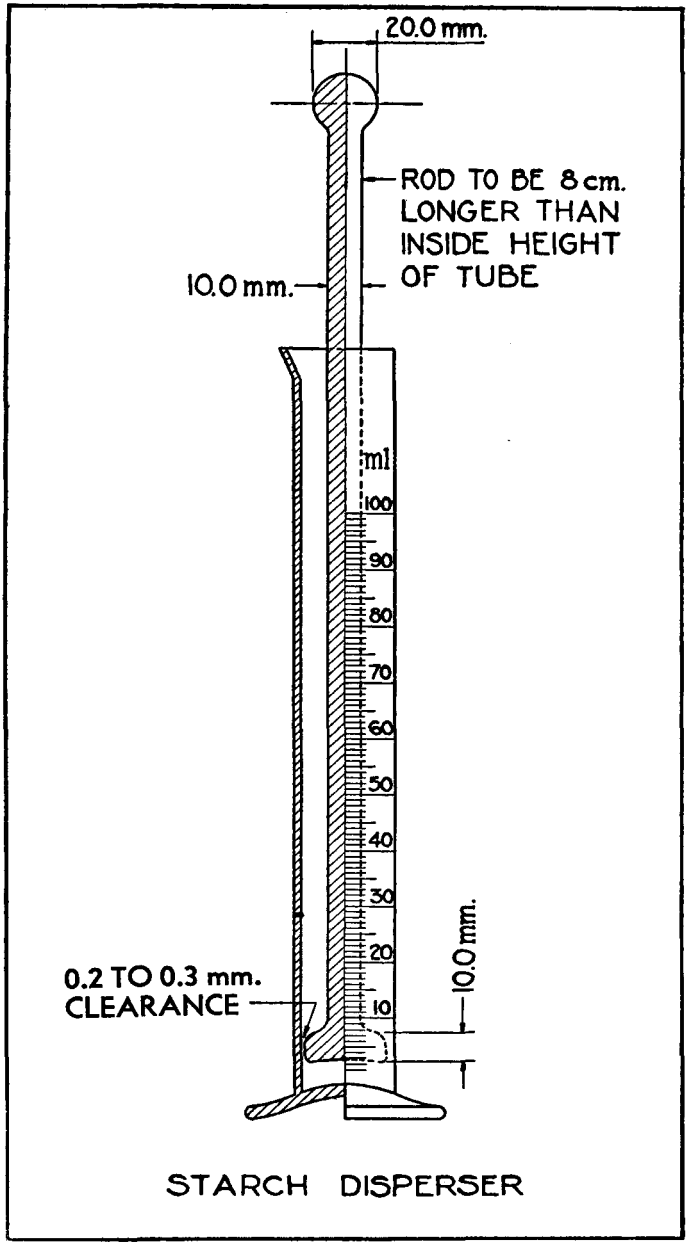
2. The A.O.A.C. tamping technique will, no doubt, eventually disperse the sample satisfactorily in the dispersing acid, but such a tamping is laborious and time-consuming and has the still more serious disadvantage that it does not enable the analyst to tell when the tamping is sufficient for such dispersion as required for a complete extraction and solution of the starch. The writer has therefore devised another reliable technic by which the sample can be dispersed rapidly with the required completeness. This technic calls for the following equipment:

An ordinary 100 ml. stock cylinder selected for accuracy of calibration, uniformity of bore and thickness of bottom and wall.

A pyrex rod about 0.9 to 1.0 cm. in diameter with one end melted into the shape of a sphere about 2.0 cm. in diameter to serve as a handle and with the other end pressed into a rather thick disc about 0.5 mm. smaller in diameter than the bore of the cylinder, the under side of the disc being preferably so concave as to articulate

* Methods of Analysis, 6th Edition, 1945, p. 249.

closely with the usually convex bottom of the cylinder. The total length of the rod is about 8 cm. greater than the depth of the cylinder. See the illustration.



