WEDNESDAY—MORNING AND AFTERNOON SESSIONS

REPORT ON ALCOHOLIC BEVERAGES

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

Our principal duty this year is to assist the Revision Committee of *Methods of Analysis, A.O.A.C.*, in revising Chapter **XIV:** Malt Beverages, Sirups and Extracts, and Brewing Materials; Chapter **XV:** Wines; and Chapter **XVI:** Distilled Liquors, and our reports will deal largely with that subject. However, additional reports on regular A.O.A.C. investigational work dealing with the interesting subjects of turbidity and color in beer and wort; spectrophotometric examination of wines; artificial coloring matter in alcoholic liquors; etc., will be reports of progress, and will be dealt with by the Association according to their individual merits.

The Referee's report will necessarily deal in large part with the revision of the three chapters and some of the reasons and necessities therefore.

The most extensive changes are in Chapter **XIV,** because the studies of methods which have been conducted by the Associate Referees on the subjects dealt with in this chapter have been supplemented extensively by the chemists of the A.S.B.C. (*i.e.*, American Society of Brewing Chemists). The Referee is able to report that the highest degree of cooperation in spirit and in fact exists between our Association and the A.S.B.C., and the methods of each Association are made available to the other, with corresponding benefit to the members of both Associations and to food chemists in general. Stephen Laufer was designated by the A.S.B.C. as coordinator of A.S.B.C. and A.O.A.C. Methods of Analysis with respect to the subject matter of Chapter **XIV,** and in that capacity has worked long and diligently to harmonize the methods as far as supporting investigational and collaborative work permits. The revisions which he has proposed have been incorporated in the writer's report either in detail or by reference to his reports as Associate Referee on Brewing Sugars and Sirups and on Wort, Spent Grains, and Yeast. The writer takes pleasure in acknowledging the assistance rendered by Dr. Laufer and his associates, especially Hugo W. Rohde, in the revision of that chapter. Likewise, the assistance of the associate referees on their respective subjects in the revision of all three chapters is acknowledged with full appreciation of their efforts.

The writer's attention was directed to the necessity of a consideration of the methods relating to the determination of alcohol in the three chapters and to the alcohol tables in the 5th edition of *Methods of Analysis, A.O.A.C.*, Chapter **XLIII, 19, 20,** and **21,** by an article "Determination of Alcohol by Volume in Distilled Liquors; Sources of Error" by L. C. Cart-

wright[1] and by correspondence in 1941 with Mr. A. Herman, the former dealing with Table 19 and the latter with Table 20. Consideration of the subject led to the appointment by the Association of a Committee on Alcohol Tables, A.O.A.C., 1944, with a membership of representatives from the Food and Drug Administration, the National Bureau of Standards, and the Alcohol Tax Unit of the Bureau of Internal Revenue. Recommended revisions of the three chapters follow.

RECOMMENDATIONS*

MALT BEVERAGES, BREWING MATERIALS, AND ALLIED PRODUCTS

It is recommended—

(1) That the revisions of Chapter **XIV** of *Methods of Analysis, A.O.A.C.* 1940, described in this year's report of the Referee on Alcoholic Beverages, be adopted.

(2) That the following tentative procedures in *Methods of Analysis, A.O.A.C.*, 1940, be dropped: Chapter **XIV, 10,** Total acidity; **28,** H-ion Concentration; **50** and **54**—Moisture; **51** and **55**—Fat; **52** and **56**—Extract.

(3) That the following methods described in this year's report of the Associate Referee on *p*H and acidity of beer be adopted as tentative: Total acidity of beer by indicator titration; total acidity of beer by potentiometric titration to *p*H 8.2, electrometric method for determination of *p*H of beer, colorimetric method for determination of *p*H of beer.

(4) That the methods described in this year's report of the Associate Referee on Cereal Adjuncts be adopted as official—first action, and that studies on them be continued.

(5) That the following methods described in this year's report of the Associate Referee on Brewing Sugars and Sirups be adopted as tentative:

Extract; Non Extract; Fermentable Extract **(a)** Regular fermentation method; **(b)** Rapid fermentation method; Protein; Diastatic Power (Malt Sirups only); Iodine reaction; Acidity; H-ion Concentration (*p*H); Ash; Total reducing sugars.

(6) That the Lane-Eynon General Volumetric Method, **XXXIV, 32, 33, 34,** and the Munson and Walker General Method, **XXXIV, 37, 38, 39,** be adopted as tentative for determination of reducing sugars in brewing sugars and sirups.

(7) That the methods for analysis of Wort, Spent Grains, and Yeast, described in this year's report of the Associate Referee on those subjects, be adopted as tentative.

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[1] *Ind. Eng. Chem., Anal. Ed.*, **14**, 237–239 (1942).

* For report of Subcommittee D and action by the Association, see *This Journal*, **28**, 63–65 (1945). Details of these revisions, as recommended by this Committee, were approved and will be incorporated in revisions of *Methods of Analysis, A.O.A.C.*, for the 6th edition, 1945.

(8) That Milos test for caramel as described in this year's report of the Associate Referee on Distilled Spirits be adopted as official for beer.

(9) That the tentative method for caramel (**XXI, 16 (f),** p. 252) be modified as described in this year's report of the Associate Referee on Distilled Spirits and adopted as tentative for beer.

(10) That the methods for turbidity and color in beer, described in this year's Associate Referee report on that subject, be studied further.

(11) That the study of the analysis of cereal adjuncts be continued.

(12) That methods for alpha- and beta-amylase be studied.

(13) That the tentative method for the determination of carbon dioxide in beer (**19,** p. 152) be further studied.

(14) That methods for essential oil in hops be studied.

(15) That study of methods for inorganic elements in beer be continued in cooperation with the Referees on Metals in Foods and on Iron in Cereals.

(16) That methods for soluble starches be studied.

WINES

It is recommended—

(1) That the revisions of Chapter **XV** of *Methods of Analysis, A.O.A.C.*, 1940, described in this year's report of the Referee on Alcoholic Beverages be adopted.

(2) That work on the spectrophotometric examination of wines be continued.

(3) That study of formol titrations be continued.

(4) That the Milos test as described in this year's report of the Associate Referee on Distilled Spirits be adopted as official for the detection of caramel in wine.

(5) That the tentative method for caramel (**XXI, 16 (f),** p. 252) be modified as described in this year's report of the Associate Referee on Distilled Spirits and adopted as tentative.

DISTILLED LIQUORS

It is recommended—

(1) That the following official methods be revised as described in this year's report of the Referee on Alcoholic Beverages and be classed as official (final action under suspension of the rules): **XVI, 6,** page 172—Extract; **9,** page 173—Fixed acids; **11,** page 173—Esters; **20,** page 175—Fusel Oil; **23,** page 176—Methanol (Modified Deniges Method).

(2) That the revisions in Chapter **XVI** of *Methods of Analysis, A.O.A.C.* 1940, proposed in this year's report of the Referee on Alcoholic Beverages be adopted.

(3) That the Marsh tests (**XVI, 35, 36,** p. 180) be modified by incor-

porating in them the cyclohexanol test as described in this year's report of the Associate Referee on Distilled Spirits.

(4) That the tentative method for caramel (**XXI, 16 (f),** p. 252) be modified as described in this year's report of the Associate Referee on Distilled Spirits and adopted as tentative.

(5) That the effect of the alcoholic content on *p*H of distilled liquors be further studied.

GENERAL RECOMMENDATION

It is recommended—

That the conclusions of the Committee on Alcohol Tables, A.O.A.C., 1944, be accepted as a basis for revision of the methods for alcohol in Chapters **XIV, XV,** and **XVI,** and of the alcohol tables in Chapter **XLIII.**

No report on malt was given by the Associate Referee.

No report on diastatic activity and alpha- and beta-amylase of malt was given by the Associate Referee.

No report on hops was given by the Associate Referee.

REPORT ON CEREAL ADJUNCTS

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

Cereal adjuncts used in the brewing industry are cereal products such as prepared flaked corn, prepared flaked rice, corn grits, corn meal, brewer's rice, refined grits, and refined flakes. Different methods for moisture and fat in cereal products occur in the chapters of *Methods of Analysis* relating to cereals and brewing materials. It seems evident that there should be no conflict or overlapping on these methods. This report aims to eliminate the possibility of confusion in the application of methods to cereal products and presents methods for these products used by the brewing industry. Since cereal products referred to above are commonly and usually known in the brewing industry as cereal adjuncts, it is recommended that the determinations on these products be under one group heading—"Cereal Adjuncts." The procedures used by the American Society of Brewing Chemists on sampling, preparation of sample, physical characteristics, and methods for the determination of moisture, oil, and extract were furnished by Stephen Laufer, of the Technical Com-

mittee and Editorial Board of the American Society of Brewing Chemists.

It is recommended* that the present tentative methods, **XIV, 50** to **57**, pp. 162–163, be dropped and the procedures for sampling, preparation of sample, physical characteristics, moisture, oil, extract, crude fat or ether extract, proteins, ash, and crude fiber be adopted as official, first action, and the studies continued.

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

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

The subcommittee on sugars and dextrins of the American Society of Brewing Chemists, under the chairmanship of Philip P. Gray, this year started collaborative testing of methods of analysis and presented its report at the May meeting of the A.S.B.C.[1] In accordance with the recommendations made last year,[2] four samples, consisting of three corn sirups, A, C, and D, and one malt sirup, B, were sent out to 12 collaborators, who began with collaborative testing of the modified Sichert and Bleyer method[3] for determination of dextrose in the presence of other reducing sugars. This method has been adopted by the Corn Industries Research Foundation and detailed instructions for use of this method were distributed to the collaborators. In addition, the collaborators were also asked to carry out the following determinations on the samples for check purposes: (a) Total reducing sugars by the Munson-Walker and Lane-Eynon methods; (b) Fermentable extract by the regular fermentation method; and (c) Fermentable extract by a rapid fermentation method proposed by the subcommittee on rapid fermentation of the A.S.B.C.

The results obtained on fermentable extract in these samples were discussed in the report of Philip P. Gray, Associate Referee on Fermentable Extract in Brewing Sugars and Sirups (see p. 441). The present report will deal only with the values obtained for dextrose and total reducing sugars.

Sample "A" was intended to represent an invert sirup, but, through an error, a corn sirup was substituted. Following the discovery of the error, collaborators were advised to disregard sample "A." This explains the limited number of results reported for this sample (Table 1).

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* For report of Subcommittee D and action by the Association, see *This Journal*, **28**, 63 (1945). Details of the methods will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.

[1] A.S.B.C. "Subcommittee Report on Sugars and Dextrins," May 1944.

[2] *This Journal*, **27**, 378 (1944).

[3] *Z. Anal. Chem.*, **107**, 328 (1936).

Table 1 presents this year's results for reducing sugars, and Table 2 summarizes the data by the Munson-Walker method for the past 5 years.

Only a few laboratories included results by the Lane-Eynon method. If some data are eliminated that deviate markedly from the rest (as indicated by*), the results show satisfactory agreement between laboratories by the Munson-Walker method and by the Lane-Eynon method as well. However, the results obtained by the Lane-Eynon method were somewhat

TABLE 1.—*Total reducing sugars*

COLLABORATOR NO.	A—CORN SIRUP		B—MALT SIRUP		C—CORN SIRUP		D—CORN SIRUP	
	REDUCING SUGARS AS DEXTROSE		REDUCING SUGARS AS MALTOSE		REDUCING SUGARS AS DEXTROSE		REDUCING SUGARS AS DEXTROSE	
	MUNSON & WALKER	LANE-EYNON	MUNSON & WALKER	LANE-EYNON	MUNSON & WALKER	LANE-EYNON	MUNSON & WALKER	LANE-EYNON
1	48.52		54.74		29.88		47.40	
2			53.20		27.51		46.15	
3			54.30	53.79	29.75	31.76	47.78	49.79
4			48.48*		26.78*		45.55	
5			54.48		29.98		49.29	
6	48.45	50.32	55.77	54.12	28.95	31.12	47.78	49.38
9			55.91		28.8		47.5	
10	48.7		46.2*		25.7*		44.2*	
11	49.8		57.1		29.8		49.0	
12				53.5		31.7		49.8
Number Reporting	4	1	9	3	9	3	9	3
High	49.8		57.1	54.12	29.98	31.76	49.29	49.8
Low	48.45		46.2	53.5	25.70	31.12	44.20	49.38
Average	48.87		53.35	53.8	28.79	31.5	47.18	49.7
Average Deviation			2.7		1.2		1.3	

* Exclusive of data which differ markedly from the rest.

higher than those by the Munson-Walker method, particularly for corn sirups. For statistical evaluation of the data obtained for 5 years, all results, including the highest and the lowest, were employed (Table 2). In general, it can be stated that the results show fairly satisfactory agreement for the Munson-Walker method. It is interesting to observe that this year's data, including all results, differs little from average deviation similarly calculated for previous years.

Table 3 presents the data obtained for dextrose by the modified Sichert and Bleyer method. The results show excellent agreement if some abnormal data are excluded. Further study of this method in collaborative testing is indicated.

TABLE 2.—*Total reducing sugars—Munson & Walker method. Summary of five years' collaborative data*

YEAR	SAMPLE		NO. OF COLLABORATORS	RESULTS			
	NO.	IDENTIFICATION		HIGH	LOW	AVERAGE	AVERAGE DEVIATION
1937	1	Sugar	9	76.60	63.72	68.56	2.2
	2	Sugar	9	40.5	27.44	36.96	2.3
	3	Sugar	9	90.36	77.24	80.81	2.4
1938	4	Sugar	10	82.44	73.20	79.34	2.4
	5	Sugar	11	82.40	72.30	79.15	1.0
	6	Sugar	11	93.20	82.60	89.98	2.0
	3	Sirup	10	31.20	25.02	28.06	1.2
	4	Sirup	8	39.86	36.50	37.95	0.9
1939	7	Sugar	11	40.30	34.48	38.11	1.2
	8	Sugar	12	72.60	67.45	69.15	1.1
	5	Sirup	12	52.10	44.30	49.55	1.3
	6	Sirup	12	26.90	22.60	24.68	1.0
1940	9	Sugar	14	101.7	87.0	97.1	3.9
	7	Sirup	12	38.3	29.0	34.9	2.5
1944	A	Corn sirup	4	49.8	48.5	48.9	
	B	Malt sirup	9	57.1	46.2	53.4	2.7
	C	Corn sirup	9	30.0	25.7	28.8	1.2
	D	Corn sirup	9	49.3	44.2	47.2	1.3

TABLE 3.—*Dextrose by modified Sichert and Bleyer method*

COLLABORATOR NO.	A—CORN SIRUP	B—MALT SIRUP	C—CORN SIRUP	D—CORN SIRUP
1	27.80	8.00	13.55	24.58*
2		10.2		33.0
3	29.70	8.32	14.83	31.55
4			15.23	34.50
5		9.1	15.6	33.8
6	29.8		14.8	32.9
9			16.2	32.2
11		10.6	15.3	33.5
12		10.6	15.0	33.2
No. Reporting	3	6	8	9
High	29.8	10.6	16.2	34.5
Low	27.8	8.0	13.6	24.6
Average	29.1	9.5	15.1	32.1
Average Deviation		1.0	0.7	1.8

RECOMMENDATIONS*

The Munson-Walker and Lane-Eynon methods are recommended for

* For report of Subcommittee D and action by the Association, see *This Journal*, 28, 64 (1945). The methods will be published in the 6th edition, *Methods of Analysis, A.O.A.C.*, 1945.

adoption as tentative for determination of reducing sugars in brewing sugars and sirups, as given in Chapter **XXXIV** of the *A.O.A.C. Methods of Analysis* (1940).

The following additional methods of analysis of brewing sugars and sirups, which have been in use by brewing chemists for a number of years and appeared in the 1944 edition of the *A.S.B.C. Methods of Analysis*, are recommended for adoption as tentative: (*a*) Diastatic Power (for malt sirups only); (b) Iodine Reaction (as in beer); (c) Acidity and *p*H (as in beer); and (d) Ash (as in Chapter **XXXIV**).

In order to avoid discrepancy with Chapter **XXXIV**, Sugars and Sugar Products, it is recommended to change the subtitle "Moisture" of the present method for brewing sugars and sirups[4] to "Non-Extract (Apparent Water)." Chapter **XXXIV** provides for determination of solids in sugar products, while the brewer is mainly interested in the extract that he can obtain from such products. Therefore, the methods of analysis of brewing sugars and sirups necessarily include the procedure for determination of extract. Extract subtracted from 100 can be labeled as "Non-Extract (Apparent Water)." If other determinations, such as solids or moisture, are required, the analyst is referred to Chapter **XXXIV**.

Slight changes are recommended in the present method for Extract, Non-Extract, Fermentable Extract, and Protein. Thus, the paragraph for Extract should be numbered "1," and the paragraph for Non-Extract should be numbered "2."

The paragraph under Fermentable Extract should be divided into two sections. The first section is to be labeled "(a) Regular Fermentation Method," which is the present method. Another section is to be included, labeled "(b) Rapid Fermentation Method," as recommended in Philip P. Gray's report.

Paragraph 4 under Protein is also to be subdivided into two sections. Section (a) will be the present method, while section (b) will give reference to determination of nitrogen as directed in Chapter **XXXIV**.

For convenience, the whole chapter dealing with the methods of analysis of brewing sugars and sirups, including the former methods as well as the new methods which are being proposed, has been re-edited and rewritten for presentation in the revised *Methods of Analysis*, 6th edition.

Further collaborative studies will be conducted on brewing sugars and sirups, including the ferricyanide procedure for reducing sugars and various differential fermentation methods for determining individual sugars in the presence of each other.

WORT ANALYSIS

Methods for analysis of wort are well established in the brewing industry and have been in use by brewing chemists for many years. They are

[4] *This Journal*, **27**, 84 (1944).

similar to methods used for analysis of beer and are based chiefly on cross-references to these methods.

The following methods are recommended* for adoption as tentative methods for analysis of wort:

Preparation of Sample; Extract of Specific Gravity; Fermentable Extract; Iodine Reaction; Total Acidity; H-Ion Concentration; Color; Protein; Total Reducing Sugars.

These methods have been adopted by the A.S.B.C. and appeared in their 1944 edition of *Methods of Analysis*.

SPENT GRAINS

For testing the efficiency of mashing operations, the brewer is interested in knowing the extract that is retained by the grains in soluble form after the last spargings have gone through them. This extract is known as "soluble extract." In addition, the brewer also wants to know the residual insoluble extract which remains in the spent grains and which could be made available by efficient mashing in the presence of malt enzymes. This extract is labeled "available extract."

The soluble extract can be estimated on the wet grains by pressing out the wort from an adequate portion of the grains and determining the extract on the filtrate. Another method is to mash a portion of the wet grains with water and then to determine the extract on the filtrate. The latter method can also be applied to dry spent grains. For determination of available extract a portion of either wet or dry grains is mashed with malt and water and the extract is determined on the resulting wort.

The methods used for making these determinations, known as brewers' analysis of spent grains, are well established among brewing chemists. In order to unify and standardize these methods, a subcommittee was appointed by the American Society of Brewing Chemists in 1941, headed by the writer. The subcommittee compiled the methods and distributed them to the collaborators. It was decided that each of the collaborators would collect his own samples and carry out the tests by the proposed methods and then submit his data and comments to the chairman. Five collaborators sent in their results on seven samples, which are presented in Table 4. It will be noted that the results for soluble extract obtained on the same sample show good agreement whether carried out on the wet or on the dry grains, either by the mashing method or by the pressure method. Samples Nos. 6 and 7 were analyzed independently by two collaborators and the results for soluble extract when made by different methods, as well as those for available extract, indicate good agreement. The methods for brewers' analysis of spent grains are recommended for adoption as tentative.*

* For report of Subcommittee D and action by the Association, see *This Journal*, **28**, 63 (1945). The methods will be published in the 6th edition, *Methods of Analysis, A.O.A.C.*, 1945.

TABLE 4.—*Collaborative analysis of spent grains*

COLLABORATOR NO.:	I		II		III		IV		V
SAMPLE NO.:	1	2	3	4	5	6	6	7	7
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Wet grains									
Preliminary Moisture:			76.00	77.03			75.00	76.30	
Total Moisture:	78.1	80.6	76.71	77.45	77.4	77.7	76.48	78.68	
Soluble Extract—									
Mashing Method									
as is	0.21	0.34			1.06	1.8	1.62	0.86	
dry basis	0.98	1.77			4.6	8.0	6.88	4.03	
Pressure Method									
as is	0.21	0.29	0.12	0.25	1.08	1.7	1.73		0.71
dry basis	0.98	1.50	0.51	1.10	4.8	7.7	7.35		3.32
Dried Grains									
Moisture in dried grains			3.10	1.81	4.65	7.0	2.30	2.05	
Soluble Extract—									
in wet grains, as is	0.21	0.33	0.12	0.24	1.04	1.58	1.51	0.80	
in wet grains, dry basis	0.98	1.69	0.51	1.04	4.6	7.1	6.42	3.77	
Available Extract—									
in wet grains, as is	1.80	1.57	0.25	0.33	2.23	2.45	2.33	1.25	
in wet grains, dry basis	8.24	8.24	1.08	1.48	9.9	11.0	9.91	5.87	

When a feed analysis is required, the analyst is referred to Chapter XXVII.

YEAST ANALYSIS

In the last few years, great interest was aroused for conservation of brewers' yeast and for production of primary yeast, owing to the fact that yeast represents an important foodstuff and feed material because of its high content in protein, vitamins (B-complex), and minerals. The development of methods for analysis of this material appears to be in order. A number of laboratories and institutions started working along these lines, among them the A.S.B.C., which appointed a subcommittee on Yeast Examination Methods—Analytical, headed by Fred P. Wilcox of the Wahl-Henius Institute, Chicago, Illinois. This subcommittee started collaborative work on methods for determination of total solids, yeast solids, minerals, protein, and vitamins. The results of this collaborative work on total solids in liquid yeast and pressed yeast appear to be quite satisfactory, and a preliminary report on it will be submitted in due course. In the meantime, the method for determination of total solids in liquid and pressed yeast, as developed by this subcommittee of the A.S.B.C., is recommended for adoption as tentative.* It has been adopted by the A.S.B.C. and appeared in their 1944 edition of *Methods of Analysis*.

REPORT ON FERMENTABLE EXTRACT IN BREWING SUGARS AND SIRUPS

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

At the last meeting of the Association, the American Society of Brewing Chemists' method for determination of fermentable extract in brewing sugars and sirups was adopted as tentative.[1] The writer early this year, as chairman of an A.S.B.C. subcommittee on this subject, carried out further collaborative studies on 3 samples of corn sirup and a sample of malt sirup. Eleven collaborators took part in this work and the results, as taken from the report this year to the A.S.B.C.,[2] are given in Table 1.

Especially with a biological method of this type where much depends upon the use of an active yeast and maintenance of favorable conditions, it is fair, in judging the method, to exclude from consideration the few results which are found to deviate markedly from the rest. If this is done and the starred items are eliminated, the range for the remaining laboratories is quite small.

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* For report of Subcommittee D and action by the Association, see *This Journal*, 28, 64 (1945). The methods will be published in the 6th edition, *Methods of Analysis, A.O.A.C.*, 1945.

[1] *This Journal*, 27, 84 (1944).

[2] Presented at the Annual Meeting of the A.S.B.C., May 1944.

The determination of fermentable extract in brewing sugars and sirups has been the subject of extensive collaborative study by the A.S.B.C for a number of years. A summary of 5 years' collaborative data, including that obtained in the present year's investigation, is shown in Table 2, also taken from the above report.

Comparing over-all variations over a 5-year period, average deviations calculated with no data excluded show consistent uniformity in the last 4 years and are of the same order of magnitude as encountered in similar collaborative studies of other widely accepted methods.

There has been in the past some criticism of this method, particularly

TABLE 1.—*Fermentable extract in brewing sugars and sirups, 1944 collaborative analyses*

(Results shown as per cent)

COLLABORATOR NO.	A—CORN SIRUP	B—MALT SIRUP	C—CORN SIRUP	D—CORN SIRUP
1	56.59	50.42	29.37	48.52
2	—	48.5	27.4	46.8
3	52.25*	47.94	29.12	46.33
4	—	47.79	28.46	48.68
5	—	47.75	27.38	47.46
6	58.70	48.51	29.67	48.90
7	57.8	47.0	29.2	49.0
8	—	41.8*	25.4*	43.3*
10	49.9*	42.1*	25.8*	43.6*
11	59.1	49.9	31.4	51.1
12	—	50.1	29.8	50.1
No. Reporting	6	11	11	11
High	59.1	50.42	31.4	51.1
Low	49.9	41.8	25.4	43.3
Average	55.77	47.44	28.45	47.62
Average Deviation	3.1	2.1	1.4	1.9

as applied to corn sirups, by various corn industries' laboratories. However, as would be expected, more satisfactory results have been obtained as greater familiarity with the method has been acquired. Private communications from Dr. Ralph W. Kerr of the Corn Products Refining Company and Dr. George T. Peckham, Jr., of the Clinton Company indicated that satisfactory results were being obtained with this method in their laboratories.

In view of the satisfactory results that have been obtained in the rather extensive series of collaborative studies that have been carried out with this method, it is recommended that the procedure for determination of fermentable extract in brewing sugars and sirups be made official, first action, and incorporated in the forthcoming edition of the *Methods of Analysis*.

The present method requires a fermentation time of at least 48 hours.

A rapid method, employing a larger amount of yeast and reducing the fermentation time to four or five hours, has recently been subjected to collaborative study by another subcommittee of the A.S.B.C. under the chairmanship of Claude F. Davis. The data presented in Mr. Davis' report[3] are given in the attached Table 3. Where nutrients were added in the case of refined sugars and sirups, as called for by the 48-hour procedure,

TABLE 2.—*Fermentable extract in brewing sugars and sirups. Summary of five years' collaborative data*

YEAR	SAMPLE	NUMBER OF COLLABORATORS	RESULTS—PER CENT			
			HIGH	LOW	AVERAGE	AVERAGE DEVIATION
1937	Corn Sugar	9	80.00	65.86	72.96	4.6
	Corn Sugar	9	44.28	25.90	36.76	4.3
	Corn Sugar	9	82.56	72.18	79.22	2.4
1938	Corn Sugar	8	82.88	74.00	79.03	1.9
	Corn Sugar	8	83.63	73.40	79.35	2.4
	Corn Sugar	8	96.30	83.57	92.74	3.6
	Corn Sirup	8	37.56	27.80	34.26	1.6
	Invert Sirup	8	90.09	80.60	87.53	2.2
1939	Corn Sugar	12	40.90	35.60	38.48	1.2
	Corn Sugar	11	67.90	59.60	64.04	1.6
	Corn Sirup	11	51.60	47.33	49.58	1.3
	Corn Sirup	12	28.80	23.00	25.04	1.7
1940	Corn Sugar	14	97.5	84.0	94.9	2.3
	Corn Sirup	14	42.6	31.1	36.3	2.3
1944	Corn Sirup	6	59.1	49.9	55.8	3.1
	Malt Sirup	11	50.4	41.8	47.4	2.1
	Corn Sirup	11	31.4	25.4	28.5	1.4
	Corn Sirup	11	51.1	43.3	47.6	1.9

excellent agreement was secured as evidenced by the results shown in the table. The rapid method, so modified, has been adopted by the A.S.B.C. In view of the advantage of the shorter time required, it is recommended that the rapid fermentation method as an alternate procedure be adopted as tentative.*

The Associate Referee wishes to express his appreciation to the following who collaborated in these analyses: B. H. Nissen, C. W. Neubauer, George T. Peckham, Jr., Max N. Landauer, Christian Weaver, J. H. Bauch, H. W. Rohde, Stephen Laufer, Kurt Becker, P. J. F. Weber, Fred Wilcox.

[3] *Ibid.*

* For report of Subcommittee D and action by the Association, see *This Journal*, 28, 75 (1945). Details of the method will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.

TABLE 3.—*Fermentable extract in brewing sugars and sirups, rapid fermentation method, 1944 collaborative analyses*
(Results shown as per cent)

COLLABORATOR NO.	WITHOUT NUTRIENTS				WITH NUTRIENTS			
	A CORN SIRUP	B MALT IRUP	C CORN SIRUP	D CORN SIRUP	A CORN SIRUP	B MALT SIRUP	C CORN SIRUP	D CORN SIRUP.
1	50.66	48.03	24.01	42.76	56.24	47.61	27.68	48.02
3	—	—	—	—	57.35	47.52	28.14	50.51
4	—	46.76	—	—	—	—	27.32	47.13
5	—	47.95	28.66	41.28	—	—	—	—
6	—	47.06	—	—	56.22	—	27.28	48.12
7	—	46.9	—	—	56.8	—	29.4	49.1
8	—	42.4*	26.4	43.0	—	—	—	—
10	48.4	46.8	19.1*	34.4*	—	—	—	—
Number Reporting	2	7	4	4	4	2	5	5
High	—	48.03	28.66	43.0	57.35	—	29.4	50.51
Low	—	42.4	19.1	34.4	56.22	—	27.28	47.13
Average	49.53	46.56	24.54	40.36	56.65	47.57	27.96	48.58
Average Deviation	—	1.2	—	—	—	—	0.6	1.0
Average by 48 hour method from Table 1	—	47.44	—	—	55.77	—	28.45	47.62

* Samples are identical with similarly marked samples shown in Table 1.

REPORT ON BEER

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

For a number of years the Associate Referee on Beer had submitted samples of beer for examination together with mimeographed copies of methods. Later, this work was limited to the determination of certain ingredients. The results of these studies were presented before this Association.

During the past two years the American Society of Brewing Chemists through its Technical Committee appointed a number of subcommittees and assigned special work to these. At the annual meeting of this Society reports were presented and discussed. Either final action was taken by the Technical Committee for the adoption of the methods of examination, or suggestions were made for continuation of the work. The Associate Referee on Beer thought it inadvisable to continue sending samples to collaborators, as this would mean a duplication of work. The President of the American Society of Brewing Chemists, with a membership now over 300, urged a closer cooperation with the Association of Official Agricultural Chemists, so that in future all reports from referees on subjects pertaining to the brewing and malting industries are first to be sent to the Chairman of the Technical Committee, A.S.B.C., before these are submitted for presentation, an action highly recommendable.

The Associate Referee on Beer at this time wishes to give a brief outline of the investigations made by the members of the A.S.B.C. during the past year, limiting himself to beer only, and omitting such investigation as would fall beyond the scope of the "Methods of Analysis" of the Association.

During the latter part of September 1944 the Associate Referee sent his report and recommendation for the chapter on beer assigned to him, to W. F. Reindollar, Chairman of the Committee on Recommendations of Referees, A.O.A.C. The manuscript for the fifth edition of *Methods of Analysis*, published in 1940, was in charge of Dr. J. A. LeClerc and the Associate Referee assisted him in adding entirely new paragraphs on malt, unmalted cereals, and rice. At that time such subjects as sirups and extracts were not ready for publication. Among the investigations which have a bearing on this chapter the following may be mentioned:

Under the able chairmanship of Philip P. Gray, the subcommittee on the accuracy of the formula for calculating the original gravity of beer, conducted collaborative work in which 9 collaborators participated. The differences between the calculated and the determined values were ± 0.1 percent for ales and ± 0.14 percent for beers. The conclusions derived from this study as submitted by the committee are these:

"In spite of a number of factors that limit the accuracy in the applica-

tion of the formula, *i.e.*, composition of the wort, point at which the sample is collected, and extent to which it is truly representative of the total brew variations in sampling procedure, evaporation during fermentation, variations in growth and removal of yeast, design of fermenting and storage equipment and practice of blending brews, it is believed that the formula is essentially correct and will give excellent checks with actually determined original extract of the wort in most cases, certainly within 0.2 percent. An individual correction factor may be devised to take care of variations caused by a particular set of conditions where there is a consistent difference. Based on the results obtained, there appears to be no reason to recommend any change in the formula at this time."

The subcommittee on "Methods for determining total acidity and *p*H" under the chairmanship of Kurt Becker began their work in 1942. Among the phases of work considered, attention was given to the decarbonation of beer in vacuum, decarbonation by shaking in an open bottle and filtration and the effects on dilution. The committee mentions that in regard to the concentration of the phenolphthalein indicator for titration of beer, greater amounts of the indicator should be used than given in the present method. Improvements may also be attained by removing the last traces of carbon dioxide from the sample by washing the beer with air at room temperature. The subcommittee completed its work this year with the result that new, revised methods for the determination of total acidity and *p*H are recommended by the Associate Referee, Kurt Becker, in his report.[1]

The subcommittee on "Turbidity and Color in Beer and Wort," under the chairmanship of B. H. Nissen, continued its investigation begun two years ago and extended its work to photoelectric methods.[2] It has been previously observed that like numbers of Lovibond Color Slides Series 52, universally used in laboratories, vary in color intensity as photometric comparisons have shown, a fact which not infrequently led to disputes. Some colored solution standards have the disadvantage of fading in the course of time and require replacement. Another disadvantage is that these are not always of the same shade as the sample under examination.

Efforts were made to develop a practical method for the determination of turbidity of beer and the development of a workable color procedure to replace the Lovibond when necessary. Difficulties were met when trying to obtain necessary parts for the electrometric color and turbidity apparatus. Visual turbidity determinations using Fuller's earth and standardizing with the Jackson Candle Turbidimeter had been previously reported.[1] Inability to obtain imported Fuller's earth called for other substitutes. In photometric measurements slight differences in the curves of

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[1] *This Journal*, **27** 379 (1944).
[2] *Ibid.*, 389.

various turbidities occurring in beer were observed. Materials, especially those which remained in suspension for long periods, were subjected to electrometric study. Charts were studied of standardized suspensions and their intermediate dilutions. Some of the charts indicate the relative suitability of such materials, while others do not. Pear's Precipitated Fuller's Earth had been a previous standard but it is not so suitable for beer turbidity as for waters. It was found that household Bon Ami and Merck's Colloidal Kaolin are very satisfactory using the Jackson Candle Method, the mixture being colored by Standard Brand dyes. The haziness of beer samples is there matched in wide-mouth bottles against a black border on a white background with a set of prepared standards. In place of the Brand dye solution, 0.01 *N* iodine may be used. Saturated solutions of mercuric chloride are added as a preservative.

A detailed report on "Turbidity and Color in Beer and Wort" is presented by Associate Referee B. H. Nissen. (See p. 462.)

During the past few years the subcommittee on Carbon-Dioxide determination under the present chairmanship of Irwin Stone has begun work. A year ago a trial pressure-temperature chart was compiled and distributed to interested persons to serve as a comparison for brewery cellar temperatures and data obtained in the laboratory. Collaborative work had been planned for the present year in order to determine whether the chart gave better agreement among the carbon dioxide results obtained at different temperatures and to establish any further corrections which might be needed. However, present conditions prevented carrying out adequate tests.

The subcommittee on Rapid Fermentation Methods under the chairmanship of Claude F. Davis submitted sirups and beers to 7 collaborators. The committee found that the rapid fermentation method is satisfactory providing nutrient salts are added to corn sirups.[3] When applied to beer the calculated original gravity of the beer after fermentation showed a tendency to be somewhat too high in both one and two hours' fermentation periods. Suggestions were made to continue the work on beer using larger samples.

The above brief résumé of reports are confined to the subject of beer as reported by the different committees to the Technical Committee of the American Society of Brewing Chemists during its last two sessions. The Associate Referee on Beer had previously made different suggestions for the sixth edition of the *Methods of Analysis, A.O.A.C.*

[3] *Am. Soc. of Brew. Chem.*, Reports of the Technical Committees, May, 1945.

REPORT ON ACIDITY AND *p*H OF BEER

By KURT BECKER (J. E. Siebel Sons' Company, Chicago, Ill.), *Associate Referee*

Guided by previous studies of basic factors influencing the determination of *p*H and total acidity of beer and related products and materials, and by the results of preliminary collaborative analyses[1], a subcommittee of American Society of Brewing Chemists carried out additional collaborative work on beer and wort during the spring of 1944.[2] Specific instructions were issued covering the analytical procedures to be followed, which were essentially those governing the 1943 collaborative work, with some modifications. Emphasis was placed on strict adherence to all details. For potentiometric determinations, crystalline, C. P. grade acid potassium phthalate was again distributed, with directions for the preparation of fresh 0.05 *M* solutions to be used for the standardization of *p*H meters and *p*H 4.00, and 20–22°C.

The Associate Referee is greatly indebted to the following group of collaborators, comprising members of both the A.O.A.C. and A.S.B.C., for their splendid cooperation:

O. R. Alexander, American Can Co., Maywood, Ill.
Philip P. Gray, Wallerstein Laboratories, New York.
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B. H. Nissen, Anheuser-Busch, Inc., St. Louis, Mo.
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I. R. Sipherd, National Distiller Products Corp., New York.
Peter J. F. Weber, E. A. Siebel & Company, Chicago.
Philipp Weisz, Jacob Ruppert Brewery, New York.
Fred A. Wilcox, Wahl-Henius Institute, Chicago.

PREPARATION OF SAMPLES

Determinations of *p*H and total acidity were carried out on undiluted as well as diluted samples of wort and beer. The procedures followed in the preparation of these samples are briefly indicated in the following. Identifying letters "A" to "D" are used to facilitate reference to the various tables.

"A"—Beer, Undiluted.—The beer was decarbonated, at room temperature, by shaking in a suitable Erlenmeyer or wide mouth bottle about 12 times at 5-minute intervals, releasing the pressure after each shaking. After this, the beer was filtered through paper.

"B"—Beer, Diluted.—25 ml of the beer decarbonated as per "A" were pipetted into 250 ml of boiling distilled water. Heating was continued for 1 minute, after which the beaker was removed from the source of heat, its

[1] Kurt Becker, "Report on *p*H and Acidity of Beer," *This Journal*, 27, 379 (1944).
[2] American Society of Brewing Chemists, Subcommittee on Acidity and *p*H, Report of May 1944 (mimeographed).

TABLE 1.—*pH of beer and wort*

	ELECTROMETRICALLY				COLORIMETRICALLY			
	BEER		WORT		BEER		WORT	
Preparation of Sample:	*A*	*B*	*C*	*D*	*A*	*B*	*C*	*D*
Dilution—Sample:	100%	10%	100%	10%	100%	10%	100%	10%
Water:	0	90	0	90	0	90	0	90
Collaborator No. 1	4.12	4.32	—	—	—	—	—	—
2	4.13	4.33	5.87	6.16	—	—	—	—
3	4.18	4.34	5.89	6.12	—	—	—	—
4	4.17	4.35	5.78	6.02	4.1	—	—	—
5	4.20	4.45	5.85	(5.75)	4.2	(4.6)	5.9	5.3
6	4.30	4.50	5.73	(6.40)	3.9	3.9	(5.4)	5.5
7	4.16	4.31	5.90	6.20	4.1	4.2	5.9	6.1
8	4.12	4.31	—	—	4.1	4.2	—	—
9	—	—	—	—	4.1	—	—	—
10	4.10	4.22	(5.60)	5.90	4.1	4.1	5.7	5.9
11	4.15	4.20	5.89	6.00	4.1	4.1	5.9	6.1
12	4.15	4.39	5.83	6.11	—	—	—	—
Average	4.16	4.34	5.84	6.09	4.1	4.1	5.9	5.8
Standard Deviation	0.05	0.09	0.06	0.11	0.1	0.1	0.1	0.3
	1.3%	2.1%	1.0%	1.8%	2.3%	2.3%	1.7%	5.2%
Maximum minus Minimum	0.20	0.30	0.17	0.30	0.3	0.3	0.2	0.8

Potentiometric Equipment used by Collaborators

Collaborator No. 1..... Coleman 3A
2..... Coleman 4C & Cambridge Inst. Co. Electron Ray
3, 4, 10, 12 Beckman G
5..... Coleman, Style 200
6..... Beckman, Model 6
7, 8..... Coleman 3C
11..... Leeds & Northrup, #7660

All collaborators used glass electrodes and calomel reference electrodes.

Potentiometers were standardized against 0.05 Molar potassium acid phthalate.

Colorimetric Equipment used by Collaborators

"Taylor" and "R & B" sets; Bromocresol Green for *p*H 3.8–5.4; Chlorphenol Red for *p*H 5.2–6.8.

Results in parentheses have not been considered in computing the tabulated summaries.

contents stirred for 30 seconds and then cooled rapidly. The prepared sample represents, approximately, a 10 per cent dilution.

"*C*"—*Wort, Undiluted*—The filtered wort was used without further preparation.

"D"—Wort, Diluted.—25 ml of the filtered wort were pipetted into 250 ml of boiling distilled water, following the procedure described above under "B." (While such "boiling" preparation may seem superfluous in the case of uncarbonated wort, it eliminates the disturbing influence of carbon dioxide possibly present in unboiled distilled water.)

H-ION CONCENTRATION—(*p*H)

Electrometric as well as colorimetric procedures—essentially identical with the methods subsequently described in this report—were followed for the determination of *p*H. The results are presented in Table 1.

Considering the complex nature of the extract of beer and wort, very good agreement is shown by the *p*H values determined electrometrically

TABLE 2.—*Total acidity of beer and wort by phenolphthalein titration*

	BEER		WORT	
	ML OF N/10 NaOH PER 25 ML SAMPLE	*p*H AT END-POINT*	ML OF N/10 NaOH PER 25 ML SAMPLE	*p*H AT END-POINT*
Preparation of Sample:	*B*		*D*	
Dilution—Sample:	10%		10%	
Water:	90		90	
Collaborator No. 1	3.60	8.15	—	—
2	3.39	8.13	(3.25)	8.33
3	3.40	8.20	2.45	8.04
4	3.40	8.32	2.60	8.16
5	3.35	8.40	2.75	8.30
6	3.40	(8.89)	2.50	(8.80)
7	3.65	8.16	2.65	8.42
8	3.70	8.09	—	—
9	3.40	8.10	—	—
10	(4.05)	(7.73)	2.75	(7.83)
11	3.35	8.15	2.85	8.22
12	3.50	(8.57)	2.70	8.40
13	3.60	8.22	—	—
Average	3.47	8.19	2.67	8.29
Standard Deviation	0.13	0.10	0.14	0.14
	3.6%	1.2%	5.1%	1.7%
Maximum minus Minimum	0.35	0.31	0.40	0.38

* Determined electrometrically.

on the undiluted samples. To some degree, this also holds true of the results obtained colorimetrically.

The *p*H values of diluted samples were included merely for the purpose of affording comparisons with the *p*H values observed in the course of titrations of total acidity; there was no intention to suggest that beer or wort should be diluted prior to determining *p*H.

In view of these results, the A.S.B.C. has adopted a revised method for the determination of *p*H.*

TABLE 3.—*Potentiometric titrations to pH 7.8*

ALL RESULTS AS ML STD. NaOH PER 25 ML EQUALIZED SAMPLE	BEER				WORT			
	A—UNDILUTED		B—DILUTED		C—UNDILUTED		D—DILUTED	
	N/10	N/100	N/10	N/100	N/10	N/100	N/10	N/100
Collaborator No. 1	3.35	29.8	3.12	36.3	—	—	—	—
2	3.17	30.9	3.31	31.2	2.57	24.7	2.36	23.6
3	3.12	32.4	3.14	33.5	2.24	23.3	2.30	25.7
4	3.12	31.3	3.13	30.8	2.21	21.5	2.12	21.0
5	3.00	28.9	2.90	30.5	2.25	23.8	2.06	20.0
7	3.24	30.9	3.19	32.5	2.35	22.8	2.12	22.2
8	3.24	31.9	3.46	35.9	—	—	—	—
10	(5.06)	(48.6)	(4.58)	(42.4)	(2.72)	23.9	(2.57)	26.0
11	3.12	31.2	3.25	32.4	2.56	22.8	2.45	23.0
12	3.13	31.4	3.04	30.5	2.28	21.5	2.10	20.6
Average	3.17	31.0	3.17	32.6	2.35	23.0	2.22	22.8
Standard Deviation	0.10	1.1	0.16	2.21	0.15	1.1	0.15	2.3
	3.2%	3.4%	5.0%	6.8%	6.5%	5.0%	6.8%	9.9%
Maximum minus Minimum	0.35	3.5	0.56	5.8	0.36	3.2	0.39	6.0

TOTAL ACIDITY BY INDICATOR TITRATION

The results of determinations of total acidity in the prepared samples of beer and wort, using phenolphthalein as the indicator and following the method described below, are presented in Table 2.

Strict adherence to all details of the method led to very satisfactory agreement of results, the "standard deviations" being only about one-half of those calculated for the 1943 collaborative work. Consequently,

* With slight abbreviation to conform to usual *A.O.A.C.* style the revised method will be inserted in the 6th edition of *Methods of Analysis*, 1945.

the A.S.B.C. has adopted a revised method for the determination of total acidity of beer by indicator titration.[3] In providing for two alternatives in the reporting of results, namely as "percent lactic acid" or as "ml 1.0 *N* alkali per 100 g of beer," concession was made to traditional practice on the one hand, while, on the other, the fact was recognized that the complexities of acidity of beer are better described by stating the amount of normal alkali required to neutralize 100 g of beer than by implying that lactic acid is the principal component of that acidity.

TABLE 4.—*Potentiometric titrations to pH 8.2*

ALL RESULTS AS ML STD. NaOH PER 25 ML EQUALIZED SAMPLE	BEER				WORT			
	A—UNDILUTED		B—DILUTED		C—UNDILUTED		D—DILUTED	
	N/10	N/100	N/10	N/100	N/10	N/100	N/10	N/100
Collaborator No. 1	3.58	32.0	3.40	38.0	—	—	—	—
2	3.42	33.3	3.54	33.6	2.59	29.4	2.90	28.6
3	3.33	34.6	3.36	36.0	2.60	27.4	2.70	30.8
4	3.35	33.4	3.37	33.1	2.57	25.5	2.53	25.3
5	3.20	30.8	3.20	33.5	2.53	29.5	2.43	25.0
7	3.59	33.2	3.38	34.8	2.77	26.8	2.60	26.0
8	3.47	34.4	3.70	38.5	—	—	—	—
10	(5.60)	(55.1)	(5.17)	(45.1)	(3.32)	(31.4)	(3.22)	(32.2)
11	3.35	34.6	3.46	35.7	2.98	26.5	2.89	27.1
12	3.36	33.5	3.15	33.0	2.67	25.4	2.55	24.9
Average	3.41	33.3	3.40	35.1	2.72	27.2	2.66	26.8
Standard Deviation	0.13	1.3	0.165	2.1	0.18	1.7	0.18	2.2
	3.7%	3.8%	4.9%	5.9%	6.6%	6.2%	6.8%	8.2%
Maximum minus Minimum	0.39	3.8	0.50	5.5	0.45	4.1	0.47	5.9
Average, Titration with Phenolphthalein	3.47				2.67			

TOTAL ACIDITY BY POTENTIOMETRIC TITRATION

In the endeavor to eliminate the personal factor involved in the judging of colorimetric endpoints, a great deal of the 1944 collaboration centered around potentiometric titrations. The feasibility of determining the total acidity in beer by potentiometric titrations was investigated along two approaches, namely, titrations to selected *p*H levels, and determinations of points of inflection.