A 50 ml. pipette recalibrated, if necessary, so that two of its deliveries will fill the cylinder to its 100 ml. mark.

The technic for using this dispersing equipment is as follows:

Fill the cylinder to around its 60 ml. mark with the dispersing acid whose temperature must not be higher than 18°C. Tear away and discard as much as possible of the filter paper which did not come in direct contact with the sample and introduce the washed and ether-dried sample and the rest of the adhering filter paper into the cylinder. By means of the flattened end of the rod, slowly and cautiously, push sample and filter paper downward into the acid in the cylinder until the flattened end reaches approximately the 10 ml. mark, being careful at this stage not to press the sample down on the bottom of the cylinder. With a vigorous and an almost violent jerk pull the rod upward until its flattened end reaches the 50 ml. mark or higher. In so doing the rod may be held in one hand while the cylinder is kept on the bench by pressing the index finger and thumb of the other hand on the base of the cylinder. Repeat about five or six times these slow downward movements of the flattened end of the rod to the 10 ml. mark and the rapid and rather violent upward movements to the 50 or 60 ml. mark.† Then, and not until then, push the flattened end of the rod downward into the cylinder as far as it will go and, with considerable pressure and a twisting motion, grind the sample and filter paper remaining underneath the flattened end until both pass completely up into the solution above the flattened end, as indicated by direct contact between the latter and the bottom of the cylinder. Finally pass the flattened end rapidly up and slowly down through the entire column of acid in the cylinder ten or twelve times. As a result, due to the 0.2 mm. clearance between the flattened end of the rod and the wall of the cylinder, the sample will be dispersed sufficiently for complete extraction and solution of the starch. Lift the flattened end up to around the 100 ml. mark and rinse it free from starch solution with about 25 to 30 ml. of dispersing acid, delivered preferably from a small beaker. Remove the rod and fill the cylinder to its 100 ml. mark with the dispersing acid and add 0.5 ml., or 10 drops more of the acid to allow for the volume occupied by the filter paper and undissolved material in the sample. Close the cylinder with a soft rubber stopper and mix its contents by inverting about 20 times. Allow the cylinder to stand 4 to 5 minutes, then invert it again 3 or 4 times.

Immediately afterward, with the aid of suction, filter about 70 ml. of its contents through a sintered glass filtering crucible fitted with a thin and dry pad of asbestos on top of which is spread about 1.0 gram of dry, loosely packed and fluffy asbestos, and receive the filtrate in a dry 250 ml. suction bottle. From this point proceed as in the original method, except that the recalibrated 50 ml. pipet must be used for measuring the aliquot of the filtrate to be delivered into the 115 ml. of alcohol. The weighed crucible for collecting the precipitated starch is a sintered glass filtering crucible fitted with a thin pad of asbestos. (Among the advantages of sintered glass crucibles over regular Gooch crucibles are that they require less asbestos and are brought to constant weights with greater ease and certainty.)

As in the original method not more than 30 minutes should elapse from the moment of the initial contact of the sample with the dispersing acid until the 50 ml. of filtrate has been mixed completely with the 115 ml. of alcohol. In the above modification 20 minutes are usually sufficient.

Results obtained by the above modification have been found to be definitely more satisfactory than those obtained by the present A.O.A.C. form of the method. Some of these results will appear in a forthcoming paper on methods for determining ethyl alcohol fermentation efficiencies.

† These upward movements of the rod, if sufficiently rapid and vigorous, will impart to the acid a physical movement sufficient to disintegrate mechanically not only the sample but also the filter paper. Associated with this mechanical action will be an extensive, and sometimes a nearly complete, solvent action of the acid on the starch.

BOOK REVIEWS

Wood Yeast for Animal Food. Bulletin 12, Northeastern Wood Utilization Council P. O. Box 1577, New Haven 6, Conn. 198 pp., paper, price $2.00.

Wood yeast can be made either from sulfite waste liquor, or from wood waste, such as sawdust, chips, etc., by separating the wood sugar from the lignin after acid hydrolysis. The commercial production of wood yeast would be an important contribution of a high protein ingredient to our national feed supply.

The bulletin is presented in two parts. Part I is entitled "Feed Value." It includes a number of papers by different authors dealing with yeast, and more particularly wood yeast. These deal with chemical composition (including minerals, vitamins, purine, and resin content, etc.), digestibility of the yeast protein, available energy content, food value based on feeding experiments on cows and pigs, and with the amino acid distribution in the yeast protein.

Part II, entitled "Production of Wood Yeast" contains 3 papers which discuss in detail (1) the production of wood sugar in Germany and its conversion to yeast and alcohol; (2) the production of alcohol from wood sugar; and (3) the production of food protein from wood sugar. The various processes are described in considerable detail, together with drawings of plant lay-outs, particularly as used in Germany and Switzerland.

This bulletin should be useful in the development of the commercial production of wood yeast in this country, and also of its use as a feed.

V. E. MUNSEY

Handbook of Analytical Methods for Soybeans and Soybean Products. By the National Soybean Processors Association. Published by the same Association, 3818 Board of Trade Building, Chicago, Ill. Paper cover, 40 pp., price $1.00.

As stated in the Foreword, the methods for the analyses of soybeans and their by-products have not heretofore been available in any single printed unit. Although these methods are not official methods of the National Soybean Processors Association, many of them are official methods, or adaptations of official methods, from the Association of Official Agricultural Chemists and the American Oil Chemists' Society, as indicated.

Methods for the following determinations are given: moisture and volatile matter in whole soybeans; moisture and volatile matter in ground soybeans, soybean oil meal and soy flour; oil in soybeans; oil in soybean oil meal and soy flour; protein in soybeans, soybean oil meal, and soy flour; crude fiber in soybeans, soybean oil meal, and soy flour; ash in soybeans, soybean oil meal, and soy flour; screen test of soy flour; water absorption of soy flour; lecithin in soy flour; free fatty acids in soybean oil; insoluble impurities in soybean oil; break test for crude soybean oil; grading soybean oil for color; bleach test for refined soybean oil; color of soybean oil; refining loss of crude soybean oil; moisture and volatile matter in soybean oil (hot-plate method); moisture and volatile matter in soybean oil (vacuum oven method); acetone insoluble matter in lecithin; acetone insoluble matter in lecithin; acid value of lecithin; total phosphorus in lecithin; kerosene insoluble matter in lecithin; and moisture in lecithin. An occasional beneficial note or comment is included in some of the methods.

This booklet should be especially valuable to chemists dealing with soybean and soybean products, as well as oil, food, and agricultural chemists concerned with this subject.

V. E. MUNSEY

SIXTY-FIRST ANNUAL MEETING

The 61st Annual Convention of the Association of Official Agricultural Chemists will be held October 20 to 22, inclusive, at the Shoreham Hotel, 2500 Calvert Street, N. W., Washington 8, D. C.

Hotel accommodations are expected not to be readily obtainable even by next fall, and it is suggested that reservations be arranged well in advance of the meeting.

NOTICE TO SUBSCRIBERS

The Association urgently needs a substantial number of copies of Vol. 29, No. 1, the Feb. 15, 1946, issue of its *Journal*. Those having copies not intended as permanent files will favor the Association by returning them. Payment of $1.60 per copy will be refunded to cover their cost and postage as 2nd class matter.

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Box 540, Benjamin Franklin Station,
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METHODS OF ANALYSIS, 6th edition, 1945

The list price of the new edition will be $6.25, domestic postpaid.

CHARLES ALBERT BROWNE, 1871–1947

CHARLES ALBERT BROWNE

Charles Albert Browne, one of America's foremost agricultural chemists, died in Washington on February 3, 1947, at the age of 76 years. In view of his outstanding place in American agriculture, it is profitable to review his life.