[3] *Am. Soc. of. Brew. Chem.* Reports of the Technical Committees, May, 1945.

Tables 3, 4, and 5 show the results of potentiometric titrations of beer and wort to 3 selected *p*H levels of 7.8, 8.0, and 8.2, respectively. Generally speaking, the degree of correlation is approximately of the same order as that of the phenolphthalein titrations (Table 2). Samples of undiluted beer and wort show somewhat better agreement than diluted samples. There is insufficient evidence in these data to justify the specifying of 0.01 *N* alkali, which had been mentioned in the literature as conducive to greater accuracy, but which is more difficult to standardize and may magnify distortions due to dilution effects.

TABLE 5.—*Potentiometric titrations to pH 8.6*

ALL RESULTS AS ML STD. NaOH PER 25 ML EQUALIZED SAMPLE	BEER				WORT			
	A—UNDILUTED		B—DILUTED		C—UNDILUTED		D—DILUTED	
	N/10	N/100	N/10	N/100	N/10	N/100	N/10	N/100
Collaborator No. 1	3.70	34.3	3.65	40.9	—	—	—	—
2	3.69	35.7	3.80	36.1	3.55	34.8	3.43	34.3
3	3.57	37.5	3.56	39.5	3.05	31.9	3.14	36.8
4	3.61	35.8	3.59	35.7	3.03	30.4	3.05	30.4
5	3.45	32.5	3.40	36.8	2.95	34.0	2.80	29.5
7	3.91	36.5	3.75	38.3	3.36	31.3	—	34.6
8	3.75	37.4	(4.08)	41.0	—	—	—	—
10	(6.25)	(61.8)	(5.86)	(51.3)	(4.24)	(40.4)	(4.02)	(41.2)
11	3.55	35.8	3.76	38.2	3.59	30.6	3.34	31.1
12	3.61	35.8	3.26	35.3	3.14	29.8	3.05	29.8
Average	3.65	35.7	3.60	38.0	3.24	31.8	3.14	32.4
Standard Deviation	0.13	1.5	0.19	2.2	0.26	1.9	0.23	2.9
	3.6%	4.3%	5.2%	5.7%	8.1%	6.0%	7.2%	8.8%
Maximum minus Minimum	0.46	5.0	0.54	5.7	0.64	5.0	0.63	7.3

Investigations by one of the collaborating laboratories appeared to reveal that the time consumed for the completion of potentiometric titrations is a factor which cannot be overlooked entirely.

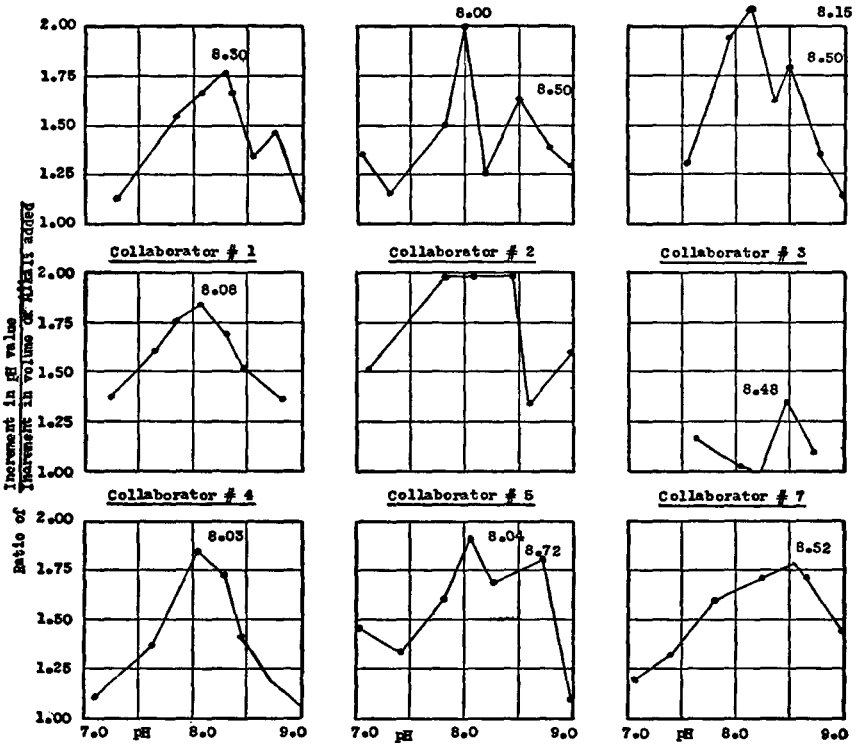
After carefully weighing the potentiometric data, the A.S.B.C. adopted an alternative method for the determination of total acidity in beer, selecting 8.2 as the *p*H level corresponding to the "end point."*

POINTS OF INFLECTION

In connection with the second phase of the potentiometric work, namely that concerned with locating points of inflections in the quest for "end

* See footnote, p. 451.

TABLE 6.—*Comparison of "Points of Inflection" determined by plotting the "differential curves" corresponding to the potentiometric titrations by 9 collaborators of the same sample of undiluted beer with 0.1 N NaOH.*



Collaborator:	1	2	3	4	7	8	11	12	Ave.	Max.-Min.
pH at Point of Inflection:	8.30	8.00 (8.50)	8.15 (8.50)	8.08	8.48	8.03	8.04 (8.72)	8.52	8.20	0.52

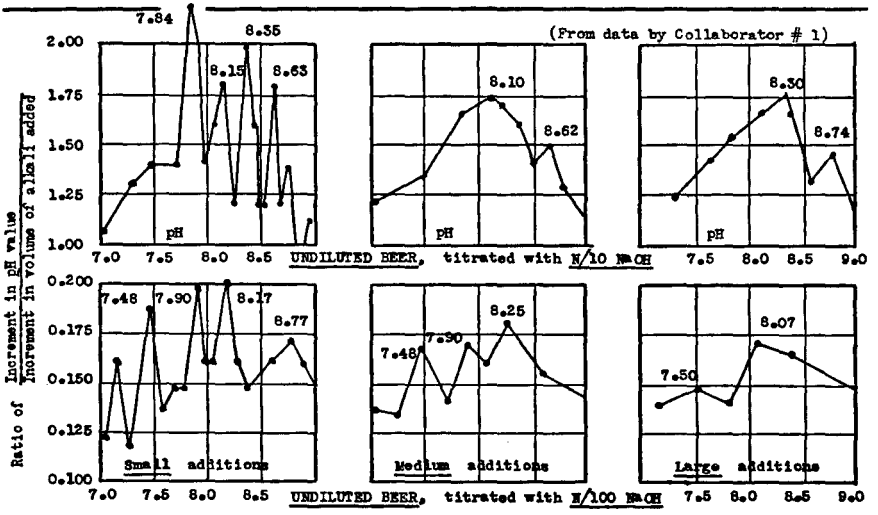
points" not subject to personal factors of evaluation, it must be emphasized that the well-known significance of potentiometric curves as expressions of the buffering properties of beer was not under investigation. As already stated, the object was simply to explore the possibilities of points of inflections as means toward more accurate and precise determinations of total acidity.

No time was lost in plotting primary potentiometric curves, because the known flatness of such curves for beer in the critical range of *p*H 7 to 9 does not make it possible to locate changes of curvature, *i.e.*, points of inflection, with any degree of accuracy. In all cases, therefore, differential curves were constructed from the original titration data furnished by the

collaborators. Because of the wealth of such data obtained in this year's work, references in this report had to be restricted to typical examples and generalized interpretations.

In Table 6, the "best," meaning least ambiguous, differential curves which could be constructed from the data of 9 collaborators, who titrated the same sample of undiluted beer with 0.1 *N* alkali, are presented. By simple inspection, the lack of agreement as to number and location of points of inflection is at once apparent. The range of *p*H 8.00 and 8.52 covered by the points of inflection in the 9 curves is entirely unsatisfactory.

TABLE 7.—*Typical effects of size of alkali-additions on points of inflection as illustrated by "differential curves" for different alkali increments in the potentiometric titrations of one sample by one experienced analyst.*



Incidentally, this work also served to demonstrate that minor factors of technic may be of far reaching consequences, again because of the peculiar flatness of the primary potentiometric beer curve. For example, Table 7 visualizes graphically how the number and location of points of inflection may be affected by traversing the *p*H span from 7.0 to 9.0 by many small or few relatively large additions of alkali, respectively, the curves plotted being based upon the data of one single, highly experienced analyst working with one sample of beer.

In summary, there was little promise in the foregoing results that greater accuracy in the determination of total acidity of beer could be achieved by resorting to the determination of points of inflection in connection with potentiometric titrations.

RECOMMENDATIONS*

It is recommended—

(1) That the tentative method for the determination of total acidity of beer (*Methods of Analysis, A.O.A.C.*, 1940, Chapter **XIV, 152, 10**) be dropped.

(2) That the method for the determination of total acidity of beer by indicator titration presented in the report of the Associate Referee on *p*H and Acidity of Beer be adopted as tentative.

(3) That the alternative method for the determination of total acidity of beer by potentiometric titration to *p*H 8.2 presented in this year's report of the Associate Referee in *p*H and Acidity of Beer be adopted as tentative.

(4) That the tentative method for the determination of H-ion concentration of beer (*Methods of Analysis, A.O.A.C.*, Chapter **XIV, 155, 28**) be dropped.

(5) That the electrometric method for the determination of *p*H of beer presented in this year's report of the Associate Referee on *p*H and Acidity of Beer be adopted as tentative.

(6) That the alternative colorimetric method for the determination of *p*H of beer presented in this year's report of the Associate Referee be adopted as tentative.

(7) That the data presented in this report be considered as sufficient basis for applying—with the necessary editorial changes—the methods referred to above under (2), (3), (5) and (6), also to wort and related materials or products.

REPORT ON INORGANIC ELEMENTS IN BEER

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

Collaborative work on methods for the determination of inorganic elements in beer has not been carried out since 1941, at which time reports were submitted by L. E. Clifcorn (2) (3) on the determination of iron and copper.

In 1940 and 1941, approximately 12 collaborators participated in the iron work, which involved wet ashing a 25 gm. sample with nitric and perchloric acids, after which the red thiocyanate color was developed in an acid methyl-cellosolve medium. In 1941, Clifcorn recommended this procedure be adopted as tentative.

Methods for copper in beer have been investigated in 1941, when Clif-

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* For report of Subcommittee D and action by the Association, see *This Journal*, 28, 64 (1945). The methods will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.

corn (3) reported on the potassium ethyl xanthate, and the dithizone-sodium diethyldithiocarbamate method of Greenleaf (4). The latter method was applied both with and without Greenleaf's provision for interference from cobalt, nickel, and bismuth. The collaborators' comments on the xanthate method, a procedure involving the addition of potassium ethyl xanthate to an almost neutral solution of the dry ashed sample, were conspicuous by their reference to an objectionable turbidity which interfered seriously with visual comparison in Nessler tubes. The dithizone-carbamate procedures gave satisfactory results, although the procedure involving the separation of bismuth, nickel, and cobalt gave somewhat higher results, in spite of the fact that lower results would be expected when interference from these elements was removed. A number of collaborators commenting on the dithizone-carbamate procedure expressed a hope that the procedure could be simplified.

No work has been done in the past on the determination of tin, the third and only other element which was considered.

IRON

Reference to the literature relative to the determination of small quantities of iron cannot be made without noticing the trend away from the use of potassium thiocyanate. In most cases, the change has been made to organic reagents and of these, the use of two similar reagents 2,2′-bipyridyl (6) and o-phenanthroline (5) predominate, although thioglycolate (8) is worthy of consideration.

In view of the fact that the potassium thiocyanate-methyl-cellosolve method in its present form is quite satisfactory, and since no improvements have been introduced during the past two years, it was not submitted for further work this year. However, in recognition of the trend to use reagents other than potassium thiocyanate, the following questionnaire was sent to the collaborators who had participated in the previous work.

1. Approximately how many determinations of iron in beer do you and your associates make each year?
2. What is the minimum accuracy that you will be satisfied with for a referee method?

   0.05 ppm
   0.10
   0.20
   0.30
   0.50
   1.0

3. If there were a tedious but reliable method available and accurate to 0.05 ppm, would it be advantageous to you to have a rapid method, but accurate to only 0.3 ppm?
4. Briefly, what procedure are you using now for determining iron in beer?
5. Are you satisfied with this method?
6. Do you prefer wet or dry ashing?

7. About what length of time does it take to conduct a single analysis by your present method? (Omit the time during which the analyst can do other work, as for example, dry ashing. Also omit time required to prepare reagents.)
8. Other comments.

Response to the questionnaire was not complete, but sufficient data were obtained to draw the following conclusions:

1. As many as 3000 iron determinations per year are made by some laboratories.
2. An accuracy of 0.05 to 0.1 ppm is desired, although some collaborators would be satisfied with 0.3 ppm.
3. Potassium thiocyanate is used by about one-half of those replying to the questionnaire.
4. Wet ashing is preferred to dry ashing.
5. 30 to 40 determinations per day per individual will be expected of a method if it is to become a popular, as well as a referee procedure.

COPPER

A survey of the literature reveals that sodium-diethyldithiocarbamate, (7) dithizone (1), or combinations of the two (4), are most commonly used for the determination of copper in small amounts. The combination dithizone-carbamate procedure was used in the 1941 collaborative work on beer, and also by Greenleaf (4) for collaborative work on foods in general. Greenleaf's procedure adequately provides for cobalt, nickel, and bismuth, which, if present, interfere with the carbamate method. Greenleaf, therefore, separates copper from these elements by extracting with dithizone at *p*H of 2, after which the dithizone complex is destroyed and the copper determined with carbamate. Bendix and Grabenstetter (1) have shown that the dithizone extraction which is used by Greenleaf to isolate copper can be used for the final determination, thereby making it an all-dithizone method.

The all-dithizone procedure of Bendix and Grabenstetter is used extensively by the powdered milk industry, and it has been suggested for collaborative work by Wichmann (9). For this reason, it seemed desirable to submit it to collaborators for the determination of copper in beer. Three laboratories, all of which had previous experience with the method for copper in milk, were selected to analyze 2 samples of beer, according to the procedure given below:

PROCEDURE

REAGENTS

Water redistilled from Pyrex should be used for all dilutions. C.P. nitric acid should be redistilled from Pyrex. All solutions should be stored in Pyrex containers.

Glassware should be cleaned with concentrated nitric acid and rinsed first with tap water, then with water redistilled from Pyrex.

Copper standard, 1 microgram of copper per ml.—Accurately weigh a 0.5000 gram sample of electrolytic sheet copper and treat with 20 ml. of 6 *N* HNO_3. Evaporate solution almost to dryness and then add 2–3 drops of glacial $HC_2H_3O_2$. Transfer soln quantitatively to a 500 ml. volumetric flask. From this stock solution, a working standard of 1 microgram of Cu per ml. should be prepared immediately before use.

Sulfuric acid, 10%.—Dilute 1 volume of conc. C.P. acid to 10 volumes.

Cresol red, 0.02 gram per 100 ml. of H_2O.

Buffer solution, pH 2.3.—Dissolve 38 g. of C.P. citric acid and 21 g. of C.P. $Na_2HPO_4 \cdot 12H_2O$ in redistilled water. Purify by shaking with a conc. soln of dithizone in CCl_4. Wash out the excess dithizone with clear CCl_4, discard the washings, and dilute the aqueous layer with redistilled water to 250 ml. When 2 ml. of this solution are added to 25 ml. of unbuffered soln, the soln is buffered at a *p*H of 2.3.

Dithizone solution.—Dissolve 15 mg. of pure dithizone in 1 liter of C.P. CCl_4.

Potassium iodide solution, 2%.—Dissolve 10 g. of C.P. KI in 450 ml. of water. Acidify with 5 ml. of 1 *N* HCl. Discharge the color caused by the presence of free I with 0.1 *N* $Na_2S_2O_3$ soln added dropwise. Shake in a separatory funnel with successive 10-ml. portions of dithizone solution until no discoloration of the dithizone occurs. Discard the CCl_4 extract and wash the aqueous layer with clear CCl_4. Dilute the aqueous soln to 500 ml. with redistilled water. Check the KI soln frequently (once a day) with dithizone solution for the presence of free I, and if necessary add more $Na_2S_2O_3$ soln. Since the KI soln may not be checked often enough to detect traces of free I, Greenleaf suggests adding some reducing agent such as H_3PO_2 or NaH_2PO to the KI soln.

Ammonium hydroxide, 1 to 200.—Dilute 1 volume of conc. C.P. NH_4OH to 200 volumes with redistilled water.

Ash a 20 g. sample of beer by any conventional wet ashing method. Dilute digestion mixture to 100 ml. Pipet suitable aliquot of sample soln into 150-ml. separatory funnel. If volume is less than 25 ml., make up to that volume with 10% H_2SO_4. Add 2 drops of cresol red soln and bring contents of flask to yellow color of indicator range with conc. NH_4OH. Add 2 ml. of buffer soln (*p*H 2.3) and exactly 10 ml. of dithizone soln. Shake for 10 minutes. Transfer CCl_4 layer to clean separatory funnel and add 10 ml. of acidic KI soln. Shake for 2 min. Transfer CCl_4 layer to another separatory funnel and add 25 ml. of 1 to 200 NH_4OH. Shake for 2 min. Determine transmittancy in the spectrophotometer at 520 millimicrons with blank at 100% transmission.

RESULTS

The results obtained by the three collaborating laboratories are given in Table 1. Table 2 shows results obtained by the same three laboratories on 5 samples of dried milk powder, which were not included in the A.O.A.C. work, but which furnish additional information on the all-dithizone procedure.

DISCUSSION

Iron.—Analysts who have the task of selecting a referee method for determining iron in beer will ultimately select a reagent which will produce a stable color. At the same time they will prefer the most sensitive reagent possible since increased sensitivity will permit the use of smaller samples, thereby decreasing the time needed for wet ashing. Orthophenanthroline or 2,2′ bipyridyl appear best suited to meet these two prerequisites. Where the accuracy of a referee method is not required, it may be possible to omit the ashing entirely and work directly with the degassed beer (1).

Copper.—The results in Table 1 are quite satisfactory, although Lab. 2

consistently obtained lower results than did the others. In Sample A, the difference on a percentage basis is appreciable. It should be mentioned that the actual amount of copper extracted in the case of Sample A was approximately one microgram, which is about one-fourth the amount most desirable for the dithizone method as used. It is evident that in the

TABLE 1.—*P.p.m. of copper in beer*

SAMPLE	LAB. NO. 1*	LAB. NO. 1**	LAB. NO. 2*	LAB. NO. 3**
A	0.20	0.38	0.11	0.26
	0.24	0.21	0.06	0.17
	0.17		0.08	
			0.07	
			0.05	
			0.06	
B	1.7	1.8	1.5	1.7
	1.6	1.8	1.4	1.7
	1.6	1.7	1.5	
			1.5	
			1.5	
			1.5	

* Ashed with H_2SO_4, HNO_3 and $HClO_4$.
** Ashed with H_2SO_4 and 30% H_2O_2.

Lab. No. 1—Army Service Forces—Q.M.C. Subsistence Research and Development Laboratory, Chicago, Ill.
Lab. No. 2—American Dried Milk Institute, Chicago, Ill.
Lab. No. 3—Continental Can Co., Inc., Chicago, Ill.

TABLE 2.—*P.p.m. copper in dried milk*

SAMPLE	LAB. NO. 1	LAB. NO. 2	LAB. NO. 3
1	0.45	0.65	0.64–0.64
2	0.75	0.75	0.88–0.88
3	1.0	0.70	1.12–1.04
4	5.9	5.1	6.40
5	19.3	19.7	19.2 –21.3

future the procedure should specify taking an aliquot which will contain a larger amount of copper.

The procedure is believed to be specific for copper except for relatively large amounts of bismuth, mercury, and some of the noble metals. If these are absent, as they most likely are in beer, the potassium iodide washing may be omitted. The Associate Referee's laboratory obtained one result with the potassium iodide wash omitted, and found a value of 1.7, which is in good agreement with the values in Table 1 for Sample B.

For beer, the direct dithizone procedure seems to offer no pronounced advantage over the direct carbamate method used in 1941. Dithizone procedures, however, have some general advantages over carbamate methods (1) dithizone is used with acid rather than neutral or slightly alkaline solutions, thus avoiding the precipitation of the hydroxides or phosphates of certain elements; (2) the red dithizonate is more readily compared visually by most individuals than is the yellow carbamate; (3) the sensitivity is slightly greater than that of the carbamate.

For a more detailed discussion of the properties and applications of dithizone and diethyldithiocarbamate, reference may be made to the excellent reference book on trace analysis by E. B. Sandell (10).

RECOMMENDATIONS*

1. It is recommended that the thioglycolate and o-phenanthroline methods be compared with the tentative methylcellosolve procedure.
2. That further work be done on the direct carbamate method and the all dithizone method with and without provision for interference from bismuth, cobalt, nickel, and the noble metals.

ACKNOWLEDGMENT

The Associate Referee is grateful for suggestions and assistance of D. Grabenstetter and N. H. Strodtz, of Continental Can Company, Inc.; J. B. Thompson of QMC, Subsistence Research and Development Laboratory; and C. M. O'Malley of the American Dried Milk Institute.

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* For report of Subcommittee D and action by the Association, see *This Journal*, 28, 64 (1945).

REPORT ON COLOR AND TURBIDITY IN BEER AND WORT

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

During the past year the Associate Referee has continued to serve as chairman for both the Color and Turbidity Committees for the American Society of Brewing Chemists. The work during this year has been directed to the study of practical methods for turbidity of beer, and the development of two workable color procedures to replace the Lovibond; one the use of electrometric colorimeters, and the other, standard color solutions.

Electrometric methods for both color and turbidity have been discussed in detail in earlier reports.[1,2] A simplified photoelectric circuit of low cost has been considered for this colorimetric work, and it was hoped that equipment of this kind could be made available for this year. However, there was difficulty in securing photoelectric cells and measuring instruments.

Some of the commercially available photometric equipment has been investigated and the new Cenco Sheard Sanford Photelometer, Type B-2, has been tested. The latter utilizes short flat-bottom half-inch test tubes; and it is quite similar to the older model but is more compact and much less expensive. By means of this instrument a standard light absorption curve can readily be prepared by which the comparable Lovibond colors can be secured. After an instrument has once been standardized in this manner it can easily be used for the determination of color of any clear beer. Other similar photoelectric colorimeters are now being examined.

Since, however, such instruments are not generally available, it was decided to also consider and develop satisfactory visual color methods as a substitute for the present Lovibond, especially where such instruments or slides are no longer available. Furthermore, a simple, easily manipulated method for turbidity and color would be of advantage where the expense of electrometric equipment is not warranted.

In pursuing the literature of turbidity methods, a report on the measurement of turbidity in sugar juices was found. In preference to other standards, this method of utilizing resuspended press cake materials was submitted as suitable redispersion material for turbidity standards. This suggested a similar study on beer turbidity. Samples of cloudy beer sediment were concentrated, and after evaporation to dryness, complete redispersion was attempted, but proved difficult and unreliable. Accordingly, an investigation of other materials more closely resembling beer turbidities was suggested.

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[1] *Am. Soc. Brew. Chem.*, Subcommittee Report on Turbidity, Annual Meeting, 1943.
[2] Nissen and Petersen, *Am. Soc. Brew. Chem. Proceedings*, 1942, p. 77.

In selecting a more suitable turbidity standard, various types of suitable materials, especially those that tended to remain in suspension for long periods of time, were subjected to electrometric study. Suspensions of the various materials were first standardized by the recommended Jackson Candle Turbidimeter[3] to a definite value of 100 ppm. turbidity. Photoelectric determinations were then made of such standardized suspensions, as well as their intermediate dilutions. Several such curves were studied.

As a result of this study and other factors, such as ease of procurement, preparation, and life of suspension, two rather suitable materials have been selected. These are Merck's Colloidal Kaolin and a readily procurable mineral household polishing powder. The polishing powder seems to be a very interesting material for turbidity standards in that it is extremely fine, and when resting for several hours, only a small amount settles out, and even this very readily returns to colloidal suspensions. Fuller's earth samples must often be shaken quite vigorously for complete reincorporation. Furthermore exactly 0.2000 grams of this polishing powder suspended in one liter of distilled water reads 100 ppm. by the Jackson Candle Method.[3] This, of course, simplifies the preparation of the standards.

In all of this turbidity work, no definite standard is used. By definition, $\frac{1}{10}$ of a gram of any completely insoluble, finely divided material suspended in one liter of water could be the correct 100 ppm. standard. The difficulty, however, seems to be to find a material which reliably contains such degree of dispersion so that direct weighing alone can be used. The number of particles present in any unit weight of material may vary greatly, depending on its fineness and insolubility. Accordingly, it has become general practice to prepare stock solutions of turbidity standards slightly more concentrated than required, and then standardize back by means of some type of physical test such as the candle turbidimeter or other light ray comparison. The Jackson Candle Turbidimeter seems satisfactory for this purpose, but some error can result, especially where the operator's eyesight fails to distinctly observe the sharp outline of the candle. It is hoped that some more reliable standard may eventually be worked out and distributed, similar to the very convenient Bureau of Standard samples used for other tests.

Using the polishing powder as a dispersing medium, therefore, our preparation of the 100 ppm. turbidity stock solution can be given as follows: 0.2000 grams of polishing powder is mixed with one liter of distilled water. This mixture is then colored either by use of the Brand dyes referred to later, or less preferably by N/100 iodine, to the desired beer color, 2.5 to 3.5° Lovibond. If Merck's Colloidal Kaolin is used,

[3] Standard Methods of Water Analysis, *Am. Publ. Health Assoc., 8th Edition*, 1936, p. 7

0.0650 grams is mixed with one liter of water to obtain the 100 ppm. standard. Brand dye, however, must be used for this turbidity standard, since the iodine does not retain its natural color.

The haziness of the beer sample is then matched in wide mouth bottles against a black border on a white background, with a set of standards prepared by diluting the above 100 ppm. mixtures to the following parts per million: 0, 1, 2, 4, 6, 8, 10, 12, 15, 20, 30, 40, and 50. To simulate the color of beer, the required amount of Brand dye solution is added to each bottle.

In lieu of Brand dye for the coloring of these turbidity solutions, 0.01 *N* iodine may be used.[4] This is prepared with crystalline iodine, potassium iodide, and distilled water; 5 to 6 ml. of this iodine solution added to 100 ml. turbidity standard gives a Lovibond color reading of approximately 3, using the $\frac{1}{2}''$ cell. The use of iodine, however, is limited, and its mixtures do not remain stable nearly as long as the Brand dye.

A saturated solution of mercuric chloride is added as a preservative, 5 ml. $HgCl_2$ solution per 100 ml. for each standard. The bottles used are square type, tall, 16 fluid ounce, wide mouth, clear glass sample bottles, $2\frac{1}{2}'' \times 2\frac{1}{2}''$ and $5\frac{1}{2}''$ to the shoulder. The bottles are well filled and closed with screw caps, paper lined (avoid metal discs).

In working with these types of turbidity standards, collaborators have obtained what might be considered quite close agreement, within 90 percent of the correct value. This is within the limit of accuracy suggested by the American Public Health Association for methods of this kind. In most cases, agreement is much closer than the 10% variation.

In the turbidity work, it was found that as a coloring material for the turbidity standards, the Brand dyes seem to be the most suitable and stable. In an effort to prepare a satisfactory substitute other than the photoelectric method for the Lovibond color glasses which are unavailable and, in many instances, apparently not uniform, colored solutions seem to be the most desirable.

There have been various types of inorganic salts[5] suggested for beer color comparison, and no doubt with careful preparation and accurate standardization, such solutions may prove very satisfactory for some beers. Some of these mixtures, notably the copper sulphate, cobalt sulphate, and ferric chloride combination have been tried out during recent months. It appears readily possible to prepare these inorganic color standards to match an individual beer color, but it was found somewhat more difficult to prepare a graduated color series, especially in the lighter ranges of the Lovibond scale.

Iodine standards have also been prepared and used in recent color work, but these fade out somewhat and therefore cannot be relied on too

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[4] Subcommittee Report on Color, *Am. Soc. Brew. Chem. Proceedings*, 1941, p. 106.
[5] U. S. Pharmacopoeia, Eleventh Decennial Revision, 1936, p. 557.

generally. Conclusions from these various studies suggest, as mentioned above, a preference for the Brand dye mixtures.

Several years ago when the study of suitable color standards was begun, an effort was made to secure the exact dyes referred to by Pawlowski[6] as required for the preparation of the so-called Brand dye. Efforts at that time resulted in a contact with the General Dyestuff Corporation, and samples of the closest resemblance to the imported dyes were obtained. Just recently contact with this source has been renewed, with the report that these dyes were still available and relatively inexpensive.

A stock solution of the Brand dye estimated at about 400 to 500 Lovibond can be prepared by dissolving the dyes in the following proportions:

0.04895 gram Patent Blue VF	0.12230 gram Resorcine Brown G
0.19600 gram Amaranth WD	0.78320 gram Tartrazine C Extra

These may be secured from the General Dyestuff Corporation, 731 Plymouth Court, Chicago, Illinois.

The above dyes are shaken in 20% ethyl alcohol in a liter flask and then diluted to one liter with 20% alcohol. It may be necessary to vary the concentrations of each dye slightly to approach exactly the color desired of any particular beer. Experiments in methods whereby the use of Brand dye standards might be more easily accomplished have been made. A series of dilutions of this stock solution resulted in the accompanying curve which represents the number of ml. of such stock solution diluted to 500 ml. of water for corresponding Lovibond values. By reference to this chart (Fig. 1), any desired color standards can readily be prepared.

For manipulation of these color standards, three different methods have been tried out. Two of these are modifications of the Pawlowski Brand Colorimeter and consist in preparing a set of uniformly shaped square bottles containing graduated amounts of the stock dye solution corresponding to the various Lovibond values. This can be easily accomplished by use of the chart on page 466. A sample of the beer for color estimation is degassed and then poured into a similar square bottle, whereafter the color can be ascertained by direct comparison with the bottled color standards, or the various colors may be poured into wide one-inch Nessler tubes and viewed in a block comparator.

A third method using the different colored solution standards, given above, is to make the comparisons by pouring the color standards and the beer into Lovibond cells in the standard Lovibond instrument, thus eliminating the color slides. The manipulation here is not quite as convenient, but the comparison of the colors is better. This could be combined with the first method, and serve as the final color comparison where greater accuracy is desired.

Another method suggested by Pawlowski is to arrange two beakers,

[6] Pawlowski-Doemens, "Die brautechnischen Untersuchungsmethoden," 5th Edition, 1938, p. 126.

one containing the wort or beer for color determination, and the other clear water. From a burette the N/10 iodine stock solution freshly prepared, or the equivalent Brand dye, is run in dropwise until a matching color is obtained. The varying colors are then expressed in the number of ml.

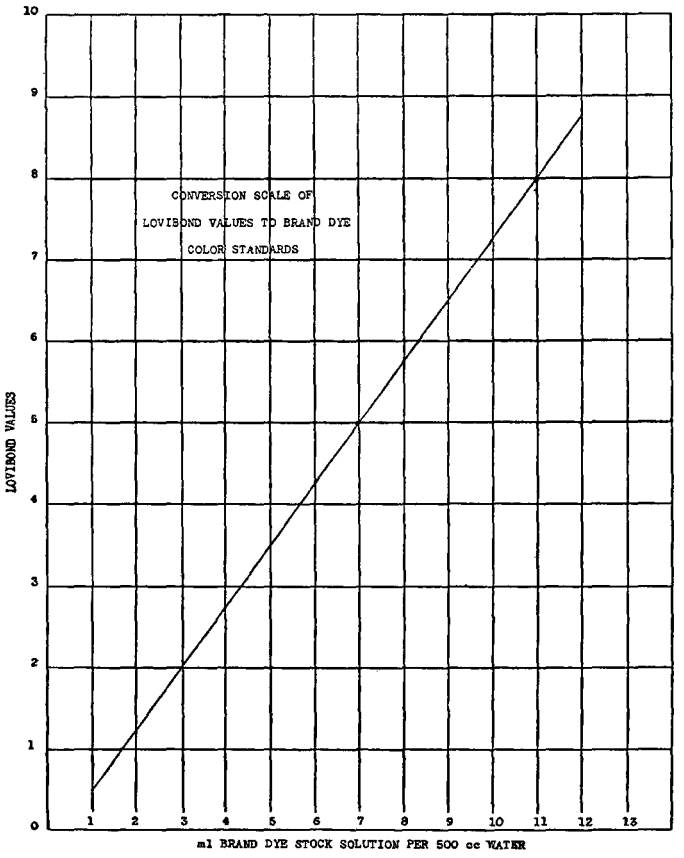

FIG. 1.—Conversion scale of Lovibond values to Brand dye color standards.

N/10 iodine solution. Other instruments and methods may no doubt suggest themselves for this color comparison.

Recently, an article by G. F. Beyer[7] entitled "Spectrophotometric Determination of Lovibond Numbers in Brown Lovibond Glass Series No. 52, Brewers Scale," corroborates our earlier findings that photoelectric methods can definitely replace the customary Lovibond procedure.

[7] *This Journal*, **26**, 164, 1943.

Of particular interest is the rather remarkable coincidence of linear relationship between the arbitrary Lovibond color numbers and Beyer's color transmittance values $(-\log T)10$. If this relationship holds generally for all series No. 52 Lovibond glasses, it should serve excellently as a reference method.

In the foregoing are outlined the most recent developments on methods for color and turbidity in beer and wort. The use of electrometric colorimeters has been suggested as one procedure for color in beer, while in addition, visual color standards are recommended where the electrometric instruments are not available. These visual methods consist mainly of the use of graduated amounts of previously prepared and standardized inorganic dye mixture. For turbidity a simple visual method is described.

The above methods are still under investigation and are currently being examined by collaborators.

No report on carbon dioxide in beer was made by the Associate Referee.

CARAMEL AND OTHER ARTIFICIAL COLORING MATTER IN ALCOHOLIC LIQUORS

By PETER VALAER, JR., Alcohol Tax Unit, Treasury Department, Washington, D. C., *Associate Referee*

Work was initiated in 1944 with view of including the Milos test for caramel (published in *Am. J. Pharm.* Vol. 114, No. 4, April 1942) as an A.O.A.C. procedure particularly for caramel in wines but to include other alcoholic liquors as well.

Effort is also being made to broaden the scope of this test, which has been applied quite successfully to wines of all kinds for the past several years, and also the present A.O.A.C. procedure to include beer, wines, and spirituous liquors as well.

It is hoped that the Milos test for caramel and the present A.O.A.C. method (5th ed., p. 252, modified as will be seen later) will be accepted eventually as official A.O.A.C. methods of analyses, as separate tests for caramel and as confirmatory tests for each other.

It is also the desire of the Associate Referee to introduce at this time for the consideration of the Association a new simple test for caramel and other coloring matter in spirituous liquor, in particular, and as a confirmatory test for caramel, in connection with the other tests already referred to above, and in the manner which will be described later.

This test was originally designed by Harlan L. Clapp, of the Alcohol Tax Unit's laboratory, Philadelphia, Pennsylvania, who prefers that we refer to it as the cyclohexanol test. The reagent used is applied in the

same manner as the Marsh test, and consists of

50 parts by volume of cyclohexanol
50 parts by volume of methyl-propyl-ketone
3 parts by volume of sirupy phosphoric acid (85%)
3 parts by volume of distilled water.

The following is a statement by Mr. Clapp to the Associate Referee, concerning his test:

Concerning Cyclohexanol Reagent for Artificial Color:

"The value of this reagent rests upon the greater solubility of 'quick-aged' color in it than takes place in either Marsh or Williams reagents when applied in a manner similar to those reagents.

"The methyl propyl ketone present is essentially a diluent to reduce cost and viscosity without affecting the color solubility in the cyclo-hexanol.

"Tests made have given conclusive evidence that if the yellow color observed in the lower layer from the Marsh or Williams reagent is due to uncharred oak or 'quick-age' color in the whiskey, the lower layer with the present reagent is in every case colorless.

"Considerable work has been done here on the partition of natural and artificial color with the various reagents. None effect an ideal color separation. Williams probably has the least solvent action on all colors, the presently discussed reagent has the greatest solvent action, while the Marsh reagent falls somewhere between. Tests with Schreiner's colorimeter on Marsh reagent indicate a part of the caramel color in the whiskey dissolves in the Marsh reagent while some of the natural color yet remains in the lower layer, while at the same time there appears to be some fading of the total amount of artificial color present. Qualitatively, tests for artificial color made with the present reagent appear to be more conclusive of the actual presence of caramel than those made with the other reagents. (This is indicated especially in the presence of certain old whiskies, also, in brandy. However, there is a possibility that color from uncharred wood may be involved here as in the 'quick-aged' whiskies.) The sensitivity of the test for caramel has not been satisfactorily determined and it is probable that traces of caramel may be present without appearing in the lower layer, but since traces of color in the lower layers of the Marsh and Williams tests should probably be disregarded the present test at least has the advantage of positive indications. Where it is desirable to compare, specifically, caramel concentrations (as for determining if rectification has occurred in addition to that originally permitted in the case of brandy) the present reagent will be of value as a supplement to the Williams and Marsh reagent."

The Associate Referee recommends that the Cyclohexanol test be included as supplementary to the present Marsh test,[1] in which the reagent sometimes indicates absence of caramel if the sample has been stored a very long time in charred or uncharred packages; or when there are present in the whiskey certain coal dyes or combinations of coal-tar dyes; or in whiskey that has been quick-aged with raw uncharred or toasted white oak chips.

Such conditions as are described may show positive reactions with the Marsh and Williams' reagents but are negative, as they should be, with

[1] *Methods of Analysis, A.O.A.C.*, 35, 180 (1940).

cyclohexanol reagent. These two tests, the Marsh and the Cyclohexanol, working together, would disclose the nature of coloring matter in the spirits in question. The aqueous color solution at the end of the tests obtained by the Milos procedures and the A.O.A.C. method with its present modification[2] may be further confirmed by the Marsh and cyclohexanol reagents, phenylhydrazine hydrochloride reagent and paraldehyde (p. 252 f.).

In order to make a beginning for this investigation and support any recommendations with the necessary collaborative results by as many chemists as possible, fairly large bottles of material were prepared and analyzed carefully before sets of six samples of each were sent out to 18 collaborators (all reporting except one).

These samples consisted of two samples of beer, two samples of red wine (blackberry), two samples of whiskey, the latter previously aged in wood. General results are as follows:

CONCLUSIONS AS TO THE PRESENCE OF CARAMEL

COLLABORATOR	1	2	3	4	5	6
Milos, New York	Neg.	Neg.	Neg.	Pos.	?	Pos.
Watson, Louisville, Ky.	Neg.	Neg.	Neg.	Pos.	Pos.	Pos.
Quillen, Baltimore, Md.	Neg.	Neg.	Neg.	Pos.	Pos.	Pos.
Fonner, Chicago, Ill.	Neg.	Pos.	Neg.	Pos.	Pos.	Pos.
Dale, Kansas City, Mo.	Neg.	Neg.	Neg.	Pos.	Pos.	Pos.
Nealon, Detroit, Mich.	Neg.	Neg.	Neg.	Pos.	Pos.	Pos.
Love, San Francisco, Cal.	Neg.	Neg.	Neg.	Pos.	trace	Pos.
Blakely, Philadelphia, Pa.	Neg.	Pos.	Neg.	Pos.	Pos.	Pos.
Holman, Atlanta, Ga.	Neg.	Neg.	Neg.	Pos.	Pos.	Neg.
Hoyt, New Orleans, La.	Neg.	Neg.	Neg.	Pos.	Pos.	Pos.
Ringstrom, Seattle, Wash.	Neg.	Neg.	Neg.	Pos.	Pos. Milos Neg. *A.O.A.C.*	Pos. *A.O.A.C.* Neg. Milos
Morawski, A. Boston, Mass.	Neg.	Neg.	Neg.	Pos.	Pos.	Pos.
Wilson, J. B., F. & D. Lab., Washington, D.C.	Neg.	Neg.	Neg.	Pos.	Pos.	Pos.
Young, J. L., Int. Rev., D.C.	Neg.	Neg.	Neg.	Pos.	Pos.	Pos.
Blaisdell, A. C., Int. Rev., D. C.	Neg.	Neg.	Neg.	Pos.	Pos.	Pos.
Valaer, Peter, Int. Rev., D.C.	Neg.	Neg.	Neg.	Pos.	Pos.	Pos.
Bunit Loren, Int. Rev., D.C.	Neg.	Neg.	Neg.	Pos.	Pos.	Pos.

[2] Will be described in *Methods of Analysis*, 6th edition, 1945.

COLLABORATORS' REPORTS

From the various collaborators (17 in number) there were obtained some very encouraging results. With a very few exceptions (see tabulated chart of results) practically all reported "no caramel" in the samples to which no caramel was added and almost all reported the presence of caramel in the samples to which caramel coloring had been added, by both the Milos and the present A.O.A.C. method (modified). This to the Associate Referee is quite encouraging, since to samples (excepting #1) were added dextrins, wood color from plain, charred, or toasted chips, and natural fruit colors, and only a minimum of caramel, for the purpose of making the samples in question more troublesome than those usually found on the market or in trade.

Among the cheaper wines, including, grape, fruit, and berry wines, there is often found added commercial caramel, which has been added to cover up imperfections, lack of color, or thinness of body due to extensive over-stretching of the fruit material. The reports contained, besides the conclusions as to the presence or absence of caramel, constructive suggestions for the improvement of the two methods and their confirmation tests. Several new tests were also suggested but not fully developed; while these reports convinced the Associate Referee of the value of the tests and their general applicability, it is not feasible to present them all, under the restrictions now in existence on printing materials. His recommendations follow:

RECOMMENDATIONS*

(a) That the Milos test be accepted as an official A.O.A.C. method for the qualitative determination of caramel coloring in wines.

(b) That the present A.O.A.C. method for caramel (p. 252, paragraph (f), 5th Ed.) be referred to as a confirmation test for Milos procedure, with the modification as outlined in the text of this report.

(c) That the Cyclohexanol test be included with the Marsh test to be conducted in the same manner, for the determination of caramel in distilled spirits.

(d) That reference be made in chapter XIV, Malt Beverages, Sirups, etc., including the analysis of beer, to the two caramel tests of **(a)** and **(b)**.

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* For report of Subcommittee D and action by the Association, see *This Journal*, 28, 65 (1945). The methods will be published in Chapters 15 and 16 of the 6th edition of *Methods of Analysis, A.O.A.C.* (1945).

REPORT ON SPECTROPHOTOMETRIC EXAMINATION OF WINES

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

This investigation was begun in order to find a differentiation between red grape wines and other red wines, especially blackberry, loganberry, raspberry, etc., and, perhaps, mixtures of grape and berry.

Most methods used heretofore, to distinguish between grape and berry wines, were based on the detection of tartaric acid, or any tartrate, since this is characteristic only of the grape. Thus, the presence of a tartrate generally indicated that the wine under examination was produced entirely, or at least partially, from grapes. However, some winemakers have fermented or stored berry wines in casks that previously contained grape wine, in which some potassium acid tartrate had caked to the bottom and walls. Thus the presence of the tartrate led to a false conclusion. Also, grape wines are produced from de-tartrated concentrates where the presence of the tartrate is somewhat difficult to detect, unless through a rather involved method requiring finesse on the part of the analyst. Grape wines are less expensive and are often substituted for the various berry wines, especially blackberry, as disclosed through the examination of a large number of wines labeled blackberry, purchased on the open market and taken from various bonded wineries. The conclusions reached in these cases resulted from finding tartrates in the wine and from chromatographic[1] tests indicating the presence of grape material. However, in a large number of samples no tartrates could be found, which gave a positive indication of grape material when the chromatographic test was applied. This situation often raised considerable doubt in the mind of the analyst examining the sample.

Therefore, in view of these conditions and circumstances, this laboratory made wine from some thirty-odd varieties of grapes obtained from California, New York, New Jersey, and Ohio, and from blackberries, both frozen and fresh, from various parts of the country, including the Far Northwest, which also furnished the loganberries. Wine was also made from elderberries, strawberries, and red and black raspberries. All of the wine was made strictly in accordance with U. S. Internal Revenue Regulations No. 7 pertaining to the production, fortification, tax payment, etc., of wine, for the purpose of setting up standards for the various products mentioned above. Some of the blackberry wine was ameliorated and sweetened with white cane sugar; some was ameliorated with brown cane sugar and sweetened with white cane sugar; while still others were ameliorated and sweetened with brown cane sugar. Some batches were made

[1] Hamill, Geo. K., and Simonds, Paul W., *This Journal*, **25**, 220–226 (1942).

containing potassium meta bisulfite, because it was known that some wineries occasionally used this chemical.

A complete chemical analysis was made of all batches, but this report deals only with the spectrophotometric examination (see Table 1).

Attention is called to the fact that the results obtained and set forth in this paper were based on new wines. It is a well-known fact that red wines lose an appreciable amount of their red pigment with age, and this loss bears a direct relation to the amount of color produced with hydrochloric acid. In fact, if permitted to age long enough no color at all is produced when the acid is added, but berry wines, in general, are not aged like grape wines but are sold soon after they are produced. Therefore, if some unscrupulous winemaker decided to use some red grape wine and label it blackberry, he would use one of recent production, since the color of the new wine would more nearly simulate the blackberry, in which case, the conclusions obtained from this investigation apply.

EXPERIMENTAL

All samples were filtered through a small filter (paper) until perfectly free from all suspended particles that might interfere with the transmission of light. The filter paper was then washed with 15% alcohol in order to extract as much of the color as possible and added to the main filtrate which was further diluted to a transmittance (T) of 25–35% in a 13 mm. cell, depending upon the color developed after the addition of hydrochloric acid. The preparation of the sample for the spectrophotometer, which was a Coleman D.M., Model 10-S, using principally a 15 mμ exit slit, was substantially as follows: After proper dilution was obtained, 10 ml. was placed in each of two graduated 10 ml. cylinders. Then 0.2 ml. of distilled water was added to one, while 0.2 ml. conc. hydrochloric acid was added to the other cylinder. The transmittance (T) was then measured at intervals of 15 to 20 mμ in the visible range of the spectrum from about 380–680 mμ, on the solution containing no acid, which takes approximately 30 minutes. By this time the acid solution will have developed a maximum amount of color, when the transmittance is measured in a similar manner. Percent transmittance (T) was then plotted as a function of wave length on regular coordinate paper, superimposing the data or curve obtained for the acid sample on the other. When this procedure was followed on all of the wines mentioned, it was noted that there was always a "hump" (minima) in the violet or blue-violet region of the spectrum of the (S-T) curve, except for the strawberry wines and those blackberry wines containing brown sugar, and for the Beacon, and one Ives grape wines. Whether this is characteristic of the Beacon grape is not known, since only one such wine was tested. The reason the Ives did not show usual curve was that some of the coloring matter had precipitated out of solution. It was also noted that the addition of hydrochloric acid

pushed this "hump" (minima) in the (S-T) curve farther into the violet end. The acid affected more of the grape wines than of the berry wines. However, whatever effect it did have, it was to shift the peak of absorption to the right or longer wave length in the green. A further examination of the transmittance curves showed that no consistent difference exists between the grape and berry wines, except where the acid and acid-free curves cross each other. It is noted that for all the red grape wines examined, except Pinot Noir, the curves cross at the right of the 590 mμ line, while for all red berry wines, they cross at the left of this line. Thus, the ratio of the transmittance factor (T) before, to (T) after the addition of hydrochloric acid at 590 mμ, is less than 1.0 for all red berry wines and greater than 1.0 for all red grape wines examined except Pinot Noir. The (S-T) curve for Pinot Noir wine made in the same manner as the others is entirely different from any berry or grape wine so far examined. And if this characteristic is consistent with this particular grape, it may be so identified. (See Table 1.)

Although the wines investigated might have been identified by the methods outlined in Hardy's "Handbook on Colorimetry" (1936), a great many involved and tedious calculations are necessary, as well as graphical plots. Therefore, this method of examination was not adopted.

Before the method used in this investigation was completed, other schemes were tried. Solvents such as normal butanol and a mixture of butanol and toluol were used in an effort to extract the color for further purification and examination. Some of the wine was dried with sand on a steam bath and subsequently extracted with absolute ethyl alcohol. The solvents from these various extracts were carefully evaporated; the residues were dissolved in 15% ethyl alcohol and the transmittance measured and plotted against wave length. The curves obtained were no more characteristic than those obtained on the untreated, diluted wine. However, extraction with these solvents indicated that more than one pigment or color was present, but the (S-T) curves failed to furnish any distinguishing characteristics other than those obtainable from the wine before extraction.

It has been known for some time that the intensity of the color, or perhaps more correctly the brightness of red wines, varies inversely with the *p*H of the solution—a property inherent in the wine itself. Since the hydrogen ion effects on the pigments of the wine are much the same as those on acid-base indicators, it was thought that perhaps an "isobestic" point could be obtained that would serve as the distinguishing feature between the two groups of wines, if not between the individual wines themselves. After experimenting with a number of different wines in both groups, it was found that the isohydric curves for the various *p*H solutions crossed at a definite wave length for some wines, while for others the crossing point varied with the *p*H of the solution.

TABLE 1.—*Consistent Differences in Spectrophotometric Data of Red Grape and Red Berry Wines as taken from their Respective (S-T) Curves. (A & C = approximate wave length of peak in "hump" (minima) of the curves. B & D = approximate wave length of maximum absorption (maxima). (X = approximate wave length of the crossing of the acid and acid-free curves. All wave lengths are in millimicrons (mμ).)*

GRAPE VARIETY OR TYPE	DILUTED WINE		DILUTED WINE +HCl		
	A	B	C	D	X
Alicanti	425–450	425	410	510	591
Alicanti raisin	440–450	415–430	410	530	597
Bailey	440–470	500	410	510	595
Beacon	—	—	395	515	593
Cabernet	410	515	395	515–525	595
Camay	410	500–510	395	510–520	595
Carignane	425–440	425–455	410	510–520	600
Clinton (hot pressed)	425	525	400	520	595
Clinton (made in lab.)	410–425	510	395	510–515	598
Concord (made in lab.)	440	520	400	520–525	603
Concord (hot pressed)	425	520	410	520–525	595
Concord (Quality Fruit Co.)	460	520	410	520–525	593
Concord (juice in lab.)	490–500	500	460	520	600
Concord (wine from above)	440	520	400	520	600
Eumelan	440–455	500–510	410	510–520	615
Fredonia (hot pressed)	425	520	410	520	595
Ives (hot pressed)	430	520	400	520	613
Ives (by Dorn)	—	—	425	520	593
Ives (made in lab.)	470	470–500	440	515	591
Ives (by Tomasello)	450–460	520	410–420	520	620
Norton (made in lab.)	425	515	395	515	605
Pinot Noir	395	455	395	510–520	582
Red Concentrate	440	500	400–410	520	593
Red Concentrate	470	515	410–420	525	593
Red Concentrate	425	500	400–410	520–525	593
Red Raisin (by Quality)	470	515	425	520–525	605
Zinfandel Raisin	485	515	440	530	597

BERRY VARIETY OR TYPE	DILUTED WINE		DILUTED WINE +HCl		
	A	B	C	D	X
Blackberry wine #15	425–440	510	395	510	583
Blackberry wine #16	—	—	425–470	500–510	585
Blackberry wine #17	455	500–510	410	510	588
Blackberry wine #18	455	500–510	425	510	580
Blackberry wine #19	455	510	425	510	585
Blackberry wine #20	455–470	500–510	410	510	583
Blackberry wine #21	470	500–510	425	510	575
Blackberry juice #22	440	500–510	395	500–510	587
Blackberry wine #22	—	—	470	500–510	575
Blackberry wine #23-A (contains K-Metabisulfite & ameliorated & sweetened with brown sugar)	—	—	440	510	580
Blackberry wine #23-B (contains no bisulfite—ameliorated with brown, but sweetened with white sugar)	470	510	425–530	510–515	580
Blackberry juice #24	455	515	395	515	588
Blackberry wine #24-B	480–485	500	410	510–515	570
Blackberry wine #24-C	470	500	410–425	515	568
Blackberry juice #21 from commercial winery	455	510	410	510–515	580
Blackberry juice #23 from Ga.	455	515	410	510–515	575
Blackberry wine made Dec. '43— ameliorated 75%	450–470	510	420–430	510–515	587
Blackberry wine from U. S. Horticultural berries	410	440–470	410	490–500	573
Blackberry wine from U. S. Horticultural berries (Eldorado)	410	440–470	410	500	570
Blackberry wine from wild Maryland berries	440–470	470–500	440	500–515	570
Blackberry juice from wild berries from nearby Maryland	440–460	510	395–410	510–515	587
Blackberry wine made by Chemist Holman, Ga.	—	—	440–470	500–515	563
Black raspberry wine	410	500–510	395	510	580
Elderberry wine (Lab.)	470	510	410	510	588
Elderberry wine from F. S. Lodge	470	530	410	510	583
Loganberry wine (1939)	410	500–515	410	515	580
Loganberry wine (1940)	425	500	410–425	515	582
Red raspberry wine (Hommel.)	430	510	410	515	583
Red raspberry wine (Lab.)	410	500–510	410	510–515	580
Strawberry wine (Dorsett)	—	—	440–460	490–500	580
Strawberry wine (Blakemore)	—	—	440–460	490–500	580

CONCLUSIONS

By using the spectrophotometer as outlined in this paper, red berry wines may be distinguished from red grape wines by calculating the ratio of the spectral transmittance at 59° mμ before and after the addition of hydrochloric acid. If this ratio be greater than 1.0, the wine is grape, and if less than 1.0 it is a berry wine. This holds for all wines examined except Pinot Noir. The crossing of the (S-T) curves also serves as a distinguishing feature.

No report on formol titrations was made by the Associate Referee.

No report on chromatographic absorption of wines was made by the Associate Referee.

REPORT ON *p*H IN DISTILLED ALCOHOLIC BEVERAGES

By M. ROSENBLATT (Schenley Research Institute Inc., Lawrenceburg, Ind.), *Associate Referee*

Continuing the work reported in 1943 (*This Journal*, **26**, 301), collaborative investigation was completed on certain features of *p*H determination which were discussed in the previous report.

Six laboratories kindly cooperated, and a set of 4 samples was sent to each laboratory with the request to determine the *p*H of the samples electrometrically. Not only was complete information concerning the standardizing buffer requested, but a standard buffer was included in the set of 4 samples.

TABLE 1.—*Description of samples*

CODE	TYPE	ALCOHOLIC CONTENT
		per cent by volume
A	Straight Whiskey	55
B	Standard Buffer (*p*H 4.00)	0
C	Spirit Blend	43
D	Straight Whiskey	43

Each collaborator was requested to follow the technic recommended by the manufacturer of the instrument used.

COLLABORATORS

The Associate Referee appreciates the cooperation of the following collaborators:

(1) M. Rosenblatt, Associate Referee
(2) J. Banks, Geo. T. Stagg Co., Frankfort, Ky.

(3) D. Dewar, Bernheim Distilling Co., Louisville, Ky.
(4) K. Becker, J. E. Siebel Son's Co., Inc., Chicago, Ill.
(5) A. C. Blaisdell, Treasury Dept., Washington, D. C.
(6) H. B. Dixon, War Food Administration, Washington, D. C.

DISCUSSION OF RESULTS

The data reported by the collaborators, together with the calculated averages and standard deviation for each sample, are shown in Table 2.

TABLE 2.—*Results of collaborative study on pH*

COLLABORATORS	SAMPLE A	SAMPLE B	SAMPLE C	SAMPLE D	INSTRUMENT
1	4.27	4.00	4.07	4.03	Coleman *p*H
	4.26	4.02	4.08	3.99	Meter Model
	4.24	3.99	4.07	4.01	4C
	4.25	4.01	4.07	4.05	
2	4.27	4.00	4.10	4.04	Coleman *p*H
	4.30	4.00	4.10	4.04	Meter Model
	4.35	4.00	4.14	4.05	3C
	4.35	4.00	4.10	4.04	
3	4.34	4.06	4.14	4.09	Coleman *p*H
	4.35	4.06	4.14	4.09	Meter Model
	4.31	4.05	4.10	4.07	3D
	4.37	4.06	4.14	4.09	
	4.35	4.09	4.13	4.10	
4	4.27	3.99	4.07	4.00	Coleman *p*H
	4.26	3.98	4.06	4.00	Meter Model
	4.27	3.99	4.04	3.98	3C
	4.28	3.98	4.08	4.00	
5	4.34	4.04	4.11	4.04	Leeds &
	4.34	4.04	4.12	4.04	Northrup
	4.34	4.04	4.11	4.05	Laboratory
	4.34	4.04	4.10	4.04	Model
	4.36	4.04	4.10	4.04	
	4.34	4.04	4.10	4.04	
	4.34	4.04	4.10	4.04	
	4.34	4.05	4.10	4.04	
6	4.32	4.00	4.08	4.03	Beckman *p*H
	4.37	4.00	4.10	4.03	Meter
	4.33	3.99	4.10	4.06	
	4.36	4.01	4.12	4.06	
	4.33	3.99	4.09	4.05	
	4.33	4.00	4.09	4.05	
Average	4.31	4.02	4.10	4.04	
Standard Deviation	0.04_6	0.02_8	0.02_3	0.02_8	

Particular emphasis was placed on the buffer used for standardizing the instrument, since the previous report (*loc. cit*) indicated that this solution may be a source of significant error. It is to be noted that the *p*H of the distributed buffer sample as determined in 5 of the 6 laboratories (1, 2, 4, 5, and 6) differed from the standard value (4.00) by an amount less than one standard deviation. It is even more striking that all of these 5 laboratories prepared the standardizing buffer in the same manner, which was also identical with the standard solution distributed: 10.209 g. of Bureau of Standards potassium acid phthalate were dissolved in 250 ml. of freshly boiled and cooled distilled water.

The sixth collaborator (3) obtained a significant variation on the distributed buffer sample (greater than two standard deviations) and, in this case, the standardizing buffer used was a prepared solution supplied by a chemical manufacturer. This confirms previous suspicions that such solutions are not as reliable as buffers prepared from a high-grade salt.

The standard deviation of the *p*H determination of whiskey as calculated from the data in Table 2 is in the neighborhood of 0.03. This deviation is about half of that found in the previous collaborative work and can probably be attributed to the use of an accurate standardizing buffer. The magnitude of the standard deviation indicates that the method in its present form is capable of yielding results on any given sample which should not spread more than about ± 0.06 *p*H unit.

The accuracy of the determination (deviation from the "true value") is dependent upon a reliable standardizing buffer and caution should be observed in the use of buffers prepared outside of one's own laboratory.

Samples A and D were identical whiskey except that, in the latter case, the alcoholic content was reduced by the addition of distilled water. A marked reduction in *p*H resulted.

This Associate Referee recommends* that collaborative work be continued to study the quantitative effect of alcoholic content on *p*H.

No report on wine was made by the Associate Referee.

No report on cordials and liqueurs was made by the Associate Referee.

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* For report of subcommittee D and action by the Association, see *This Journal*, 28, 65 (1945).

REPORT ON CACAO PRODUCTS

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

The work anticipated last year covered four subjects, namely (1) fat in chocolate beverage bases, etc.; (2) lecithin; (3) chocolate constituents; and, (4) shell content by means of pectic acid. Some work has been done on two of these subjects.

Fat in chocolate beverage bases and other refractory samples.—The Associate Referee on this subject made a comparative study of two methods of fat determination on a number of chocolate beverage bases, mainly chocolate malted milks; the methods were the Hillig method and a Roese-Gottlieb method which was modified by the Associate Referee. Results on the various samples are given in the report of the Associate Referee. Significantly higher results were obtained on all samples by the Hillig method.

Lecithin.—No work was done the last year on lecithin. The method reported in 1941 should be studied collaboratively.

Chocolate constituents.—No laboratory work was done on chocolate constituents the past year. Some search of the literature was made, however, and several methods selected which should be of considerable value in arriving at the cacao content of products containing such materials as flour solids, soya grits, or other similar matter. The methods referred to are: The determination of the tannins in cacao kernel (*Analyst*, **63**, 27 (1938)) and the "Determination of Anthocyanins in Cocoa and Chocolate" originally published in *Z. Untersuch Lebensm.*, **82**, 20 (1941) and reviewed in *Chem. Abstracts*, **38**, 2289 (1944). The first method is based on the extraction of tannin with 40 percent acetone solution and the precipitation of the tannin with Steansy's reagent composed of HCl water and 40 percent formaldehyde solution.

The second method, on the determination of anthocyanins, is dependent upon the color produced by heating with a hydrochloric acid alcohol solution (15 parts concentrated hydrochloric acid, and 85 parts 95 percent alcohol). The solution is read colorimetrically.

These methods should be investigated to determine their merit as measures of the cacao content of samples.

Shell content by pectic acid.—The determination of cacao shell in chocolate products has had a considerable history. Among the various means tried and suggested have been the coloring matters, pentosan content, levigation method, crude fiber, cellulose, various microscopic methods, and finally pectic acid. Of these the crude fiber and pectic acid are most used today.

When Adan[1] estimated pentosan content by the furfural method of

[1] *Bull. Soc. Chim. Belg.* **21**, 211 (1907); *Internat. Cong. App. Chem.*, 1909, Sect. VIII C, 203–204.

Tollens[2] he believed he had found a satisfactory method. Subsequent investigation, however, showed that the difference in furfural containing substance between shell and nib were not as great as at first supposed. The one factor which Adan failed to note, however, which might have made the method successful was the fact that a considerable part of furfural obtained from the shell was contributed by pectin, while the furfural from the nib was contributed mainly by pentosans and very little by pectin. A separation of these materials would probably have rendered the method successful. The presence of pentosan prevents determination of pectin also by furfural determination.

During the past several years considerable work has been done on methods for the determination of pectic acid in cacao, and a procedure for that constituent in bitter and sweet chocolate and for milk chocolate was published. The procedures have been rather long and tedious and considerable effort has been made to shorten them and also to remove or separate more effectively pectin from interfering substance. An effort was made to remove pentosans with the enzyme pentosanase but preparations tried did not prove effective, probably because of their low content of this enzyme. A good source of this has not been found. In previous methods an ammonium oxalate extract of the cacao containing the pectin and other materials has been evaporated to low volume, and pectin, together with other substances precipitated by addition of several volumes of acidified alcohol. In order to avoid the rather laborious evaporation of the extract and at the same time effect a better separation (since boiling results in some change, apparently lactone formation of pentose containing substance, which makes separation more difficult) of pectin and gums, etc., the saponification of the extract is made in a volume of about 200 ml. and in the presence of the ammonium oxalate. To accomplish this a considerably higher concentration of alkali was used, followed by a larger acid concentration than formerly used for the conversion of pectin to pectic acid. A small amount of alcohol was used to aid in precipitation of the pectic acid, which was then separated by centrifugation, using some filtercel to aid in collection. The results have been very satisfactory, effecting a considerable shortening of time and a much better preliminary separation of other matters.

The Referee submits the revised procedure with the recommendation that it be adopted as tentative.*

REVISION OF THE CHAPTER ON CACAO PRODUCTS

Sampling of such products as chocolate liquor, sweet chocolate, or milk chocolate, and the various samples, can be done more advantageously in

[2] *Zeitsch. Angew Chem.*, 1896, 712, 749.

* For report of Subcommittee D and action by the Association, see *This Journal*, 28, 65 (1945). The details of the method will be published in the 6th edition of *Methods of Analysis, A.O.A.C.* (1945).

the liquid condition. The procedure proposed for adoption will be given in the 6th edition of *Methods of Analysis.*

SACCHARINE INGREDIENTS

The recent use of corn sirup, corn sugar, and the increased use of dextrose as sweeteners in cacao products has created a need for methods for determining these materials, when present in chocolate products. The polariscopic method on the inverted solution at 87° for commercial glucose, in the chapter on sugars, is suitable for use on cacao products after the proper preparation and clarification of the sample solution. It is recommended that this method be included by reference to the sugar chapter.

There are several methods of determining dextrose in the presence of other sugars and the Referee has been informed that one of these will be included in the chapter on sugar and sugar products. This also would be applicable to cacao products after a preliminary preparation of the solution, and it is recommended that the method selected be included in the cacao products chapter by reference.

There is need for methods for determining malt solids in cacao products and work should be done on this subject.

The sucrose heading in the present book should be changed to "Sucrose —Official," as the method was subjected to collaborative study for several years and good agreement between collaborators was obtained.

DAIRY INGREDIENTS

Under dairy ingredients should be grouped lactose, butterfat, casein, and milk ash.

The present heading of lactose contains no designation of the status of the method and this should be changed to "Lactose—Official (in absence of other reducing sugars)." Collaborative work was done on this method with good results.

The calculation of butterfat from the R.M. number makes use of a formula based on values of 0.5 for cacao butter and 24 for butterfat. The Referee has constructed a general formula using the terms X and Y which it is recommended be substituted in place of the present one. The new formula would allow the substitution of the most up-to-date values in place of the old values. The proposed formula is:

$$\text{Percentage butterfat} = \text{Percentage total fat} \times \frac{c-y}{x-y}$$

where c = R.M. number of fat sample; x = R.M. number of butterfat; and, y = R.M. number of cacao fat.

FAT

The determination of total fat and its examination should be placed under the common heading of fat.

OTHER METHODS

One of the characteristic substances of cacao is its theobromine content. At present there is no method in *Methods of Analysis, A.O.A.C.*, for the determination of this ingredient. It becomes necessary at times to determine this substance, and since it is so characteristic of chocolate, the Referee believes the chapter should contain a method for its determination. Of the methods with which the Referee is acquainted, that of Wadsworth (*Analyst*, 1921, p. 32, and 1922, p. 153) appears to be the most reliable. The theobromine extracted by this procedure is unusually pure, according to the author of the method and others who have used it. Recently, the Referee had occasion to determine theobromine by this method and submitted the results to the Microanalytical Division, where it was found to be unusually pure. Results are also higher than by most other methods. It is recommended that it be adopted as tentative.

RECOMMENDATIONS*

It is recommended—

(1) That the method for determination of pectic acid be adopted as tentative and further studied.

(2) That work on the determination of lecithin and fat in chocolate products, where ordinary extraction methods are difficult, and on shell be continued, and that work on malt solids be initiated.

(3) That associate referees be appointed to investigate methods for the determination of cacao constituent in various cacao products and the determination of lactose in the presence of other reducing sugars in chocolate products.

(4) That the heading for the method for determination of sucrose be changed from sucrose to "Sucrose—Official."

(5) That the heading of the method for lactose in milk chocolate be changed to read "Lactose in Milk Chocolate—Official" (in absence of other reducing sugar).

(6) That the last sentence of the method under Fat—Method I, Official "the fat-free sample may be used for crude fiber determination," be deleted, as the crude fiber is no longer determined in that way.

(7) That the Wadsworth method (using tetrachlorithane) referred to in this report for the determination of theobromine in cacao products be adopted as tentative.

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* For report of Subcommittee D and action by the Association, see *This Journal*, 28, 65 (1945).

(8) That the formula proposed for calculation of butterfat in mixed fat be substituted for the present one.

(9) That the method proposed in the Associate Referee's report for sampling chocolate liquor, sweet chocolate, skim-milk chocolate, buttermilk chocolate, or milk chocolate, be included as a tentative method in *Methods of Analysis, A.O.A.C.*

(10) That references be made in Chapter **XIX** to methods for corn sirup and dextrose in Chapter **XXXIV**.

No report on lecithin in cacao products was made by the Associate Referee.

The report on pectic acid in cacao products was incorporated in the Referee's report.

No report on chocolate constituents was made by the Associate Referee.

REPORT ON FAT IN MILK CHOCOLATE OR CACAO

By MARIE L. OFFUTT (U. S. Food & Drug Administration, New York, N. Y.), *Associate Referee*

The Associate Referee appointed last year for the determination of fat in cacao products, such as milk chocolates, chocolate malted milks, etc., has been able to do some work along this line using the two methods given below.

The Modified Hillig Method used is based on Hillig's "Unified Method for the Determination of Fat in Foods with Special Reference to the Evaluation of their Butterfat Content," published in *This Journal, A.O.A.C.*, **18**, 454 (1935). This method was modified, submitted to collaborators and partially reported in the *Journals*, **19**, 380 (1936); **20**, 341, (1937); **21**, 366 (1938), and **22**, 486 (1939). The results obtained by collaborators were very good, but the detailed modified method was not printed in any of these reports, and after 1939 the work seems to have stopped. The modified method is, therefore, given here.

MODIFIED HILLIG METHOD

Weigh accurately into a 400 ml beaker 10 gm milk chocolate (50 grams chocolate malted milk or chocolate powders containing milk), add 30 ml water and 25 ml concentrated hydrochloric acid. Heat 30 min. on steam bath, stirring frequently; add 5 g filter aid and 50 ml ice water and chill 30 min. in ice water. Fit a heavy piece of crude fiber quality linen into 8-cm Büchner funnel and moisten with water; apply gentle suction and completely overlay the linen with filter aid (3 g of filter aid suspended in 30 ml water poured over funnel and let drain). Filter the hydrolyzed mixture by gentle suction, rinsing the beakers three times with ice cold water (but do not let the pad be sucked dry until transfer and washing are complete). Finally,

wash 3 times with ice cold water, tamping tightly with a flattened stirring rod after the last washing and suck dry. Strip the linen from the cake and transfer the cake to the original beaker. With a small piece of filter paper, transfer to the beaker any material adhering to the funnel. Wash funnel with petroleum ether and add to cake in beaker. Evaporate ether on steam bath. Break up the cake with stirring rod and allow to remain on steam bath until the contents pulverize easily and appear to be dry. Place in oven at 100°C for 1 hour. Add 15 g of powdered anhydrous sodium sulfate and mix well. Transfer this dry mass to a large Knorr tube (about 175 ml capacity fitted with ½ inch perforated metal disk, over which is laid a dry asbestos pad about ⅜ inch thick). Wash the beaker with 50 ml of ether, pouring the rinsings into the tube. Extract the material in tube with six 50 ml of petroleum ether. Stir sample thoroughly with each 50 ml of petroleum ether by means of glass rod, taking care to crush all the lumps. Let stand 2 min. and drain by gentle suction into tared 250 ml flask. Evaporate the petroleum ether on steam bath and dry the fat to constant weight.

MODIFIED ROESE-GOTTLIEB METHOD

Take 25 g of the chocolate milk powder (10 g milk chocolate); add 25 ml of water and 25 ml of concentrated hydrochloric acid and heat for 30 min. on steam bath. Transfer to large separatory funnel with 100 ml of 95% alcohol. Extract in 50 ml portions with 100 ml of mixture of petroleum and ethyl ethers (1+1). Draw off the clear solutions of ethers into tared flask. Evaporate solvent on steam bath, dry fat in oven at 100°C to constant weight.

The proportions used in the above modified Roese-Gottlieb and the length of time for heating were found to give higher results after trying a number of other proportions at different times. The fat was determined on 5 different samples and results were as follows:

NO.	PRODUCT	HILLIG METHOD	R.G. METHOD
56153-F	Choc. Malted Milk	5.09	3.71
57248-F	Choc. Malted Milk	1.93	0.93
66179-F	Choc. Malted Milk	3.49	1.17
56152-F	Malted Milk	6.13	5.06
—	Milk Chocolate	32.62	31.06

The Roese-Gottlieb method gave about 1% lower fat results in each case.

The Associate Referee recommends* that further work be done on fat by the Modified Hillig Method and by the suggested Modified Roese-Gottlieb Method; and that the best method then be used for collaborative work.

REPORT ON CEREAL FOODS

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

The report this year covers the recommendations made in connection with the revision of the cereal chapter in the *Methods of Analysis A.O.A.C.*,

* For report of Subcommittee D and action by the Association, see *This Journal*, 28 65 (1945).

for the sixth edition, and also the recommendations on the work of the associate referees.

RECOMMENDATIONS*

The following recommendations are in connection with the subjects assigned to the associate referees. It is recommended—

(1) That the tentative method for iron on which collaborative work was reported by the Associate Referee be adopted as official, first action, and study be continued.

(2) That the tentative method for calcium on which collaborative work was reported by Associate Referee be adopted as official, first action, and study continued.

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

(4) That the electrometric procedure for the determination of hydrogen ion concentration in cereal products, *This Journal*, **26**, 109, be adopted as official, first action, and study continued.

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

(6) 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.

(7) That the procedure for reducing and non-reducing sugars in flour, page 215, section 20, now official, first action, be made official, final action, and its application to the determination of sugar in bread and other cereal products be studied.

(8) That the study of the tentative method for the determination of benzoyl peroxide in flour, page 223, section 44, be continued.

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

(10) That methods for the determination of lactose in bread, *This Journal* **25**, 630, be further studied.

(11) That the tentative method for the estimation of milk fat in bread, page 229, section 65, be further studied.

(12) That the method with proposed changes of the Associate Referee for the determination of proteolytic activity of flour be further studied.

(13) That the study on the methods for the determination of water, ash, nitrogen, crude fiber, and ether extract in soya flour and other soya products be continued.

(14) That studies be made of the detection and determination of soya flour and cereal products by immunological methods or other suitable means of estimation.

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* For report of Subcommittee D and action by the Association, see *This Journal*, **28**, 66 (1945). These revisions, as recommended by this Committee, were approved and will be incorporated in revisions of *Methods of Analysis*, *A.O.A.C.*, for the 6th edition.

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

(16) 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.

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

(18) That methods for the determination of moisture and fat by acid hydrolysis in fig bars and raisin filled crackers as submitted in the Associate Referee's report be adopted as official, first action, and study continued.

(19) That further study be made of the modified distillation methods with benzene or other suitable immiscible solvents for the determination of moisture in all flour-like products containing sodium bicarbonate as one of its constituents.

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

(21) That the method for apparent viscosity measurement of flour be studied.

(22) That the method for "Detection of small amounts of tartrazine (F.D.C. Yellow No. 5) in alimentary pastes" reported by C. F. Jablonski, Referee on Coloring Matter in Foods, 1944, be included in the Cereal chapter as tentative.

CALCIUM IN ENRICHED SELF-RISING FLOUR AND ENRICHED BREAD

This report is a continuation of the collaborative study on the determination of calcium in enriched self-rising flour, enriched bread, and calcium solution by the same method as used last year.[1] The enriched self-rising flour (No. 3) was prepared according to a typical commercial formula and the enriched bread (No. 4) was of a typical composition with 6 per cent of dry skim milk The results from 15 collaborators are given in Table 1. The statement "Ca present" at the bottom of the table merely indicates the composition based on analysis of the component parts by the Associate Referee. The results of the collaborators are all within 5 percent of the average which very closely represents the amount of calcium present.

The collaborative assistance of the following chemists is appreciated: W. H. King, Department of Health, New Orleans, La.; J. J. Winston, Jacobs Cereal Products Lab., Inc., New York, N. Y.; F. H. Luckman, The Best Food, Inc., Bayonne, N. J.; C. Hoffman, Ward Baking Co., New York, N. Y.; J. S. Andrews, General Mills, Minneapolis, Minn.; W. Reeder and K. L. Fortmann, Campbell Taggart Research Corp., Kansas City, Mo.; M. Howe, Russel-Miller Milling Co., Minneapo-

[1] *This Journal*, 27, 400 (1944).

lis, Minn.; H. Shuman, A. G. Buell, C. C. Cooley, F. J. McNall, M. A. Braun, J. Carol, all of various stations of the Food and Drug Administration.

It is recommended (1) that the method for calcium in enriched self-rising flour and enriched bread be adopted as official, first action and (2) that the study be continued.*

TABLE 1.—*Results of calcium in enriched self-rising flour, enriched bread, and calcium solution*

COLLABORATOR	CALCIUM SOLUTION		SAMPLE 3	SAMPLE 4
	MG/ML	MG/LB[3]	MG/LB	MG/LB
1	0.200	454	1372	460
2	0.206	468	1365	498
3	0.207	470	1373	427
4	0.203	461	1413	472
5	—	—	—	—
6	0.203	461	1380	481
7	0.209	474	1332	473
8[1]	0.199	452	1422	496
9	0.202	459	1410	476
10	—	—	—	—
11[2]	0.202	459	1391	467
12	0.204	463	1399	480
13	0.207	470	1424	489
14	0.198	450	1381	490
15	0.200	454	1362	481
16	0.199	452	1399	478
17	0.204	463	1403	481
Minimum	0.198	450	1332	427
Maximum	0.209	474	1424	498
Average	0.203	461	1388	477
Ca present	0.207	470	1425	463

[1] Single determination.
[2] Average of 3 determinations.
[3] Ca results calculated in mg/lb assuming solution obtained from 10 g sample of flour following the method.

IRON IN ENRICHED FLOUR, ENRICHED SELF-RISING FLOUR, AND ENRICHED BREAD

This report is a continuation of the collaborative study on the determination of iron in enriched flours, enriched self-rising flour, enriched bread, and iron solution, by the same method as used in last year's report[1] with the exception of one slight, essentially editorial, change.

* The details of the method will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.
[1] *This Journal*, **27**, 396 (1944).

Two enriched flours, one enriched self-rising flour, enriched bread, and iron solution were submitted to the collaborators. The two enriched flours were prepared in the laboratory with commonly used commercial enrich-

TABLE 2.—*Iron results on enriched flours, enriched self-rising flour, enriched bread, and iron solution by collaborators*

COLLABORATOR	IRON SOLUTION		SAMPLE 1	SAMPLE 2	SAMPLE 3	SAMPLE 4
	MG/ML	MG/LB[4]	MG/LB	MG/LB	MG/LB	MG/LB
1	.0100	13.6	15.3	14.5	16.1	16.2
2	.0100	13.6	14.6	13.5	17.0	16.1
3	.0096	13.1	14.3	13.7	16.5	15.0
4	.0100	13.6	16.1	14.7	17.9	17.5
5	.0100	13.6	16.0	14.9	18.0	17.4
6	.0103	14.0	14.8	14.2	17.2	15.9
7	.0099	13.5	14.8	14.4	17.5	16.0
8[1]	.0098	13.4	14.9	14.5	16.3	15.9
9	.0105	14.3	14.3	13.8	17.0	16.4
10	.0102	13.9	15.5	15.4	17.4	16.3
11[2]*	.0103	14.0	14.1	13.7	17.1	16.6
**	.0105		14.2	14.0	16.9	16.2
12	.0103	14.0	15.0	14.6	16.4	15.5
13	.0100	13.6	13.2	14.3	17.7	16.6
14**	.0100		13.6	13.5	16.3	15.5
*	.0103	14.0	14.1	13.9	16.7	16.0
15**	.0993	14.0	13.4	13.1	16.2	15.6
*	.0103		14.1	13.6	16.8	16.1
16	.0099	13.5	14.8	14.7	17.1	16.8
17[3]	—	—	14.7	14.3	17.0	16.4
Minimum	.0096	13.4	13.2	13.5	16.1	15.0
Maximum	.0105	14.3	16.1	15.4	18.0	17.5
Average	.0101	13.8	14.8	14.3	17.0	16.3
	σ	0.30	0.67	0.52	0.55	0.62
Iron present	.0100	13.6	14.8	14.6	17.7	14.0

[1] Single determination.
[2] Ave. of triplicate.
[3] Ave. of 10 determinations.
[4] Calculated on basis this solution obtained from 10 gm flour samples. Results reported with alpha alpha dipyridyl not included in the averages.
* Orthophenanthroline.
** Alpha alpha dipyridyl.

ment mixtures. Sample No. 1 contained sodium iron pyrophosphate, Sample No. 2, ferrum reductum, enriched self-rising flour. No 3 contained ferrum reductum, and enriched bread; No. 4, sodium iron pyrophosphate. Results were obtained from 17 collaborators as given in Table 2.

The results given at the bottom of the table as "Iron present" may not be the absolute iron content of the flour and bread samples, but merely

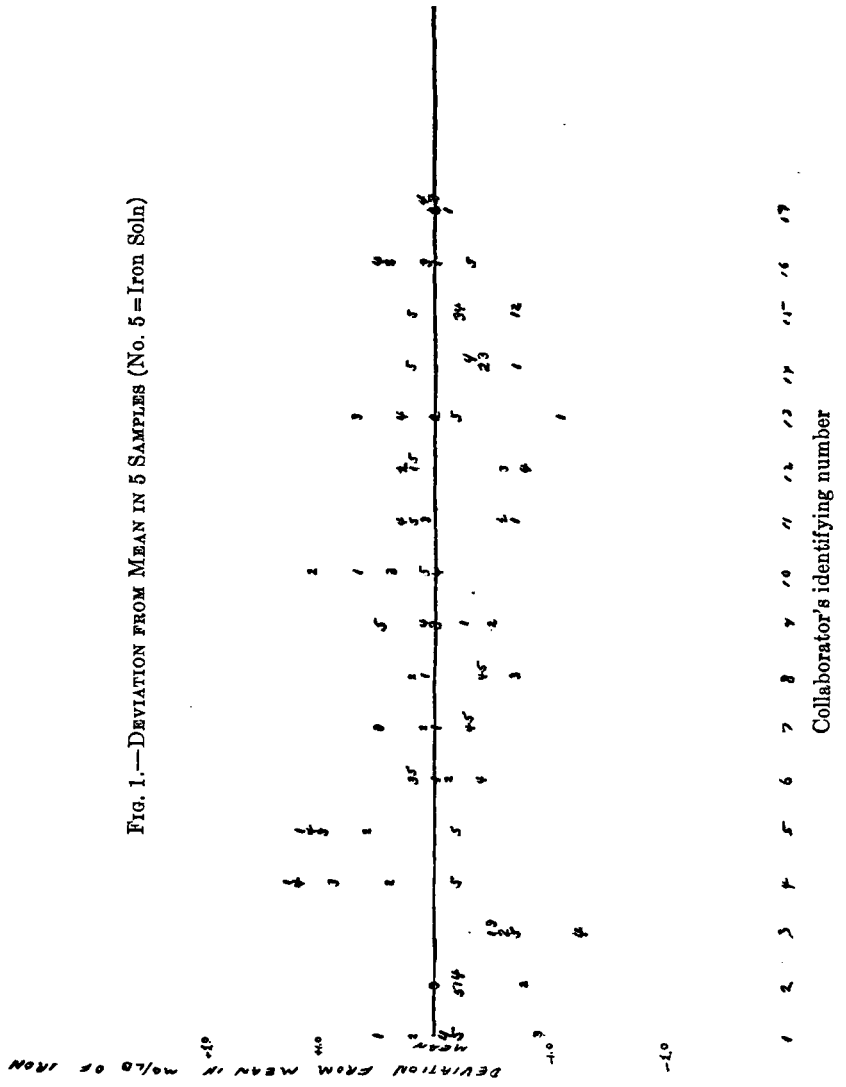

FIG. 1.—DEVIATION FROM MEAN IN 5 SAMPLES (No. 5 = IRON SOLN)

represent the calculated amount present based on the analysis by the Associate Referee of the component parts of the samples The average results of the collaborators agree closely with this calculated amount present with the exception of the bread, Sample 4. Frequently, as in this work, the determination of iron in enriched bread is higher than the estimated amount present. In the results of the collaborators the variation from the average is within 5 per cent on the iron solution. Over 82 per cent of the results on the flour and bread are within 5 per cent, and all results, except on one sample by one collaborator, are within 10 per cent. The variations from the mean plotted for each individual collaborator in

TABLE 3.—*Iron results on enriched flours, enriched self-rising flour, and enriched bread in mg/lb*

	SAMPLE 1	SAMPLE 2	SAMPLE 3	SAMPLE 4
	14.65	14.35	16.90	16.42
	14.65	14.55	17.30	16.30
	14.75	14.08	17.30	16.64
	14.91	14.08	17.10	16.74
	14.45	14.20	16.50	16.66
	14.70	14.31	16.78	16.85
	14.66	14.08	16.80	16.12
	14.71	14.35	16.90	16.03
	14.50	14.12	17.16	16.07
	14.62	14.50	16.85	16.21
Mean	14.66	14.26	16.96	16.40
σ	0.128	0.177	0.254	0.300

Fig. 1 give a graphic presentation of the trend of these results. It is evident that some of the collaborators, namely, 4, 5, and 10, tend to get high results; others 2, 3, 14, and 15, tend to obtain low results; and 1, 4, 5, and 13 tend to have a greater range in their variation than the other collaborators.

The uniformness of distribution of the small amounts of added material for enrichment in the preparation of enriched flour samples is always under suspicion. In order to obtain some information on the uniformity of these collaborative results, the Associate Referee made 10 determinations on each of these samples with the results, together with standard deviations, reported in Table 3. It is mentioned here that since these determinations were made over a period of time along with other work the results may not be as representative as if they had been done in a series at one time.

The procedure for adding and mixing the enrichment ingredients differs in commercial practice among the mills and also differs from the labora-

tory procedure used on these samples. Some information is presented on the uniformity of enriched flours from two large mills and one small mill. The results and standard deviations are given in Table 4.

The results in Tables 3 and 4 indicate the uniformity is similar and that a lack of uniformity is probably not the only factor in the variation obtained by the collaborators. The collaborative results on the iron solution

TABLE 4.—*Iron results on commercial flours from two large mills and one small mill in mg/lb*

	A	B	C
	15.59	14.82	12.40
	15.55	15.01	13.03
	15.34	14.70	12.66
	15.54	14.96	12.54
	15.38	14.90	12.75
	15.56	14.90	13.03
	15.80	15.15	12.66
	15.55	15.19	12.40
	15.70	14.95	12.50
	15.64	15.00	12.44
	15.65		
Mean	15.57	14.96	12.64
σ	0.132	0.144	0.236

in which the uniformity factor may be eliminated has a much lower standard deviation than the flour and bread samples.

WET DIGESTION APPLICATION

There appears to be some interest in the wet digestion method for the determination of iron in enriched flour and enriched bread. The following method has been applied to these samples and also submitted to a few collaborators.

REAGENTS

Nitric acid, sulfuric acid, ammonium hydroxide:—These chemicals can be obtained labeled as iron free.

PROCEDURE

Transfer 10 gms. of flour or air-dried ground bread into a 800-ml Kjeldahl flask, add 25 ml. H_2SO_4, immediately and vigorously rotate flask to mix the sample and acid. Place on digestion equipment, add 25 ml of HNO_3, allow reaction to subside. Heat with low flame for a few minutes until SO_3 fumes begin to evolve, add, drop-wise, 25 ml. HNO_3, heat until SO_3 fumes evolve and the digestion is complete (pale yellow). Add 5 ml. of HNO_3, drop-wise, heat until SO_3 fumes evolve (digestion period should be about one hour), cool, add two glass beads, iron free, 100 ml. water, boil slowly until SO_3 fumes begin to evolve, cool, add cautiously at first, drop-wise, 50 ml (1+1) NH_4OH and occasionally cool under water. Continue addition of NH_4OH (about 40 ml.) until litmus just turns blue, add H_2SO_4 (1+1), drop-wise, to just turn litmus red. Add 3.9 ml. HCl, filter (11 cm. free flowing filter paper), into 200-

ml. volumetric flask. Make to volume and proceed as under procedure for dry ashing beginning "pipet 10 ml. into 25-ml. volumetric flask." Run blank on reagent and deduct before calculation of iron in mgm. per pound. (If digestion is done on the regular Duriron digestion equipment, care must be taken to prevent iron contamination.)

Results of the determination of iron on the collaborative samples by the wet digestion method are given in Table 5. These results average slightly higher than those obtained by the dry ashing procedure. These

TABLE 5.—*Results of iron in enriched flours, enriched self-rising flour, and enriched bread in mg/lb*

COLLABORATOR	SOLUTION	SAMPLE 1	SAMPLE 2	SAMPLE 3	SAMPLE 4
9	0.0103	14.2	14.0	17.2	16.4
11[1]	—	14.9	15.1	18.7	16.8
13	—	—	14.5	17.7	—
17[2]	0.0105	15.1	14.4	17.4	17.0
Average	0.0104	14.7	14.5	17.7	16.7

[1] Single determination.
[2] Average 4 determinations.

higher results may not be due to more iron in the samples. Experiments with this method show that even after allowance for iron in the reagents some iron appears to be dissolved from the Pyrex flasks during digestion. The wet digestion method is considerably more time-consuming, requires more reagents than the dry ashing procedure, and requires more study.

It is recommended* (1) that the dry ashing method be adopted as official, first action, and (2) that the study be continued.

The collaborative assistance of the following chemists is appreciated: W. H. King, Department of Health, New Orleans, La.; J. J. Winston, Jacobs Cereal Products Lab., Inc., New York, N. Y.; J. C. Finley, The Great A & P Tea Co., New York, N. Y.; F. H. Luckman, The Best Food, Inc., Bayonne, N. J.; C. Hoffman, Ward Baking Co., New York, N. Y.; J. S. Andrews, General Mills, Minneapolis, Minn.; W. Reeder and K. L. Fortmann, Campbell Taggart Research Corp., Kansas City, Mo.; M. Howe, Russel-Miller Milling Co., Minneapolis, Minn.; and H. Shuman, A. G. Buell, C. C. Cooley, F. J. McNall, M. A. Braun, D. M. Ottens, J. Carol, all of various stations of the Food and Drug Administration.

No report on rye flour in rye bread and in flour mixtures was made by the Associate Referee.

No report on H-ion concentration was made by the Associate Referee.

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* For report of Subcommittee D and action of the Association, see *This Journal*, 28, 66 (1945).

REPORT ON STARCH IN RAW AND BAKED CEREALS

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

Last year the Hopkins revision of the Mannich-Lenz procedure[1] for the determination of starch in raw and baked cereals was adopted as a tentative method by the A.O.A.C. (*This Journal*, **27**, 404). This year the Associate Referee wished to work on the factor from glucose to starch, as well as the specific rotation for starch by the polarimetric method. But since there was lack of agreement as to procedures, this phase of the work was postponed.

Owing to the great interest of the flour chemists, it was thought that Clendinning's stannic chloride procedure should be compared with the tentative method, on some flour samples; and it was deemed advisable to send out another commercial starch, for estimation of its starch content.

Since the samples were sent out late, only three samples were selected for study, and the following were sent to collaborators: No. 1, Idaho potato starch; No. 2, soft wheat flour; No. 3, hard wheat flour.

The Idaho potato starch was furnished gratis to the Association by Stein Hall and Company, of New York. The soft and hard wheat flours were obtained from a local bakery in Starkville, Miss., being used by them in baking cake and bread, respectively. The samples were allowed to come to moisture equilibrium in the Mississippi State Chemical Laboratory before being sent out.

They were sent to 12 collaborators with instructions to analyze the Idaho potato starch by the tentative method adopted by the Association last fall. The two flour samples were to be analyzed by this method and compared also to the stannic chloride procedure according to Clendenning Report No. 21-4-44 (unpublished). The principal difference in the latter method, only applicable to wheat products, is that, instead of washing out proteins with alcohol before dispersion, they are precipitated out with 5 ml. of a 4% stannic chloride solution.

Reports were received from 8 collaborators. The Associate Referee wishes to thank each one for the continued interest necessary to make this report possible. The results shown in the tables were contributed by:

V. E. Munsey, Food and Drug Administration, Washington, D. C.
R. T. Milner, Northern Regional Research Laboratory, Peoria, Ill.
J. D. Guthrie and C. L. Hoffpauir, Southern Regional Research Laboratory, New Orleans, La.
L. R. Brown, A. E. Staley Manufacturing Company, Decatur, Ill.
Marilyn R. Cooney, General Mills, Minneapolis, Minn.
K. A. Clendenning, National Research Council, Ottawa, Can.
C. O. Willits, Eastern Regional Research Laboratory, Philadelphia, Pa.
The W. E. Long Company, Chicago, Ill.

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[1] *Can. J. Research*, 11, 751–758 (1934).

TABLE 1.—*Starch in potato starch and wheat flours*

	IDAHO POTATO STARCH	SOFT WHEAT FLOUR		HARD WHEAT FLOUR	
ANALYST	TENTATIVE METHOD	TENTATIVE METHOD	CLENDENNING PROCEDURE	TENTATIVE METHOD	CLENDENNING PROCEDURE
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
(1)	87.7	72.0	73.2	69.5	71.0
	87.9	71.8	73.2	69.7	70.8
Av.	87.8	71.9	73.2	69.6	70.9
(2)	86.7	71.8	71.2	68.6	68.6
	86.8	71.5	71.4	69.0	68.7
	87.0	71.9	72.0	69.1	69.2
	86.9	71.6	71.7	68.6	69.5
Av.	86.9	71.7	71.6	68.8	69.0
(3)	87.0	72.4	71.2	69.2	68.8
	87.1	72.4	70.9	69.1	68.7
	87.2		71.2		68.2
			71.0		68.6
Av.	87.1	72.4	71.1	69.2	68.6
(4)	86.3	73.9	73.5	70.5	70.7
	86.3	73.9	73.5	70.5	70.7
Av.	86.3	73.9	73.5	70.5	70.7
(5)	87.0	71.7	72.1	69.2	69.6
	86.9	72.1	71.8	68.7	69.2
	87.1	71.8	72.3	68.9	69.5
Av.	87.0	71.9	72.1	68.9	69.4
(6)	87.1	72.0	71.9	69.3	69.4
	87.1	72.0	71.9	69.3	69.4
Av.	87.1	72.0	71.9	69.3	69.4
(7)	86.2	70.8	72.1	67.3	69.2
	86.1	70.8	72.1	67.3	69.3
Av.	86.2	70.8	72.1	67.3	69.3
(8)	86.9	72.1	71.7	68.4	69.0
	86.7	72.2	71.7	67.8	69.0
	87.1	71.8	71.7	69.8	68.8
	86.8	71.7	71.5	68.2	69.0
Av.	86.9	72.0	71.7	68.6	69.0
(*9)	86.5	70.5	71.0	67.0	67.2
	86.5	70.5	71.0	67.0	67.2
	86.5	70.5		67.0	
Av.	86.5	70.5	71.0	67.0	67.2

* Too late to be included in discussions.