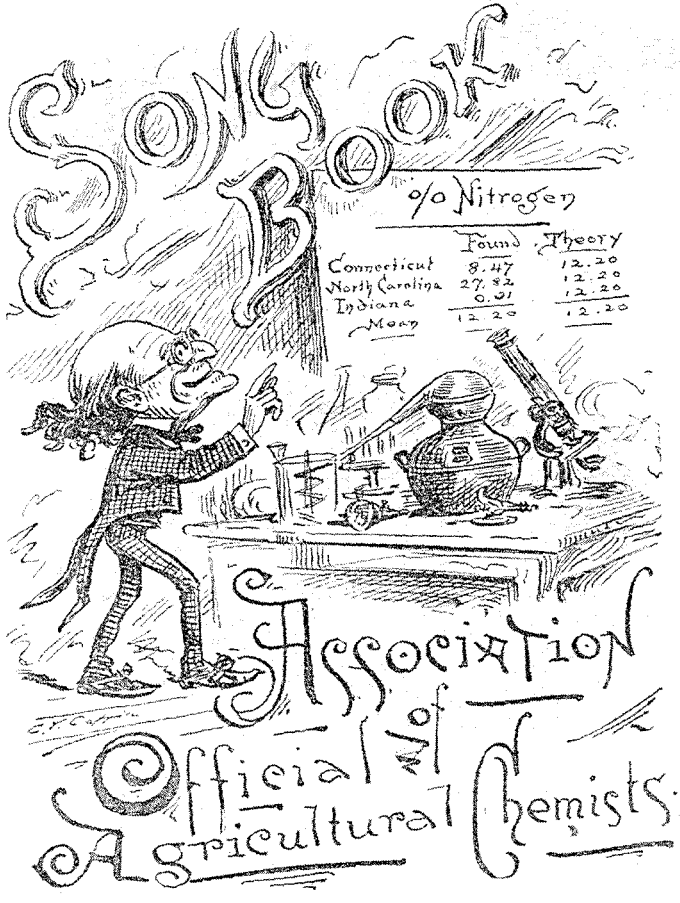
In this age of minute specialization, Dr. Browne's work is conspicuous for its inclusive outlook, and its generality of application. Quite possibly he will be thought of as the last American to be conversant with agricultural chemistry as an integral field, rather than with the details of a part of it.

Albert Browne was a New England boy of Colonial ancestry. This is particularly interesting in view of the pioneering nature of much of his work. The old urge to discover had not disappeared; it was merely diverted into other channels. His father was an electrical expert, a farmer, and a chemist. He developed much pleasure and some profit from chemical experimentation on explosives and their manufacture. He also lost his eyesight thereby through an explosion with copper fulminate. Charles Albert's environment was thus an ideal one for the development of an agricultural chemist. However, during his education at Williams College he developed another interest, in addition to chemistry. This interest was in Greek, and it became a very serious one. He has often told how he decided between the two professions by making up his mind to accept the first suitable job that offered itself,—probably an even chance in those days. His first real offer was in chemistry, and he became an assistant to one of New York's most successful consulting chemists. Not long after, he went to Pennsylvania State College, doubtess influenced in that move by his agricultural background; the next step was to the University of Göttingen for graduate work. At Göttingen, Geheimrat Tollens gave him a doctor's degree—the first of three he received. Under Tollens, his interest in sugars was further stimulated, to be retained all his life. But the midnight oil spent on Plato and Aeschylus was not wasted. From it came Dr. Browne's extraordinary competence in history, and his appreciation of the timelessness of man's problems. Incidentally, he was able to make himself understood in modern rural Greece after a little practice.

On his return to America, Dr. Browne devoted himself to sugar chemistry, first as research chemist of the Louisiana Sugar Experiment Station, next in the Federal Department of Agriculture under Harvey W. Wiley, and finally in New York City where he established and directed the New York Sugar Trade Laboratory.