DISCUSSION OF RESULTS

Only two analysts checked closely by the two methods, these two having had more experience with both of the methods. Three other analysts checked themselves reasonably closely. Two analysts obtained much higher results by the Clendenning procedure, while one obtained lower. There was not as wide spread of results by the Clendenning procedure as by the tentative method.

The Iowa potato starch analyzed over a 100 per cent on dry basis, as had all the other starches analyzed by the Association. This indicates that the specific rotation of 200 is too low.

Referee Munsey and J. D. Guthrie also analyzed the Idaho potato starch by the stannic chloride procedure, but the results were lower. This is in agreement with Clendenning's assertion that the method is not applicable to pure starches.

TABLE 2.—*Moisture results*

ANALYST	METHOD	IDAHO POTATO STARCH	SOFT WHEAT FLOUR	HARD WHEAT FLOUR
		per cent	*per cent*	*per cent*
1	100° vacuum 6 hr. (Humid day)	13.39	11.20	11.21
2	105° vacuum 20 hr.	14.01	11.98	11.97
3	105° vacuum 18 hr.	13.99	11.82	11.95
4	100° vacuum 5 hr.	13.93	11.52	11.49
5	100° vacuum 16 hr.	13.90	11.76	11.74
6	100° vacuum	13.77	11.81	11.92
7	80° vacuum 5 hr.	13.80	11.46	11.68
8	vacuum	13.62	11.38	11.29

Guthrie also used the method of Steiner and Guthrie (unpublished) and obtained lower results on all three samples. Milner also compared the analysis of the two flours with a Northern Regional Laboratory method, to be published, and obtained almost identical results as he had by both Clendenning and tentative methods.

MOISTURE RESULTS

Moisture results are shown in Table 2. While they agree reasonably well, it will be observed that analysts 2 and 3 check almost identically, both using 105° for 20 and 18 hrs., respectively. Assuming this additional loss to be moisture, it is believed that the additional time is necessary, for the nearest approach to these was analyst No. 5, with a 16 hr. drying period.

WORK ON SPECIFIC ROTATION

Last year, collaborators were asked to do some specific rotation work on the corn and wheat starch samples submitted. As stated, only two besides the Associate Referee did any work on these samples. This year more results have been sent in. However, owing to the variance of procedure, as well as of results, no report on these results will be made at this time. It is understood that papers are coming out on this score by Milner and Guthrie for their respective regional laboratories, and also by Clendenning of the National Research Council, Ottawa.

COMMENTS OF COLLABORATORS

Analyst 2.—Samples foamed badly, but, this was cut down by octanol.

Analyst 3.—Foaming was controlled with cetyl alcohol. In addition to Celite, uranyl acetate was used to good advantage.

Analyst 5.—The use of a 400 ml. beaker instead of Erlenmeyer flask cuts down foaming.

Analyst 8.—The Clendenning procedure is much faster and simpler than tentative method.

CONCLUSIONS

It is believed that the work this year warrants consideration by the Association for the use of the Clendenning procedure in flour analyses because of its greater ease and simplicity and, apparently, good results by a majority of analysts.

It is believed that as soon as the Association has advantage of the published work on specific rotation, it should immediately consider revising the specific rotation of 200 upwards.

Also, sincet he diastase-hydrochloric procedure is the oldest official method, the Association should consider further study of it, first, for enzyme conversion; second, quantitative methods for estimation of glucose; third, glucose-starch factor.

No report on fat acidity (grain, flour, corn meal, and whole wheat flour) was made by the Associate Referee.

No report on sugar in bread and other cereal foods was made by the Associate Referee.

No report on benzoyl peroxide in flour was made by the Associate Referee.

No report on carbon dioxide in self-rising flour was made by the Associate Referee.

REPORT ON MILK SOLIDS AND BUTTERFAT IN BREAD

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

Numerous experiments have been tried on the extraction of the fat from milk bread for the purpose of estimating the amount of butterfat present. Some of the solvents tried for the direct extraction of fat from the air-dried bread, such as acetone, dioxane, trichlorethylene, ether, or alcohol benzene mixture, recovered only about two-thirds of the fat. A modified Roese-Gottlieb method did not give promising results. Extraction of the milk bread hydrolyzate following digestion with aqueous hydrochloric acid in liquid extractors with ether did not give the desired results for a large sample of material such as 50 grams of air-dried bread. An attempt was made to estimate the amount of butterfat by the determination of the butyric acid content by steam distillation without extraction of the fat. The sample was hydrolyzed with sulfuric acid, heated on the steam bath for three hours with a large excess of potassium hydroxide, slightly acidified with sulfuric acid and steam distilled for volatile acids. The large amounts of acids, essentially formic and acetic, distilled along with the butyric, indicated considerable doubt in the accuracy and practicability of this procedure. Additional work has resulted in a modification of the tentative procedure given in the *Methods of Analysis*, 5th ed., page 229, section 65, for future collaborative study.

It is recommended* that the study be continued.

REPORT ON PROTEOLYTIC ACTIVITY OF FLOUR

By SUTTON REDFERN (Fleischmann Laboratories, 810 Grand Concourse, New York, N. Y.), *Associate Referee*

A further study of the semi-autolytic method using hemoglobin as an auxiliary substrate which was proposed by the former referee, Dr. Landis (*This Journal*, **25**, 631 (1942)), has been made. The method as originally given was found to be quite satisfactory except for two minor changes. It was found that in preparing the hemoglobin substrate, the solution foamed so badly that it was not accurate to prepare it by making to a specified volume. Therefore, the directions are being changed so that it is made by weight.

It was thought originally that 10 minutes was a satisfactory minimum time to insure solution of non-precipitable proteins from the flour. Further work showed that it was desirable to increase the initial extraction time from 10 minutes to 25 minutes. At 10 minutes the soluble nitrogen was still increasing quite rapidly, but between 20 and 30 minutes the rate

* For report of Subcommittee D and action by the Association, see *This Journal*, **28**, 67 (1945).

leveled off slightly, making this a better base point. After this time the soluble nitrogen again increased more rapidly due to proteolysis.

RECOMMENDED CHANGES IN METHOD*

Under Reagents (*c*): change " . . . dilute to 100 ml." so as to read "dilute to 100 grams."

Under determination in 7th line: change "After 10 minutes . . . " so as to read "After 25 minutes. . . ."

Some collaborative work has been started, but too late to be included in this report. It is recommended that investigation of this method be continued.

No report on soybean flour was made by the Associate Referee.

No report on soybean flour in foods (immunological tests) was made by the Associate Referee.

The paper entitled "Method for Differentiating Between Egg Lecithin and Soybean Lecithin in Macaroni and Noodle Products," by James K. Winston and B. R. Jacobs, is published in *This Journal*, p. 607.

No report on phosphated flour was made by the Associate Referee.

No report on noodles and egg-containing products was made by the Associate Referee.

REPORT ON BAKED PRODUCTS OTHER THAN BREAD

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

Last year the Associate Referee submitted a report on moisture and fat determinations on baked products other than bread, which contain fruit (*This Journal*, **26**, 305). Two samples were selected for this work, Sample I, Fig Bars, which contain approximately 25 percent of figs, and Sample II, Raisin-filled Crackers, which contain approximately 50 percent of raisins. Samples such as these present considerable difficulties in handling and it was thought advisable to continue the collaborative study of methods on the same type of samples, following outlined procedures carefully.

The method which was sent to the collaborators for determining the

* For report of Subcommittee D and action by the Association, see *This Journal*, **28**, 67 (1945).

moisture content of these samples is essentially the same as the official method for moisture determinations on liquid and semiliquid sugar products (*Methods of Analysis*, Fifth Edition, 1940, Chapter XXXIV, 5).

The method for determining fat which was sent to the collaborators was the modification (*This Journal*, **26**, 306) of the official method for determining fat in flour by the acid hydrolysis method, as given in the accompanying *Methods of Analysis*, *A.O.A.C.*, 1940, Chapter **XX**, 11.

The detailed data from this collaborative study are presented in the table.

COLLABORATORS' COMMENTS ON METHODS

1. Suggested procedures followed.

2. A most important step in determining fat by the acid hydrolysis method is the thorough disintegration of samples such as these with the 95% alcohol. The charge should be thoroughly dispersed by warming for 15–20 minutes with the alcohol. The acid is then added and digestion carried out according to directions. This step should be emphasized.

3. When determining fat by the acid hydrolysis method on samples of the consistency of these, emphasis should be placed on the thorough dispersion of the charge with 95% alcohol before proceeding with the acid digestion.

4, 5, 6 and 9. Suggested procedures followed.

7 and 8. Used aluminum beakers for purification of the fat as recommended; No. 8 also used wide mouth Pyrex flasks. The glass flasks were used in the hope that constant results would be obtained in less time. No difference was found in the time it took to obtain constant weights. Glass flasks delayed the evaporation of the solvents by suction. Both analysts noted that drying of the purified fat for one hour at 100°C. in the air oven did not give constant weight. Extension of the drying time in the 100°C. air oven to 2 to 3 hours until weight remains constant is recommended.

10. There was no further loss of moisture after 16 hours drying time in vacuum oven at 70°C.

In determining fat, rubber stoppers are not satisfactory for use on account of solvent action of the alcohol and ethers. More accurate results are secured by using cork stoppers with small end cut fine.

Control Blank found, using cork stoppers .0011 gram residue.
Control Blank found, using rubber stoppers .0060 gram residue.

There is less danger of loss from the creeping action of ethers when a flat-bottomed, wide-neck, round, extraction flask with vial type mouth is used in place of the 250 ml. beaker.

11. Suggested procedures followed. It is important to thoroly disperse the charge with alcohol before adding the digestion acid when determining fat by the acid hydrolysis method on samples such as these.

Considering the comments and suggestions of the collaborators, the instructions for determining the fat content by the acid hydrolysis method (*This Journal*, **26**, 306) on samples such as these were revised.

DISCUSSION

The individual samples, representing a variety, which were sent to

TABLE 1.—*Collaborative results—moisture and fat determinations (per cent)*

COLLABORATOR	SAMPLE I, FIG BAR				SAMPLE II, RAISIN FRUIT			
	MOISTURE		FAT		MOISTURE		FAT	
	CHECKS	AV.	CHECKS	AV.	CHECKS	AV.	CHECKS	AV.
1		17.80		6.57		9.40		7.05
2			6.66 6.74	6.70			6.73 6.81	6.77
3	18.05 18.04	18.05	6.58 6.58	6.58	9.49 9.48	9.49	6.89 6.90	6.90
4	18.05 18.05	18.05	6.45 6.71 6.45 6.66	6.57	9.39 9.41	9.40	6.64 6.78 6.75 6.72	6.72
5	17.93 17.95	17.94	6.61 6.56 6.59	6.59	9.22 9.22 9.25	9.23	6.55 6.53 6.46	6.51
6	18.08 18.10	18.09	6.60 6.61	6.61	9.54 9.48	9.51	6.81 6.85	6.83
7	18.02 17.99	18.01	6.52 6.59	6.56*	9.38 9.35	9.37	6.60 6.58	6.59*
Ext. fat dried 2 hrs. 100°C. air oven.			6.41 6.54	6.48*			6.53 6.47	6.50*
Ext. fat dried 1 hr. 100°C. vacuum oven.			6.27 6.42	6.35			6.39 6.39	6.39
8	17.92 17.99	17.96	6.35 6.55	6.45*	9.39 9.35	9.37	6.62 6.61	6.62*
Ext. fat dried 3 hrs. 100°C. air oven.			6.20 6.42	6.31*			6.47 6.47	6.47*
Ext. fat dried 1 hr. 100°C. vacuum oven.			6.28 6.44	6.36			6.50 6.50	6.50
9	18.12 18.24	18.18	6.35 6.39 6.34	6.36	9.38 9.40	9.39	6.56 6.57 6.60	6.58
10	18.06 18.08 17.99	18.04	6.57 6.55 6.58	6.57	9.33 9.29 9.23	9.28	6.62 6.64	6.63
11	17.72 17.90	17.81	6.63 6.63	6.63	9.11 9.22	9.17	6.65 6.66	6.66
Final Average		17.99		6.54		9.36		6.69
Maximum		18.18		6.70		9.51		7.05
Minimum		17.81		6.35		9.17		6.39

* Not included in averages.

collaborators for this work, were taken from a parent batch which was ground and thoroughly mixed to insure equal distribution of moisture and fat contents.

The collaborative results obtained are quite satisfactory, indicating that if these procedures are followed, reasonable checks between analysts should be expected. Therefore, the procedure as outlined for determining moisture, and the procedure as outlined for determining fat by the acid hydrolysis method, in baked products other than bread which contain fruit, are recommended by the Associate Referee to be made official, first action.*

COLLABORATORS

J. C. Finley, The Great Atlantic & Pacific Tea Company, A & P Bakeries Laboratory, New York, N. Y.

James J. Winston, Jacobs Cereal Products Laboratory, Inc., New York, N. Y.

Frederic H. Luckman, Best Foods, Inc., Bayonne, N. J.

Aaron Friedman, Robert Laster, and Nat Nash, Joe Lowe Corporation, New York, N. Y.

E. A. Tjarks and Henry Klaessig, The Borden Company, New York Control Laboratory, New York, N. Y.

Gaston Dalby, Ward Baking Company, New York, N. Y.

R. W. Harter, E. K. Spotts, and E. G. White, National Biscuit Company Laboratory, New York, N. Y.

REPORT ON MOISTURE IN SELF-RISING FLOUR, AND IN PANCAKE, WAFFLE, AND DOUGHNUT FLOURS

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

In continuing the work of the previous associate referee, L. H. Bailey,[1] further consideration was given the distillation method, again using benzene as the distilling agent. The water is carried over with the benzene and is collected in a special trap and measured.

For this work a sample of self-rising flour was prepared by mixing together 1000 grams of soft wheat flour, 17.5 grams of salt, 12.5 grams of sodium bicarbonate, and 15.6 grams of monocalcium phosphate. After thoroughly mixing the ingredients with the flour, the mixture was run through a sieve 10 times and stored in air-tight containers.

Moisture was determined on the flour component of the prepared mixture in a 100°C. vacuum oven for 5 hours and the average of three determinations was 13.71 percent. The calculated true moisture of the prepared self-rising flour was 13.13 percent.

A problem involved in any determination of moisture by a distillation

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* For report of Subcommittee D and action by the Association, see *This Journal*, 28, 67 (1945). Details of the method will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.

[1] L. H. Bailey, *This Journal* 25, 648, (1943).

method is the optimum length of time to carry on the distillation process. Sair and Fetzer,[2] in their extensive work in the wet milling industry, discovered that the official method[3] consistently gave low results. They found that as many as 80 hours of distillation were needed to reach equilibrium in some grain products using benzene as the distilling agent.

Accordingly, determinations were made in an attempt to discover the equilibrium point in the benzene distillation of self-rising flour. The results are shown in Figure 1. It is seen that nearly 5 hours of distillation are needed to reach stability. One distillation was carried on for 10 hours with no detectable change after the first 4½ hours.

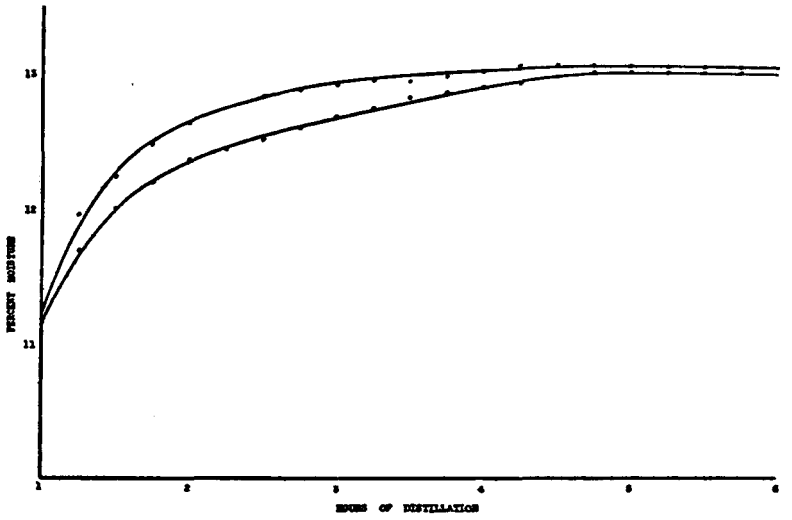

FIG. 1.—Hours of distillation.

Three moisture determinations were made by the following benzene distillation method:

METHOD

Add 25 grams of self-rising flour to a 300 ml. Erlenmeyer flask containing ca 25 previously dried glass beads. With 70 ml. of redistilled benzene (benzene redistilled over $NaHCO_3$) nearly fill the Bidwell-Sterling receiving tube, and pour the balance on flour in Erlenmeyer flask. Connect receiving tube to flask and condenser, and apply a suitable source of heat to flask. The distillation is started quite slowly (1–2 drops per second) but can be hastened after first half-hour period to about 2–4 drops per second. The distillation is so conducted that the distillate is condensed in the bottom two inches of condenser. After the first hour, and then from time to time, a

[2] Sair, L., and Fetzer, W. R., *Cereal Chem.*, XIX, 5 (1942).
[3] *Methods of Analysis, A.O.A.C.*, 4, 353 (1940).

few ml. of benzene is poured into the top of condenser to wash down any droplets of water that may have formed inside the tube. Continue the distillation for 5 hours, at end of which time condenser is thoroughly washed down with benzene. If any water remains in condenser or receiving tube remove it by brushing down by means of a copper wire with a small piece of rubber tubing attached to the end. Allow receiving tube to come to room temp. Read volume of water and calculate to percentage.

The results are given in Table 1.

TABLE 1.—*Moisture determinations (per cent)*

APPARENT MOISTURE	CALCULATED MOISTURE
13.20	13.13
13.16	
13.16	

Close agreement was obtained between the calculated moisture and the apparent moisture.

Moisture was also determined by the 70° vacuum oven method. These samples were placed in the oven for total of fifteen hours with weighings being made at five hour intervals. There was no change in weights after ten hours. The average of the three determinations was 13.09% which compares favorably with the calculated moisture.

However, these results are not conclusive and further extensive investigations are necessary. This will be done next year, when the method will be tried by collaboration.

It is also planned to use carbon tetrachloride as a distilling agent. Since carbon tetrachloride is heavier than water a different type of moisture trap will have to be employed. Close agreement should be obtained between the benzene and carbon tetrachloride distillations, since they boil at nearly the same temperature and are equally insoluble in water.

It is recommended* that the studies on the determination of moisture in all flour-like products that contain bicarbonate of soda as one of their constituents be continued.

No report on proteins in flour was made by the Associate Referee.

No report on bromates in flour was made by the Associate Referee.

No report on apparent viscosity measurement was made by the Associate Referee.

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* For report of Subcommittee D and action by the Association, see *This Journal*, **28**, 67 (1945).

REPORT ON BAKING POWDERS AND BAKING CHEMICALS

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

There are no associate referees on this subject. Referee's recommendations are confined to revisions and additions to Chapter **XVII,** "Baking Powders and Baking Chemicals." Associate Referee Clifford on fluorine and also lead has included baking powder in his studies and reports published in *This Journal* which should be identified with this chapter in the revision of *Methods of Analysis,* 1945.

RECOMMENDATIONS*

It is recommended that (1) the title "fluorine—Tentative" be added to this chapter, see Chapter **XXIX,** "Metals in Foods—general method on fluorine" (Referee, *This Journal* **27,** 90); and (2) the title "Lead—Tentative" be added, see Chapter **XXIX,** "Metals in Foods" sections 14, 17, 18, 19, and 20; or 14, 17, 21, 22, and 23. Special directions for sample preparation are given in *This Journal* **21,** p. 438, and **22,** p. 340.

(3) According to some authoritative references another procedure for the determination of neutralization value of monocalcium acid phosphate is used at the present time instead of the tentative procedure, *Methods of Analysis,* 1940, page 188, section 11. It is recommended that this section 11 be dropped and the procedure of "Cereal Laboratory Methods" of the A.A.C.C., 4th edition, 1941, be substituted.

(4) Sodium acid pyrophosphate is one of the acid reacting materials used in the manufacture of baking powder. In order to complete the methods for the determination of neutralizing values on acid reacting materials a method for the determination of the neutralizing value of sodium acid pyrophosphate should be added. (Method taken from S. Mendelsohn, Book on Baking Powder, Chemical Publishing Company, New York, 1939.)

(5) This chapter has qualitative methods for aluminum and tartrates in baking powder and should have a qualitative test for phosphoric acid. The test which has been used for many years by the Food and Drug Administration is recommended as a tentative method.

(6) That the following statement for expressing the neutralizing value be added to page 188, section 10; "Express results as parts of sodium bicarbonate equivalent to 100 parts of the acid reacting material."

(7) That lines 8 and 9, page 189, section 15, be deleted and substitute "Not more than 0.15 ml of 0.1 *N* alkali should be required to neutralize 100 ml of a mixture of 50 ml of chloroform and 150 ml of the saturated alcohol."

* For report of Subcommittee D and action taken by the Association, see *This Journal,* **28,** 67 (1945). Details of methods will be published in the 6th edition of *Methods of Analysis, A.O.A.C.,* 1945.

(8) That the following statement on purification pf phenylhydrazine be inserted after "redissolved," page 190, section 23 (b), line 3. "Unless freshly repurified, phenylhydrazine may not form a crystalline precipitate in which case it may be purified by distillation under reduced pressure of not more than 60 mm, discarding the first distillate until the temperature becomes constant."

A baking powder is on the market which contains a relatively large amount of calcium carbonate as a stabilizer in place of starch. The official gasometric method, page 187, section 6, may not be satisfactory without some modification on this type of baking powder. Some evidence indicates that the (1+5) sulfuric acid should be replaced with dilute hydrochloric (1+2). Study should be continued before a definite recommendation can be made to the Association.

REPORT ON FLAVORS AND NON-ALCOHOLIC BEVERAGES

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

At the 1943 meeting of the Association, it was recommended that collaborative study be continued upon the methods listed below:

(1) Determination of beta-ionone when 1 to 10 mgs are present.

(2) Determination of aldehydes in lemon oils and extracts by the Ripper method.

(3) Determination of esters in lemon extracts by the method published in *This Journal*, **25**, 154.

(4) Determination of esters in lemon oils by the Seeker-Kirby method.

(5) Determination of isopropyl alcohol in mixtures containing acetone.

(6) Photometric determination of vanillin and coumarin.

(7) Determination of glycerol, vanillin, and coumarin in vanilla and imitation vanilla, with special reference to the automatic extraction of vanillin and coumarin.

(8) The study of emulsion flavors.

(9) Studies of maple concentrates and imitations.

In addition, work was to be initiated upon the collaborative study of the following methods:

10. Determination of peel oil in citrus juices by the reflux method using the modified oil distillation trap.

11. Determination of diacetyl by the method published in *This Journal*, **25**, 255.

In view of the fact that insufficient collaborative work has been reported, on any of the above methods, to make a recommendation for adoption

at this time, the Referee recommends* that all of these studies be continued during the coming year.

No report on beta-ionone was made by the Associate Referee.

No report on lemon oils and extracts was made by the Associate Referee.

No report on organic solvents in flavors was made by the Associate Referee.

No report on glycerol, vanillin, and coumarin in vanilla and imitation vanilla was made by the Associate Referee.

No report on emulsion flavors was made by the Associate Referee.

No report on maple flavor concentrates and imitations was made by the Associate Referee.

No report on diacetyl was made by the Associate Referee.

REPORT ON FRUITS AND FRUIT PRODUCTS

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

RECOMMENDATIONS†

It is recommended:

(1) That directions for the preparation of sample for the determination of volatile and lactic acids, as given in the Report of the Associate Referee on those subjects be adopted as official, first action (with reference to the appropriate chapter for the determinations) and that the method (*This Journal*, 27, 210 (1944)) for the determination of volatile and lactic acids be made official, first action.

(2) That the magnesium uranyl acetate method for sodium (12, 18) be incorporated by reference and that the method be made tentative.

(3) That the reference to "Chlorine in ash, tentative" (Chap. XII, 35 and 37) be changed to "XII, 34, 35, and 37."

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* For report of Subcommittee D and action by the Association, see *This Journal*, 28, 68 (1945).

† For report of Subcommittee D and action by the Association, see *This Journal*, 28, 68 (1945). Details of the methods will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.

(4) That sodium chloride (rapid method) *Methods of Analysis*, XXXV, 25, page 521, be incorporated by reference and that the method be adopted as tentative.

(5) That methods for the determination of sodium and chloride be further studied and that these methods be subjected to collaborative work with a view to making them official.

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

(7) That the Hartmann methods for the determination of citric, malic, and tartaric acids (*This Journal*, **26**, 444) be subjected to collaborative study.

(8) That the volumetric procedure for the determination of P_2O_5 (*This Journal*, **25**, 441) be adopted as official, first action.

(9) That the gravimetric procedure for P_2O_5 (**38**, p. 347) be dropped, final action.

(10) That the gravimetric chloroplatinate procedure for the determination of potassium (*This Journal*, **26**, 326) be adopted as official (final action).

(11) That the gravimetric cobaltinitrite procedure for the determination of potassium (*This Journal*, **26**, 330) be adopted as official (final action).

(12) That the unified procedure for the determination of potassium (*This Journal*, **26**, 326) be subjected to collaborative study.

(13) That the investigation of methods for sampling cold-pack fruit be continued.

(14) That reference be made to the method (*This Journal*, **27**, 102, 1944) of determination of ascorbic acid (vitamin C) in citrus juices and tomato juice chapter on vitamins and that it be made official, first action.

(15) That the revised procedure of the report of the Referee for this year for determination of water-insoluble solids be adopted as tentative.

(16) That the study of the electrometric determination of titratable acidity of fruit acid solutions relatively high in phosphate (.01 *M*) be continued.

(17) That the following specification be added in the tentative method for titratable acidity by glass electrode (*This Journal*, **25**, 89): "The *p*H values used for interpolation should lie in the range 8.10 ± 0.2." This specification should follow the last sentence of the paragraph on "determination."

(18) That "neutralized" distilled water be made an alternate medium for dilution in the official method for total acidity (**24**, 341).

(19) That the official phenolphthalein powder, outside indicator method, be deleted because of the usually high and variable results of the collaborators.

(20) That the official .05% azolitmin solution, outside indicator method, be deleted because this indicator changes color at *p*H 7 instead of 8.

(21) That the extreme dilution method for the precise titration of colored solutions with phenolphthalein inside indicator be adopted as tentative.

No report on sodium and chlorides was made by the Associate Referee.

No report on polariscopic methods was made by the Associate Referee.

REPORT ON TITRATION OF ACIDS

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

When a colored fruit juice is titrated with the aid of a colored indicator, the quantity of standard alkali consumed per unit volume of sample depends largely upon the precision with which the end point can be detected. Deeply colored solutions, especially of red or purple shades, almost obscure the phenolphthalein end point of the official method for total acidity. The alternate outside indicators, .05 percent azolitmin solution or a dry powder mixture (1+99) in phenolphthalein and potassium sulphate, are likewise unsatisfactory. For this reason many workers have proposed methods for reducing the color interference by various means, such as adsorption or precipitation of the coloring matter, or otherwise. However, the most simple and popular methods involve the extreme dilution of the pigments so that the phenolphthalein color will predominate. Then various differential procedures are used for determining the titration value corresponding to the end point. The question at once arises as to whether the various methods of estimating the end point may not influence the results.

Subcommittee D in 1942[1] recommended: (4) "That methods for the titration of total acidity of fruits and fruit products with indicators be subjected to collaborative study with a view to unifying and standardizing this procedure."—and, further, (5) "That the titration of acidity with the commercial glass electrode *p*H meter to an end point of *p*H 8.10, be adopted as tentative."

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[1] *This Journal*, 25, 73 (1942).

This year the collaborative work has a two-fold purpose: (1) to further test the glass electrode method and (2) to study certain variations of the official indicator method.

COLLABORATIVE WORK

Collaborators were supplied with (1) a vial of National Bureau of Standards No. 84b potassium biphthalate prepared according to the directions of that Bureau (*i.e.*, crushing and drying for 2 hours at 120°C) and (2) five solutions of "unknowns."

Unknown A—.04991*M* potassium biphthalate (C.P.) prepared according to the N.B.S. directions for a buffer solution of *p*H 4.00 (20°C.).

Unknown B—Unknown A colored with 5 mg./liter of F.D.&C. Red No. 2 (Amaranth).

Unknown C—5 gms. of citric acid monohydrate (C.P.) and 1.1. gm. of potassium dihydrogen phosphate (C.P.) per liter.

Unknown D—canned orange juice (filtered solution 150 gms./liter) with acidity increased by adding 4.2 gms. of citric acid monohydrate (C.P.) and 0.9 gms. of potassium dihydrogen phosphate (C.P.) per liter.

Unknown E—filtered solution (150 gms./liter) of commercial product labeled "Grape Puree—ingredients—fruit, citric acid, sugar, grape extract, artificial color." The acidity was increased by adding 4.2 gms. of citric acid monohydrate (C.P.) and 0.9 gms. of potassium dihydrogen phosphate (C.P.) per liter of solution. The artificial color was F.D.&C. Red No. 2.

DIRECTIONS TO COLLABORATORS

The collaborators were requested to prepare a solution of *p*H 4.00 from the N.B.S. solid potassium biphthalate and to titrate the unknown solutions electrometrically to *p*H 8.10 after standardizing the *p*H meter against the standard buffer solution.

The same unknown solutions were also to be titrated by a number of variations of the official indicator methods, as follows:

(1) The official phenolphthalein (inside indicator) method followed as closely as possible.

(2) Complete the same as (1) but using "neutralized" instead of "recently boiled" distilled water.

(3) Same as (1) but using color standards to judge the end point by showing the change in color due to the dye and/or fruit ingredients near the *p*H of the phenolphthalein end point.

(4) The official phenolphthalein potassium sulphate (outside indicator) method with the indicator in the dry state.

(5) Same as (4) except that the outside indicator was moistened with neutral 95 percent alcohol.

(6) The official method using .05 percent azolitmin solution as an outside indicator.

(7) The method proposed by L. W. Ferris, of the Buffalo Station of the U. S. Food and Drug Administration, in a private communication.

ANALYST	LABORATORY	INSTRUMENT
(1) A. J. Tarr	Truesdail Laboratories, Inc. Los Angeles, Calif.	Beckman—Model G
(2) P. A. Mills	U. S. Food and Drug Administration, Seattle, Wash.	Beckman—Model F
(3) H. W. Gerritz	U. S. Food and Drug Administration, San Francisco, Calif.	Coleman-Style 200
(4) J. F. Armstrong	U. S. Food and Drug Administration, Los Angeles, Calif.	Coleman-Model 3C
(5) H. M. Bollinger	U. S. Food and Drug Administration, Los Angeles, Calif.	Coleman-Model 3C
(6) L. C. Jones	U. S. Food and Drug Administration, Los Angeles, Calif.	Coleman-Model 3C

COLLABORATIVE RESULTS

Table 1 shows the results of titrations of the unknowns by the alternative official indicator procedures and certain apparently desirable variations therefrom.

Tables 2 and 3 give a summary of the collaborators' data for the electrometric titrations.

ELECTROMETRIC TITRATIONS

Unknowns A and B were identical in composition except that B was colored with 5 mg. per liter of FD & C. Red No. 2 (Amaranth). These solutions were prepared so as to be .04991 *M* (20°C) in potassium biphthalate. This is the exact composition specified by the National Bureau of Standards for a buffer solution of *p*H 4.00 at 20°C or *p*H 4.01 at 25°C. Good results were obtained on the measurement of the initial *p*H values of these solutions. Each collaborator's data for the electrometric titration of solutions A and B were plotted (*p*H vs. ml 0.1*N* NaOH (20°C), individually, after adjustment of the buret readings to correspond to an aliquot of exactly 100 ml at 20°C. The results obtained by the collaborators are recorded in Table 2. The average of the concentrations from a total of 16 determinations by 6 collaborators was .04990 *M* (20°C.) with a minimum value of .04983 *M* and a maximum of .05002 *M*, $\sigma = \pm .000043\ M$.

At selected *p*H values, which well define the electrometric titration curves of the collaborators, the corresponding titrations were read. The ratio of these titration values to the value at *p*H 8.10 is recorded as "percentage neutralization" in Table 2. If all the collaborators' curves had exactly the same curvature in the *p*H range 6.8 to 9.0 the percentage neutralization value at a given *p*H should be the same for each. From this data a curve with the average characteristics of all the individual curves was constructed, and the deviations of the individual collaborators were considered. The standard deviations of the percentage neutralization values at the selected *p*H values show that the curves are all nearly iden-

tical with the exception of collaborator No. 3. In this case, subsequent calibration of the *p*H meter against Clark and Luf's buffers of known *p*H (range 7.6–9.0), based on standardization with N.B.S. potassium biphthalate buffer of *p*H 4.01, showed that the meter was operating satisfactorily. At present the Associate Referee is at a loss to explain the observations of Collaborator 3. These data were omitted from all the comparisons except calculation of acidity at *p*H 8.10.

The individual titration curves were slightly adjusted parallel to the titer axis so that each passed through the point (*p*H 8.10, titration 49.90 ml. 0.1 *N* NaOH) and then were compared with the "average titration curve." It was found that the maximum variation in *p*H from the average was ±.06 *p*H in the range below *p*H 8.10. At *p*H 8.50 this variation

TABLE 1.—*Acidity by indicator titrations*
(mls. 0.1 *N* NaOH/100 mls. of A and B or 50 mls. of C, D, and E (20°C.)

COLLABORATOR NO.	UNKNOWN	ALIQUOT ML. (20°C.)	METHOD						
			1	2	3	4	5	6	7
1	A	100	50.02	49.92		50.1			
	B	100	49.94	50.05	49.92	50.1			
2	A	100	49.85	49.90		50.2	50.0		
			49.85	49.90					
	B	100	49.95	49.90	49.90	50.3	50.0		
			49.95			50.0	50.0		
3	A	50	49.80	49.74			50.0		
	B	50	**	**	49.96		50.4		
4	A	100	49.94	49.94		50.3			
	B	100	49.93		50.09*	50.7			
5	A	100	49.96	49.96		50.1	50.2	48.9	49.90
	B	50	50.03		49.95	50.2	50.1	**	49.96
6	A	100	49.94	49.89					
	B	100	50.07	50.05		50.4		50.2	
Average	A		49.91	49.89		50.2	50.1	48.9	49.90
	B		49.96	49.97	49.96	50.3	50.2	50.2	49.96
1	C	50	42.24	42.18		42.6			
2	C	50	42.46	42.46	42.31	42 9	42.9		
			42.46	42.41	42.36	42.9	42.9		
3	C	50	42.04	41.87			42.0		
4	C	50	42.43	42.32		43.0			
5	C	50	42.37	42.35		42.8	42.6	41.0	
6	C	50		42.24					
Average			42.31	42.23	42.34	42.8	42.5	41.0	

TABLE 1.—*Continued.*

COLLABORATOR NO.	UNKNOWN	ALIQUOT ML. (20°C.)	METHOD						
			1	2	3	4	5	6	7
1	D	50	42.2†	41.1	41.0	41.4			
2	D	50	41.2 41.2	41.2 41.2	41.0 41.0	41.5 41.7	41.5 41.5		
3	D	50	41.6	41.3	41.2		41.3		
4	D	50	41.3	41.3	41.3	42.1			
5	D	50	41.3	41.2	41.0	41.5	41.3	39.0	41.0
6	D	50		41.1					
Average			41.4	41.2	41.1	41.7	41.4	39.0	41.0
1	E	50	**		**	**			42.3
2	E	10 5	42.1 41.5	42.1 41.5	41.5 41.5	43.0 44.0	42.5 43.0		
3	E	10	**	**	**		**		
4	E	10	41.2	41.8	41.5	48.4 47.6			
5	E	10	42.1	41.8	41.2	42.9	42.0	**	42.3
6	E	10	41.8		**	43.2*	44.0*	**	42.0
Average			41.7	41.8	41.4				42.3

* 50 ml aliquot of sample.
** Color interference.
† The Collaborator says the difference is probably due to overtitration of difficult end point—omit from average.

amounted to ±0.12 and at *p*H 9 the value was ±0.2. The above comparisons indicate that the variations in calculated acidity at *p*H 8.10 are probably due largely to volumetric errors, rather than to errors in *p*H measurement.

Similar treatment of the electrometric titration data for Unknowns C, D, and E (see Table 3) was not possible because of the uncertainty introduced by the various dilutions used by the collaborators. These solutions were prepared with the following points in mind: (1) To contain a relatively large concentration of phosphate ion (ca .01 *M*) so as to demonstrate the effects of dilution upon the final calculated acidity corresponding to *p*H 8.10; (2) the slope of *p*H-titer curve at *p*H 8.10 should be approximately 1 *p*H/ml of 0.1 *N* NaOH. This is a common value for the slope of this curve in the titration of fruit products electrometrically and enables *p*H differences to be judged fairly accurately; (3) the slope of the *p*H-titer curve in the vicinity of *p*H 8.10 should change rapidly so that errors due to

TABLE 2.—*Acidity by electrometric titration—unknowns A and B*

(mls. 0.1 *N* NaOH per 100 mls. (20°C.)

COLLABORATOR NO.	UNKNOWN	ROOM TEMPERATURE °C.	INITIAL pH AT B. T.	ACIDITY AT pH 8.10		"PERCENTAGE NEUTRALIZATION" AT pH—						
						6.80 (0.44)*	7.00 (0.65)	7.30 (1.3)	7.50 (1.8)	8.10 (5.5)	8.50 (5.5)	9.00 (3.2)
					Average							
1	A	26	4.01		49.89	98.04	98.82	99.42	99.64	100.00	100.09	100.24
	B	26	4.01		49.83	98.17	98.73	99.36	99.64	100.00	100.08	100.22
2	A	23	4.00	49.85		98.14	98.90	99.52	99.72	100.00	100.12	100.24
			4.02	49.88	49.87	98.09	98.84	99.52	99.74	100.00	100.10	100.22
	B	23	4.00	50.02		98.14	98.86	99.48	99.68	100.00	100.14	100.32
			4.00	49.95	49.99	98.10	98.82	99.46	99.64	100.00	100.10	100.26
3**	A	25	4.11	49.84†		97.76†	98.54†	99.20†	99.42†	100.00†	100.46†	—
		26	4.03	49.90	49.90	—	98.32	99.00	99.42	100.00	100.52‡	—
	B	25	4.03	49.88		—	98.36	99.04	99.28	100.00	100.5‡	—
		26	4.01	49.87	49.88	—	—	—	—	100.00	—	—
4	A	25	4.00		49.87	98.20	98.86	99.44	99.68	100.00	100.12	100.42
	B	25	4.00		49.82ǂ	98.05	98.76	—	—	—	100.16	100.36
5	A	25	4.01		49.93	97.99	98.76	99.40	99.62	100.00	100.14	100.36
	B	25	4.00		49.92	97.95	98.72	99.38	99.62	100.00	100.12	100.36
6	A	26	4.00		49.91	97.74	98.56	—##	99.58	100.00	100.12	—
	B	25	4.03		49.91	—	—	99.37	99.62	100.00	100.12	100.36
Average				49.90		98.05₈	98.78₈	99.43₈	99.65₈	100.00	100.11₈	100.30₈
Minimum				49.83		97.74	98.56	99.36	99.58	—	100.08	100.22
Maximum				50.02		98.20	99.90	99.52	99.74	—	100.16	100.36
Standard Deviation				±.04		±0.13	±.09	±.06	±.05	—	±.02	±.08

* The values in parentheses are the ca. slopes (pH/ml. 0.1 *N* NaOH) of the titration curves at the given pH.

** All data except acidity at pH 8.10 are omitted from the averages, since the titration curves are obviously different from those of all the other collaborators. The curves of this collaborator intersect the average curve near pH 8.10. Subsequent calibration of the pH meter against Clark and Lubs buffers indicated that it was working satisfactorily.

† When these data are adjusted to an initial pH 4.01, the acidity at pH 8.10 calculates 49.89 ml. 0.1 *N* NaOH. Percentage neutralizations were then calculated on the basis of the adjusted data.

‡ Extrapolated values.

ǂ Incompatible data from pH 7.2 to 8.4—smoothed curve gives acidity of 49.87 ml. 0.1 NaOH; percentage neutralization calculated from original data and 49.87 ml. titration.

"Bump" in titration curve, data omitted.

interpolations, based on the assumption of a straight line relationship, will be exposed.

Several times the collaborators were able to take buret readings at exactly *p*H 8.10, or very close thereto, so that the errors of interpolation were negligible. In other cases the *p*H range between consecutive readings which bracketed *p*H 8.10 was substantial; the largest errors due to interpolation were of the order of −.05 ml. The maximum error that could result would be in sample C for the unusual case where the consecutive *p*H values were 7.75 and 8.50; the corresponding error would be −0.14 ml. Therefore, it seems that a specification is necessary to make the interpolation error for this unusual type of curve as small as practicable. Such a specification would be that the *p*H values on which the interpolation is based fall within a *p*H range of 8.10 ± 0.2. This allowable range would result in a maximum error of −.04 ml.

INDICATOR TITRATIONS

Unknown A—Colorless solution with "sharp" end point by all indicator methods.
Unknown B—Deep pink solution, otherwise identical with A.

Acidities based on all the phenolphthalein inside indicator methods, using either boiled distilled water or neutralized distilled water, are substantially the same. The values range from 49.74 to 50.09 ml 0.1 *N* NaOH per 100 ml aliquot ($\sigma = \pm .07$ ml). The collaborator's remarks concerning color interference in B ranged from "no interference" to "impossible end points." Comparison of the average results for A and B show that there was a slight tendency to over-titrate the colored solution.

The average results of phenolphthalein outside indicator titrations tend to be slightly higher than the inside indicator methods in these solutions in which the *p*H is changing rapidly at the end point.

The single result with .05 percent azolitmin solution shows that this indicator is not satisfactory since the color change takes place near *p*H 7.

Unknown C—Colorless solution with "slow" end point.
Unknown D—Yellow colored solution with "slow" end point.
Unknown E—Deep purple colored solution with "slow" end point.

Results of all the phenolphthalein inside-indicator methods* are substantially the same. Neutralized distilled water appears to be a satisfactory substitute for "recently boiled distilled water." The titrations for unknown C average 42.27 and range from 41.87 to 42.46 ml ($\sigma = \pm 0.17$ ml); for D the range is 41.0−41.6; for E the range is 41.2−42.1. The yellow color in sample D usually resulted in a slight over-estimation when the titration procedure was not supplemented by some means of minimizing the color interference. The color of E was so deep that most of the collaborators used a 10 ml aliquot diluted with 200 ml of water; calculation of these results for a 50 ml aliquot thus multiplies the titration error by a factor of five.

* Methods 1, 2, and 3.

TABLE 3.—*Acidity by electrometric titration—unknowns C, D, and E*
(mls. 0.1 *N* NaOH per 50 mls. aliquot (20°C.)

COLLABORATOR NO.	UNKNOWN	ROOM TEMP., °C.	INITIAL pH AT R.T.	ACIDITY AT pH 8.10		"PERCENTAGE NEUTRALIZATION" AT pH—						
						7.50	7.90	8.10	8.30	8.60	8.80	9.10
						(0.45)*	(0.84)	(c = 1.2) (D&E = 1.1)	(c = 1.7) (D&E = 1.4)	(D&E = 1.3)	(c = 3.2)	(D&E = 0.9)
					Average							
1	C†	25	2.54†									
			2.66		42.17	97.60	99.50	100.00	100.31		100.73	
2	C†	23	2.63	42.46		97.86	99.51	100.00	100.31		100.80	
			2.71	42.66	42.56	97.77	99.51	100.00	100.40		100.94	
3**	C	25	2.55	41.99		97.28	99.36	100.00				
			2.69	41.93	41.96		99.43	100.00				
4	C	26	2.50		42.24	97.85	99.50	100.00	100.28		100.78	
5	C	25	2.49		42.31	97.87	99.53	100.00	100.31		100.76	
6	C‡	25	2.66		42.29	97.47	99.41	100.00	100.42			
Average Range					42.26 41.93–42.66							
			2.98††									
1	D†	22	3.02		40.84	97.62	99.41	100.00	100.39	100.91		101.91
			3.01	40.98		97.71	99.46	100.00	100.39	100.90		101.88
2	D†	23	2.98	40.91	40.95	97.85	99.46	100.00	100.39	100.88		101.86

			3.08	41.31		97.53	99.32	100.00	100.56			
3	D**	25	3.00	41.19	41.25		99.29	100.00	100.80			
4	D	25	2.89		40.97	98.00	99.49	100.00	100.37	100.93		102.00
5	D	23	2.89		41.05	97.91	99.46	100.00	100.37	100.88		101.95
6	D‡	25	3.03		40.93	97.48	99.36	100.00	100.44			
Average					41.00							
Range					40.84–41.31							
			2.82††									
1	E†	23	2.90		40.96	97.88	99.39	100.00	100.46	101.07		101.17
			2.87	41.11		97.91	99.51	100.00	100.46	101.05		102.16
2	E†	23	2.84	14.12	41.12	98.01	99.56	100.00	100.44	101.00		102.19
			2.73	41.70		97.79	99.38	100.00	100.58			
3	E**	23	2.90	41.79	41.75		99.40	100.00	100.60			
4	E	26	2.77		41.10	98.05	99.51	100.00	100.36	101.00		102.34
5	E	24	2.78		41.18	98.08	99.47	100.00	100.36	100.90		102.36
6	E‡	24	2.93		41.12	97.69	99.44	100.00	100.44			
Average					41.10							
Range					40.96–41.79							

* See corresponding footnote of Table 2.
** See corresponding footnote of Table 2. Results for unknown E omitted from average.
† 50 mls. of water added before titration.
†† Initial *p*H before addition of water.
‡ 75 mls. of water added before titration.

The phenolphthalein outside indicator results are all higher (ca 1 percent) and have a wider range than those of the inside indicator methods.

Azolitmin indicator solution when usable gives the wrong end point for these samples.

EXPERIMENTAL

Effect of variable dilutions.—Variations in the degree of dilution of samples C, D and E by the collaborators introduced uncertainties which made it impossible to accurately compare the electrometric titration data over the *p*H range from 7.5–9.0. Therefore, it seemed advisable to determine the magnitude of the effect of very high dilutions upon the titratable acidity. A series of dilution experiments on these samples showed that 100-fold dilutions do not change the calculated acidity results by more than −0.6 percent, and intermediate-fold dilutions have a smaller effect.

Effect of CO_2 in neutralized distilled water.—Before proposing the alternate use of neutralized distilled water in place of "recently boiled distilled water" for dilutions, additional data concerning the buffer effect of the bicarbonate ion was obtained. In the Associate Referee's experience the highest values for CO_2 content of distilled waters have been equivalent to 3 to 4 ml 0.1 *N* NaOH per liter by phenolphthalein titration. The buffering effect of a hypothetical water solution of CO_2 titrating 10 ml 0.1 *N* NaOH per liter would certainly be about as high as might be encountered.

A saturated CO_2 solution was allowed to stand overnight in contact with air. 100 ml of this solution titrated 10.4 ml 0.1 *N* NaOH (25°C.) at *p*H 8.10. The slope of the titration curve from *p*H 7.8 to 8.5 was nearly constant at 1.05 *p*H/ml. From this data an estimate of the slope of the titration curve of 100 ml of the above hypothetical water sample calculates (1.05) (10.4) = 11*p*H/ml 0.1 *N* NaOH. This slope is far greater than any slopes encountered in the usual titration of fruit products or the pure fruit acids, thus the CO_2 content of the hypothetical water sample would not offer any disadvantage in either the electrometric or indicator titrations. The probable CO_2 content of a normal distilled water would certainly have a negligible effect.

SUMMARY

The collaborative results by indicator titrations show that "neutralized" distilled water is a satisfactory substitute for the "recently boiled" distilled water specified in the present official method for the total acidity of fruits and fruit products. In addition, the buffer characteristics of dilute CO_2 solutions were studied briefly. It was found that any concentration of bicarbonate ion likely to be found in normal "neutralized" distilled waters was so low as to have a negligible buffer effect in the titration.

A comparison of the use of the outside indicators, 1 percent of phenolphthalein powder +99 percent of potassium sulfate powder mixture, or .05 percent azolitmin solution shows that these indicators give different results from those obtained with the aid of the inside indicator (1 percent phenolphthalein) solution. The dry phenolphthalein indicator is usually slow in changing color. This results in over-titration. The magnitude of this over-estimation depends on depth of color of the sample and its buffer characteristics.

Azolitmin indicator solution was found to give low results because the color change takes place near *p*H 7. This latter finding is in accord with

the results reported by Roberts,[2] the Associate Referee in 1935.

When the inside phenolphthalein indicator method is supplemented by a means of judging the appearance of the end point, results which are more concordant with the electrometric method are obtained. Such supplemental methods are those proposed by Ferris and the Associate Referee. The former depends on extreme dilution of a small aliquot of the titration mixture so that the indicator color predominates. The latter depends on auxiliary color standards which show the color change of the dye and/or fruit ingredients near the end point *p*H. The appearance of the pink phenolphthalein color can then be judged by reference to these standards.

The results of 16 electrometric titrations of a .04991*M* potassium biphthalate solution by 6 analysts averaged .04990*M*; minimum .04983*M*, maximum .05002*M*; standard deviation ± .000043*M*. This standard deviation is about half of the corresponding deviation for the phenolphthalein titration. Such a finding is in accord with the collaborative results of the preceding report (1942).[3] In addition, the curvatures of the individual collaborators titration curves were compared in the *p*H range from 6.8–9.0 (omitting the data of Collaborator #3). It was found that the maximum variations in *p*H were of the order of .06 *p*H in the range below *p*H 8.10. At *p*H 8.50 the variation amounted to 0.12 *p*H and at *p*H 9.0 the variation was 0.2 *p*H. The Associate Referee is unable, at this time, to explain the anomalous results of Collaborator #3.

A similar graphical analysis of the electrometric titration data on solutions C, D, and E was not possible because of the uncertainty introduced by variable dilutions at *p*H values other than near 8. The wide range of titration values at *p*H 8.10 reported on these solutions is more than could be accounted for by the sum of the usual volumetric errors and the *p*H errors indicated by the foregoing calculations related to solutions A and B. It seems that some other explanation must be sought for the variations which are common to the three solutions, C, D, and E. These solutions are different in composition from each other except that the phosphate content is relatively high (ca .01*M*). They are also different in composition from the fruit solutions analyzed collaboratively in the previous Associate Referee's report (1942), in that the latter contained no added potassium dihydrogen phosphate.

RECOMMENDATIONS*

It is recommended—

That the study of the electrometric determination of titrable acidity of fruit acid solutions, relatively high in phosphate, be continued.

[2] W. L. Roberts, North Dakota Regulatory Department, Bismarck, N. Dak. *This Journal*, 18, 599 (1935).

[3] *This Journal*, 25, 412 (1942).

* For report of Subcommittee D and action by the Association, see *This Journal*, 28, 69 (1945). The details of the approved methods will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.

That the following directions be added in the tentative method for titratable acidity by glass electrode: "The *p*H values used for interpolation should lie in the range 8.10 ± 0.2. This should follow the last sentence of the paragraph on "determination."

That "neutralized" distilled water be made an alternate medium for dilution in the official method for total acidity.

That the official phenolphthalein powder, outside indicator method for highly colored solutions, be deleted, and the official .05 per cent azolitmin solution, outside indicator method, be deleted, because this indicator changes color at *p*H 7 instead of 8.

That the extreme dilution method (*e.g.* the Ferris method) or an alternative method with color standards (*e.g.*, the procedure described in this report) as recommended by the Referee for the precise titration of colored solutions with phenolphthalein inside indicator, be adopted as tentative.

No report on fruit acids was made by the Associate Referee.

No report on phosphoric acid was made by the Associate Referee.

No report on potassium was made by the Associate Referee.

REPORT ON SAMPLING COLD PACK FRUIT

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

The work done last year was repeated and extended as recommended.

A trier approximately 1 inch inside diameter and 40 inches long, of stainless steel tubing, equipped with a cap allowing the use of an electric motor, was obtained.

The packing of the following barrels was supervised in a commercial packing plant:

1 barrel straight strawberries
1 barrel 3+1 strawberries
1 barrel straight red raspberries
1 barrel straight Evergreen blackberries
1 barrel straight Evergreen blackberries containing 10% added water.

During the time each barrel was being filled 42 quart jars of fruit being packed were taken, except that the two barrels of blackberries were filled

simultaneously and only one sample was obtained to represent both barrels.

Each 42-jar sample was divided and treated as follows:

Sub. A, 12 jars, was placed in storage with the barrel, frozen two days at 0° F. and stored at 10° F.

Sub. B, 6 jars, was immediately heat-processed and held in storage at 8° F.

Sub. C, 6 jars, was held 12 hours at 65° F, processed and stored at 8° F.

Sub. D, 6 jars, was held 12 hours at 65° F, 24 hours at 40° F, processed and stored at 8° F.

Sub. E, 6 jars, was held 12 hours at 65° F, 24 hours at 40° F, 24 hours at 8° F, processed and stored at 8° F.

Sub. F, 6 jars, was held 12 hours at 65° F, 24 hours at 40° F, and stored at 8° F without processing.

This treatment was designed to approximate conditions in the freezing barrel and was based on freezing data contained in Tech. Bull. 148, U. S. Department of Agriculture, by H. C. Diehl, *et al.*, 1930. It was believed that heat processing would arrest the ripening of the fruit at the times desired.

Each sub. was then analyzed as time permitted.

SAMPLING OF BARRELS

Each barrel was sampled from both ends, *i.e.*, top and bottom.

Using the small, or 1-inch trier, 3 cores, spaced equally around the circumference, were taken diagonally through the length of the barrel from both top and bottom. The three cores from the top were composited and likewise those from the bottom. All cores were spaced in such a manner as not to coincide with another except at the center of the barrel.

Using the 2-inch trier, 3 cores were taken from each end straight down in the barrel midway between the edge and the center and evenly spaced around the circumference. These cores were approximately 26 inches long and lacked about 4 inches of extending the full depth of the barrel. Each of these large cores was analyzed separately and all results are given in Table 1.

DISCUSSION OF RESULTS

In this series of samples there were found no significant differences in the analyses of the fresh fruit samples. Possibly the fruits packed were more mature at the time of packing than last year. There appears to be a slight increase in the ash and potash values and a greater increase in the P_2O_5 and water insoluble solids values of samples GT and GB (from barrels) compared with corresponding fruit samples in the Mason jars (A to F). The small variations may be due to evaporation of small amounts of moisture from the barrelled fruit during freezing. The greater amounts of P_2O_5 in the samples from barrels appear anomalous.

TABLE 1.—*Trier sampling of barrels*

SUB	SAMPLE	INSOL. SOLIDS	SOLUBLE SOLIDS, COR.	TOTAL SUGARS AS INVERT	TOTAL ACID AS CITRIC	ASH	MG/ 100 GM K_2O	MG/ 100 GM P_2O_5	CALCULATED TO SUGAR-FREE BASIS				
									INSOLUBLE SOLIDS	TOTAL ACID AS CITRIC	ASH	MG/ 100 GM K_2O	MG/ 100 GM P_2O_5
		%	%	%	%	%			%	%	%		
	Inv. 93853E Strawberries												
B		2.38	8.25	5.72	0.674	0.357	160	25.0	2.52	0.715	0.379	170	26.5
C		2.22	8.33	5.63	0.650	0.326	166	28.2	2.35	0.689	0.345	176	29.9
D		2.36	8.05	5.38	0.614	0.353	168	30.2	2.49	0.649	0.373	178	31.9
E		2.42	7.85	5.33	0.616	0.332	167	28.9	2.53	0.651	0.351	176	30.5
F	Stored 8°F—8 months	2.40	7.96	5.14	0.607	0.331	161	28.4	2.53	0.640	0.349	170	29.9
A	Stored with bbl.—10°F—9 months	2.33	7.94	5.39	0.653	0.303	166	27.9	2.46	0.690	0.320	175	29.5
	Average (6)	2.35	8.06	5.43	0.636	0.334	165	28.1	2.48	0.672	0.353	174	29.7
	BBl. Straight Frozen.												
GT	3—1 inch cores from top of bbl. Composited	2.41	8.54	5.72	0.708	0.328	170	33.1	2.56	0.751	0.348	180	35.1
GB	3—1 inch cores from bottom of bbl. Composited	2.34	8.01	5.43	0.650	0.312	161	32.3	2.47	0.687	0.330	170	34.2
G1	2 inch core from top of bbl.	2.57	8.18	5.55	0.638	0.335	163	29.8	2.72	0.675	0.355	173	31.6
G2	2 inch core from top of bbl.	2.53	8.19	5.47	0.640	0.342	163	30.1	2.68	0.677	0.362	172	31.8
G3	2 inch core from top of bbl.	2.55	8.02	5.48	0.665	0.303	162	30.0	2.70	0.703	0.321	171	31.7
G4	2 inch core from bottom of bbl.	2.14	8.31	5.75	0.713	0.319	172	31.8	2.27	0.756	0.338	182	33.7
G5	2 inch core from bottom of bbl.	2.57	8.66	5.91	0.713	0.327	174	32.8	2.73	0.758	0.348	185	34.9
G6	2 inch core from bottom of bbl.	2.40	8.30	5.78	0.706	0.336	173	32.4	2.55	0.749	0.357	184	34.4
	Average (6)	2.46	8.28	5.66	0.679	0.327	168	31.1	2.61	0.720	0.347	178	33.0

	Inv. 93854E Strawberries											
B		2.10	8.27	5.53	0.640	0.333		26.7	2.22	0.677	0.352		28.3
C		2.05	8.34	5.50	0.620	0.318	157	27.9	2.17	0.656	0.337	166	29.5
D		2.27	7.79	5.28	0.598	0.339	162	26.0	2.40	0.631	0.358	170	27.4
E		2.21	7.73	5.26	0.609	0.330	161	26.7	2.33	0.643	0.348	170	28.2
F	Stored 8°F—8 months	2.21	7.98	5.24	0.616	0.330	160	27.7	2.33	0.650	0.348	169	29.2
A	Stored with bbl.—10°F—9 months	2.27	7.75	5.43	0.638	0.316	162	27.0	2.40	0.675	0.334	171	28.6
	Average (6)	2.19	7.98	5.37	0.620	0.328	160	27.0	2.31	0.655	0.347	169	28.5
	Bbl. 3+1 Frozen												
GT	Comp. 3—1 inch cores from top.	2.38	29.08	26.97	0.548	0.262	137	26.4	3.26	0.750	0.359	188	36.1
GB	Comp. 3—1 inch cores from bottom	2.06	29.45	26.70	0.522	0.242	128	24.0	2.81	0.712	0.330	175	32.7
G1	2 inch core from top	2.16	29.41	26.48	0.524	0.245	130	23.0	2.94	0.713	0.333	177	31.3
G2	2 inch core from top	2.09	29.44	27.38	0.524	0.237	126	21.7	2.88	0.722	0.326	174	29.9
G3	2 inch core from top	2.62	17.20	14.45	0.621	0.299	152	28.7	3.06	0.726	0.350	178	33.5
G4	2 inch core from bottom	2.21	28.33	26.38	0.527	0.255	130	23.2	3.00	0.716	0.346	177	31.5
G5	2 inch core from bottom	2.65	27.21	25.13	0.530	0.269	132	23.8	3.54	0.708	0.359	176	31.8
G6	2 inch core from bottom	2.07	26.82	25.10	0.530	0.255	133	22.9	2.76	0.708	0.340	178	30.6
	Average (6)								3.03	0.716	0.342	177	31.4
	Inv. 93857E Red Raspberries												
B		5.87	9.83	6.64	0.956	0.398	171	37.6	6.29	1.024	0.426	183	40.3
C		5.89	9.89	6.72	0.908	0.411	174	40.2	6.31	0.973	0.441	187	43.1
D		5.93	10.14	6.99	0.890	0.360	178	35.9	6.38	0.957	0.387	191	38.6
E		5.95	9.79	6.48	0.914	0.357	175	36.8	6.36	0.977	0.382	187	39.3
F	Stored 6 months—8°F.	5.85	10.31	6.58	0.948	0.352	174	38.9	6.26	1.015	0.377	186	41.6
A	Stored with bbl.—10°F.—8½ months	5.75	10.30	7.07	0.942	0.357	170	41.6	6.19	1.014	0.384	183	44.8
	Average (6)	5.87	10.04	6.75	0.926	0.373	174	38.5	6.30	0.993	0.400	186	41.3

TABLE 1.—*Continued*

SUB	SAMPLE	INSOL. SOLIDS	SOLUBLE SOLIDS, COR.	TOTAL SUGAR AS INVERT	TOTAL ACID AS CITRIC	ASH	MG/ 100 GM K_2O	MG/ 100 GM P_2O_5	CALCULATED TO SUGAR-FREE BASIS				
									INSOLUBLE SOLIDS	TOTAL ACID AS CITRIC	ASH	MG/ 100 GM K_2O	MG 100 GM P_2O_5
		%	%	%	%	%			%	%	%		
	Bbl. Straight Frozen												
GT	Comp. 3—1 inch cores from top	6.28	10.52	7.13	1.001	0.379	178	46.3	6.76	1.078	0.408	192	49.9
GB	Comp. 3—1 inch cores from bottom	5.91	10.25	6.73	0.969	0.364	174	44.8	6.34	1.039	0.390	187	48.0
G1	2 inch core from top	6.28	10.58	7.14	1.016	0.384	182	41.9	6.76	1.094	0.414	196	45.1
G2	2 inch core from top	6.50	10.86	7.22	1.031	0.386	185	46.4	7.01	1.111	0.416	199	50.0
G3	2 inch core from top	6.24	10.77	6.93	1.003	0.372	173	47.2	6.70	1.079	0.400	186	50.7
G4	2 inch core from bottom	6.22	11.01	7.24	1.040	0.386	181	46.3	6.71	1.121	0.416	195	49.9
G5	2 inch core from bottom	6.07	10.61	7.19	1.048	0.392	183	46.1	6.54	1.129	0.422	197	49.7
G6	2 inch core from bottom	6.29	10.88	7.51	1.067	0.406	189	46.7	6.80	1.154	0.439	204	50.5
	Average (6)	6.27	10.79	7.21	1.034	0.388	182	45.8	6.75	1.115	0.418	196	49.3
	Inv. 93860E Blackberries												
B		6.52	9.04	5.70	0.761	0.402	204	40.2	6.91	0.807	0.426	216	42.6
C		7.19	8.89	5.65	0.728	0.396	202	37.9	7.62	0.772	0.420	214	40.2
D		7.18	8.91	5.74	0.646	0.384	198	40.0	7.62	0.685	0.407	210	42.4
E		6.96	8.88	5.66	0.648	0.394	202	40.8	7.38	0.687	0.418	214	43.2
F	Stored 8°F.—5½ months	7.00	9.10	5.46	0.655	0 401	204	41.6	7.40	0.693	0.424	216	44.0
A	Stored with bbl.—10°F.—8 months	7.35	8.57	5.80	0.728	0.394	200	38.6	7.80	0.773	0.418	212	41.0
	Average (6)	7.03	8.90	5.67	0.694	0.395	202	39.9	7.46	0.736	0.419	214	42.2

	Bbl. Straight Frozen												
GT	Comp. 3—1 inch cores from top	7.37	8.57	5.58	0.732	0.436	210	51.1	7.81	0.775	0.462	222	54.1
GB	Comp. 3—1 inch cores from bottom	7.55	8.55	5.62	0.711	0.417	203	48.1	8.00	0.753	0.442	215	51.0
G1	2 inch core from top	7.34	8.90	5.93	0.709	0.397	202	47.0	7.80	0.754	0.422	215	50.0
G2	2 inch core from top	7.77	8.37	5.52	0.743	0.407	204	48.3	8.22	0.786	0.431	216	51.1
G3	2 inch core from top	7.98	8.42	5.51	0.775	0.410	211	49.4	8.45	0.820	0.434	223	52.3
G4	2 inch core from bottom	7.53	8.21	5.36	0.741	0.427	214	47.3	7.96	0.783	0.451	226	50.0
G5	2 inch core from bottom	7.66	8.61	5.70	0.735	0.424	208	48.2	8.12	0.779	0.450	221	51.1
G6	2 inch core from bottom	7.61	8.79	5.82	0.724	0.398	203	46.5	8.08	0.769	0.423	216	49.4
	Average (6)	7.65	8.55	5.64	0.738	0.411	207	47.8	8.11	0.782	0.435	220	50.7
	Bbl.—10% added water												
GTW	Comp. 3—1 inch cores from top	7.06	8.20	5.26	0.674	0.385	196	46.9	7.45	0.711	0.406	207	49.5
GBW	Comp. 3—1 inch cores from bottom	6.88	7.75	5.02	0.659	0.363	184	42.9	7.24	0.694	0.382	194	45.2
G2W	2 inch core from top	6.45	7.47	4.84	0.687	0.356	181	39.8	6.78	0.722	0.374	190	41.8
G3W	2 inch core from top	6.48	7.76	5.13	0.640	0.356	181	39.8	6.83	0.675	0.375	191	42.0
G4W	2 inch core from bottom	7.07	7.65	4.90	0.685	0.350	187	42.3	7.43	0.720	0.368	197	44.5
G5W	2 inch core from bottom	6.76	7.49	4.67	0.685	0.365	181	40.0	7.09	0.719	0.383	190	42.0
G6W	2 inch core from bottom	7.00	7.53	4.78	0.700	0.368	181	42.3	7.35	0.735	0.386	190	44.4
	Average (5)	6.75	7.58	4.86	0.679	0.359	182	40.8	7.10	0.714	0.377	192	42.9

Inspection of the results of analysis indicates that a sample taken diagonally through the length of the barrel from the bottom represents the package quite well. Juicing of the fruit during packing results in a condition where the proportion of juice to fruit increases toward the bottom of the barrel and it is impossible to obtain a representative sample from the top unless the trier is bored completely through the bottom of the barrel. However, if the package is sampled from the bottom the air space left at the top furnishes a space which insures complete sampling. These data do not include advantages of diagonal sampling over cores taken straight through. Neither can any conclusions be drawn as to the minimum number of cores that should be taken. The Associate Referee is of the opinion that not less than 3 should be taken with the one-inch trier in order to obtain a sample large enough for convenient analysis.

Samples taken with the 2-inch trier did not prove representative because much of the barrel was represented twice. Cores 26 inches long taken from each end result in a sample of 4 inches from each end and a duplicate of the 18 inches in the center. A more representative sample could be obtained by boring only half way from each end. Study of the analyses of individual cores indicates that one core halfway through the barrel from each end, midway between the side of the barrel and its center and from opposite sides should result in a representative sample. This method should be especially effective on sugar packs, where the material is not frozen solid, because the core from the top would not extend to the sirup layer, and the core from the bottom would contain all the sirup trapped by a plug of fruit.

The experiment with blackberries in which two barrels were packed, one straight and one with 10 percent added water, showed on comparison of the analyses of the cores taken diagonally through the barrels from the bottom an average lowering of the various constituents of 9.91 percent, and, when compared to the sample of fruit stored with the barrel, a lowering of 9.13 percent (excluding phosphoric acid which showed an increase in the barrels).

In this experiment there was no evidence of unequal freezing from one side to the other or toward the center. All of the barrels appeared to have been frozen top up. It is believed that diagonal sampling or sampling from opposite sides from both ends of the barrel will compensate for any variations encountered.

METHODS OF ANALYSIS USED

All samples were prepared by passing them 3 times through a sausage mill.

Soluble Solids—Abbé Refractometer.

Insoluble Solids— *Methods of Analysis,* **XXVI,** 7 (1940).

Total Sugars after inversion—*Methods of Analysis*, **XXXIV, 24c, 38, 39** (1940).

Total Acidity—Direct titration using *p*H meter at 8.1 as an indicator.

Ash—*Methods of Analysis*, **XXVI, 9** (1940).

Phosphoric acid—*This Journal*, **25**, 437 (volumetric).

Potassium oxide—*This Journal*, **26**, 324 (short gravimetric).

RECOMMENDATIONS FOR FURTHER WORK

Instead of collecting glass jars to represent the barrel being packed, a large sample consisting of 2 or 3 No. 10 tins should be taken. Where fruit-sugar packs are made, the proper proportion of fruit and sugar should be placed in the tins and the tins should be frozen and stored with the barrels. Where barrels containing added water are packed, the same proportionate quantity of water should be added to the representing sample at the time of packing and frozen and stored with the barrels.

After storage for a desirable period each barrel should be sampled from the bottom with the 1-inch trier, taking 3 cores diagonally and 3 cores straight through, each 3 to be composited for analysis.

It should be sufficient to confine this work to strawberries and blackberries which would include that fruit most commonly packed with sugar and one that would represent other fruits most usually packed straight.

The excellent cooperation extended by the National Fruit Canning Co., Seattle, Wash., in preparing the samples and packs, and by H. O. Fallscheer, of the Seattle Station, in analytical assistance, is gratefully acknowledged.

LACTIC AND VOLATILE ACIDS IN FRUITS AND FRUIT PRODUCTS

By FRED HILLIG, *Associate Referee*, and L. L. RAMSEY
(Food and Drug Administration, Federal Security Agency, Washington, D. C.)

At the meeting last year an associate refereeship was established on the above subject. Since this is a new project it is believed that a résumé should be given of the work already performed on the determination of volatile and lactic acids in food materials other than fruit products, and it should be shown what application, if any, this work may have to the solution of the project under consideration.

Accurate methods for the determination of volatile acids have been proposed (1) and developed (2). They have been applied to fish products and have been found of value in confirming decomposition (3, 4, 5). The most recent application is to eggs and egg products (6).

A colorimetric method for the determination of lactic acid has been developed and applied to dairy (7, 8) tomato (9) and fruit products (10). The most recent application is to fish and fish products (11).

Attention is called to a paper (2) in which a method is proposed by which individual volatile acids can be quantitatively determined. The method calls for the use of an apparatus previously described (1). In the proposed procedure distillation data on volatile acids of known purity must be obtained on each apparatus. These distillation data are necessary for use in setting up the simultaneous equations that are employed in computing the quantity of volatile acid or acids present. With the exception of the preparation of the material for steam distillation the method is the same for all food products to which it is applicable. Therefore, attention can be called to the highly satisfactory collaborative data collected on various mixtures or pure volatile acids reported on previously. The data are just as applicable to fruit and fruit products as to fish and fish products under which they were reported to the Association. In the same report calibration data obtained by 20 collaborators on their own apparatus are given (11).

In two previous papers report was made on the determination of lactic acid in tomato products (9) and in fruit and fruit products (10), using the colorimetric method. In the paper on tomato products, tables showing recoveries of varying quantities of lactic acid added to tomato juice and catsup are given. The paper also shows that sound tomato juice contains but small quantities of lactic acid. The paper on fruits and fruit products contains information as to the recovery of lactic acid added to grape juice and apply jelly. A table is given showing the quantity of lactic acid found in various authentic fruits as well as in various samples of commercial jams and jellies. A procedure is also given for the application of the method to wines.

In attempting to apply the volatile acid method to fruits and fruit products, it soon became evident in the case of those products high in carbohydrates or fruit solids that some means must be found to make a preliminary separation of the acids from the solids. This is necessary because of inability to use a sample of sufficient size to yield a satisfactory quantity of the acid for determination and also because of possible decomposition of sugars on distillation from acid solution yielding small quantities of volatile acids. Also, concentration of solids in the distillation flask will increase the distillation rate of the acids, thus giving erroneous results. Since there is no satisfactory reagent for removing carbohydrates from solution, as phosphotungstic acid and other reagents remove proteins, solvent extraction was considered the logical answer for separating and simultaneously concentrating volatile acids from carbohydrate materials.

A special extractor of similar construction to the lactic acid extractor previously described (8), but of four times the capacity, was constructed.

If the large extractor is not available the lactic acid extractor could be used, extracting four 50 ml portions to get the same results. With each extractor preliminary experiments must be conducted to determine the length of time necessary to effect complete extraction. Lactic acid can be determined on an aliquot of the ether extract.

It is realized that it is not possible to lay down a set of rules for preparation of sample that would be applicable to all fruit products. In some cases it will be necessary for the analyst to exercise his own ingenuity in surmounting difficulties that may arise. However, procedures have been found which are of general application where it is necessary to make a preliminary separation of the volatile acids from the solids prior to steam distillation.

In the case of some fruit juices, such as grape fruit and orange juice or other juices low in solids, direct distillation with possible filtration or centrifuging can be made. It is realized that any concentration of solids in the distillation flask might have a tendency to increase the distillation rate of the acids. However, it is believed that such an increase will not materially affect the final results, in the case of materials where the soluble solids content is below 15 percent, and where only formic and acetic acids are present. If salt has been added it is removed with silver perchlorate prior to distillation.

In connection with the work on tomato products, several kinds of rots including soft, black, oospora, green, anthracnose, and mucor were collected at 2 different tomato canneries, by carefully cutting the rot from the tomato. The rots were transferred to cans and given the cannery process without the addition of salt. Volatile and lactic acids were later determined in these rots. The volatile acid was found to consist principally of acetic acid with traces of formic acid. These rots in quantities of 0.5, 1.0, 2.0, and 5.0 percent were added to good quality tomato juice and the resulting mixtures were analyzed for acetic and lactic acids. In Table 1 will be found the analysis of the various rots, analysis of the tomato juices to which the rots were added (blanks), as well as net recoveries of the rots added, computed from the quantity of acetic and lactic acid contained in the portion of rot added and from the quantities found in the mixtures. The recoveries of rot added in most cases, as determined by the quantities of acetic and lactic acids found, are very satisfactory.

The data in Table 1 are not submitted to show that the methods are an absolute means of determining the quantity of rot in a tomato product. They are submitted at this time to show the quantities of acetic and lactic acids that have been found in various kinds of tomato rots, and also to indicate the accuracy of the methods as applied to the recovery of acetic and lactic acids contained in different quantities of various rots added to a rot free tomato juice.

During the past year opportunity presented itself to determine the

TABLE 1.—*Recovery of rot added to tomato juice, based on acetic and lactic acid contents*

KIND	JUICE (BLANK)			ROT			EQUIVALENT IN ACIDS			ACETIC ACID FOUND			LACTIC ACID FOUND		
	ACIDS IN JUICE			ACIDS IN ROT			% ROT ADDED	OF ROT ADDED		TOTAL	NET	NET AS ROT %	TOTAL	NET	NET AS ROT %
	ACETIC	LACTIC	FORMIC	ACETIC	LACTIC	FORMIC		ACETIC	LACTIC						
Soft	2.5	2.1	1.5	128	127	1.8	0.5	0.6	0.6	3.2	0.7	.5	2.1	0	0
							1.0	1.3	1.3	4.2	1.7	1.3	3.0	0.9	0.7
							2.0	2.6	2.5	4.8	2.3	1.8	6.3	4.2	3.3
							5.0	6.4	6.4	8.6	6.1	4.8	10.0	7.9	6.2
Black (Alternaria)	2.9	2.3	1.6	77	68	5.2	.5	.4	.3	3.3	.4	.5			
							1.0	.8	.7	3.7	.8	1.0			
							2.0	1.5	1.4	4.3	1.4	2.0			
							5.0	3.9	3.4	5.6	2.7	3.5			
Oospora	3.1	—	3.2	176	276	3.2	.5	.9	1.4	3.1	0	0			
							1.0	1.8	2.8	4.5	1.4	.8			
							2.0	3.5	5.5	6.5	3.4	1.9			
							5.0	8.8	13.8	12.1	9.0	5.1			
Green	2.7	2.3	1.5	62	48	6.8	.5	.3	.2	3.1	.4	.7	3.0	.7	1.8
							1.0	.6	.5	3.4	.7	1.2	3.6	1.3	2.7
							2.0	1.2	1.0	4.5	1.8	3.0	3.0	.7	1.4
							5.0	3.1	2.4	5.7	3.0	4.8	3.6	1.3	2.7
Anthracnose	3.0	2.3	1.5	94	482	4.7	.5	.5	2.4	3.8	.8	.9	5.1	2.8	.6
							1.0	.9	4.8	4.2	1.2	1.3	7.9	5.6	1.2
							2.0	1.9	9.6	5.2	2.2	2.3	9.7	7.4	1.5
							5.0	4.7	24.0	7.7	4.7	5.0	28.9	26.6	5.5
All Soft Tomato				79	60										
Soft	2.9	4.5	1.1	158	193	3.6	.5	.8	1.0	3.6	.7	.4	6.6	2.1	1.1
							1.0	1.6	1.9	4.6	1.7	1.1	7.5	3.0	1.6
							2.0	3.2	3.9	7.5	4.6	2.9	7.5	3.0	1.6
							5.0	7.9	9.7	11.2	8.3	5.3	13.6	9.1	4.7
Black	2.3	4.5	1.3	67	509	5.8	.5	.3	2.5	3.4	1.1	1.6	6.5	2.0	.4
							1.0	.7	5.1	3.6	1.3	1.9	10.5	6.0	1.2
							2.0	1.3	10.2	4.8	2.5	3.7	14.3	9.8	1.9
							5.0	3.4	25.0	6.2	3.9	5.8	36.0	31.5	6.2
Oospora	2.7	3.4	1.2	171	297	5.1	.5	.9	1.5	4.6	1.9	1.1	5.4	2.0	.7
							1.0	1.7	3.0	5.0	2.3	1.3	7.2	3.8	1.3
							2.0	3.4	5.9	6.2	3.5	2.0	10.0	6.6	2.2
							5.0	8.6	14.9	12.3	9.6	5.6	19.3	15.9	5.4
Mucor	3.4	2.3	2.5	104	145	2.5	.5	.5	.7	4.5	1.1	1.1	3.4	1.1	.8
							1.0	1.0	1.5	5.2	1.8	1.8	3.4	1.1	.8
							2.0	2.1	2.9	5.2	1.8	1.9	4.8	2.5	1.7
							5.0	5.2	7.3	9.9	6.5	6.5	7.6	5.3	3.6
Soft				124	228	2.8									

acetic and lactic acids contained in a number of tomato products of good quality. The data are presented in Table 2.

TABLE 2.—*Acetic and lactic acid in tomato products*
(Mgs/100 grams)

SAMPLE	JUICE		PUREE		CANNED	
NO.	A	L	A	L	A	L
1	3.4	0.6	7.2	3.4	2.5	1.2
2	2.0	2.8	4.5	2.3	2.6	3.5
3	2.0	2.8	4.3	0.0	3.0	1.8
4	3.7	0.6	3.1	1.1	2.6	1.8
5	3.7	0.0	4.2	0.0		
6	2.3	0.0	4.2	2.3		
7	3.5	0.6	6.0	5.7		
8	3.2	2.6	6.5	0.0		
9	1.8	1.2	7.0	0.5		
10	1.8	2.8	6.5	5.7		
11	3.1	0.0	4.3	2.3		
12	3.9	3.4	4.1	4.6		
13	3.1	0.0				
14	3.3	1.1				
15	3.3	1.7				
16	3.6	1.7				
17	2.5	3.4				
18	1.6	0.6				
19	2.1	2.3				
20	1.6	2.6				
21	1.8	1.7				
22	3.0	2.7				
23	3.0	2.7				
24	2.5	3.2				
25	2.2	1.9				
26	3.6	2.6				

The volatile and lactic acid methods in principle are the same for all food products to which they are applicable, the only difference being in the preliminary preparation of the sample either for ether extraction in the case of lactic acid, or steam distillation in the case of volatile acid. The method for lactic acid as applied to dried skim milk is now tentative and the results this year are such as to justify that it be made official, final action. The method for volatile acids has been subjected to a rather exhaustive study, with excellent results. In view of the satisfactory results previously obtained it is believed a recommendation that the two methods be adopted as official for fruit and fruit products is in order and it is so made. Inasmuch as the sixth edition of the *Methods of Analysis* is

to appear next year, it is recommended that the official action be made final for publication in that edition.*

Acknowledgment is made to Mae Carstensen of the Food and Drug Administration for her assistance in the analysis of a number of the samples, and to W. I. Patterson, also of the Food and Drug Administration, for his helpful suggestions and advice.

TABLE 3.—*Analysis of fruits and fruit products*
(Mg/100 grams)

MATERIAL	ACETIC ACID	LACTIC ACID
Grape fruit juice	0.7	2.5
Grape fruit juice	1.1	3.5
Apple	1.1	3.4
Apple	0.9	4.2
Apple	4.0	4.4
Apple jelly juice	3.2	2.4
Apple jelly juice	5.5	7.2
Apple jelly juice	77.0	25.0
Apple honey	253.0	121.0
Strawberry juice	3.0	9.1
Strawberry juice	7.0	29.0
Frozen figs (sour)	352.0	647.0
Frozen figs (sour)	327.0	613.0
Pineapple preserves	3.5	8.6
Pineapple preserves	187.0	293.0
Pineapple preserves	253.0	402.0

In Table 3 there are presented analyses of a few fruits and fruit products. These data are presented merely to show the application of the methods to materials of this nature.

REFERENCES

(1) *This Journal*, **21**, 684 (1938).
(2) *Ibid.*, **25**, 176 (1942).
(3) *Ibid.*, **21**, 688 (1938).
(4) *Ibid.*, **22**, 116 (1939).
(5) *Ibid.*, **22**, 414 (1939).
(6) *Ibid.*, **27**, 204 (1944).
(7) *Ibid.*, **20**, 130 (1937).
(8) *Ibid.*, **25**, 253 (1942).
(9) *Ibid.*, **20**, 303 (1937).
(10) *Ibid.*, **20**, 605 (1937).
(11) *Ibid.*, **27**, 237 (1944).

* For report of Subcommittee D and action by the Association, see *This Journal*, **28**, 79 (1945). Details of the methods will be published in the 6th edition, *Methods of Analysis, A.O.A.C.*, 1945.

REPORT ON SUGAR AND SUGAR PRODUCTS

By CARL F. SNYDER (National Bureau of Standards, Department of Commerce), *Referee*

Reports will be presented by the Associate Referees on the following subjects: Drying Methods, Densimetric and Refractometric Methods, Honey and Honeydew Honey, Reducing Sugars, Corn Sirup and Corn Sugar, and Color and Turbidity in Sugar Products. In regard to the recommendations made in these reports, the Referee concurs. The Associate Referees and the other members who have cooperated are to be congratulated on the progress made.

At the last meeting of this Association it was recommended that a method for the preparation of a standard invert sugar solution be studied with the view of including it in the official *Methods of Analysis*. Lane and Eynon give directions for the preparation of a standard invert sugar solution which they found to be stable over a considerable period of time. A number of these standard solutions were prepared by the Referee and analyzed by several collaborators over a period of several months. No deterioration could be detected, which confirmed the findings of Lane and Eynon. It is therefore recommended that the following be incorporated in the Book of Methods, **XXXIV, 32**:

> "*Standard Invert Sugar Solution.* To soln of 9.5 g of pure sucrose add 5 ml HCl (sp. gr. 1.19) and dilute with H_2O to ca 100 ml. Let stand at room temp. for several days (*e.g.*, about 7 days at 12–15° or 3 days at 20–25°), and then make up to 1 liter. The acidified 1% soln of invert sugar is very stable over period of several months. Neutralize known volume of standard soln with NaOH and dilute to desired concentration immediately prior to use."

The official method for the determination of copper in reducing sugar determinations by the Munson and Walker and other methods by thiosulfate solution directs that this volumetric solution be standardized against pure copper. It was suggested at the last meeting that a standard sample of pure copper be made available for this purpose. Analyses were made on a number of samples of copper from various sources. Electrolytic copper in the form of sheet or foil was found satisfactory, after the exposed surfaces had been subjected to suitable cleansing. The results of this study will be included in a separate report submitted for publication in *This Journal*.

In connection with the revision of *Methods of Analysis* certain changes will be made in accordance with the official actions of the Association. These include the deletion of the columns headed "Copper" and "Levulose" in the Munson and Walker table and the insertion of the Hammond table with the column headed "Cuprous Oxide" deleted. The details of these changes are explained in the report of the Associate Referee on Reducing Sugars, see p. 536, together with appropriate recommendations.

The Lane-Eynon volumetric method for the determination of reducing sugars (**XXXIV,** 32–34) has been the subject of extensive study. The close agreement between the original work and the results obtained by various investigators warrants the adoption of this method as official, in accordance with the recommendation of the Associate Referee.

The studies on unfermented reducing substances in molasses which have been so ably conducted by the Associate Referee, F. W. Zerban, have been interrupted by reason of unavoidable conditions. It is hoped that they may be resumed at an early date.

The polarimetric determination of commercial glucose by the two official methods has been the subject of some criticism. On account of the wide variation in the composition of corn sirups the results obtained are questionable, and it is therefore recommended that they be made "tentative" instead of "official." In fact, the heading for this section, **XXXIV, 30,** designates the determination as "Approximate," although the methods themselves are designated "Official."

There appears to be a definite need for a method for the selective determination of dextrose, particularly in mixtures containing other reducing substances. Numerous inquiries and suggestions have been received by the Referee regarding the introduction of such a method into the official *A.O.A.C. Methods of Analysis.* The methods which have been considered are the following: Steinhoff's, Sickert and Bleyer's, and Zerban and Sattler's, and certain modifications of them. Further details are covered in the Report on Reducing Sugars.

The method of Quisumbing and Thomas for the determination of reducing sugars is official in Chapter **XII,** 44. It has been suggested that this method be placed in Chapter **XXXIV.** Since this method employs heating in a water bath at 80° instead of boiling over a gas burner or on a hot plate, it is especially suitable for the analysis of materials which cause foaming when boiled. It was developed as the result of an extensive research by its authors; however, since the Referee is unaware of any collaborative work verifying the values given in the table accompanying the method, it seems desirable that the method be subjected to collaborative study to establish the accuracy of this table. It is so recommended.

The Associate Referee on Reducing Sugars has recommended the tentative adoption of the Ofner method for the determination of small quantities of reducing sugar in the presence of sucrose.

The Referee concurs with the recommendations of the Associate Referees regarding studies on methods of moisture determination; and on the collaborative study of color and turbidity measurements on sugar products; on refractometric methods; and on the study of methods for the determination of resinous glaze in confectionery.

The proposed work on honey consists of methods for the determination

of dextrin and free acid, the details of which are incorporated in the report of the Associate Referee on Honey.

There is a need in the *Book of Methods* for analytical procedures applicable to starch conversion products. The progress made is covered in the report of the Associate Referee on this subject. The Referee wishes to express his appreciation of the cooperation of the Analytical Committee of the Corn Industries Research Foundation.

The Referee concurs in the recommendation of the Associate Referee that the subject "Ash and Sucrose in Molasses" be dropped.

RECOMMENDATIONS*

It is recommended that—

(1) The Lane-Eynon volumetric method for the determination of reducing sugars (**XXXIV, 32**) be made official, final action.

(2) The Hammond table[1] for determination of reducing sugars by the Munson and Walker method be made official, final action.

(3) The determination of copper by electrolytic deposition (**XXXIV, 44**) be made official, first action.

(4) The Jackson-Mathews method (**XXXIV, 52**) for the determination of levulose be made official, first action.

(5) The volumetric thiosulfate method (**XXXIV, 40**) making alternative procedure mandatory, be made official, final action.

(6) The Steinhoff method as modified by Zerban and Sattler[2] for the determination of dextrose alone and in mixtures be adopted as tentative.

(7) A collaborative study be made of selective methods for the determination of dextrose by means of the copper acetate reagent.

(8) The reducing sugar method of Quisumbing and Thomas be subjected to collaborative study.

(9) The study of unfermented reducing substances in molasses be continued.

(10) A critical study be made of the existing data on refractive indices of dextrose and related starch conversion products.

(11) The determination of moisture in sugar products be continued, including methods employing the Brabender tester, and the method using the Karl Fisher reagent.

(12) The study of methods for the determination of resinous glaze in confectionery be continued.

(13) The methods for the determination of dextrin in honey be continued.

(14) The free acid in honey be subjected to collaborative study.

* For report of Subcommittee D and action by the Association, see *This Journal*, **28**, 70 (1945).

[1] *This Journal*, **27**, 101 (1944). The table will be published in the 6th edition, *Methods of Analysis, A.O.A.C.*, 1945.

[2] *Ind. Eng. Chem., Anal. Ed.*, **10**, 669 (1938).

(15) The measurement of color and turbidity in sugar products be subjected to collaborative study.

(16) The study of methods applicable to starch conversion products be continued.

(17) The subject "Ash and Sucrose in Molasses" be dropped.

The paper entitled "Errors in the Sampling and Analysis of Cane Molasses," by F. W. Zerban, is published in *This Journal*, p. 616.

REPORT ON DRYING METHODS

By LESTER D. HAMMOND (National Bureau of Standards, Department of Commerce), *Associate Referee*

No collaborative work has been done during the year on this subject. However, preliminary work has been started on determination of moisture in various sugar products by means of the Brabender semi-automatic moisture tester. This apparatus has the advantage of rapidity, since dishes are not removed from the oven during the operation of weighing. It is therefore recommended that methods employing this apparatus be subjected to study.

The errors involved in the determination of moisture in carbohydrate products by the heating method are well known. A recent contribution to the determination of moisture is by electrometer titration using the Karl Fisher reagent. It is recommended* that this method be subjected to study.

The report on densimetric and refractometric methods is included in the report of the Referee on Sugar and Sugar Products.

REPORT ON HONEY AND HONEYDEW HONEY

By GEORGE P. WALTON (Special Commodities Branch, Office of Distribution, War Food Administration, Washington, D. C.), *Associate Referee*

Development of a specific and reasonably facile method for the determination of dextrin in honey will, it is believed, largely solve the problem of distinguishing between true honeys and those derived from honeydews.

At the beginning of the year we were faced with a problem in what may be called "Service of Supply." Authentic samples of floral-nectar honey and of honeydew honey had to be obtained, and even suitable jars in

* For report of Subcommittee D and action by the Association, see *This Journal*, 28, 70 (1945).

which to distribute sub-samples for collaborative work had to be procured.

Sufficient quantities of three straight honeys and of a cedar honeydew honey—upon which collaborative work by this Association has previously been done[1]—have been made available through the cordial cooperation of the Division of Bee Culture of the U. S. Department of Agriculture; and sample jars have been generously provided by the Food Container Division, Owens-Illinois Glass Company, Toledo, Ohio.

Before arrangements were made for the planned collaborative study of features of the present A.O.A.C. tentative method, however, an article published by K. T. Williams, and C. M. Johnson[2] entitled "Determination of Soluble Pectin and Pectic Acid by Electrodeposition," suggested to this Associate Referee an entirely new line of approach to the determination of dextrin. The gravimetric determination of the dextrin after electrodeposition upon the cathode of an electrolysis system, under controlled conditions, appears to offer a possible direct method for determining the content of this troublesome complex.

Detailed information as to the type and cost of equipment that will be required to test the method has been collected; and collaborators in two laboratories have signified interest and willingness to undertake the needed exploratory work. Recommended work for 1945 is as follows:

RECOMMENDATIONS*

In addition to the recommendations made by this Associate Referee at the 1943 meeting,† it is recommended—

(1) That a study be undertaken of the applicability of electrodeposition to the direct quantitative determination of dextrin in honey and honeydew honey.

No report on sucrose and ash in molasses was made by the Associate Referee.

REPORT ON CONFECTIONERY

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

It appears the most immediate need in the section on confectionery is a method for the determination of harmless resinous glaze. Section 402 D of the Federal Food, Drug, and Cosemetic Act permits the use of this glaze when "not in excess of four-tenths of 1 per centum." A search of the

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[1] *This Journal*, 25, 681 (1942).
[2] *Ind. Eng. Chem., Anal. Ed.*, 16, 23 (1944).
* For report of Subcommittee D and action by the Association, see *This Journal*, 28, 70 (1945).
† *This Journal*, 27, 480 (1944).

literature reveals only one method, that of Molnar and Grumer[1] for estimation of lac on glazed candies. In this procedure, the lac is dissolved in alcohol and the lac acid titrated with standard sodium carbonate, calculation being based on an average factor for acidity of the lac acids. However, Molnar warns that bleached lac, if used on candy, may change into an alcohol and alkali insoluble form. Investigation indicates the use of bleached lac is quite common in the industry. Therefore, the solubility and titre of representative bleached lacs was examined in the laboratory. Both solubility and titre were found to vary too widely for use as a method for lac determination when the bleached lac is employed for glazing.

Although the Molnar method may be very useful in checking the lac content of candies glazed with light orange refined lac, it would not be suitable for use in regulatory work where bleached lac may very probably be encountered.

Shaeffer, Weinberger, and Gardner,[2] in a study of solvents for lac, find ethyl lactate to be one of the best materials for this purpose. Preliminary tests indicate this solvent may be suitable for removing bleached lac from candies. Traces of sugar, fat, theobromine, and the like that may be carried along in the extraction can probably be eliminated by addition of a suitable liquid to precipitate the lac in a form for drying and weighing.

It is recommended* that the study of this topic be continued.

REPORT ON CHEMICAL METHODS FOR REDUCING SUGARS

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

(1) Lane and Eynon General Volumetric Method (page 498, paragraph 32, of *Methods of Analysis*) is now a tentative method. It is recommended that this be made an official method for dextrose, levulose, invert sugar, and invert sugar plus sucrose.

A collaborative study was reported by J. F. Snell at the Eighth Meeting of the Canadian Committee on Sugar Analysis, in which samples of clarified and unclarified maple sirups were analyzed by Munson and Walker's and by Lane and Eynon's methods.