It was doubtless his association with Wiley in 1906–7 that contributed materially to Dr. Browne's life-long interest in the Association of Official Agricultural Chemists. The two 10-year indexes of this Journal list some 25 addresses, reports, book reviews, and other writings, but only those who saw and talked with Dr. Browne at the annual meetings of the Association can have a full appreciation of his devotion to the A.O.A.C. He was president of the Association in 1925. His presidential address is full of wisdom and seasoned with Attic salt.

Perhaps two of the most important bonds between Wiley and Browne were their classical learning and their impish humor. It gives one a certain nostalgic pleasure to note that they combined the two in the "Song Book of the A.O.A.C.," the cover of which is here reproduced. To me it is a pinnacle of clever chemical cartoons (not excepting the celebrated "Berichte der Durstigen Chemischen Gesellschaft"). As a masterpiece of understatement, it should be "required reading" for any candidate for Ph.D. The same may be said for the humorous propaganda song in Latin verse, which is at the end of the book. The first stanza is reproduced here, not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North Carolina 27.82 12.20
Indiana 0.91 12.20
Mean 12.20 12.20
Association
of
Official
Agricultural Chemists

that it will be understood by specialists of today "with little Latin and less Greek," but because it is reminiscent of that vanished time when there were broadly educated scientists:

Gaudeamus igitur
'Gricolae dum sumus
Post arenam per molestam
Post argillam induratam
Nos delectat humus.

In 1923, more than 15 years after his first connection with the Department of Agriculture, Dr. Browne made his last move, back to that Department as Chief of the Bureau of Chemistry.* As years went on, Dr. Browne gradually gave up administrative work, to continue the guidance of research until he retired in 1940. Thereafter, as a Collaborator, he spent his time in study and literary work.

There is no opportunity here to review all of Dr. Browne's very numerous scientific achievements, nor to list his publications, which run into the hundreds. Even their relative importance is hard to estimate, for some seem destined to develop greater importance with the years. Because so many of Dr. Browne's best known publications deal with sugar, it is sometimes overlooked that he was deeply interested in the biochemistry of agriculture, and regarded sugar as a substance to be studied in that connection. He by no means neglected other phases of agricultural chemistry, and invariably preferred to think of them in terms of the general principles involved. For example, he was one of the very first to realize the importance of studying the action of enzymes in agricultural products. Here again he was less interested in the catalysts themselves than in the extent to which they determine biochemical processes by giving such priority to one chemical reaction that others become insignificant in comparison. The concept of a substance directing cellular processes because it was capable of "discriminatory" chemical reactions is an old concept, but it was Dr. Browne who showed me how very old it is. He possessed one of the earliest printed editions of Paracelsus, and in this I read how it worked (allegorically expressed of course; just as our ideas might appear to readers, if any, four centuries hence).

Among Browne's early agricultural studies with enzymes, the following will serve as examples: On the lipase of rice (1), a paper we have recently had to study for its bearing on the spoilage of rice oil; on peroxidase (2), still interesting for the physiological significance attributed to the enzyme; on dilatometry (3), since then the subject of very many papers; on invertase (2) and the financial losses it caused in sugar cane.

Extensive travel, and the critical and systematic observation of agriculture all over Europe, Egypt, Australia, New Zealand, the West Indies, and the United States, made Browne an expert on the practice of agriculture, as his contribution to revising and editing Wiley's compendium on the "Principles and Practice of Agriculture" shows. His efforts to introduce a perfume industry into semi-tropical parts of the United States were the result of observations made on his travels in Southern Europe (4). His voluminous travel notes, all carefully edited and arranged, have often been of great help to other agriculturalists who planned to visit any of the countries he had studied. In his later years the social implications of agriculture

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* 1862–1901—Division of Chemistry,
1901–1927—Bureau of Chemistry,
1927–1938—Bureau of Chemistry and Soils,
1938–1943—Bureau of Agricultural Chemistry and Engineering,
1943–1947—Agricultural Research Administration, Bureau of Agricultural and Industrial Chemistry.

concerned him deeply, as is indicated by his papers on Thomas Jefferson (5) and on Robert Owen's communal farm colony at New Harmony, Indiana (6).