The average results obtained by the two methods on any one sample are in good agreement. In all cases, except in the sample designated C.M.S.Co. #2 sirup, the results of the three analysts were in better agreement when Lane and Eynon's method was used than when Munson and Walker's

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[1] *Ind. Eng. Chem., Anal. Ed.*, VII, p. 673, 1939.
[2] *Ind. Eng. Chem.*, 30, 451 (1938).
* For report of Subcommittee D and action by the Association, see *This Journal*, 28, 70 (1945).

method was employed. The following table has been prepared from that report:

TABLE 1.—*Reducing sugars, as invert sugar, in maple products**
(Percent of Dry Substance)

SAMPLE	ANALYST	METHOD			
		MUNSON & WALKER		LANE & EYNON	
		UNCLAR.	CLAR.	UNCLAR.	CLAR.
Q.M.P.	Whaley	1.30	1.26	1.24	1.28
No. 1	Landry	1.17	1.12	1.33	1.29
Sirup	Janson	1.48	1.53	1.36	1.36
	Mean	1.32	1.30	1.31	1.31
Q.M.P.	Whaley	6.02	5.99	6.13	6.14
No. 2	Landry	6.10	5.74	6.11	6.07
Sirup	Janson	6.54	6.38	6.06	6.09
	Mean	6.22	6.04	6.10	6.10
P.S.E.Q.	Whaley	1.27	1.27	1.23	1.28
No. 1	Landry	1.05	0.94	1.30	1.29
Sirup	Janson	1.50	1.52	1.32	1.30
	Mean	1.27	1.24	1.28	1.29
P.S.E.Q.	Whaley	7.65	7.47	7.60	7.99
No. 2	Landry	7.67	7.21	7.69	7.86
Sirup	Janson	7.95	7.95	7.75	7.77
	Mean	7.76	7.54	7.68	7.87
C.M.S.Co.	Whaley	1.26	1.18	1.19	1.19
No. 1	Landry	1.25	1.05	1.23	1.20
Sirup	Janson	1.07	1.33	1.09	1.13
	Mean	1.19	1.19	1.17	1.17
C.M.S.Co.	Whaley	10.26	10.27	10.40	10.28
No. 2	Landry	8.98	9.00	9.14	9.37
Sirup	Janson	8.91	8.78	8.69	8.69
	Mean	9.38	9.35	9.41	9.45

* A portion of Table 1 presented before the Eighth Annual Meeting (1944) of the Canadian Committee on Sugar Analysis, by J. F. Snell.

Lane and Eynon's method was studied by Jackson and Mathews[1] in the determination of levulose, dextrose, and mixtures of the two sugars. They report that this method is "more expeditious and in most cases more precise than the gravimetric estimation of total reducing sugar."

Dr. Snell has pointed out that in the *Methods of Analysis*, under maple

[1] *J. Research, Nat. Bur. Standards*, **8**, 420 (1932). (RP 426.)

products, Chapter **XXXIV**, par. 114, "Reducing Sugars as Invert Sugar," an official method, refers to Lane and Eynon's method, which is tentative. The same inconsistency exists in the case of beer and of honey. Making Lane and Eynon's method official will remove these discrepancies.

(2) It is recommended that the following changes be made, all concerning Munson and Walker's General Method:

RECOMMENDED CHANGES*

(a) Hammond's table for Cu-sugar equivalents to be included in Chapter **XLIII.** The copper amounts need be given in even number of milligrams of copper only. The column headed Cu_2O in the table as published (*J. Research National Bureau Standards*, **24**, 579 (1940). RP1301) to be deleted. This column was obtained by computation from the amount of copper determined and hence does not take account of the impurities present when Cu_2O is determined gravimetrically; it should have a footnote, "to be used when copper is determined as pure copper."

(b) Table 9, Chapter **XLIII**, page 671: the columns headed "copper" should be deleted. These values were obtained by computation, while the Cu_2O was obtained experimentally. Table 9, Chapter **XLIII**, the column headed "levulose" to be deleted. The values in this column were determined by calculating the Cu_2O equivalents of the copper values determined by Hammond.

This table should have a footnote saying "to be used when Cu_2O is determined by direct weighing."

(c) Paragraph 44, line before last, page 502: Insert after ". . . break current": "This washing may be accomplished by using a syphon, water being added as the solution is removed. The displacement of the nitric acid solution by water is complete when the current ceases to flow."

Paragraph 44, following the last line on page 502: "The electrolyte may be stirred by a rotating anode or a mechanical stirrer in which case the time required for the complete deposition of copper is shortened to approximately one hour."

Electrolysis may be carried out from a solution containing HNO_3 plus a drop of HCl, with a rotating anode as described in Chapter **VI.**

CONCERNING MAPLE PRODUCTS

(3) It is recommended that par. 114 (a) read " . . . 110, the solution may be unclarified or only neutral Pb acetate used for clarification."

Page 114 (b), read " . . . 111 (a), the solution may be clarified or only neutral Pb acetate used for clarification."

Collaborative work has been done by the Canadian Committee on Sugar Analysis, in which clarified and unclarified samples of maple sirup were analyzed. Their results demonstrate that clarification of solutions of maple products is unnecessary as a preliminary to the determination of reducing sugars.

Tables 1 and 2 of this report show the results of these collaborative studies.

(4) It is recommended that the determination of copper by electrolytic deposition from nitric acid solution be made an official method.

The electrolytic determination of copper is widely used for copper

* For report of Subcommittee D and action by the Association see *This Journal*, **28** 70 (1945).

analysis of various materials. Chapter VI, paragraph 14, describes an official method for the electrolysis of copper from a nitric acid solution by use of a rotating anode in a platinum dish. In the same chapter, paragraph 71 describes an official method for determining copper by electrolysis from a nitric acid solution to which hydrogen peroxide has been added. A rotating anode is used here. The advantage of using a rotating anode is that the time required for the electrolysis is reduced from about 16 hours, as recommended in Chapter **XXXIV**, paragraph 44, to 45 minutes.

TABLE 2.—*Invert sugar in unclarified and clarified maple sirups**

COLLABORATOR	SAMPLE NO. 1		SAMPLE NO. 2		SAMPLE NO. 3		SAMPLE NO. 4	
	UNCL.	CL.	UNCL.	CL.	UNCL.	CL.	UNCL.	CL.
Quebec Maple Products	1.14	1.19	3.25	3.50	4.48	4.54	1.00	1.07
N. Bowers	1.10	1.13	3.37	3.46	4.51	4.50	1.00	1.07
Laval Univ.	1.26	1.26	3.78	3.78	4.71	4.72	1.38	1.41
E. Bois	1.28	1.29	3.79	3.81	4.78	4.81	1.47	1.48
Les Producteurs de Sucre d'Erable de Quebec	1.09	1.09	3.53	3.22	4.52	4.44	0.72	0.79
A. Roberge	1.09	0.87	3.53	3.26	4.48	4.40	0.72	0.79
Central Exp. Farm	1.09	1.14	3.51	3.46	4.49	4.51	0.93	0.94
J. T. Janson, Dept. of Agri.	1.19	1.10	3.38	3.39	4.12	4.08	1.16	1.04
M. Lessard	1.20	1.11	3.37		4.05	4.11	1.16	1.03
United Maple Products	1.00	0.97	3.35	3.33	4.54	4.53	0.87	0.84
F. M. Rees, Cary Maple Sugar Co.	1.04	1.00	3.24	3.22	4.26	4.31	0.88	0.95
A. Conlin, Macdonald College J. F. Snell	1.19	1.19	3.54	3.57	5.07	5.03	1.22	1.12
Average	1.13	1.11	3.45	3.43	4.49	4.49	1.02	1.04

* From a report by A. Conlin presented at the Fifth Annual Meeting of the Canadian Committee on Sugar Analysis, Montreal, Jan. 1941.

Collaborative work on the determination of copper by the electrolytic method has been conducted by several of the referees on sugar. The results of this work, which confirm the findings of previous investigations, will be presented as a separate report.

(5) It is recommended that the thiosulfate procedure for determining copper as described by Jackson and McDonald[2] be included in the thiosulfate method.

[2] *J. Research Nat. Bur. Standards*, **27**, 244 (1941) RPI 417.

This procedure calls for the adding of a sodium acetate solution to the copper nitrate solution, rather than the addition of a sodium hydroxide solution to slight turbidity followed by the addition of a few drops of acetic acid. The above authors have shown that the precision of the method is not altered by this change of procedure. The saving of time and work involved is appreciable.

(6) It is recommended* that Ofner's method be made a tentative method for the determination of small amounts of invert sugar in the presence of sucrose.

No report on corn sirup and corn sugar was made by the Associate Referee.

REPORT ON COLOR AND TURBIDITY IN SUGAR PRODUCTS

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

No cooperative results on this subject are to be reported this year, but it is highly desirable that collaborative work be done in order that procedure may be unified. Some of the details touched upon in outline in the report for 1943, although not new, are deemed sufficiently important to the work of this Association to warrant fuller discussion.

1. A SYSTEM FOR THE MEASUREMENT OF COLOR

Some twenty years ago it was recommended that the spectrophotometric method be used for the measurement of color in solutions of sugar products. This, in simple terms, means the measurement of the percent of light from a source transmitted or absorbed by a layer of sugar solution of known thickness and of known concentration of colored dry substance of various wave lengths. Or, as later simplified, at one particular wave length. The results of observation are reported in terms of the absorption, at each wave length calculated to unit thickness of layer and unit concentration of dry substance per milliliter. The soundness of the spectrophotometric method is generally recognized among sugar chemists and the method is applicable to all color problems.

2. INSTRUMENTS

Good spectrophotometers, both visual and photoelectric, American made, are available. There are also simpler instruments of certain types that, with little change, may be adapted to the measurement of sugar color at certain wave lengths. Regardless of the type of instrument used it

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* For report of Subcommittee D and action by the Association, see *This Journal*, 28, 70 (1945). Details of methods will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.

should be tested against some known transmission standard. A calibrated colored glass is suitable for this. It may be advisable for collaborators to check readings by an interchange of such standards.

In the case of highly transmitting solutions of white sugars it is necessary to interpose a calibrated absorbing medium such as a colored glass in the light beam between the solution and the photometer in order to obtain easily readable values. The total absorption is then corrected by deducting the absorption of the standard at the given wave length.

It should be stated here that in measuring the transmittancy of white sugar solutions extra long absorption cells (10–15 cm.) must be used. In most photoelectric photometers cell space has been sacrificed for compactness and such instruments are incapable of yielding satisfactory results with white sugars.

3. PREPARATION OF SOLUTIONS FOR OBSERVATION

It is contemplated to employ the method of R. T. Balch[1] for the measurement of turbidity. In this method the solution of the sugar product is mixed with filter aid and filtered with suction. It is general practice in the measurement of transmittancy to filter the solution using either asbestos or diatomaceous earth as filter aid.

A comparison of transmission results using both these filter aids should be obtained and a study made of the quantity of each necessary to produce acceptable filtrates.

For future collaborative work of this Association on color and turbidity in sugar products the following recommendations are submitted:

RECOMMENDATIONS*

1. That the spectrophotometric method for the measurement of color in solutions of sugar products be used and that a complete absorption curve ($-\log t$) at intervals of 20 mμ between $\lambda = 420$ and $\lambda = 700$ be reported if possible. Where a spectrophotometer is not available, measurements should include $-\log t$ at $\lambda = 560$.

2. That the solutions for transmittancy measurement be filtered with asbestos and with diatomaceous earth and that results be reported for each.

3. That the method of Balch be used for the measurement of turbidity.

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[1] Balch, R. T., *Ind. Eng. Chem., Anal. Ed.*, **3**, 124 (1931).

* For report of Subcommittee D and action by the Association, see *This Journal*, **28**, 71 (1945).

REPORT ON WATERS, BRINE, AND SALT

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

RECOMMENDATIONS*

It is recommended:

(1) That methods for the determination of iodide (58, p. 368, and *This Journal*, **26**, 440, 1943) be used as alternate methods as recommended in Referee's report and be adopted by the Association as official, final action.

(2) That methods for the determination of fluorine in salt be studied collaboratively.

(3) That studies on boron be continued.

No report on boron in water was made by the Associate Referee.

No report on fluorine in salt was made by the Associate Referee.

REPORT ON IODIZED SALT

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

Method for the Determination of Iodine.—Following the recommendation of last year, the two volumetric methods for the determination of iodine in iodized salt were subjected to collaborative study. The principles involved in the two methods are similar. The directions for collaborative work were given as alternate procedures for the same determination.

The sample submitted for collaborative study contained 0.01 percent of added potassium iodide (based on sodium chloride dry weight).

It appears from data in Table 1 that both procedures give satisfactory results.

Several of the collaborators obtained values by both procedures which are in very close agreement with the amount of potassium iodide actually added, and most of the collaborators obtained practically the same results with both of the procedures. One collaborator (No. 12) obtained low results with the B procedure.

Recoveries by 3 of the collaborators are 10–20 percent higher than theoretical.

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* For report of Subcommittee D and action by the Association, see *This Journal*, **28**, 71 (1945). Details of the methods will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.

TABLE 1.—*Collaborative results—Iodine in iodized salt solution*
(0.1% KI added to 20% NaCl solution)

COLLABORATOR NO.	PROCEDURE		COLLABORATOR NO.	PROCEDURE	
	A	B		A	B
1	.012	.012	7	.010	.010
	.012	.012		.010	.010
	.012			.010	
2	.012	.012	8	.010	.010
	.011	.012		.010	.010
	.011	.011			
			9	.010	.010
3	.011	.012		.010	.010
	.011	.011			
			10	.010	.009
4	.010	.010		.010	.009
	.010	.010			.009
		.010			.009
5	.011	.010	11	.010	.010
	.011	.010		.010	.010
		.010		.010	.010
		.009		.010	.010
6	.010	.010	12	.010	.005
	.010	.010		.010	.004
		.010			

It is recommended* that these two procedures be adopted by the Association as official, final action.

REPORT ON VITAMINS

By CHESTER D. TOLLE (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Referee*

Vitamin A.—The Referee approves the recommendation of the Associate Referee that the proposed spectrophotometric method for the determination of vitamin A in fish liver oils be adopted as a tentative method.

Vitamin B_1.—The Referee approves the recommendation of the Associate Referee that the proposed fermentation method for the determination of vitamin B_1 be adopted as a tentative method.

Vitamin B_2 (Riboflavin).—The first recommendation of the Associate Referee, which reads as follows, is approved:

* For report of Subcommittee D and action by the Association, see *This Journal*, 28, 71 (1945). Two alternate methods will be published in the 6th edition, *Methods of Analysis, A.O.A.C.*, 1945.

(1) That fluorometric methods for riboflavin be studied.

It is also recommended that the Associate Referee be instructed to study the microbiological method further to see if it can be made more nearly identical with the U.S.P. method than that proposed by Dr. Kemmerer in his report (see p. 560).

Vitamin C.—The Referee approves of the recommendation of the Associate Referee that the proposed method as amended be adopted as official, first action.

Vitamin D, Milk.—No report was received.

Vitamin D, Poultry.—There was no report. For several reasons there has been no work in this field, (1) that the Associate Referee's time was too limited; (2) that it would have been difficult to get collaborators; (3) the A.V.R.C., which is interested and active in this field, did not present a proposed diet for A.O.A.C. collaborative studies.

Vitamin K.—No report was received.

Nicotinic Acid.—No report was received. However, the Associate Referee has been active and collaborative study is in progress, the results of which will be reported next year.

Pantothenic Acid.—The Referee approves the recommendation of the Associate Referee that the proposed microbiological method be further studied.

Carotene.—The Referee approves the recommendations numbered 6 and 7 of the Associate Referee, and recommends that the Associate Referee on Carotene be instructed to give consideration to the statement prepared by E. M. Nelson regarding carotene methods, which follows this report. In this connection it may be well to consider appointing two associate referees next year to study the different aspects of the carotene problem, as outlined in Dr. Nelson's statement.

STATEMENT ON CAROTENE

By E. M. Nelson

Since the study of a method for carotene was undertaken there has arisen a need for a method that has general application to plant products. There has been rapid progress in development of methods for carotene and it is now possible to make much more accurate determinations than were possible a few years ago. It seems desirable to review the carotene methods from the viewpoint of their broad application before considering the details of the methods which may need modification or study.

The Associate Referee on Carotene was first appointed under the Feeding Stuffs Section and for a number of years did not report to the Referee on Vitamins. Not until 1942 was the Associate Referee on Caro-

Note.—Details of the above methods will be found in the reports of the Assistant Referees, *This Journal*, 28, pp. 547–567, 1945.

tene so instructed. The first interest in carotene apparently arose from the need for a method for determining carotene in hays such as alfalfa. Since that time there has been a tremendous change with respect to the importance of a method for carotene, because determination of beta carotene now constitutes the common method for determining vitamin A activity in plant materials. In adopting a method for carotene the objective therefore must be one that is suitable for application to a wide variety of materials; and there has been much progress in perfecting these methods for carotene in recent years. A method which only determined "crude carotene" may have been satisfactory a few years ago for determining the carotene content of hays, but much more precise methods for determining beta carotene can now be applied just as readily.

A method for the determination of beta carotene can be divided into three sections, each representing what appear to be independent problems.

(1) Extraction and saponification.
(2) Purification of the extract.
(3) Measurement of the carotene content of the purified extract.

Extraction and saponification cannot be separated. In most instances saponification can be carried out following extraction, but in some instances saponification prior to extraction is necessary to permit complete extraction of the carotene. Different methods of extraction and saponification must be prescribed for different types of materials and possibly in some instances for individual products. The present Associate Referee on Carotene has worked out for a number of products methods of extraction which appear to be quite satisfactory. This type of investigation must be continued and methods subjected to collaborative study as they become available. The part of the method which deals with extraction and saponification should whenever possible appear in the *Methods of Analysis, A.O.A.C.*, in the section dealing with the product to which this procedure applies. The remainder of the method should appear in the "Vitamin" chapter.

The most important developments in improving methods for carotene determination have come through use of adsorbing material for separating carotene from other plant pigment. Phasic separation of carotene from other pigments by the use of immiscible organic solvents is now recognized as being unsatisfactory for quantitative separation. Absorbing materials are now in use which appear to be satisfactory for separating beta carotene from pigments found in various extracts. Magnesium oxide and magnesium carbonate have both proved satisfactory in the hands of a number of investigators. It appears that either one of these compounds could be specified for use in purification of the extract, but obviously even better reagents may be found. Specifications for the type of column that would be most practicable need further study. There is also need for study of the most suitable solvent and most suitable eluent.

The most precise method for determining concentration of carotene in a solution is undoubtedly the spectrophotometric method. Not only will this procedure measure the concentration of carotene with a high degree of accuracy, but the procedure can be applied to demonstrate the degree of purity of the unknown solution being studied. However, this analyst doubts the wisdom of including in the A.O.A.C. method the requirement that carotene shall be determined spectrophotometrically. This would impose on many laboratories the necessity for acquisition of expensive equipment that probably cannot be well afforded. There is objection, also, to the use of more than one specific procedure for the measurement of carotene, for the obvious reason that different procedures may yield divergent results unless far more time is taken to study minute details than now seems practicable. It is recommended that no specific method for measurement of carotene be prescribed, and that it be pointed out that the most precise method for measurement in the purified extract is the spectrophotometric method. It could be stated in addition that if precise measurements are not necessary, carotene content can be determined by colorimetric comparison with a pure beta carotene solution or with potassium dichromate. It seems unnecessary to state whether this comparison shall be made by a visual or a photoelectric method. If desirable, further details relating to the spectrophotometric method can be given. Comparison can, of course, be made with a pure beta carotene standard solution, or absorption curves for beta carotene in different solvents can be provided.

If the approach to the problem as outlined above is followed each of the three sections can be modified as occasion demands without disturbing the others, and assignments can be made to a referee or to referees on that basis.

As far as can be determined, this is the present status of carotene methods:

1. *Tentative methods have been specified* (see *This Journal*, **27**, 108 (1944) for the preparation of extracts in materials other than dried hays and plants and enumerated as follows: Fresh green materials, fresh green materials which have been preserved in alcohol, fresh carrots and apricots, dehydrated sweet potatoes and carrots, dehydrated green leafy vegetables, canned foods, egg yolk, butter and other fats, and blood plasma.

2. *Carotene in hays and dried plants.*—Official, first action, *This Journal*, **25**, 46 (1942). This method includes the preparation of extracts. This method also provides for the colorimetric determination of carotene, and the Associate Referee in his last report wants to introduce a photoelectric method for this measurement.

3. *Method of selective adsorption adopted as tentative.*—*This Journal*, **25**, 92 (1942). This method involves shaking the plant extract with mag-

nesium carbonate to remove interfering pigments. If the magnesium carbonate adsorbs too much carotene it is treated with water before being used in the method.

4. *Complete chromatographic method* (*This Journal*, 27, 107 (1944)), involving the use of a long column of calcium hydroxide adopted as tentative.

It appears that an analyst would have difficulty in selecting from these a method which would be regarded as an official method for determination of carotene in a particular substance. It is believed that it will be necessary to reorganize these methods in the manner indicated above. One of the most important applications of A.O.A.C. methods is for enforcement work. For this purpose it is not practicable to provide alternate methods for determination of beta carotene on individual products. There should be one method for purification of the extract for any one food. While the use of a long column of calcium hydroxide may be desirable for the complete separation of plant pigments, it does not appear necessary or even desirable to use this procedure in routine determinations of beta carotene. If it is not practicable to specify one method for the determination of beta carotene in the purified extracts, a preferred method should be specified which can be recognized in case of contest, and other methods can be provided if a precise method is not needed.

REPORT ON VITAMIN A

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

Since the last A.O.A.C. report on the vitamin A determination, given in 1940, many events have happened that can have an important bearing on the matter. New compounds and results have appeared and many practical aspects of the determination have developed.

Some of the newer compounds should first be mentioned. Cyclized vitamin A was studied by Shantz, Cawley, and Embree (1) and as a result of these studies the compound was renamed anhydro vitamin A. Its importance for the determination of the relative values of vitamins A_1 and A_2 (2) was reindicated. The application of the compound for the estimation of vitamin A in the presence of interfering materials such as occur in blood plasma was demonstrated.

Another vitamin-A-like material, designated as subvitamin A, has been obtained from shark liver oil. It was found to have a maximum absorption at 290 mμ, greater solubility in 83 percent menthanol than vitamin A_1 or A_2. The anhydrous derivative possessed similarity but not exactness with the corresponding vitamin A_1 and A_2 anhydro derivatives. The subvitamin vitamin A was reported to have no biological potency but to

respond to the antimony trichloride test in the same manner as vitamin A. This appears to be evidence against the too general acceptance of the antimony trichloride method for vitamin A.

It was believed by the investigators that the compound might be similar to vitamin A_1 but with one less HC=CH group. The molecular weight still indicated a greater weight than for vitamin A, and as a consequence the possibility of the compound being an oxygenated derivative was expressed.

Reference has also been made to a provitamin A kitol from whale liver oil. Kitol appears to be a dihydric alcohol closely related to vitamin A (3). Its absorption maximum is at about 290 mμ. Its antimony trichloride reaction product has peak absorptions at 428, 505, and 580 mμ, respectively. It has no biological potency. However vitamin A_1 apparently can be derived from kitol, since the thermal decomposition product has a peak absorption at 328 mμ, the antimony chloride reaction product exhibits maximum absorption at 620 mμ, and it also possesses the required biological potency.

Baxter and Robeson (4), using a different purification technique than that of Holmes and Corbet, obtained pure crystalline vitamin A melting at 64°, rather than 7.5–8° the material originally designated as crystalline vitamin A by Holmes and Corbet (5). Baxter and Robeson also presented evidence that the 7° crystals were of a different structure and contained 1 molecule of methyl alcohol per molecule of vitamin A. The extinction coefficient of the pure material E(1%/1 cm)−325 was found to be 1780 which, when multiplied by the usually accepted commercial factor of 2000 would result in 3,560,000 units of vitamin A per gram of the pure material. Harris, collaborating with Baxter and Robeson, found a biological value 4,300,000 units of vitamin per gram, which would require a conversion factor of 2460. No valid explanation for the discrepancy is available. The value of 3,560,000 would be consistent with findings from some of the crystalline material of Holmes and Corbet. Baxter and Holmes found their crystallized vitamin A gave an antimony trichloride E(1%/1cm.) value of 4800 measured at 622 mμ, and an L value at 622 of 3990 when an Evelyn photoelectric colorimeter was used; L was found to be a linear function for galvanometer readings between 30 and 70.

It appears that further work will be necessary before a truly acceptable conversion factor for crystalline vitamin A will be available.

Baxter and Robeson also have prepared several vitamin A esters including the acetate, palmitate, and di-vitamin A succinate (6). The biological potencies of the esters adjusted for differences in molecular weight are indicated to be the same as that of crystalline vitamin A. The attainable purity, the duplicability, ease of preparation, the inherent stability, together with the demonstrated biological potency, have definitely indicated the possibilities of one of these as a standard to replace the U.S.P. Reference Oil, or perhaps ultimately the original beta carotene.

Largely through the original interest of Dr. Embree it appears that the U. S. Pharmacopoeia will soon start a study of the use of one of these esters as a possible standard for vitamin A. The palmitate would be a good compound from the biological viewpoint but it seems that the acetate might be better from the standpoint of preparation and stability.

Vitamin A in its normal form possesses a typical green fluorescence (7) (8) (9) (10). This has been used as a basis for identification of vitamin A in tissues and has been used in our laboratory for the routine-chromatographic control of vitamin A in oleomargarine and similar products (11).

It has also been used as an end point control in the determination of vitamin A by titration with oxidizing agents such as picric acid or bromine (12).

Calibration curves made in our laboratory have demonstrated the feasibility of using fluorescence in the fluorometer for the determination of vitamin A.

Sobotka and coworkers (13) have demonstrated the possibility of determining vitamin A in a photoelectric fluorimeter. Of still greater interest is their demonstration (14) of determining the degree of esterification by virtue of difference in behavior of the esters and vitamin A itself under the influence of ultraviolet irradiation.

At this time it is also interesting to note that the other independent efforts have been made to separate vitamin A from its esters (15) (16) by means of both phasic and chromatographic methods. It appears that all of these methods will work with purified compounds or with specific materials. However, the real utility of any of these methods appears to depend upon studies showing effect of likely impurities upon the results obtained. It has been demonstrated repeatedly that phasic separations are not universally applicable.

A modified antimony trichloride reaction as used by Rosenthal and Weltner (17) has been adopted for the estimation of vitamin A from shark livers (18). A 0.5 percent solution of guiacol in chloroform is added to the reagent as a stabilizer for the reaction with vitamin A. This modified reagent reacts with vitamin A within 45 seconds, and the resulting color is stable for 20–30 minutes.

It has been reported that ordinary daylight may cause deterioration of vitamin A solutions (19). Amber glass has therefore been advocated to prevent much deterioration. Others as well as ourselves have not found this precaution to be necessary. However it appears wise for each laboratory to check this matter under the existing conditions.

Vitamin A, as usually obtained from the nonsaponifiable fraction of a fish oil, has a very typical absorption curve which is especially significant when the absorption values are expressed in ratios, a fact which appears to have been first emphasized by Oser, Melnick, and Pader (20). It has also been recognized for sometime that the more potent fish oils without sapon-

ification would give a curve more closely approaching that of the purer vitamin A than the less potent oils.

A move in industry, to regulate fish liver oil sales in part by the degree of absorption at 328 mμ without saponification, was inaugurated and has gained considerable headway but with the compromise provision that the absorption ratios of densities 300 mμ/328 mμ and of densities 350 mμ/328 mμ be those or similar to those of typical vitamin A curve within specified limits. That is, the ratio E 300 mμ/E 328 mμ was specified to be not greater than 0.73 and the ratio E 350 mμ/E 328 mμ was to be not greater than 0.65.

The plan had considerable advantage as a time saver and appeared to have a degree of soundness, and since the need was immediate the plan was put into operation without extended investigation. It was put into operation through a War Food Administration purchase specification (21). The scheme may be expedient and possesses a degree of soundness, but there are possibilities of inaccuracies in the determination of the ratio, especially the short wave ratio 300/328, because of lack of standardized spectrophotometric practice in this region. For example, when an incandescent source is used, as appears to be commonly the case, the possibilities of excessive stray light in the 300 mμ region may be great, and such a procedure ordinarily would not be unqualifiedly recommended by the spectrophotometer manufacturers.

This matter is now being studied by the interested parties and no doubt a satisfactory solution will be found.

This brings us to the consideration of apparatus. Several new types have appeared since the 1940 publication of *Methods of Analysis, A.O.A.C.* These include photoelectric spectrophotometers, photoelectric photometers, and fluorimeters. Many of these are available as manufactured instruments, others are specially constructed instruments and only available in certain laboratories. Most of this equipment seems to give good service, but failures and poor operation will happen even with the best equipment. While it is not the function of this report to go into the details of these matters, there is one item of general importance to the operation of ultraviolet spectrophotometers important enough to be considered here. This refers to the operation of the hot cathode hydrogen discharge lamps.

This laboratory has been operating this type of lamp for about 5 years. However, we did experience early lamp failure in the sense that after a period of disuse it would not start as it did originally. A high frequency discharge of several thousand volts applied to the anode, however, provided a never-failing start during an estimated 2500 hours, for a three-and-a-half-year period, and the lamp is still going strong. This lamp operated directly from the 110-volt D.C. current. Some of the newer

equipment is also provided with electronic rectification and regulation; however even in such a case it has been possible to apply high frequency, high voltage excitation by interposing a high frequency choke coil in the anode lead from the power supply to obtain an extended life from the hot cathode hydrogen tube. We find that the hydrogen lamp provides steadier operation than the incandescent lamp and is regarded as practically essential for wave lengths less than 320 mμ. More attention should be given to slit widths, and width of wave bands used, than has been customary in most vitamin A investigations.

Since the definition and standard promulgated (22) for oleomargarine has specified not less than 9000 U.S.P. units of vitamin A per pound, means for physical or chemical assay of this product have become increasingly important.

Although this assay is more difficult than for fish liver oils, either colorimetrically or spectrophotometrically, it appears that considerable progress has been made along both of these lines. Oser, Melnick, and Pader (20) have described a colorimetric method applicable to oleomargarine. Neal and Luckmann (23) have described a light destruction spectrophotometric method. We have found a direct chromatographic-spectrophotometric method to have a special merit. The latter work was reported in detail in a contributed paper (11).

The last report in 1940 dealt largely with a U.S.P. collaborative study which had just been completed. This study was made under somewhat more exacting conditions than previous studies that had dealt with vitamin A standardization. As a consequence the potency of the Reference Oil #2 as determined biologically was found to be 1700, resulting in a conversion factor of 2140. Subsequent determinations by independent investigators (24) to (30) essentially confirm figures close to 2000. British workers have also continually attempted to determine a correct conversion factor for vitamin A. Their most recent report was published in 1943 in *Nature* (31). This study was based upon collaborative work using the vitamin A beta-naphthoate ester. The conversion factor determined upon was 1720 compared with factor of 1570 and 1820 in two preceding studies. But, as has been previously pointed out, collaborative work and multiple assays in this country have demonstrated repeatedly that the correct factor should be in the region of 2000. In fact, this factor has been largely adopted in this country as a basis for commercial transactions and has sometimes been designated "the commercial standard conversion factor." The International standard unit of vitamin A remains 0.6 microgram of beta carotene. The British investigators claim that our factor is high because our original U.S.P. Reference Cod Liver Oil perhaps was evaluated high on the basis of the 1931 standard, which was not pure beta carotene. British claims do not appear to be entirely consistent with the available facts.

Our laboratory has been able to repeatedly check U.S.P. Reference Cod Liver Oil #1 at 3000 units/gram and U.S.P. Reference Cod Liver Oil #2 at 1700 units/gram by direct comparison with the 1934 International standard checked against independently purified beta carotene. This may be regarded as substantiating the conversion factor of 2000, since the corresponding E values on the nonsaponifiable fraction were found to be 1.5 and 0.85, respectively. The U.S.P. bio-assay procedure as practiced appears to be further supported by such data. No evidence of any deterioration was found in the International standard when comparisons were made against the purest beta carotene obtainable. Increased precision of instruments now available, together with new nutritional knowledge as concerned with accessory or synergistic factors, should enable the beta carotene vitamin A bio relationships to be established with greater certainty than has heretofore been possible (32) to (37).

The factor of 2000 as accepted for commercial purposes in the United States has been checked sufficiently to be within the limits of biological error as the assay is now constituted. It may be considered to have been so established within such an error if averages of values are considered rather than the extreme values which sometimes may have been given too much weight.

Spectrophotometric vitamin A determinations can be duplicated in various laboratories with greater accuracy and ease than the corresponding bio-assays; and even if exact agreement between the spectrophotometric determinations and bio-assays is impossible under present conditions there appears to be no logical reason for not accepting E(1%/1 cm) 325 mμ×2000 as an acceptable value for vitamin A in fish liver oils or similar material. In such a case it would be necessary to use the nonsaponifiable material, uniform saponification, and extraction procedure, and accept the results only in case a typical vitamin A curve could be obtained. The results should be expressed in spectrophotometric vitamin A units, leaving any interpretation in terms of actual biological units to the desires and background of those concerned. The need and regard for such a method has been previously expressed by others.

Zscheile and Henry have shown that the vitamin A absorption coefficient of the crystalline material at 320 mμ, 326 and 328 mμ is affected quite markedly by the nature of the solvent used (28). An apparently significant difference was always found between ethanol and propanol and cyclohexane. Typical extinction coefficients were 1712 for ethanol, 1660 for 2 propanol, and 1590 for cyclohexane (38). Morgareidge further emphasized the importance of solvents in a study with both esters of vitamin A in the form of natural oils and with vitamin A as obtained after saponification of fish liver oils. His findings were concordant with those of Zscheile in that alcohol solvents consistently resulted in higher E values than less polar materials as cyclohexane.

The effect was found to be most pronounced for the nonsaponifiable fractions of oils or vitamin A alcohol.

This work appears to be of real importance for the introduction of greater needed uniformity in the practice of spectrophotometric vitamin A determinations. As a consequence of the above considerations a procedure and calculation is proposed for tentative adoption.

Details of apparatus and method will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.

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RECOMMENDATIONS FOR VITAMIN A*

The Associate Referee makes the following recommendations:

(1) That the spectrophotometric method for vitamin A in fish liver oils described herein, involving the use of 2000 as the factor for converting E(1%/1 cm.) 325 mμ values to spectrophotometric vitamin A units, be adopted tentatively.

(2) That a collaborative study be made of the spectrophotometric method for vitamin A in oleomargarine.

(3) That vitamin A studies be continued with reference to new materials and methods.

(4) That studies be made in order to more closely correlate the vitamin A potency of beta carotene to the E(1%/1 cm.) value of vitamin A.

The paper entitled "Spectrophotometric Procedure for the Estimation of Vitamin A in Oleomargarine," by J. B. Wilkie and J. B. De Witt, was published in *This Journal*, 28, 174 (1945).

REPORT ON VITAMIN B_1

By O. L. KLINE (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

At the last meeting of the Association action was taken to adopt, as a tentative procedure, the thiochrome method for the determination of thiamine.[1] In accordance with the recommendations of the Associate Referee this method was stated to be suitable for cereal and vegetable products. The Associate Referee was instructed to study the procedure further with a view to adapting it to the assay of meat products. During the past year, although increased experience has shown that the method in some laboratories is reasonably suited to this purpose, no modification that might be subjected to collaborative study has been presented. It is planned to conduct a collaborative study during the coming year which may serve as a basis for official adoption of this method.

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* For report of Subcommittee A and action by the Association, see *This Journal*, 28, 40 (1945).
[1] *This Journal*, 27, 103 (1944).

It is understood that in studying the problems relating to the determination of the thiamine content of foods, the stability of thiamine during the processing of foods and under varying conditions of storage as well as the body requirement for thiamine, additional methods of approach are of great value. There is no question of the fact that a chemical procedure may possess a higher degree of precision than a biological method, but may at the same time be much less specific, and specificity has been an important consideration in dealing with determinations of the vitamins. It was from this point of view that consideration was given to the study of the microbiological procedure for thiamine developed in the Fleischman Laboratory by Frey, Atkins, and Schultz.

This method, which depends upon the stimulation by thiamine of the rate of fermentation of yeast, has been under study in the laboratory of the Associate Referee for more than a year, and extensive correlation of results with those obtained by thiochrome and rat-curative methods have indicated after some modification of the procedure, that it possesses a satisfactory degree of precision and specificity.

During the past summer 11 laboratories responded to an invitation to participate in collaborative study with a request for samples. Invitations were extended to all laboratories in this country known to have a fermentometer. Three samples—dried bread, flour, and yeast—were distributed with a procedure described in detail. Request was made to carry out at least four determinations on each of the samples, following in detail the procedure submitted. It was also suggested that where time permitted the samples be assayed by the current procedures being used in each of the collaborator's laboratories.

The method submitted for study was based essentially upon that published by Schultz, Atkin, and Frey.[2] Friedman and Kline, in unpublished studies, have demonstrated that yeast fermentation is stimulated markedly in the presence of added pyridoxine, and hydrolyzed casein, as well as with added thiamine. The fermentation medium in the proposed A.O.A.C. procedure includes pyridoxine and hydrolyzed casein, and in addition defines specific conditions under which the assay is to be conducted.

Results of this study were reported from eleven laboratories and are tabulated in Table 1. In this table are given the thiamine values reported for the three samples assayed, flour, bread, and yeast, expressed as micrograms of thiamine per gram of sample. In each laboratory the moisture content of each sample was determined. Since these vary only slightly, the results given are on the basis of the samples as received.

In response to the request for at least four determinations on each sample, the number reported varies from 4 to 9. Having these values made it

[2] *J. Ind. Eng. Chem., Anal. Ed.*, 14, 35 (1942).

possible to calculate the standard deviation for each laboratory. This figure is given in Table 1 for each collaborator and each sample so that direct comparisons of error within each laboratory may be made. This figure is considered preferable to the standard error of the average determination at each laboratory, which would have been affected by the number of determinations made. Also given in Table 1 is the weighted average of the thiamine values for each sample. This weighted average was obtained by weighting the result from each laboratory by the reciprocal of the square of the standard deviation and according to the number of observations. In other words, the laboratory having the most determinations and the smallest standard error was given the greatest weight.

TABLE 1.—*A.O.A.C. collaborative study of fermentation method for thiamine*

LAB. NO.	FLOUR			BREAD			YEAST		
	NO. OF DETNS.	AV. B_1 GAMMA PER GM	STAND. DEV.	NO. OF DETNS.	AV. B_1 GAMMA PER GM	STAND. DEV.	NO. OF DETNS.	AV. B_1 GAMMA PER GM	STAND. DEV.
1	6	4.14	0.111	8	3.47	0.221	5	20.8	1.03
2	5	3.27	.490	5	2.86	.266	7	20.9	2.36
3	5	4.38	.121	4	3.57	.123	4	21.0	1.26
4	9	3.07	.390	6	3.29	.347	4	20.0	.66
5	6	3.85	.198	6	3.56	.091	6	21.9	1.12
6	6	4.67	.334	5	3.98	.265	6	22.9	1.21
7	5	3.99	.085	5	3.76	.315	4	24.1	.32
8	4	3.30	.054	4	2.97	.257	4	21.3	.67
9	4	3.50	.213	4	3.00	.281	6	20.1	1.61
10	5	3.03	.185	6	2.76	.204	4	21.4	1.39
11	4	4.30	.065	4	3.42	.089	4	21.7	.32
Weighted Av.	$3.75 \pm .149$			$3.44 \pm .102$			$22.7 \pm .55$		
Standard Dev. between Labs.	.446			.307			1.64		
Thiochrome Values:									
Mickelsen	3.98			3.61			23.2		
Hall	4.00			3.35			19.9		
Rat-curative Values	3.8			3.4			20.0		

The standard deviation between laboratories has also been computed and is given here. With the exception of a few cases the deviation between laboratories is greater than within any one of them. One may conclude, however, that for a study such as this good agreement was obtained.

It will be noted that the weighted average values for the samples studied compare favorably with values obtained by the thiochrome method reported from two laboratories. Those reported by W. L. Hall of the Food and Drug Administration were obtained by the procedure which includes the base-exchange step. Rat-curative assays of these samples conducted by L. Friedman gave results that fall well in line with those of the thiochrome and fermentation methods.

TABLE 2.—*A.O.A.C. thiamine collaborative study*

(Total, residual, and corrected thiamine values in micrograms thiamine per gram of sample)

LAB. NO.	FLOUR			BREAD			YEAST		
	TOTAL FERM.	RESIDUAL FERM.	TRUE THIAMINE	TOTAL FERM.	RESIDUAL FERM.	TRUE THIAMINE	TOTAL FERM.	RESIDUAL FERM.	TRUE THIAMINE
1	4.65	0.51	4.14	4.38	0.92	3.47	26.3	5.5	20.8
2	3.85	.70	3.27	3.76	.90	2.86	25.3	4.4	20.9
4	4.35	1.28	3.07	4.60	1.31	3.29	28.3	8.3	20.0
5	4.42	.57	3.85	4.54	.98	3.56	28.5	6.6	21.9
6	4.91	.24	4.67	4.47	.49	3.98	28.5	5.6	22.9
7	4.46	.47	3.99	4.27	.51	3.76	28.5	4.4	24.1
8	4.35	1.05	3.30	3.98	1.02	2.97	26.3	5.0	21.3
9	4.17	.67	3.50	3.81	.81	3.00	25.8	5.7	20.1

Yeast is known to synthesize thiamine from the specific pyrimidine and thiazole parts of the molecule. It is known also that yeast fermentation is stimulated by the pyrimidine portion as well as by the thiamine itself. In the fermentation method pyrimidine and other non-thiamine stimulation is taken into account by conducting a sulfite-blank determination, in which thiamine is converted to non-stimulating compounds. The difference, then, between the total fermentation, and the residual fermentation obtained with the sulfite-blank, is the true thiamine value. In Table 2 are given these three values for each sample from each of seven laboratories. It seems clear from the uniformity of the total fermentation values, and the variation in results from the residual fermentation that a critical and difficult step in the method is the preparation of the sulfite-blank. This is true particularly for the flour sample which becomes gelatinous and difficult to handle in the sulfiting process. This difficulty was commented upon by several of the collaborators, and needs further study.

In Table 3 is a comparison of results by the proposed procedure with those obtained by procedures being used in the respective laboratories. These "other" methods departed from the proposed procedure in small details only, but in the case of two laboratories results obtained in par-

ticular on the flour sample were more in line with the average value when determined by their own familiar method. Rewording of the directions of the proposed procedure to prevent any misinterpretation may be in order.

In general, it may be concluded that without question the proposed method is suitable for determining thiamine in the types of foods studied. The error between laboratories and within laboratories is within the range ordinarily met with in this type of work, and there is promise of improvement with further clarifications of the description, and with further study of the critical steps of the method. Deutsch[3] has recently pointed out, in working with pure solutions, that the sulfite cleavage products of thiamine may give a small degree of stimulation to fermentation of yeast.

TABLE 3.—*A.O.A.C. thiamine collaborative study*

(Results of proposed and other methods in micrograms thiamine per gram)

LAB. NO.	FLOUR		BREAD		YEAST	
	PROPOSED METHOD	OTHER METHOD	PROPOSED METHOD	OTHER METHOD	PROPOSED METHOD	OTHER METHOD
3	4.38	4.41	3.57	3.53	21.0	21.5
4	3.07	3.87	3.29	3.27	20.0	19.7
5	3.85	3.82	3.56	3.52	21.9	22.2
8	3.30	3.93	2.97	3.15	21.3	21.9
9	3.50	3.43	3.00	2.92	20.1	20.7

Although in the Fleischmann Laboratories, and in our own, substantially complete removal of stimulation with sulfite treatment has been demonstrated, it is desirable to direct further study to this point, using the purified sulfonic acid derivatives. Very recently Scrimshaw and Stewart[4] have pointed out the desirability of using the internal standard technic in order to properly account for either depression or stimulation of fermentation by non-thiamine constituents of the sample. It is interesting that in their hands the use of sulfited liver-extract in the fermentation medium accomplished the same purpose. One can conclude that in the proposed A.O.A.C. method the use of added pyridoxine and casein hydrolysate and maintenance of similar sample size in total and residual fermentation bottles circumvents the need for the internal standard technic. It is desirable, however, to conduct further studies along this line.

RECOMMENDATIONS*

It is recommended—

(1) That collaborative study of the thiochrome method be conducted

[3] *J. Biol. Chem.*, **152**, 431 (1944).
[4] *Ibid.*, **155**, 79 (1944).
* For report of Subcommittee A and action by the Association, see *This Journal*, **28**, 40 (1945).

during the coming year with a view to obtaining results that may serve as a basis for official adoption of the method.

(2) That collaborative study of the thiochrome method include the assay of meat products, as well as of cereal and vegetable materials.

(3) That the proposed fermentation method be adopted as a tentative method for the determination of thiamine in cereal products and in yeast.

(4) That further study be carried out during the coming year of the fermentation method modified in accordance with experience obtained in the study reported here.

ACKNOWLEDGMENTS

The following collaborators took part in the study:

(1) H. J. Deuel, Jr., and C. H. Johnston. University of Southern California, Los Angeles.
(2) L. Friedman, Vitamin Division, Food and Drug Administration, Washington, D. C.
(3) C. Hoffman, Ward Baking Co., New York, N. Y.
(4) A. Keys and O. Mickelson, Laboratory of Physiological Hygiene, U. of Minn.
(5) H. R. Kreider, Research Laboratory, Mead Johnson & Co., Evansville Ind.
(6) H. Lawford, Standard Brands, Ltd., Toronto, Canada.
(7) G. McGuire, Laboratory of Industrial Hygiene, New York, N. Y.
(8) R. B. Meckel, American Institute of Baking, Chicago, Ill.
(9) A. S. Schultz, Fleischmann Laboratories, New York, N. Y.
(10) M. R. Speck, Research Laboratory, Sealtest Inc., Baltimore, Md.
(11) E. G. White, National Biscuit Co., New York, N. Y.

The Associate Referee wishes to express his appreciation to Lila F. Knudsen for her help in reviewing the collaborative results, and to the collaborators for their interest and cooperation in this work.

REPORT ON ASCORBIC ACID (VITAMIN C)

By WALLACE L. HALL (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

It is recommended*—

That the tentative method for ascorbic acid (vitamin C), as published in the *Journal of the Association of Official Agricultural Chemists*, **27**, 102 (1944), be adopted as official (first action), after the addition of the following:

(1) Page 102, following the line "Ascorbic acid (Vitamin C)" change to "Applicable to orange, grapefruit, lemon, lime, and tomato juice provided the juices do not contain ferrous iron, SO_2, sulfite, or thiosulfate."

(2) Page 103, 2d line, after "filter" add: "rapidly through a loose texture folded filter. (The Eaton-Dikeman Company's paper No. 195 folded filter 18½ cm. size or equivalent.)"

* For report of Subcommittee A and action by the Association, see *This Journal*, **28**, 41 (1945).

No report on vitamin D Milk was made by the Associate Referee.

No report on vitamin D Poultry was made by the Associate Referee.

No report on vitamin K was made by the Associate Referee.

REPORT ON RIBOFLAVIN

By A. R. KEMMERER (Agricultural Experiment Station, College Station, Texas), *Associate Referee*

Last year it was recommended, *This Journal*, **27**, 48 (1944), that the microbiological method for riboflavin be further studied to improve the basal medium and also with a view to making the procedure identical with the procedure that has been adopted by the U. S. Pharmacopoeia. A sample of whole wheat flour that had previously been studied collaboratively, *This Journal*, **26**, 81 (1943), was sent out again this year and analyses for riboflavin were made by a number of collaborators by the U.S.P. method with slight modifications. This method will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.

In a previous report, *Ibid.*, **24**, 421 (1941), one of the collaborators found that all of the riboflavin could not be extracted from dehydrated alfalfa leaf by autoclaving the sample one time in 0.1 *N* HCl for 15 minutes at 15 lb. pressure. A second period of autoclaving was needed. Work in our laboratory has confirmed this finding (unpublished data). In order to further study this problem and to eliminate the second period of autoclaving, if possible, the following experiment was conducted:

EXPERIMENTAL PROCEDURE

One to 2 gm of 4 different samples of alfalfa leaf meal were autoclaved; 2 times, for 15 minutes each with 125 ml. 0.1 *N* HCl; 1 time for 30 min. with 125 ml. HCl; and one time for 30 min. with 250 ml. HCl. The data from this work are given in Table 1. Autoclaving the samples in 250 ml. of 0.1 *N* HCl for 30 min. gave approximately the same results as autoclaving with 125 ml. 0.1 *N* HCl 2 times for 15 min. Autoclav-

TABLE 1.—*Effect of amount of acid and number of autoclavings on riboflavin content of alfalfa*

NUMBER	RIBOFLAVIN—P.P.M.—		
	AUTOCLAVED 2 TIMES FOR 15 MIN. IN 125 ML. 1 *N* HCl	AUTOCLAVED 1 TIME 30 MIN. WITH 125 ML. .1 *N* HCl	AUTOCLAVED 1 TIME FOR 30 MIN. WITH 250 ML. .1 *N* HCl
1 Average of (2)	9.7	8.3	9.6
2 Average of (3)	10.4	8.5	9.8
3 Average of (2)	14.4	11.3	13.8
4 Average of (3)	8.1	7.6	8.1

ing one time for 30 min. with 125 ml. acid did not remove all of the riboflavin. In the extraction of riboflavin from dried leafy materials the amount of solvent seems to be an important factor.

DISCUSSION OF COLLABORATIVE RESULTS

In Table 2 are shown the results of the collaborative study. The mean riboflavin content of the whole wheat flour was 1.17 parts per million as compared with a mean value of 0.92 obtained in 1942, *Ibid.*, **26**, 81 (1943). The maximum variation from the mean was 54 percent; 3 of the 10 collaborators obtained results that agreed within 20 percent of the mean. This is not nearly as good agreement as was obtained in the collaborative study conducted in 1942 (*Ibid.*, **26**, 81 (1943)), in which 14 of 16 collaborators obtained results that agreed within 20 percent. The Associate Referee cannot account for the wider variation obtained this year. The methods used were essentially the same. In this year's study the amount of sugar was increased in the basal medium to make it conform with the medium used in the U.S.P. method. Also, for the same reason, the sample extracts were adjusted to a *p*H of 4.5 with sodium acetate instead of sodium

TABLE 2.—*Results of the collaborative study on riboflavin (ppm)*

COLLABORATOR	PROPOSED A.O.A.C. METHOD	OTHER METHODS
1	1.10	
2	.88	1.04
3	1.24	
4	.80	.74
5	1.03	1.10
6	1.80	1.52
		1.81
7	.92	
8	.78	
9	1.50	
10	1.63	
Mean	1.17	1.24

hydroxide, and the extracts were filtered at *p*H 6.6 and 4.5, instead of only at 4.5. None of these changes should in any way make the method more variable. From the collaborative results received this year and last year, the Associate Referee believes that microbiological method changed to read as given in this report should be adopted as tentative. This method proposed differs slightly from the U.S.P. method for the reason that a method adopted by the A.O.A.C. must be applicable to all types of feeds and especially to feeds that contain low amounts of riboflavin. The U.S.P. method was developed primarily for materials high in riboflavin. The

proposed method differs in the following respects from the U.S.P. method.

(1) In the yeast extract solution, powdered yeast extract (Difco) may be used in place of fresh bakers' yeast. The use of yeast extract saves time because it can be used directly without the treatment required by the fresh bakers' yeast, which may also be difficult to secure or may not be uniform.

(2) Quantities of sample used in making up the test solution and the dilutions differ because, as mentioned above, the A.O.A.C. method usually is applied to material low in riboflavin.

(3) The test solutions are assayed at 2 or more levels instead of 4. For routine assays in which the approximate riboflavin content of the sample is known, 2 levels are ample.

(4) In the assay procedure 3 or 4 tubes are used on each level instead of 2 tubes. In this way there should be at least two tubes on each level that check.

(5) For the sake of clarity, the calculation is written differently.

The Associate Referee appreciates the generous cooperation of the following collaborators:

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J. R. Foy, National Oil Products, Harrison, N. J.
C. N. Frey, Fleischmann Laboratories, New York, N. Y.
C. O. Gourley, The Beacon Milling Co., Cayuga, N. Y.
O. I. Struve, Eastern States Cooperative Milling Corp., Buffalo, N. Y.
W. L. Kellogg, U. S. Department of Agriculture, Bureau of Animal Ind., Washington, D. C.
H. C. Schaefer, Ralston Purina Co.. St. Louis, Mo.
B. L. Oser, Food Research Laboratories, Long Island City, N. Y.

COMMENTS OF COLLABORATORS

John R. Foy. The collaborative method for riboflavin was as satisfactory as any used to date.

C. O. Gourley. Measurement of turbidity instead of the titration of lactic acid has been used for some time in this laboratory. Assaying at a *p*H of 5.7 instead of 6.8 has given better and more even growth response.

O. I. Struve. The procedure used this year appeared to result in greater uniformity than the procedure studied in 1942 (*This Journal*, **26**, 81 (1943).

RECOMMENDATIONS*

It is recommended—

(1) That fluometric methods for riboflavin be studied.

(2) That microbiological method for riboflavin adopted as tentative, *This Journal*, **24**, 416 (1941) be changed to read as given in this report in

* For report of Subcommittee A and action by the Association, see *This Journal*, **28**, 40 (1945).

order to conform as nearly as possible with the method adopted by the U. S. Pharmacopoeia and remain as tentative.

No report on nicotinic acid was made by the Associate Referee.

REPORT ON CAROTENE

By A. R. KEMMERER (Agricultural Experiment Station, College Station, Texas), *Associate Referee*

Last year the recommendations of the Associate Referee were adopted by this Association, *This Journal* **27**, 107 (1944).

In the work this year the collaborators were asked to analyze two samples: No. 1, dehydrated alfalfa leaf meal, and No. 2, dehydrated carrots. The alfalfa was analyzed for carotenes by the method of selective adsorption, *This Journal*, **25**, 92 (1942), and by the abridged chromatographic method adopted as tentative last year for carotene in high lycopene-containing materials, *Ibid.*, **27**, 107. The collaborators were also asked to determine the constituents of the crude carotene from the alfalfa by the complete chromatographic method which was adopted as tentative last year, *Ibid.*, **27**, 107. The dehydrated carrots were analyzed for crude carotene by a method given in the recommendations, and for carotenes by the method of selective adsorption and by an abridged chromatographic method.*

The Associate Referee appreciates the generous cooperation of the following collaborators:

A. F. Morgan, University of California, Berkeley.
J. B. DeWitt, Food and Drug Administration, Washington, D. C.
C. H. Robinson, Department of Agriculture, Ottawa, Canada.
O. I. Struve, Eastern States Cooperative Milling Corp., Buffalo, N. Y.
J. W. Nelson, Cargill, Inc., Minneapolis, Minn.
C. N. Frey, Fleischmann Laboratories, New York, N. Y.
K. Derby, F. E. Booth and Co., Emeryville, Calif.
B. L. Oser, Food Research Laboratories, Long Island City, N. Y.

COMMENTS OF COLLABORATORS

A. F. Morgan. The samples were assayed by methods regularly used in this laboratory. The standard used was beta-carotene and the filter was for a wave length of 440 millimicrons.

James B. DeWitt. With a wide variety of materials extraction by refluxing with ethanol according to the proposed method for carrots gives higher and more consistent results than are obtained by extraction with ethanolic potassium hydroxide in the cold; or by refluxing with ethanolic potassium hydroxide.

In the abridged chromatographic method, difficulty may be encountered in

* Details of the method will be published in the 6th edition of *Methods of Analysis*, A.O.A.C., 1945.

quantitatively eluting carotene from the cotton plug. To overcome this a tube fitted with a fritted glass filter of medium porosity may be employed. More consistent results were obtained by replacing the magnesium carbonate with a mixture of 1 part reagent grade magnesium oxide and 3 parts filter aids (Celite). Rapid elution from this column is obtained by use of a 5 per cent solution of acetone in petroleum ether. The method of selective adsorption seems unduly cumbersome, less efficient and generally less desirable than other adsorption methods.

The time required for the complete chromatographic method makes the method unsatisfactory for routine work. Loss of carotene may occur when solutions are allowed to remain in contact with the adsorbent for long periods and in the removal and subsequent elution of the pigments. The separation of zones of neo-beta carotenes U and B was not sharp and distinct. It was not possible to quantitatively remove the beta-carotene zone without disturbing the zone of neo-beta-carotene B.

C. H. Robinson.—Grinding the residues with sand and solvent after the first refluxing removes more carotene than does a second refluxing.

O. I. Struve.—Considerable difficulty was encountered in finding calcium hydroxide that would act as a satisfactory adsorbent. Bakers U.S.P. calcium hydroxide was found to be the most acceptable for use without filter aid.

C. N. Frey.—The methods submitted gave very reproducible results. The results at the various wave lengths would be in better agreement if the measurements were made at 465 and 475 millimicrons instead of 470 and 480 millimicrons. Also, it seems unnecessary to remove the xanthophyll by extraction with 90% methanol before passing the crude carotene extract through a column of calcium hydroxide or magnesium carbonate.

B. L. Oser.—Difficulty was experienced in differentiating neo-beta-carotene U from beta-carotene on the calcium hydroxide column.

DISCUSSION OF RESULTS

In Tables 1 and 2 are given the results obtained by the various collaborators for the carotene in the alfalfa and the dehydrated carrots. Both

TABLE 1.—*Collaborative work on carotenes—alfalfa*

COLLABORATOR NO.	CRUDE CAROTENE—P.P.M.		CAROTENES (PURIFIED CAROTENE)—P.P.M.				
	A.O.A.C. METHOD	OTHER METHODS	SELECTED ADSORPTION	ABRIDGED CHROMATOGRAM	OTHER METHODS		
1				108.0	90.0	102.5	
2	128.0	158.0	112.0	105.0	111.0	108.0	116.0
3	98.0	110.0	100.0	96.0		108.0	
4	120.4		108.5	104.5			
5						328.0*	
6	113.2		97.3	82.0*			
7	110.0		94.0	95.0			
Mean	114	134	102.	100.			

* Omitted from Mean.

the method of selective adsorption and the abridged chromatographic method give a fair degree of purification of crude carotene extracts from either the alfalfa or dehydrated carrots. From the data obtained this year

TABLE 2.—*Collaborative work on carotene—dehydrated carrots*

COLLABORATOR NO.	CRUDE CAROTENE P.P.M.		CAROTENES (PURIFIED CAROTENE)—P.P.M.			
	A.O.A.C. METHODS	OTHER METHODS	SELECTIVE ADSORPTION	ABRIDGED CHROMATOGRAM	OTHER METHODS	
1				152.0	136.0	165.5
2	125.0		123.0	122.0	119.0	
3	109.0			107.0		
4	363.8*			336.0*		
5					80.0*	
6	160.8		148.0	126.0		
7	271.0			254.0*		
Mean	131.6		136	127.0		

* Omitted from Mean.

and from the data in a previous report, *This Journal*, **26**, 77 (1943), it is the opinion of the Associate Referee that the abridged chromatographic method adopted as tentative for tomatoes and watermelon, *Ibid.*, **27**, 107 (1944), should be extended to other materials. The entity obtained by either this method or the method of selective adsorption should not be termed pure carotene because at best it is a mixture of several stereoisomers. The term "carotenes" would be more appropriate.

The Associate Referee regrets that more collaborative data could not be obtained on the method for dehydrated carrots. However, from the data obtained and because fairly consistent results were obtained in a collaborative study conducted by the National Research Council for the same method (Pavcek unpublished data, 1944), it is the opinion of the Referee that the method should be adopted as tentative.

In Table 3 are the results of the study on the complete chromatographic method adopted as tentative last year, *Ibid.*, **27**, 107. The agreement between results of the 4 collaborators is poor. This method should be studied further, collaboratively. Since this method was developed the pigment carotenoid X was found to be neo-beta-carotene U, and neo-beta-carotene was found to be neo-beta-carotene B (*J. Am. Chem. Soc.* **66**, 305 (1944).

TABLE 3.—*Collaborative work on complete chromatographic method*

COLLABORATOR NO.	PERCENTAGE OF CONSTITUENTS OF CRUDE CAROTENE FROM ALFALFA			
	IMPURITY A	NEO-BETA CAROTENE U	BETA CAROTENE	NEO-BETA CAROTENE B
1	30.6	11.6	67.8	—
2	4.1	4.2	91.7	—
3	13.2	15.0	64.2	7.6
4	18.3		72.3	9.4

In Table 4 are shown the results of a spectroscopic study carried out by Dr. Frey and his coworkers. From these data it is evident that the absorption coefficients in the method for crude carotene, *Methods of Analyses, A.O.A.C.*, 5th Ed., p. 369 (1940), do not give results that agree at the 3 wave lengths selected, for either the crude carotene solutions or for the purified solutions. For beta-carotene solutions the results obtained at the 3 wave lengths agree as well as can be expected. It is the opinion of the Associate Referee that these absorption coefficients and other details should be reinvestigated before any spectrophotometric method is officially adopted.

TABLE 4.—*Spectroscopic study by Dr. Frey of carotene extracts*

WAVE LENGTH-MILLIMICRONS	CRUDE CAROTENE P.P.M.		CAROTENES BY SELECTIVE ADSORPTION P.P.M.	CAROTENES BY ABRIDGED CHROMATOGRAPH P.P.M.	BETA-CAROTENE BY COMPLETE CHROMATOGRAM
	1	2			
Alfalfa					
450	117.5	114.0	98.5	53.9	50.0
470	118.5	115.5	99.5	65.5	50.0
480	108.5	105.5	94.0	76.5	49.0
Dehydrated Carrots					
450	165.0	160.0	152.0	124.6	
470	180.0	171.5	159.0	139.0	
480	148.0	140.5	133.0	115.5	

RECOMMENDATIONS*

It is recommended—

(1) That spectrophotometric method, *Methods of Analyses, A.O.A.C.*, 5th Ed., p. 370 (1940), be tentatively discontinued, and that further work be done on the absorption coefficients and other details.

(2) That the method for crude carotene in hays and dried plants, *Methods of Analyses, A.O.A.C.*, 5th Ed., p. 369 (1940), with the spectrophotometric procedure eliminated, be adopted as official, final action. First action, *This Journal*, **25**, 46 (1942).

(3) That the photoelectric colorimetric method adopted as tentative, *This Journal*, **24**, 80 (1941), be adopted as official, first action.

(4) That the abridged chromatographic method for purification of crude carotene extracts adopted as tentative for high lycopene-containing materials, *This Journal*, **27**, 109 (1944), be extended to include other materials and then adopted as tentative.

(5) That the term "pure carotene" in the title of the method of selec-

* For report of Subcommittee A and action by the Association, see *This Journal*, **28**, 40 (1945). Details of the method will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.

tive adsorption adopted as tentative, *This Journal*, **25**, 92 (1942), be discontinued and the term "carotenes" substituted as tentative.

(6) That some of the modifications of the method for crude carotene that were adopted as tentative last year (*This Journal*, **27**, 107, (2) (1944), to extend the utilization of the method to other materials, be revised and adopted as tentative.

REPORT ON PANTOTHENIC ACID

By HENRY W. LOY, JR. (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

During the past few years the role of pantothenic acid in normal nutrition has been investigated extensively. The importance of the assay for pantothenic acid from the standpoint of control problems has been emphasized by the appearance of representations for this vitamin in pharmaceutical preparations presented for human use, and in products designed for animal feeding. Surveys of the nutritional value of American dietaries also have placed an additional responsibility on the research biochemist for the development of satisfactory procedures for determining pantothenic acid and other of the various members of the vitamin B complex.

Biological methods for the assay of pantothenic acid involving the use of both rats and chicks have been used for a number of years. However, these are time-consuming. The more recently proposed microbiological procedures have been studied extensively and may be satisfactory for those preparations that contain the vitamin in the crystalline form where the extraction process is relatively uncomplicated. These procedures, when applied to food products, have required modifications in the extraction process. Yeast methods in which growth is measured and procedures involving the response of certain types of bacteria as measured by acid production or degree of turbidity have both been reported.

At the last meeting of the Association, the Associate Referee was appointed, with instructions to make a survey of pantothenic acid assay methods, and to conduct a collaborative study of the method that appeared to be best suited to A.O.A.C. problems. Such a survey was made and it was indicated by a review of the literature that although several methods have been used for the assay of food products, the microbiological procedure first described by Strong, Feeney, and Earle,[1] and later modified in the same laboratory, is the procedure most widely accepted. Experience in the laboratory of the Associate referee served as a basis for outlining, for collaborative study, a specific proposed procedure suited to control problems.

[1] *Ind. Eng. Chem., Anal. Ed.*, **13**, 566–570 (1941).

A communication was addressed to 29 persons inviting them to collaborate in a study designed to evaluate the microbiological assay procedure for pantothenic acid as a suitable A.O.A.C. method. Replies were received from 16 persons stating that they wished to take part in this work. The proposed procedure was based upon that described by Strong, Feeney, and Earle,[1] as modified by Neal and Strong,[2] and it included certain additional modifications. The collaborators were asked to compare this specified procedure, followed in close detail, with any other procedure that was being used routinely in their laboratories. Collaborators also were asked to suggest any modifications to the specified procedure that they might wish to include. The study included the assay of 3 samples, representative of food products, the approximate potency of which was indicated as given in Table 1.

TABLE 1.—*Expected approximate potency in pantothenic acid*

SAMPLE	PRODUCT	EXPECTED APPROXIMATE POTENCY (MICROGRAMS OF PANTOTHENIC ACID PER GRAM OF SAMPLE ON THE AS-RECEIVED BASIS)
1	Ground Whole Wheat	9
2	Dry Skim Milk	30
3	Dried Brewers Yeast	115

These samples had been reduced to as fine a condition as necessary for assay. However, because of the possibility of moisture changes in the samples between the different laboratories, collaborators were asked to report the moisture content of the samples as assayed.

Twelve laboratories, including the Food and Drug Administration, submitted results. Since some reports were received late, it was impossible before this meeting to discuss any of the results with the collaborators, with a view toward reassay of the samples. As a whole, the results did not lead to the recommendation of the method for adoption this year. Approximately one-half of the values reported for the proposed procedure were within a range of ± 10 percent of the average. There was a variance in the moisture content of the samples between the laboratories, and it was impossible to calculate the true average pantothenic acid content because 2 of the collaborators failed to report on moisture results. However, the approximate average of the potencies reported are shown in Table 2.

As calculated from Table 2, about 52 percent of the results for the proposed procedure are within a range of ± 10 percent of the average, while about 38 percent of the results for other procedures are within this range.

[2] *Ibid.*, 15, 654–657 (1943).

There is no obvious explanation for the wide divergence of the results reported. In the Associate Referee's laboratory, acceptable results have been obtained with the proposed procedure with repeated assays of a series of samples. With present knowledge, it is not possible to be sufficiently specific with regard to the enzyme treatment. Undoubtedly, further attempts to improve the basal medium also will prove profitable. These points will be given full consideration in further studies.

CONDENSED COMMENTS OF COLLABORATORS

C. O. Gourley.—In our method, instead of measuring titration values, we measure the turbidity in a Beckman spectrophotometer at a wave length of 580 millimicrons. We have not found it necessary to digest samples in the autoclave. Also, we have

TABLE 2.—*Average potency in pantothenic acid*

SAMPLE NO.	PROPOSED PROCEDURE			OTHER PROCEDURES		
	NO. OF COLLABORATORS REPORTING RESULTS	APPROXIMATE AVERAGE POTENCY FOUND (MICROGRAMS OF PANTOTHENIC ACID PER GRAM OF SAMPLE ON THE DRY BASIS)	NO. OF COLLABORATORS REPORTING RESULTS THAT WERE WITHIN ±10% OF THE APPROXIMATE AVERAGE POTENCY FOUND	NO. OF RESULTS REPORTED	APPROXIMATE AVERAGE POTENCY FOUND (MICROGRAMS OF PANTOTHENIC ACID PER GRAM OF SAMPLE ON THE DRY BASIS	NO. OF RESULTS THAT WERE WITHIN ±10% OF THE APPROXIMATE AVERAGE POTENCY FOUND
1	9*	10.7	5	10	11.1	3
2	11	32.5	6	11	32.3	6
3	11	117.0	5	11	121.0	3

* One result was omitted in the calculation, since it was almost 3 times higher than the average shown.

found that we get much more uniform results by keeping the *p*H at 5.7 throughout the entire procedure.

H. H. King and *M. J. Caldwell.*—We were able to get considerably better checks with the proposed procedure than with the Neal and Strong procedure we have been using.

H. J. Cannon and *L. Rosner.*—We wish to suggest a change in technic in enzyme treatment of the sample. Clarase, we find, is not the best enzyme preparation to use in pantothenic acid assays. Higher results are obtained with either Mylase or Polydase. Further, Clarase contains more pantothenic acid than either of the other 2 enzymes. We have also found the treatment to be more effective at an incubation temperature of 45° for 24 hours than at 37° for 72 hours. Higher results are also obtained by using an equal weight of enzyme and sample.

C. W. Sondern.—Similar results were obtained with the A.O.A.C. medium with the omission of glutamic acid. Also, 3 percent glucose medium as recommended in the A.O.A.C. medium is unnecessary, as similar results were obtained with 1 percent glucose.

P. B. Pearson.—We have spent considerable time on the microbiological method for pantothenic acid as outlined, but in our hands the method has not been satisfactory because of high blanks. Even after adsorbing the yeast extract a third time with Darco G-60 there is still excessive growth in the blanks. Using essentially the

medium described by Neal and Strong, there was virtually no difference in results with and without ether extraction.

L. Atkin.—The preparation of the yeast extract solution could not be completed in the 2 hours stipulated. We would prefer to express results in terms of pantothenate, *i.e.*, based on the calcium salt. The enzyme preparation, Mylase P, was used.

K. Morgareidge and *J. R. Foy.*—We are glad to see this subject being given recognition by the A.O.A.C. Our own opinion is that the methods, as they now stand, need a great deal of study and modification before they can be considered satisfactory for adoption by the A.O.A.C. We found that on the preparation of the yeast extract the removal of carbon was quite difficult. Darco G-60 could not be completely removed and Norit A was finally used.

A. Arnold.—Sample No. 1 was contaminated with mold after autolysis, so that no results are available. This would suggest that 2 ml. of toluene, as given in the procedure, is not sufficient for the autolysis stage.

F. M. Strong.—We feel that the blank (2–3 ml.) obtained in the collaborative method was fairly high, and believe that the peptone still retains some pantothenic acid. Probably a 12-hour alkali treatment would be preferable in this respect. The A.O.A.C. yeast supplement, however, seems very good, and gives a lower blank than we have been able to get. The standard curve obtained in the A.O.A.C. method extended from about 2.5–12 ml., but was markedly rounded and appeared to be usable only up to about 9 ml., which was reached at a level of 0.125 microgram of pantothenic acid. We have regularly been getting much steeper standard curves, which are more nearly linear, and reach a maximum at about 0.08 microgram of pantothenic acid. We cannot see any advantage in increasing the glucose to 3 percent in the medium, and also question the necessity of autoclaving the samples both before and after enzyme digestion. Incidentally, I feel sure that we do not yet know enough about this enzyme digestion to correctly specify the time, temperature, *p*H, kind of enzyme, and amount of enzyme needed to secure the best results. In view of such uncertainties, it is probably too soon to adopt a method for pantothenic acid at this time.

COLLABORATORS

C. O. Gourley, The Beacon Milling Co., Inc., Cayuga, N. Y.
B. L. Oser, Food Research Laboratories, Inc., Long Island City, N. Y.
H. H. King and M. J. Caldwell, Kansas State College, Manhattan, Kan.
H. J. Cannon and L. Rosner, Laboratory of Vitamin Technology, Chicago, Ill.
C. W. Sondern, White Laboratories, Inc., Newark, N. J.
P. B. Pearson, Texas Agricultural Experiment Station, College Station, Texas.
N. B. Guerrant, The Pennsylvania State College, State College, Pa.
L. Atkin, The Fleischmann Laboratories, New York, N. Y.
K. Morgareidge and J. R. Foy, National Oil Products Co., Inc., Harrison, N. J.
A. Arnold, Winthrop Chemical Co., Inc., Rensselaer, N. Y.
F. M. Strong, University of Wisconsin, Madison, Wis.
H. W. Loy, Jr., Food and Drug Administration, Washington, D. C.

The Associate Referee wishes to express appreciation to the collaborators and to their organizations for their cooperation in this study.

RECOMMENDATIONS*

It is recommended—

(1) That no method be adopted at this time.

* For report of Subcommittee A and action by the Association, see *This Journal*, 28, 41 (1945).

(2) That further studies be conducted on the microbiological method.

(3) That a comparison be made of results obtained by the microbiological and chick growth methods.

REPORT ON INSECTICIDES AND FUNGICIDES

By J. J. T. Graham (War Food Administration, Office of Distribution, Livestock and Meats Branch, Insecticide Division, Beltsville, Md.), *Referee*

The Referee has given careful consideration to the reports of the Associate Referees on Fluorine, Nicotine, and D.D.T., and approves their recommendations.

The Associate Referee on Rodenticides has been unable to conduct collaborative work and has therefore submitted no formal report. He has, however, made a survey of the possibilities in this field and finds that there are already methods adopted for insecticides, that are applicable to analysis of rodenticides, *i.e.*, methods for arsenicals, fluosilicates, thallous sulphate, etc.

He recommends that the work on rodenticides be continued and that attention for the present be directed to methods for zinc phosphide preparations. The Referee approves this recommendation.

Last year the Referee gave consideration to the value of the factor used in the Mercury Reduction Method (1) for determination of Pyrethrin I in pyrethrum powder and extracts.

The factor in that method proposed by Wilcoxon[1] and adopted by this Association[2] was 1 ml of 0.01 *M* potassium iodate = 4.4 mgs Pyrethrin I. Following a reinvestigation of the factor value, by Graham and LaForge,[3] and collaborative analysis of pure chrysanthemum monocarboxylic acid, under the direction of the Referee, the value 1 ml of 0.01 *M* $KIO_3 = 5.70$ mg of Pyrethrin I was adopted as "Official, first action" at the 1944 meeting of the Association. All work reported prior to 1944 was carried out at room temperature.

Early this year the Referee received a communication from Dr. S. H. Harper[4] of the University College, Southampton, England, giving the results of an investigation he had made of the Mercury Reduction Method using synthetic dl-trans-2:2-dimethyl-3-isobutenylcyclopropane-1-carboxylic acid. This is the racemic form of the natural acid and should be indistinguishable from it in chemical reactions. In carrying out his investigations, Harper employed several different temperatures and reaction

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[1] Wilcoxon, Frank, *Contrib. Boyce Thompson Inst.*, **8**, 175 (1936).
[2] *This Journal*, **28**, 41 (1945).
[3] Graham, J. J. T., and LaForge, F. B., *Soap*, **19**, 111 (1943).
[4] Harper, S. H., Private communication.

TABLE 1.—*Analysis of pure chrysanthemum monocarboxylic acid by the mercury reduction method showing the ratio mgs of Pyrethrin I/mls. of 0.01 M KIO_3 solution*

TEMP.	REACTION PERIOD			
	½ HR.	1 HR.	2 HRS.	3 HRS.
	MGS. PER ML	MGS. PER ML	MGS. PER ML	MGS. PER ML
0°	6.87	6.81	6.17	6.18
		6.59	6.30	
Av.		6.70	6.24	
15°	6.01	5.88	5.76	
	6.01	5.88	5.85	
		5.96	5.92	5.74
Av.	6.01	5.91	5.84	
20°	5.88	5.82	5.73	
	5.88	5.94	5.70	
		5.92	5.78	5.69
		5.85	5.66	5.72
Av.	5.88	5.88	5.72	5.71
25°	5.76	5.73	5.61	
	5.82	5.73	5.59	
		5.74	5.56	5.48
		5.74	5.56	5.45
Av.	5.79	5.74	5.58	5.47
30°	5.73	5.59	5.53	
	5.70	5.56	5.54	
		5.62	5.50	5.42
		5.62	5.50	5.42
Av.	5.72	5.60	5.52	5.42

periods. He found that reduction is not complete in 1 hour at temperatures below 25°, but that at 25° there was no further significant reduction for periods up to 3 hours. He recommended that the reduction be carried out at a standardized temperature of 25° in a thermostat for a period of 1 hour and the remainder of the method to be unchanged. According to his analytical results, the factor would be 5.8 mgs of Pyrethrin I per ml of 0.01 *M* KIO_3.

After receiving Harper's communication the Referee reinvestigated the reaction, employing several temperatures and periods of reduction, using

one sample* of the pure acid that was used by Graham and LaForge in their investigation. The results are given in Table 1, and in Figs. 1, 2, and 3.

The results show that equilibrium is not reached at any temperature or period of time investigated, although the rate of reduction at temperatures of 20° to 30° and beyond 1 hour is very slow. The factor at 25°,

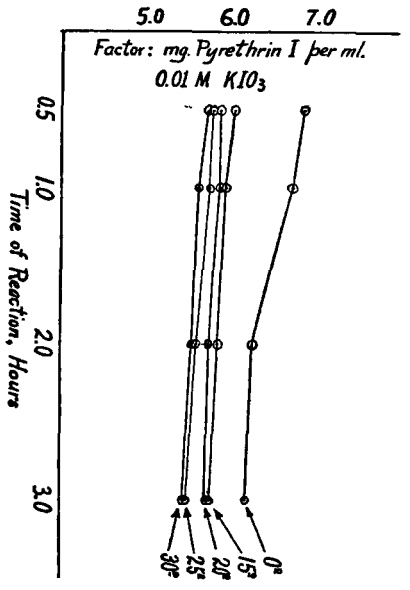

FIG. 1

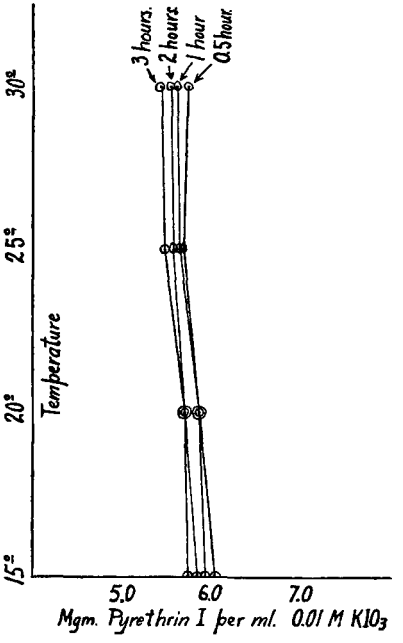

FIG. 2

derived from this study, is the same as that found in the previous work reported by Graham and LaForge.

With the permission of Dr. Harper, the results of his investigation are included in this report, and are given in Table 2.

The factor derived by Harper, using a reduction period of 1 hour at 25°, is 5.80 mgs which is substantially the same as that derived by the Referee in his investigation.

In consideration of the work of Harper and also that of the Referee, it seems necessary to define the time and temperature of the reduction. As previously mentioned, Harper recommends reduction for 1 hour at a

* The Referee wishes to express his appreciation to Dr. F. B. LaForge for furnishing this sample of pure chrysanthemum acid for the investigation.

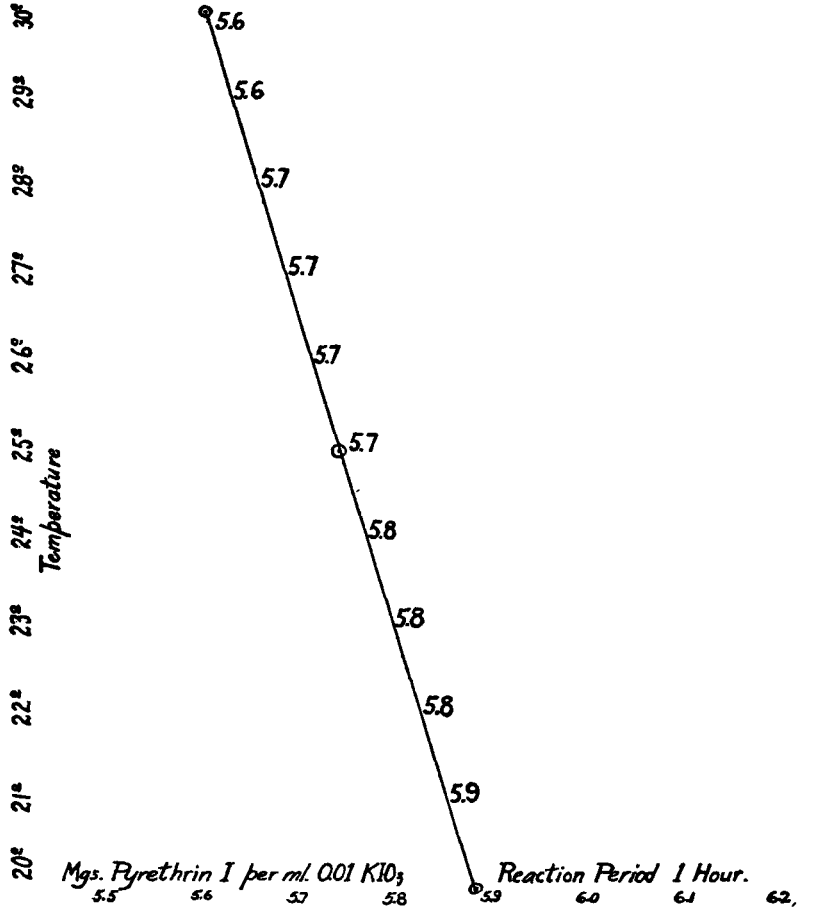

FIG. 3

TABLE 2.—*Analysis of pure synthetic dl-trans-2:2-dimethyl-3-isobutenylcyclopropane-1-carboxylic acid by the mercury reduction method showing the ratio mgs. of Pyrethrins I/mls. of 0.01 M KIO_3 solution*

TEMPERATURE	REACTION PERIOD		
	1 HR.	2 HRS.	3 HRS.
0.0°	7.08	6.51	6.40
20.0°	6.08	6.01	5.94
25.0°	5.80	5.84	5.84
30.5°	5.78	5.49	5.60

temperature of 25°. Considering all the data these conditions appear to be the most appropriate. However, in view of the fact that Fig. 3 shows a variation of only 0.1 mg over a range of 4°, it appears that the purposes of definition would be served if the temperature was defined as 25° ± 2°. This would allow a working temperature covering a range from 23° to 27°, and over a large area, room temperature would fall within these limits much of the time.

In reference to the value of the factor, Graham and LaForge arrived at a value of 5.7 mgs and the work of the Referee here reported confirms that value. Harper's value, derived from a study of the synthetic acid, is 5.80, and this difference is not appreciable.

Figure 3 shows that over a range of 4° the factor varies from 5.7 to 5.8 mgs. To show that no significant difference would be found if a variation of ±2 were allowed, the following hypothetical analyses are given:

Fly Spray —100 ml charge, 80 ml aliquot, weight of aliquot 62 gms., titration 6.0 ml of 0.01 M KIO_3 solution.
Factor 5.7—0.055% Pyrethrin I
Factor 5.8—0.056% Pyrethrin I
Pyrethrum Concentrate, same titration, aliquot weight 3 gms.
Factor 5.7—1.14% Pyrethrin I
Factor 5.8—1.16% Pyrethrin I

RECOMMENDATIONS*

It is recommended—

(1) That in the Mercury Reduction Method for determination of Pyrethrin I in pyrethrum powder (*Methods of Analysis*, **VI**, 113) and in pyrethrum extracts in mineral oil (**VI**, 116) the reduction be carried out at temperature of 25° ± 2° for a period of 1 hour, and that the factor 1 ml of 0.01 *M* KIO_3 = 5.7 mgs of Pyrethrin I be used and made official, final action.

(2) That the method for determination of thallous sulfate in ant poisons adopted as official, first action, in 1941, be adopted as official, final action, for this determination in ant poisons and rodenticides.

REPORT ON FLUORINE COMPOUNDS

By C. G. DONOVAN (War Food Administration, Office of Distribution, Livestock and Meats Branch, Insecticide Division, Beltsville, Md.), *Associate Referee*

During the past year, collaborative investigations of the analysis of fluorine in special samples containing very large quantities of organic

* For report of Subcommittee A and action by the Association, see *This Journal*, 28, 72 (1945).

matter were conducted by both the official lead chlorofluoride and the tentative distillation methods.

At the 1943 meeting of the A.O.A.C., a revised lead chlorofluoride method divided into five procedures was adopted for the determination of fluorine under different conditions.[1] When the method was devised, procedures were included that were applicable for the analysis of fluorine in most commercial insecticides. Recently, there have appeared some proprietary insecticide samples containing very large quantities of organic matter, for example 90–95 percent of dried apple peel and pulp with 5–10 percent of sodium silicofluoride. Without a special preliminary ashing of such samples, a carbonate fusion is impracticable, and the distillation as directed in procedure (5) appears unsatisfactory. Because of excessive foaming and distillation of extraneous matter, high results are obtained by the lead chlorofluoride precipitation. Since the use of these products or others having similar characteristics will probably continue, it is deemed advisable to slightly revise the official lead chlorofluoride method to include a new procedure for their analyses.

GENERAL MODIFICATION

It is proposed to modify procedure (2) to include directions for the analyses of samples containing large quantities of organic matter, now applicable to the present procedure (5), and also for those of the nature of apple peel and sodium silicofluoride. It is also proposed to incorporate in procedure (4) the remaining part of procedure (5) pertaining to the determination of fluorine in the presence of "appreciable quantities of sulfates." The present specified procedure for samples containing appreciable quantities of sulfates and for those applicable to procedure (4) is to remain unchanged; so the incorporation could be accomplished by changing the title of procedure (4) to include "appreciable quantities of sulfates (over 5%)." These changes would delete procedure (5) from the method.

This proposed modification of the procedure pertaining to large quantities of organic matter has been used by the Associate Referee during the past year. Its basic principles for destroying organic matter have been employed for many years for the determination of small quantities of fluorine in foods and spray residues.

The tentative distillation method as published on pages 51 and 52 of the Fifth Edition of *Methods of Analysis, A.O.A.C.* was adopted in 1939.[2] It contains two procedures **(a)** In absence of organic matter and **(b)** In presence of organic matter. The procedure for the determination of fluorine in the presence of organic matter was devised for the analysis of fluorine insecticides containing only moderate quantities (not above 50

[1] *This Journal*, 27, 48, 549 (1944).
[2] *This Journal*, 23, 547 (1940).

percent) of organic material. As has been previously mentioned, the formulae of the various fluorine compounds are changed from time to time and it appears advisable to revise the present procedure **(b)** so as to include instructions for the determination of fluorine in the presence of both moderate and large quantities of organic matter.

ANALYSES OF TEST SAMPLES

Two specially prepared samples containing large quantities of organic matter, one consisting of dried apple material (peel and pulp) and sodium silicofluoride, and the other containing pyrethrum flower powder and sodium fluoride, were used for collaborative analysis.

Table 1 shows the composition of the samples and the results obtained by the two collaborators with the use of the proposed lead chlorofluoride modified procedure 2.

TABLE 1.—*Collaborative results on fluorine insecticides (obtained by use of proposed modified procedure 2—lead chlorofluoride method)*

	SAMPLE 1				SAMPLE 2			
Composition (per cent)	Sodium silicofluoride 10.00 Dried apple peel and pulp 90.00				Sodium fluoride 15.00 Pyrethrum powder 85.00			
Theoretical values (per cent)	Total fluorine 6.02 Sodium silicofluoride 9.93				Total fluorine 6.70 Sodium fluoride 14.81			
Analyst	Total F	Av	Na_2SiF_6	Av.	Total F	Av.	NaF	Av.
C. G. Donovan, Beltsville, Md.	5.92 5.89	5.91	9.77 9.72	9.75	6.66 6.58	6.62	14.72 14.54	14.63
J. J. T. Graham, Beltsville, Md.	5.96 5.88	5.92	9.83 9.70	9.77	6.48 6.52	6.50	14.32 14.41	14.37

The results obtained by the collaborators with the same samples, using the proposed distillation procedure **(b)** for the determination of fluorine in presence of large quantities of organic matter, are shown in Table 2.

RECOMMENDATIONS*

It is recommended—

(1) That in the lead chlorofluoride method for the determination of total fluorine, procedure (5) be deleted; that the proposed modification of

* For report of Subcommittee A and action by the Association, see *This Journal*, 28, 41 (1945). The proposed revisions will be incorporated in the *Methods of Analysis*, 6th edition, 1945.

TABLE 2.—*Collaborative results on fluorine insecticides (Obtained by use of proposed procedure* **(b)***—Distillation method)*

	SAMPLE 1				SAMPLE 2			
ANALYST	TOTAL F	AV.	$NaSiF_6$	AV.	TOTAL F	AV.	NaF	AV.
C. G. Donovan	6.02		9.93		6.64		14.68	
	5.88	5.95	9.70	9.82	6.59	6.62	14.57	14.63
J. J. T. Graham	6.00		9.90		6.59		14.57	
	5.92	5.96	9.77	9.83	6.59	6.59	14.57	14.57

procedure (2) and the proposed change of title of procedure (4) be adopted; and the method as modified to continue as official, first action.

(2) That in the tentative distillation method for the determination of total fluorine, the proposed revision of procedure **(b)** be adopted.

No report on rodenticides was made by the Associate Referee.

REPORT ON NICOTINE

By C. VERNE BOWEN (Division of Insecticide Investigations, Bureau of Entomology and Plant Quarantine, Beltsville, Md.), *Associate Referee*

Methods of analysis of nicotine have not been studied by this Association since the present official silicotungstate method (1) was adopted on the recommendation of Roark (2) in 1916. The data presented at that time indicated that the method was capable of accurate results for the samples tested.

In 1940, however, Markwood (3) in analyzing fresh tobacco leaves compared the results obtained by using phosphotungstic acid as the precipitant with results obtained by a colorimetric method. He found that the fresh leaves tested contained a "relatively nonvolatile" alkaloid, which he identified as nornicotine. About 95 percent of the total alkaloid present was nornicotine. Other data (see ref. 4 to 12) have shown that nornicotine is commonly associated with nicotine in tobacco and that it is likely to be found in nicotine products.

Nornicotine is less volatile with steam than is nicotine, but it may be quantitatively recovered in a steam distillate if sufficient time is allowed. Nornicotine is more easily volatilized by steam from a strongly alkaline solution or from a solution containing sodium chloride and alkali (8, 10). The collection of a large volume of distillate, using a large sample, and

maintenance of the distilland at a small volume are procedures that aid in giving consistent results where small amounts of nornicotine occur with nicotine. Avens and Pearce (13) observed that "excessively large amounts of sodium hydroxide give abnormally high results" in the analysis of tobacco powders and stated that "it seems probable that the higher results obtained with large excesses of sodium hydroxide are due to something besides nicotine." They recommended that "for more consistent results the sodium hydroxide should be limited to a slight excess over that necessary to produce an alkaline reaction or the use of solid barium hydroxide should be adopted." For the same reason magnesium oxide is also used in some laboratories to make the sample alkaline before distillation. This phase of the analysis should be investigated.

At the time of the investigation that resulted in the present official method, the use of a Gooch crucible and direct weighing of the anhydrous nicotine silicotungstate was considered, but was discarded with the statement that "The ignition method is quicker and in general more accurate" (2). In the same reference it was stated that dry nicotine silicotungstate was very hygroscopic, requiring special manipulation in weighting. Rapp, Woodmansee, and McHargue (14) in 1942 presented a modification of the official method in which direct weighing of the nicotine silicotungstate was substituted for weighing of the ignited oxides. The results obtained by them were such as to demand consideration of their method.

In a micro method for nicotine by Spies (15, 16) the nicotine silicotungstate is ignited at 650°C. for ½ hour. Accordingly, some laboratories are now filtering the silicotungstate into a Gooch crucible, washing and igniting in a muffle furnace at 650°C., and calculating the nicotine from the ignited oxides as in the official method.

In view of the foregoing the objects of the investigation on nicotine analysis were first, to compare the distillation using strong alkali and sodium chloride with the official method; second, to compare drying at 105°C, weighing the anhydrous nicotine silicotungstate, and igniting in a Gooch crucible at 650°C. with the official method of ignition; and third, to study the procedure for the determination of nicotine and nornicotine when present together.

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.

R. Jinkin, Insecticide Division, Office of Distribution, War Food Administration, Chicago, Ill.

W. Ralston, Tobacco By-Products and Chemical Corporation, Richmond, Va.
K. E. Rapp, Kentucky Agricultural Experiment Station, Lexington, Ky.
F. A. Spurr, Insecticide Division, Office of Distribution, War Food Administration, Beltsville Research Center, Beltsville, Md.

RESULTS

The materials investigated were designated as follows:

Sample 1, powdered Maryland Medium Broadleaf tobacco.
Sample 2, powdered Burley tobacco from Kentucky.
Sample 3, commercial nicotine dust.
Sample 4, commercial nicotine sulfate solution.

The results of analyses by the official (A.O.A.C.) method reported by the collaborators are presented in the first column under each sample in Table 1.

The agreement among collaborators in the analyses for Samples 1 and 2 is very poor. No doubt slight variations in manipulations have resulted in consistent results by an individual chemist but lack of agreement between chemists. Sample 2 was a low-nicotine tobacco with a reported analysis of about 0.02 per cent nicotine. Sample 1 was also a low-nicotine tobacco and, as the table shows, no nicotine was reported by one collaborator. Samples 3 and 4 contained nornicotine but in an amount relatively small as compared with the nicotine; consequently, a better agreement in analyses was obtained by different chemists. Sample 3 had been previously analyzed by J. J. T. Graham, R. Jinkins, and N. L. Knight, of the Insecticide Division of the Office of Distribution, War Food Administration. From their results, which are shown in Table 2, it is evident that the volume of the distilland and the alkalinity, which is controlled by the dilution of the distilland, are critical factors where nornicotine it present. This need for control is recognized in the official method where it is stated, "when distillation is well under way heat distillation flask to reduce volume of liquid so far as practicable without bumping or undue separation of insoluble matter."

The volatilization of nornicotine by steam is greatly accelerated by sodium chloride and strong alkali. Consequently, the official method was modified in the present investigation by use of 10 grams of sodium chloride and 10 ml. of sodium hydroxide (30 per cent by weight) in the distillation flask. Results higher than in the A.O.A.C. method were obtained on calculation of the steam-volatile alkaloids as nicotine—up to nearly 200 per cent higher in Sample 1 and 133 per cent in Sample 2. There still exists a lack of agreement between the collaborators but a consistency in the results of individual chemists, as shown in the second column under each sample in Table 1. One of the collaborators states in a letter that his laboratory "has always used considerable excess of sodium hydroxide in the steam distilling flask and added salt and sometimes a little paraffin to reduce tendency to foam."