It may profit us to consider what subjects Dr. Browne regarded as important in agricultural research. Among them, as most of us know, was the loss of sugar (and so of food value) in hay after it is cut and before it is dried. This loss reaches an astronomical figure, even at the present low price of sugar. Another great economic loss that interested him was the so-called spontaneous combustion of hay in lofts and stacks. The study of spontaneous combustion which the Bureau of Chemistry made under Dr. Browne's direction (7) did much to clarify the problem, and not a little to reduce the loss.

Dr. Browne interested himself also in plant nutrition. He was greatly impressed with the empirical progress made in ancient and medieval times, and in his "Source Book of Agricultural Chemistry," (8) he traces this progress until it became part of modern science. Dr. Browne felt very strongly that two approaches to the problems of plant nutrition were being neglected in this country: Foliary diagnosis and the effect of trace elements. Ten or 12 years ago he tried hard to get the Government to invest heavily in trace element research. Later events have certainly shown the wisdom of the idea.

No reference to Dr. Browne's life and work would be complete without some mention of his charming and rather complex personality. He was an exact, painstaking, and very systematic worker. As an administrative officer he frequently exhibited an uncanny discernment. To those who knew him, however, this was coupled with a sense of humor so broad that it sometimes bordered on the comic. He loved Aristophanes—and not in ignorance. At times this combination of wisdom and a saving sense of the ridiculous quite naturally led to remarkable situations. Obviously, such a man would enjoy practical jokes; and in fact in his youth he had a reputation along that line. The same characteristic was still visible in the older man, though modified, as such things should be, by maturity. For example, I once asked Dr. Browne's advice on a certain matter; got it; thanked him for it. Whereupon he said in effect: "The best way to advise people is to find out what they want to hear and then tell it to them; such advice is always most welcome." To this day I wonder about that advice.

I was not the only one to seek advice of Dr. Browne: everybody did. He had a decided gift for psychology, which he systematized as he did everything. The result was a sort of prophetic outlook, reminiscent of the reputation of Thomas Carlyle. I have seen this power of Browne's to estimate the future become all too well justified by subsequent events that may not properly be cited here. Part of his "prophetic instinct" was owing to an ability to discern cryptic motives and to reason a chain of events stepwise therefrom. He had a cool logical mind with oddly warm corners in it that gave him a great sympathy toward everyone and an outstanding loyalty to all who had any right to claim it.

Philosophically, Dr. Browne was an Epicurean, a follower of the middle way. He contemplated life as a cyclic, not a linear function. In his Government work he achieved high place, and could have acquired higher. Instead, he intentionally and deliberately stepped down. Gracefully and sincerely he made room for younger men. Because he felt that the curve should turn, he turned it himself. He desired to do what he thought he could do best at each period of his life. First, to work hard; next to achieve, and then to direct others to achievement; last to contemplate and, in the true sense, to teach. Thus, Charles Albert Browne spent 76 years; a pleasant, fruitful, gracious, enviable life.

ARNOLD KENT BALLS

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WILLIAM HORACE ROSS, 1875–1947

WILLIAM HORACE ROSS

William Horace Ross, Principal Chemist in the U. S. Department of Agriculture, who had served thirty-four years as research chemist engaged in soil and fertilizer problems, died after a short illness on May 16, 1947, in his 72nd year. Burial was at Arlington National Cemetery.

He was born of Scotch parents, December 27, 1875, on a farm at River John, Nova Scotia, Canada. He is survived by his widow Catherine Allen, and two sons, Allen Murray and William Horace Ross, Jr.

Growing up in Nova Scotia, young Ross became familiar with the farming practices of the region, but he was most impressed by the things that he did not understand. Two years before he entered high school, he had determined that he would study problems in agriculture. Dalhousie University conferred the B.S. degree in 1903 after three years, and the M.S. degree the following year. He accepted for the next year, a fellowship at Johns Hopkins University. After one year he transferred to the University of Chicago, as private assistant to Professor H. N. McCoy. He received his Ph.D. degree in 1907, with a dissertation on the radioactivity of thorium and uranium. While on this work he was impressed by soil problems appearing in publications from the Bureau of Soils by Milton Whitney, F. K. Cameron, Oswald Schreiner, and others, and his imagination so visualized the possibilities in soil chemistry that he thereby resolved to become a member of that Bureau.

His initiation to agricultural work was in 1907 when he joined the staff of the Arizona Agricultural Experiment Station as assistant chemist investigating the composition of irrigation water. While there he gathered material published later by the Carnegie Institution of Washington on "The annual variation of concentration of saline components in water of the Salton Sea."

His service in the Bureau of Soils beginning January 1912, was dedicated, from that day to his retirement, to investigations on some phase of fertilizers, with the exception of a part year as Captain in the Chemical Warfare Service during World War I. While in the Department, Dr. Ross was author or co-author of 135 publications. Previously he had published sixteen, including his doctor's dissertation. His first agricultural paper was on "The Nutritive Value of Cholla Fruit," while at Arizona.

In the Department of Agriculture, his investigations concerning fertilizers embraced sources of materials, methods of analysis, preparation of new materials, formulation of mixed fertilizers, granulation of fertilizers, secondary elements, mechanical distribution, and other phases. His objective in all was to produce better fertilizers at lower cost to the farmer. Among his notable achievements were the first production of phosphoric acid by the electric furnace methods; the detection of boron toxicity in American potash material and the determination of permissible quantities for fertilizer use; the preparation of high analysis fertilizers; economics in the use of these and double strength fertilizers; reactions due to ammoniation of superphosphate and control of the reactions in mixed fertilizers; improvements in mixed fertilizers; and most recently, 1943–45, leadership in the conditioning of ammonium nitrate for making an acceptable material in commerce and for the farmer. At his death he was an outstanding authority on mixed fertilizer, and through all his work had given much attention to chemical methods for determining the availability of plant food in fertilizers.

Of his 151 publications, 47 dealt with methods or special equipment used in analysis. This indicated his intense interest in the A.O.A.C., which he served for many years as associate referee on phosphoric acid. It was highly fitting that he was President of the A.O.A.C. during his last year with the Department.

After his retirement in December 1945, Dr. Ross was an active collaborator in the Division of Soils, Fertilizers, and Irrigation of the Department of Agriculture and was also consultant to the National Fertilizer Association.

On the personal side, Dr. Ross always took a deep interest in those working under his direction. Frequent talks, discussions and conferences kept him always well informed on the progress of their work and gave him the opportunity to discuss their problems in a manner that endeared him to his associates. While accomplishing much, he was never too busy to discuss any problems with others.

He did not confine himself entirely to his scientific investigations. From the beginning of Chemical Abstracts, he had been an abstractor, and part of the time was Associate Editor. He was a member of the American Association for the Advancement of Science; the American Chemical Society; the American Society of Agronomy; the American Institute of Chemists; the Association of Official Agricultural Chemists; and Sigma Xi. He was a Presbyterian and had been an officer in his church for many years. His life was centered about his home, his wife Catherine Allen, and his two sons, Allen Murray and William Horace, Jr.

His Scotch Presbyterianism was accompanied by the traditional steadiness of purpose and firmness in opinions. But once converted to another viewpoint, he espoused the new position as firmly as the first. The intensity of his concentration often resulted in an apparent absentmindedness or disdain for commonplace things. These personal characteristics only endeared him the more to his associates. His counsel among his colleagues and in the meetings of the A.O.A.C. will be greatly missed.

R. O. E. Davis