TABLE 1.—*Collaborative results on nicotine*

CHEMIST[2]	PER CENT NICOTINE[1]							
	SAMPLE 1		SAMPLE 2		SAMPLE 3		SAMPLE 4	
	A.O.A.C.	NaCl–NaOH	A.O.A.C.	NaCl–NaOH	A.O.A.C.	NaCl–NaOH	A.O.A.C.	NaCl–NaOH
a	0.26	0.56	0.71	0.94	14.07	14.06	40.38	40.41
b	0.15	0.25	0.43	0.73	13.96	13.99	40.04	40.13
	0.10	0.29	0.46	0.63	13.94	13.92	40.02	39.96
c	0.18	0.47	0.38	0.89	13.98	14.05	40.15	40.19
	0.20	0.46	0.38	0.88	13.98	14.07	40.20	40.25
d	0.13	0.39	0.42	0.95	14.07	13.94	40.00	40.20
	0.13	0.38	0.44	0.96	14.02	13.94	40.14	40.39
e	0.00	0.36	0.51	0.84	13.79	14.02	40.23	40.16
f	0.10	0.27	0.33	0.55	13.91	13.87	40.18	40.31
	0.11	0.23	0.39	0.55	13.94	13.99	40.16	40.30
					14.01		40.23	40.32
					13.98			
					13.85			
g	0.20	0.19	0.38	1.24	13.98	14.15	40.57	40.85
	0.19		0.38		13.93		40.50	40.83
h	0.16	0.37	0.71	1.00	13.90	13.93		
	0.15	0.37	0.70	1.00				
i		0.31		0.71				
		0.29		0.74				
Average	0.147	0.345	0.473	0.841	13.957	13.994	40.215	40.331
Range	0.00–0.26	0.19–0.56	0.33–0.71	0.55–1.24	13.79–14.07	13.87–14.15	40.00–40.57	39.96–40.85
j[3]		0.48		1.02				40.54
		0.52		1.00				40.51

[1] Steam-volatile alkaloids reported as nicotine.
[2] The designation of the chemist does not conform with the order of the collaborators as previously given.
[3] A steam-distillation apparatus not conforming to that used in the A.O.A.C. method was employed.

In order to compare the results obtained by drying according to the method of Rapp *et al.*, with ignition to oxides in a Gooch crucible at 650°C. in a muffle, and with the A.O.A.C. method, the collaborators used aliquots from distillates of the samples. One aliquot was determined according to

TABLE 2.—*Additional analyses of sample 3*

CHEMIST[1]	VOLUME OF DISTILLAND	PER CENT NICOTINE
k	15–25 ml. throughout	14.14 14.09
l	70–80 ml. at start reduced to about 40 ml.	13.23 13.01
m	Maintained at 40 ml.	14.02 13.88
	Maintained at 40 ml.	13.97 13.99
n	(Volume not reported, MgO used to make basic)	13.67

[1] The designation of the chemist does not conform with the order of the names as previously given.

TABLE 3.—*Comparison of percentage nicotine obtained by different procedures of drying and ignition*

TEST	A.O.A.C.	DRYING AT 105°C.	IGNITION IN GOOCH CRUCIBLE IN MUFFLE AT 650°C.
1	0.26	0.23	0.22
2	0.15	0.13	0.10
3	0.10	0.12	0.11
4	0.18	0.20	0.19
5	0.20	0.20	0.19
6	0.13	0.13	0.13
7	0.13	0.13	0.13
8	0.56	0.60	0.60
9	0.25	0.28	0.29
10	0.29	0.30	0.29
11	0.47	0.44	0.47
12	0.46	0.44	0.45
13	0.39	0.40	0.41
14	0.38	0.41	0.41
15	0.36	0.39	0.34
16	0.71	0.74	0.73
17	0.43	0.42	0.39
18	0.46	0.45	0.43
19	0.38	0.38	0.36
20	0.38	0.38	0.38
21	0.42	0.42	0.41
22	0.44	0.44	0.44
23	0.51	0.55	0.48

TABLE 3—*Continued*

TEST	A.O.A.C.	DRYING AT 105° C.	IGNITION IN GOOCH CRUCIBLE IN MUFFLE AT 650°C.
24	0.94	1.09	1.09
25	0.73	0.74	0.71
26	0.63	0.63	0.61
27	0.89	0.88	0.91
28	0.88	0.91	0.87
29	0.95	0.93	0.93
30	0.96	0.94	0.94
31	14.07	14.01	14.04
32	13.96	14.00	13.89
33	13.94	13.98	13.92
34	13.98	14.08	13.93
35	13.98	14.08	13.85
36	14.07	14.07	14.06
37	14.02	14.04	14.03
38	13.79	14.32	13.59
39	13.91	13.87	13.80
40	13.94	13.90	13.95
41	14.06	14.04	14.06
42	13.99	14.05	13.92
43	13.92	13.99	13.91
44	14.05	14.12	13.96
45	14.07	14.15	13.96
46	13.94	13.92	13.89
47	13.94	13.94	13.93
48	14.01	13.85	13.81
49	13.98	13.79	13.77
50	13.85	13.85	13.83
51	40.38	40.33	40.34
52	40.04	40.29	40.06
53	40.02	40.06	39.93
54	40.15	40.18	40.03
55	40.20	40.30	40.06
56	40.00	39.99	40.01
57	40.14	40.03	40.02
58	40.23	40.16	39.89
59	40.41	40.37	40.31
60	40.13	40.42	40.08
61	39.96	40.13	39.93
62	40.19	40.31	40.09
63	40.25	40.31	40.12
64	40.20	40.26	40.25
65	40.39	40.39	40.37

the A.O.A.C. method. Another aliquot, after precipitation and digestion in the regular manner, was filtered into a tared Gooch crucible, washed with hydrochloric acid (1+1000) until the filtrate gave no test for silicotungstic acid, dried at 105°C. for 3 hours, cooled, weighed, and calculated as nicotine (factor=0.1012). This gave the percentage of alkaloid based on the weight of anhydrous nicotine silicotungstate. The crucible was then ignited at 650°C. (dull red heat) in a muffle until no carbon remained, cooled, and weighed. The percentage of nicotine was calculated using the factor for the oxides as in the official method.

Table 3 shows the data on 65 such comparisons. The close agreement between the values of most of the groups is a clear indication that the methods are equally accurate.

The four nicotine samples were also analyzed for nicotine and nornicotine by some of the collaborators, and the results indicate that further work should be done along this line.

The Associate Referee acknowledges the cooperation of the collaborators and the assistance and interest of R. C. Roark, J. S. McHargue, A. W. Avens, and J. J. T. Graham.

RECOMMENDATIONS*

It is recommended—

(1) That the use of a large excess of strong sodium hydroxide and sodium chloride in the distillation flask instead of "a slight excess of NaOH soln.," the use of Gooch crucibles and drying at 105°C. for 3 hours, and the use of a Gooch crucible with ignition at 650°C. be made tentative.

(2) That the work on the determination of nicotine and nornicotine be continued.

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* For report of Subcommittee A and action by the Association, see *This Journal*, **28**, 42, 72 (1945).

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REPORT ON METHODS OF ANALYSIS OF DDT

By ELMER E. FLECK (Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, U. S. Department of Agriculture, Beltsville, Md.), *Associate Referee*

In the absence of a method for the direct determination of 2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane, better known as DDT, use has been made of two indirect methods. The first of these is based on the determination of the total chlorine. The second method is based on the observation that alcoholic caustic will remove 1 mole of hydrochloric acid from DDT to form 2,2-bis(p-chlorophenyl)-1,1-dichloroethylene.[1]

DDT BY TOTAL CHLORINE

DDT contains 50.01 percent of chlorine. The methods that have been used for the determination of total chlorine in DDT include the Carius, the modified Winter[2] and the Parr bomb, the Willard and Thompson,[3] and the Umhoefer.[4] Of this group the modified Winter method has received the most attention, and because it is readily adapted to the determination of DDT in solution it was selected for collaborative study. For this purpose a sample of pure DDT dissolved in acetone was analyzed by the collaborators. The solution was made up by each collaborator from the same sample of DDT, and samples were pipetted from this stock solution for analysis. The results are shown in Table 1.

MODIFIED WINTER METHOD

The sample to be analyzed is volatilized and burned in a flame of ordinary illuminating gas. The chlorine-containing combustion products are absorbed in an alkaline solution of sodium arsenite. This solution is then titrated for chloride ion by the Volhard method using standard solutions of silver nitrate and ammonium thiocyanate.

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TABLE 1.—*Collaborative results on 2 percent of pure DDT in acetone solution*

ANALYST	MODIFIED WINTER METHOD		ALCOHOLIC CAUSTIC METHOD	
	DDT FOUND	RECOVERY	DDT FOUND	RECOVERY
	percent	*percent*	*percent*	*percent*
Robert K. Preston	1.74	87.0	2.09	104.5
	1.73	86.5	2.03	101.5
	1.76	88.0	2.02	101.0
Average	1.74	87.2	2.05	102.3
S. A. Hall	1.80	90.0	1.94	97.0
	1.76	88.0	2.00	100.0
Average	1.78	89.0	1.97	98.5
J. J. T. Graham	2.00	100.0	1.96	98.0
	1.99	99.5	1.93	96.5
			2.00	100.0
			1.93	96.5
			1.89	94.5
			1.93	96.5
Average	1.99	99.8	1.94	97.0
Elmer E. Fleck	1.99	99.5	1.91	95.5
	1.96	98.0	2.08	104.0
	1.97	98.5	2.07	103.5
Average	1.97	98.7	2.02	101.0
Roscoe H. Carter	0.996	99.6	1.00	100.0
(1% solution)	0.983	98.3	1.02	102.0
			1.02	102.0
			1.01	101.0
Average	0.990	99.0	1.01	101.2
General Average		94.7		100.0

APPARATUS

The apparatus consists of a U-shaped glass tube, *ABC*, with a short length of 1.5 mm. capillary tubing sealed to one end (see Fig. 1). The heating element is about 10 feet of No. 28 nichrome resistance wire spaced about 10 turns to the inch. The wire, *W*, is held in place by a narrow strip of braided asbestos tape, *T*, between the wire and tube, and by two or three single loops, *L*, of wire made tight by twisting the ends together. The heating is controlled by passing the current through a Variac variable transformer, *V*. The chimney, *D*, is made from a short piece of 18 mm. inside diameter pyrex tubing joined to 9-mm. tubing, and is connected by a short length of rubber tubing, *F*, to the inlet of the gas-washing bottle, *G*. Sufficient gas-washing solution, *S*, is placed in the washing bottle to cover the fritted glass disk, and the outlet is connected by a piece of rubber tubing, *R*, to a water aspirator (not

shown). Only a moderate flow of air through the apparatus is needed to draw the combustion products from the chimney through the absorbing solution. A pinch clamp, *P*, over the rubber tubing is used to regulate the rate of suction.

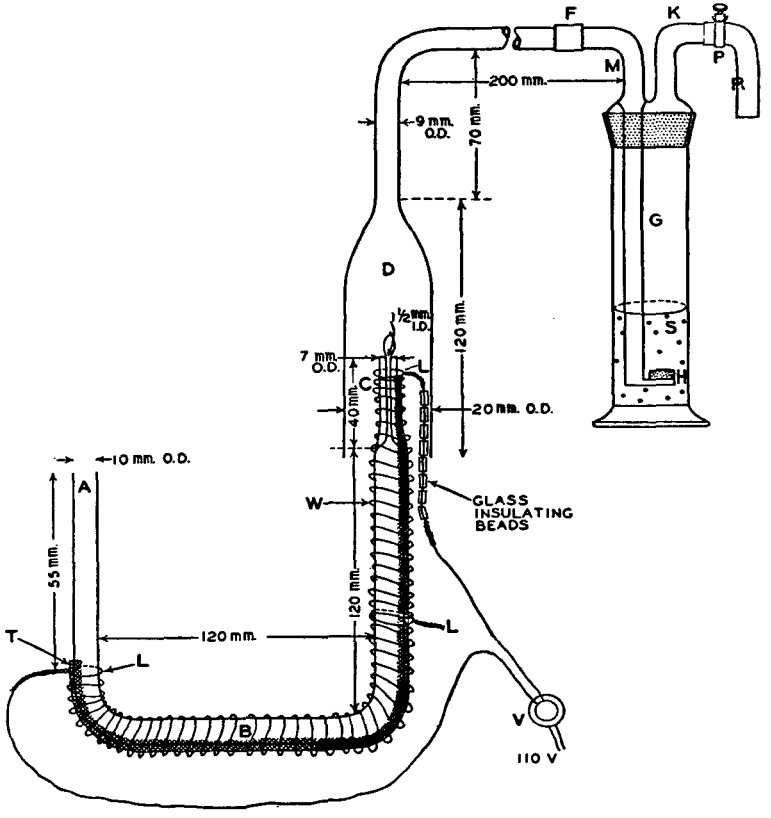


FIG. 1

REAGENTS

(*a*) *Absorbing solution.*—Dissolve 5 g of As_2O_3 in 100 ml. of a soln containing 8 g of NaOH and dilute to 1 liter with distilled water.

(*b*) *Standard silver nitrate solution* (0.0282 *N*).—Dissolve ca 4.791 g of $AgNO_3$ (analytical reagent grade) in H_2O and dilute to 1 liter. Standardize by titration against pure NaCl. One ml. of a 0.0282 *N* $AgNO_3$ soln = 1 mg of Cl.

(*c*) *Ammonium thiocyanate solution* (0.0282 *N*).—Dissolve approximately 2.147 g of NH_4CNS in H_2O and dilute to 1 liter. Standardize by comparing with the standard solution of $AgNO_3$ under the same conditions as obtained in the determination.

(*d*) Concentrated nitric acid (halogen-free).

(*e*) Powdered ferric sulfate.

(*f*) Nitrobenzene.

PROCEDURE

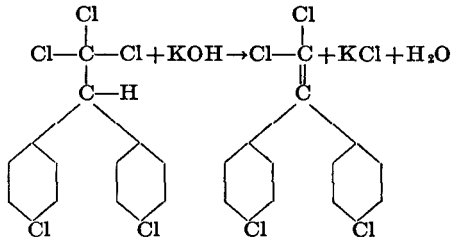
With 50 ml. of the absorbing soln in the gas-washing bottle, *G*, and with the apparatus connected up as shown, pipet a sample of the soln or emulsion containing from 5–50 mg of DDT through the opening *A* into the portion *B* of the U-tube. Connect U-tube at *A* by means of rubber tubing (not shown in Fig. 1) to an outlet of illuminating gas. Before turning on the gas and igniting at capillary opening, *C*, lower the U-tube and remove from chimney, *D*. With Variac set at 15 volts, ignite gas at *C* and adjust flame to height of ca 15 cm. After 10 min., when solvent carrying the DDT is largely burned, reduce flame to about 1 cm. in height. By manipulation of the pinch clamp, *P*, adjust the suction from water aspirator so that when U-tube is raised to insert the flame in chimney, *D*, sufficient air is drawn through system to support combustion. During the course of about 45 min. increase the heat input gradually by raising voltage from Variac. Avoid soot flame by not increasing heat too rapidly or having insufficient air drawn through the system. It will be observed that flame becomes quite luminous as the DDT is volatilized. For the last 10 min. the setting of the Variac should be about 110 volts. This setting should deliver current sufficient to impart a dull red glow to the nichrome wire.

Turn off the gas, remove the inlet tube, *M*, and rinse the adhering soln quantitatively through the disk *H* by means of a wash bottle into container *G*. Transfer the solution in *G* quantitatively to a 500-ml. Erlenmeyer flask. Likewise rinse the chimney, *D*, after allowing to cool, with a little H_2O into the flask. Add $AgNO_3$ soln in excess of the amount necessary to precipitate all the Cl to the alkaline absorbing soln in the flask. Add in succession ca 3 ml. of HNO_3, 0.5 gram of $Fe_2(SO_4)_3$, and 5 ml. of $C_6H_5NO_2$. Swirl the flask to coagulate most of the precipitate. Then back-titrate the excess of $AgNO_3$ with the HN_4CNS soln until a faint pink color appears. Cross-titrate with both standard solns, as recommended by Winter, crossing the end point in each direction. The end point, which is not too sharp, is more easily perceived in this way.

ALCOHOLIC CAUSTIC METHOD

For collaborative studies the same stock acetone solution was used as in the total-chlorine method.

This method of analysis is based on the following equation:



The amount of DDT present is calculated from a Volhard titration of the chloride ion formed.

DISCUSSION

Both of these methods are dependent to a certain extent upon the purity of the DDT. The technical grade of DDT contains approximately 75 percent of DDT; the remainder consists chiefly of DDT isomers. This technical grade is currently required to contain between 48 and 51 percent

of organically bound chlorine. It is therefore desirable to run controls upon the particular sample of DDT used.

It is known that at least some of the isomers of DDT react with alcoholic caustic in the manner outlined above for DDT. In some cases, at least, the action does not stop with the removal of 1 mole of hydrochloric acid. This gives values for the technical grade of DDT that are somewhat high and erratic.

Since this method is based upon the removal of hydrochloric acid from DDT, it is necessary to prevent this reaction from taking place during the concentration of DDT solutions prior to combustion. There are a number of catalysts that may cause this reaction to occur, such as iron, iron oxide, and anhydrous ferric, aluminum, and chromic chlorides.[5] At 115°C. as little as 0.01 percent of the anhydrous chlorides will cause the elimination of 1 mole of hydrochloric acid as a gas during the course of 15 minutes. Care should therefore be taken not to superheat the residue obtained during the removal of solvent prior to determination of DDT.

The alcoholic caustic method has an advantage over the total chlorine method in that it determines DDT and isomers and excludes 2,2-bis(p-chlorophenyl)-1,1-dichloroethylene. The latter product is the most likely decomposition product of DDT and its value as an insecticide is lower than that of DDT.

RECOMMENDATIONS*

It is recommended—

(1) That both the modified Winter method and the alcoholic caustic method be adopted as tentative methods for the determination of DDT.

(2) That studies be continued to determine the effect of the impurities of technical DDT on the results obtained.

(3) That new and more sensitive methods for the determination of DDT and DDT insecticides be sought and subjected to collaborative testing.

The paper on gravimetric determination of phenothiazine was published as a Note, *This Journal*, **28**, p. 429.

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[5] Fleck and Haller, *J. Amer. Chem. Soc.*, 66 2095 (1944).

* For report of Subcommittee A and action by the Association, see *This Journal*, 28, 41, 72 (1945). Details of the method will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.

REPORT ON DISINFECTANTS

By C. M. BREWER (War Food Administration, Office of Distribution, Livestock and Meats Branch, Insecticide Division, Beltsville, Md.), *Referee**

Within recent years emphasis has been placed on dermatophytic infections, especially so-called "Athlete's Foot," as a public health consideration. As a consequence, numbers of preparations purporting to be effective against the offending fungal agents have appeared.

In 1941 Dr. E. G. Klarmann presented to this Association a fungicidal testing method based upon the phenol coefficient germicidal test. This method has been used in conjunction with other tests in routine enforcement of the Insecticide Act, it is included in the Bureau of Ships and the Medical Department of the Army specifications for fungicides, and was adopted by the Disinfectant Scientific Section of the National Association of Insecticide and Disinfectant Manufacturers.

Since publication of the method in 1941[1] several important features of the test have been found unsatisfactory. Within the past two years the American Public Health Association appointed a committee to study fungicidal testing. Those members of the committee who have actively engaged in the study, modification, and improvement of the procedure are: C. W. Emmons, Referee, National Institute of Health; E. G. Klarmann, Vice Pres., Director of Research, Lehn & Fink, Inc.: G. G. Slocum, Food and Drug Administration; C. M. Brewer, Referee, A.O.A.C. The modified procedure resulting from these studies has been submitted to the A.P.H.A. as an acceptable method. The principal changes from the older method are the test organism employed, the method of growth and preparation of the test-culture, and the concentration of the inoculum. The so-called *Trichophyton rosaceum* used in the original test was found to be a non-pathogenic, soil habitant. With many fungicidal preparations it did not give results commensurate with those obtained with pathogenic strains of *Trichophyton interdigitale* prescribed for use by the present modification. The procedure, as modified, has now superseded other methods for the examination of fungicides in the enforcement of the Insecticide Act.

Using the recommended procedure to estimate fungicidal qualities four different laboratories obtained the following results with each of five separate compounds. The figures in the columns indicate the minimum concentrations found to "kill" in 10 minutes.

* Indebtedness to Dr. C. W. Emmons, Referee, and other members (mentioned in the text) of the A.P.H.A. Committee for the Examination of Fungicides, is acknowledged, for permission and encouragement to issue this report.

[1] Klarmann, E. G., *J. Bact.*, 42 (2) 222–229.

TABLE 1.—*Minimum concentrations in 5 preparations*

LABORATORY	COMPOUND					
	Phenol	1	2	3	4	5
A	1:50	1:30	1:400	1:400*	1:30	3
B	1:50	1:30	1:200	1:50	1:20	Undil
C	1:60	1:40	1:400	1:100	1:20†	—
D	1:50	1:30	1:200	1:100	1:30	2

* Not killed; greater concentrations not tested.
† Weaker concentrations not tested.

Compounds:

(1) Cresylic Disinfectant (Bureau of Standards Spec. CS 71–38).
(2) Mixture of Na Salts of ortho phenyl phenol and chloro phenols to meet the following specification—

Na orthophenyl phenate
Na 2 chlor 4 phenyl phenate, not over 50% of total phenols
Na 2 chlor 6 phenyl phenate, not less than 4% of total phenols
Total phenols 25%, isopropyl alcohol 20%, soap not over 10%

(3) 20% solution of Na benzyl phenols and chlorobenzyl phenols (proportions unknown)
(4) 10% solution alkyl-dimethyl-benzyl-ammonium chloride.
(5) Pine Oil Disinfectant (Bureau of Standards Spec. CS 69-38).

As can be observed from the table consistent results were obtained with preparations (1) and (4). With the latter compound more uniform results may have resulted had dilutions of 1:30 been tested in every case. Laboratory "C," and judging by the submitted data, Laboratory "B," did not test the product at this dilution. It might be mentioned that the results with this compound show a regularity ordinarily not obtained by the official phenol coefficient method of testing its germicidal properties. The variations in results with preparations (2) and (3) may not indicate the degree of accuracy obtainable by the test, since it is a frequent practice to double each succeeding dilution in arranging a series of diminishing concentrations when testing for the effective concentration. On the other hand, closer agreement would not be expected with these compounds on the basis of the variability frequently exhibited by halogenated phenols in germicidal testing by the phenol coefficient methods. The variable results reported with preparation (5) can be accounted for by the inhibition, rather than true fungicidal action, exerted by the heavy concentrations required. Actually when re-transfers were made, to eliminate inhibitory action, by three of the four collaborators, two found the undiluted pine oil emulsion failed to kill. Results of re-transfers are not here recorded because of failure of one of the participating laboratories to perform this operation (specified in the test) and irregular attention to this detail by some of the others.

Judged by chemical standards the above results appear to exhibit considerable disparity. Compared with the phenol coefficient germicidal method, and particularly with reported results obtained on similar compounds when other methods have been used, the results are gratifyingly consistent.

The current need for a method of estimating and comparing fungicidal properties is urgent and such a method would be especially appreciated, by a number of testing and public health agencies.

The method in question has had the benefit of extensive investigation; its technique is simple; and it supplies a means of obtaining relatively dependable and reproduceable results.

It is recommended* that the method described in this report be adopted as an "Official method, first action."

REPORT ON LEATHERS AND TANNING MATERIALS

By I. D. CLARKE (Bureau of Agricultural and Industrial Chemistry, Department of Agriculture, Eastern Regional Research Laboratory, Philadelphia, Pa.), *Referee*

The *Methods of Analysis, A.O.A.C.*, relating to Leathers and Tanning Materials are all tentative, as there never has been sufficient interest by members of the Association to do the committee work necessary for their adoption as official. A need for official methods has not been great, especially since committee work on methods for analysis of leather and tanning materials and the adoption of official methods is being done by the American Leather Chemists Association. Retention of methods for these materials in the Association of Official Agricultural Chemists' *Methods of Analysis*, however, has been considered advisable.

No changes in methods have been made since 1936 (*This Journal*, **19**, 392). In order that the methods may conform more nearly to those of the American Leather Chemists Association, certain changes are recommended.†

It is also recommended† that Chapters "X, Leather," and "XI, Tanning Materials" be combined.

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* For report of Subcommittee A and action by the Association, see *This Journal*, 28, 42 (1945). The details of the method will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.

† For report of Subcommittee A and action by the Association, see *This Journal*, 28, 42 (1945). These changes will be made in the forthcoming revision of *Methods of Analysis, A.O.A.C.*, 6th edition, 1945.

REPORT ON STANDARD SOLUTIONS

By R. L. VANDAVEER (Food and Drug Administration, Federal Security Agency, Chicago, Ill.), *Referee*

New Subjects.—Eight standard solutions have been studied to date. They include most of those which are important and which are more frequently used in food, drug, and cosmetic analysis. However, in the judgment of the Referee, bromide-bromate solutions and titanium tri-chloride solutions are sufficiently valuable in drug and color work, respectively, to warrant their study by this Association.

Standard Buffers.—Of great interest to chemists everywhere will be Dr. Manov's report on standard buffer solutions which anticipates that soon buffer salts may be available from the National Bureau of Standards for use in preparing buffer solutions of known *p*H. In his hands, sodium tetraborate decahydrate and acid potassium phthalate have been found useful for callibrating *p*H equipment. The Referee concurs with Dr. Manov that standard buffer solutions be further investigated and certain salts subjected to collaborative study.

Thiocyanate Solutions.—Mr. Deal has observed variable results in standardizing a thiocyanate solution when silver nitrates from different manufacturers were prepared by different procedures. By a special method of purification he has obtained concordant results even when silver nitrate from different sources is used. This subject should be continued.

Standard Sodium Thiosulfate Solutions.—Mr. Johnson subjected to collaborative study the procedure reported in 1942 for preparation and standardization of sodium thiosulfate solutions. The maximum variation from the actual normality was three parts per thousand in eleven collaborative results. Seven collaborators participated. The average variation of the results is only one part per thousand. On the basis of these data the method should be adopted as official.

Potassium Permanganate Solutions.—Because Referee on synthetic drugs first observed that the official A.O.A.C. procedure for standardizing potassium permanganate gave higher results than the procedure recommended by the Bureau of Standards, the subject of standardization of permanganate solutions has been reopened. Mr. Duggan's preliminary experiments confirm the findings of the Referee on synthetic drugs. Further work is in progress for determining the appropriate procedure for standardizing potassium permanganates. In view of the results of two chemists in different laboratories it is being recommended that studies on this subject be continued.

RECOMMENDATIONS*

It is recommended—

(1) That the preparation and standardization of bromide-bromate solutions be studied;

(2) That the preparation and standardization of titanium trichloride solutions be studied;

(3) That the studies on buffer solutions be continued;

(4) That studies on the preparation and standardization of thiocyanate solutions be continued;

(5) That preparation and standardization of sodium thiosulfate solutions be closed and that the method reported in (*This Journal*, **25**, 659, 1942, be adopted as official, first action; and

(6) That methods for standardization of potassium permanganate solutions be studied further.

REPORT ON SODIUM THIOSULFATE SOLUTIONS

By GEORGE M. JOHNSON (Food and Drug Administration, Federal Security Agency, St. Louis, Mo.), *Associate Referee*

The collaborative work on standard sodium thiosulfate solutions recommended in the previous paper[1] on this subject has been completed. The method of preparation and standardization is the same as that given in the

COLLABORATORS	VOL. OF UNKNOWN	VOL. AT 20°C.	VOL. OF $Na_2S_2O_3$ USED AT 20°C.	NORMALITY OF THIOSULPHATE AT 20°C.	NORMALITY OF UNKNOWN	VARIATIONS
	c.c.					
1	50	49.91	41.95	0.1023	.0860	−.0001
2	50	49.91	42.09	0.1023	.0863	+.0002
3	50	49.88	43.61	0.0986	.0862	+.0001
4	50	49.94	40.56	0.1061	.0862	+.0001
	50	49.94	40.54	0.1061	.0861	±.0000
5	50	49.96	43.92	0.0980	.0862	+.0001
	50	49.96	43.94	0.0980	.0862	+.0001
6	50	49.88	38.31	0.1118	.0859	−.0002
	50	49.88	38.29	0.1118	.0858	−.0003
7	50	49.89	42.49	0.1011	.0861	±.0000
	50	49.89	42.48	0.1011	.0861	±.0000

report. Collaborators were asked to prepare and standardize their solution by this procedure and in addition to titrate a solution which, unknown to them, consisted of potassium iodate, .08611 *N* by weight.

There follows the reports of the collaborators. Bureau of Standards calibrated glassware was used and all volumes were corrected to 20°C.

* For report of Subcommittee A and action by the Association, see *This Journal*, **28**, 42 (1945).

[1] *This Journal*, **25**, 659 (1942).

DISCUSSION OF RESULTS

The results seem to be in good agreement. The maximum variation from the actual normality is 3 parts in 1000 and this in only one case. The average variation is one part per thousand and the average normality is .0861. This shows that the standardization is dependable and gives accurate results. Experiments conducted by the Associate Referee have shown that the solution, prepared as described, is stable and maintains its normality for nine months or more.

The Associate Referee wishes to thank the following members of the U. S. Food and Drug Administration for their cooperation as collaborators in this work: They are H. P. Bennet and H. W. Conroy, Kansas City, Mo.; E. C. Deal, New Orleans, La.; W. Horwitz, Minneapolis, Minn.; D. M. Ottens, St. Louis, Mo.; I. Shurman, Cincinnati, Ohio.

RECOMMENDATIONS*

It is recommended that the method of preparation and of standardization be adopted as official, first action, and that the subject be closed.

REPORT ON THIOCYANATE SOLUTIONS

By E. C. DEAL (Food and Drug Administration, Federal Security Agency, New Orleans, La.), *Associate Referee*

During the year 1942, work was undertaken to determine the substance best suited for use as a primary standard in the direct standardization of silver nitrate solutions.[1] A comparison of results obtained by the use of various silver and mercury products showed nitrate of silver to offer the greatest possibilities. The Associate Referee was able to obtain results agreeing within one part in one thousand of the theoretical value using a specially prepared silver nitrate. However, when samples of a thiocyanate solution were sent out to collaborators, the results of different collaborators varied as much as 5 parts in one thousand. No explanation was offered for these differences.

This year, work was undertaken in an attempt to discover the cause of the wide variations in the collaborative results. A thiocyanate solution of known strength was prepared from recrystallized, vacuum-dried potassium thiocyanate. Various commercial samples of silver nitrate were titrated with the thiocyanate solution. Only one of the commercial C.P. grade products produced results agreeing with the theoretical value. (No A.R. or primary standard grades were obtainable.) The other samples gave results always too high, showing an impure or hydrous product. Upon drying the samples at 110°C., all except the one sample darkened badly.

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* For action of Subcommittee A and action by the Association, see *This Journal*, 28, 42 (1945).

[1] *This Journal*, 25, 661 (1942).

This sample darkened only slightly, proved to be anhydrous and gave theoretical values after drying. A standard silver nitrate prepared by triple recrystallization, drying at 110°C. and fusing at 250°C., gave erratic results. The sample showed darkening which presumably was due to reduced silver. Further experimenting showed that the darkening (decomposition) could be prevented by drying the recrystallized silver nitrate at 100°C. and then fusing by placing in a cold muffle furnace and allowing the temperature to rise until the silver nitrate melted (maximum temp. 250°C.). Samples prepared in this manner, and then pulverized and redried at 100°C., did not darken appreciably, and gave results generally agreeing within one part in one thousand with the theoretical value. Such variations as were obtained were always on the high side. No value was obtained below the theoretical, which may have been due to a tendency to undertitrate.

Experiments were conducted to determine the influence of titrating into cooled solutions of the standard silver nitrate since it is stated that, mercury titrations are effected by temperature changes.* No effect on the results could be detected by varying the solution temperature between 15° and 35°C.

It is recommended† that the subject be further studied and that new samples be sent to collaborators with directions incorporating the observations of the Associate Referee.

REPORT ON POTASSIUM PERMANGANATE SOLUTIONS

By R. E. DUGGAN (Food and Drug Administration, Federal Security Agency, New Orleans, La.), *Associate Referee*

Warren[1] observed that the strength of a potassium permanganate solution determined by the official A.O.A.C. procedure[2] was greater than the strength determined by the procedure recommended by the National Bureau of Standards.[3] Therefore, the Referee on Standard Solutions reopened the investigation of the standardization of permanganate solutions.

The present official procedure is Kolthoff's[4] modification of a procedure originally outlined by McBride,[5] subsequently studied and subjected to collaborative treatment by Johnson,[2] who, however, did not compare that procedure with the one recommended by the National Bureau of Stand-

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* Kolthoff and Furman, "Volumetric Analysis," Vol. II, p. 263. John Wiley and Sons, New York (1929).
† For report of Subcommittee A and action by the Association, see *This Journal*, **28**, 42 (1945).
[1] Warren, L. E., General Referee on Synthetic Drugs. Private communication.
[2] *Methods of Analysis, A.O.A.C.*, 1940, 653; and *This Journal*, **23**, 543 (1940).
[3] Fowler, R. M., and Bright, H. A., R.P. 843, *J. Res. N. Bur. Standards*, **15** (1935).
[4] Kolthoff, Volumetric Analysis, Vol. II, p. 282 (1929).
[5] McBride, R. S., *J. Am. Chem. Soc.*, **34**, 393 (1912).

ards for use with their oxidimetric standard sodium oxalate Standard Sample 40c.[3]

Fowler and Bright[3] investigated the standardization of permanganate solutions with sodium oxalate for the National Bureau of Standards. After comparing the results obtained using McBride's procedure with a procedure devised by themselves using primary standards other than oxalate, they concluded that McBride's procedure yielded titers too high for the most exact work and recommended the use of their own procedure, submitted with the National Bureau of Standards sodium oxalate oxidimetric standard.

The primary difference in the two procedures is that the A.O.A.C. procedure directs titration at a temperature of 85°C., while the National Bureau of Standards procedure requires the addition of 90–95 percent of the permanganate solution at room temperature, heating to 55–60°C. and completing the titration.

Fowler and Bright present data showing that the titers of permanganate solutions vary significantly with but slight deviations from the procedure of McBride—*e.g.*, rate of addition of permanganate, acidity of solution, and temperature. It is reasonable to assume that these criticisms may be applied to the A.O.A.C. procedure. Their data indicates that similar deviations from their procedure do not produce significant changes in the titer.

Permanganate solutions have been carefully standardized by the Associate Referee using the A.O.A.C. and National Bureau of Standards procedures. The titer obtained by the A.O.A.C. procedure was found to be 0.2–0.4 percent higher than titers obtained by the National Bureau of Standards procedure. A fundamental difference is illustrated since each set of determinations was made on the same day using the same buret and sodium oxalate; and the precision of each procedure is 0.1 percent.

Work is in progress to make intercomparisons of the results obtained by use of primary standards other than sodium oxalate with those obtained by the two procedures using sodium oxalate.

It is recommended* that the subject be continued.

REPORT ON BUFFER SOLUTIONS

By GEORGE G. MANOV (National Bureau of Standards, Department of Commerce, Washington, D. C.), *Associate Referee*

In the statistical analysis on *p*H values of unknowns the standard deviations which are obtained are a measure not only of the precision attainable by the worker, but also of the errors due to partially defective

* For report of Subcommittee A and action by the Association, see *This Journal*, 28, 42 (1945).

meters, old electrodes, thermal and electrical hysteresis of electrodes, liquid-junction potentials, and faulty preparation of the buffers or subsequent contamination with carbon dioxide.

This year, the Association has inaugurated a study of methods for diminishing these sources of error. It is anticipated that the *p*H Standards Section of the National Bureau of Standards will assist collaborating members of the Association and others by making available pure, solid buffer standards, together with adequate directions for preparing aqueous solutions whose *p*H values are known with an accuracy of ± 0.002 unit over the temperature range of 0° to 60°C.

In the calibration of the scale of the *p*H meter, the use of a single buffer standard frequently gives a false sense of security. The meter is adjusted to read the correct value for the selected buffer, but there is no assurance that the measured *p*H of the unknown does not involve additional errors. Borax, sodium tetraborate decahydrate, has a *p*H of 9.18 in 0.01 *M* solution at 25°C. and forms a useful standard just below the point at which the alkali error of the 015 glass electrode becomes appreciable. A 0.05 *M* solution of acid potassium phthalate (NBS Standard Sample 84b) has a *p*H of 4.01 at 25°C. These standards furnish two widely-spaced reference points along the *p*H scale which are quite suitable for calibration purposes.

Twenty-five pounds of reagent-grade borax were recrystallized from conductivity water and dried in such a manner that the product was the pure decahydrate. Analyses of the various factions, some of which were recrystallized as many as four times, indicated that the purity of the salt was independent of the number of recrystallizations after the first, and that an error of less than 0.001 *p*H unit could be ascribed to residual impurities in the finished product. This borax is to be bottled in approximately 60-gram lots. A formal announcement will be made by the Bureau of Standards when this standard sample is available for distribution.

The preparation of moderate quantities of other standards is now in progress.

When a sufficient number of these *p*H standards is available, collaborative measurements of the observed *p*H of these buffer solutions will be started to obtain information on the condition of the *p*H meters and of the glass and calomel electrodes in use by cooperating members of the Association.

An intercomparison was made of the various buffer standards recommended by the Bureau of calibrating glass-electrode meters. The work involved the determination of the differences in the potentials at the liquid junctions between various standard buffers and the saturated potassium chloride of the calomel electrode. It was found that the liquid-junction potentials of phthalate and of borax buffers in contact with saturated potassium chloride differed by less than 0.01 *p*H unit. This small correction can be safely ignored when commercial *p*H meters are used.

The *p*H values from 0° to 60°C. of solutions of borax ($Na_2B_4O_7 \cdot 10H_2O$)

and sodium chloride are taken from the data of Manov, DeLollis, and Acree[1] and presented in the table below.

Each of the solutions in the table may be used as a *p*H standard. An error of 1 percent in the total concentration corresponds to less than 0.001 in *p*H, and it is therefore permissible to make up the solution to a total volume of 1 liter instead of weighing out the water separately. A solution composed of 0.01 mole (3.814 g) borax and 0.02 mole (1.169 g) sodium

TABLE 1.—*p*H *values of borax-sodium chloride buffers at rounded values of the concentration*

TEMPERATURE, °C.	TOTAL CONCENTRATION IN MOLES PER KILOGRAM OF WATER[a]				
	0.01	0.02	0.03	0.04	0.05
0	9.465	9.449	9.439	9.431	9.425
5	9.394	9.379	9.368	9.361	9.355
10	9.334	9.320	9.310	9.302	9.295
15	9.278	9.267	9.256	9.247	9.240
20	9.231	9.219	9.208	9.200	9.193
25	9.185	9.174	9.164	9.155	9.147
30	9.144	9.132	9.123	9.114	9.106
35	9.109	9.097	9.088	9.079	9.070
40	9.073	9.063	9.054	9.046	9.039
45	9.046	9.035	9.026	9.017	9.009
50	9.019	9.009	9.001	8.993	8.985
55	8.992	8.984	8.976	8.968	8.959
60	8.964	8.960	8.953	8.944	8.934

[a] Total concentration =2 (moles of borax) +moles of sodium chloride.
Moles of NaCl =2 moles borax for each solution.

chloride per liter of solution is readily prepared and has a *p*H of 9.155 at 25°C.

Electrometric *p*H equipmenent such as glass-calomel and hydrogen-calomel assemblies and special glass electrodes for determining the *p*H of highly alkaline materials may be calibrated by means of these solutions. During the past 2 years several hundred comparisons have been made between the *p*H of a 0.05-*M* solution of potassium acid phthalate (NBS Standard Sample 84a) and borax-chloride solutions, using various commercial vacuum-tube *p*H meters. In practically all cases the calibrations of the meter with phthalate and with borax, performed by using these two standards in either order, agreed to within 0.01 *p*H unit, the limit of error of the best instrument. However, if the glass electrode was old or had been mishandled, the *p*H-emf relationship departed from linearity, and the agreement between the calibrations then differed by as much as 0.06 unit.

[1] *J. Research NBS*, 33, 287 (1944) RP 1609.

REPORT ON FROZEN DESSERTS*

By F. LESLIE HART (Food and Drug Administration, Federal Security Agency, Los Angeles, Calif.),
Associate Referee

The imminent publication of the 6th edition of *Methods of Analysis, A.O.A.C.*, necessitates a review of methods so far adopted for ice cream and other frozen desserts. The 5th edition listed an official method for preparation of sample of plain ice cream, and for determination of nitrogen (official), fat (official), and coloring matter (tentative). Since then there has been adopted a tentative method for determination of total solids in plain ice cream, and an official method for preparation of sample of ice cream containing insoluble ingredients.

The present tentative method for coloring matter in ice cream, **135,** p. 306, appeared first in the 2nd edition of *Methods of Analysis*. Incidentally the 5th edition perpetuates an error in reference to the method of detection of annatto first made in the 4th edition. The reference "under **XXI** particularly—21" should refer to **16 (b)** instead of **21**. This is the appropriate reference for the 3rd edition, but succeeding editions of *Methods of Analysis* listed the annatto test in error. It is believed this method should be made official, after proper correction of references, particularly since the detection of coloring matter in milk, **31,** p. 279, and butter, **105,** p. 299, are official, and the method for ice cream directs that the procedures in **31** and **105** be followed. The Referee on Coloring Matter in Foods concurs in this recommendation.

Some experimental work on detection of gums in ice cream has been done by the writer as Referee on Gums in Food Products, and they will be more fully reported under that heading. Algin (sodium or ammonium alginate) has to a large extent replaced locust bean gum and other gum stabilizers. While algin is probably not a true "gum," since it is a manufactured soluble alginate rather than a water-extract of plant material, it resembles true gums in many of its reactions. There is one important difference. Acids, including trichloracetic, precipitate insoluble alginic acid. A method has been devised in which algin is separated from other gums by treating separate portions of ice cream with trichloracetic and with tannic acid to remove proteins. The gums are precipitated from the two filtrates with alcohol, and presence of gums is demonstrated by appropriate tests on the precipitate. It is hoped that the proposed method can be submitted to collaborators before the next meeting of the Association.

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* This report was presented in the section on Dairy Products at the Wednesday morning session, Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 25, 26, 1944. It should have appeared in the proceedings as published in Vol. 28, No. 2.

DETERMINATION OF WEIGHT PER UNIT VOLUME OF FROZEN DESSERTS

This Association recommended[1] that "studies on the analysis of frozen desserts . . . . be continued, and that methods for determining the apparent volume of frozen desserts be included." The need for this action is evident when one considers the nature of ice cream and other frozen desserts. The incorporation of air, by churning or beating, is necessary to produce these products. An excess of air results in products below official or generally accepted standards.

Regulatory units have attempted various ways to limit the amount of air in frozen desserts, especially in ice cream. Some states limit the amount of "over-run," which is the per cent increase in volume of the ice cream over the volume of the original mix; others set a minimum figure on food solids content; still others require a minimum weight per gallon of ice cream. Lucas[2] in a report of the Joint Standard Methods Committee A.P.H.A., proposed a method for the determination of over-run, which involved measurement of the volume of a retail unit of a frozen dessert, and the volume of the melted dessert, with certain precautions to ensure the expulsion of air from the melted mix. From the figures obtained may be calculated either the over-run or the weight per unit volume. Trial of this method revealed that certain desserts, with low solids and comparatively high amounts of stabilizer, were very difficult to de-aerate. The Lucas method was devised only for the conventional retail bricks or cups, of regular shape, or desserts in water-tight containers. In working with bulk ice-cream, preliminary measurements were necessary at the factory. The method is not applicable for "novelty" packages.

After some experimentation, the Referee devised an "over-flow" can to measure the liquid displaced when a brick or container of frozen dessert was totally immersed in the liquid. Kerosene of known density was selected as the liquid. The weight per gallon of the frozen dessert, regardless of its shape, can then be calculated from the weight of the frozen dessert and the volume of the displaced liquid.

Packaged ice cream may vary in over-run, hence in density, from unit to unit, even when all the units are packaged from one batch. It was therefore not deemed practicable to prepare one series of collaborative samples for distribution. Then again, data were desired on bulk ice cream, and on packages filled from the bulk ice cream tested. Collaborators were therefore requested to apply the method to the following variations from the same churn of ice cream; (a) a wedge-shaped piece of about 1 pint volume cut from bulk ice cream (they were directed to use a sharp knife, avoiding side pressure, in cutting out this wedge); (b) a 1-pint cup or brick of packaged ice cream; and (c) a 1 pint cup dipped by hand from

[1] *This Journal*, 25, 67 (1942).
[2] *Am. J. Pub. Health*, 33, 595.

the frozen bulk ice cream. They were instructed to surround the samples in dry ice, and keep them hard-frozen until the time their weight per unit volume was to be determined.

It is recommended that the method for determination of weight per unit volume of frozen desserts be adopted as tentative.*

INSTRUCTIONS TO COLLABORATORS

It is impossible to satisfactorily ship collaborative samples of ice cream for this determination. Collaborators are requested, therefore, to furnish their own samples, according to one or more of the following procedures:

1. If ice cream in bulk and package is available *from the same mix:*
   (a) Cut a wedge-shaped piece, equivalent to about 1 pint, from the bulk ice cream, trim off the edge of the wedge, wrap in wax paper, and immediately freeze in dry ice as directed in the method. Use a sharp knife in cutting out the wedge, avoiding side pressure as much as possible.
   (b) Freeze a 1-pint package of packaged ice cream, made from the same mix, in dry ice, and proceed as directed in the method.
   (c) Dip out a 1 pint cup or carton from the bulk ice cream sampled under (a), in the usual counter fashion, freeze in dry ice and proceed as directed in the method.
2. If two collaborators can work simultaneously on a sample:
   (a) Cut a wedge, as directed under 1(a), equivalent to 1 pint to 1 quart. Immediately, trim off edge of wedge, cut into two approximately equal pieces, wrap in wax paper, and freeze in dry ice. Each collaborator shall use one of these pieces to determine weight/unit volume according to the proposed method.
   (b) If packaged ice cream can be obtained from a manufacturer packed consecutively from the same mix, each collaborator shall determine weight/unit volume on two or more packages.
3. Directions for a single collaborator:
   (a) Cut a trimmed wedge of ice cream into two approximately equal pieces and determine weight/unit volume on each piece as directed under 1(a).
   (b) If packaged ice cream can be obtained under conditions outlined under 2(b), determine weight/unit volume on at least 3 units.

Describe in detail the shape and type of packaging used in the packaged ice creams. Collaborative work is particularly desired on fancy shapes.

EXPERIMENTAL WORK

The overflow can first designed had the overflow orifice extending at a slight downward angle from near the top of the can. It was found that this was faulty in that waves disturbed the flow of liquid. The orifice was then moved to the bottom of the can and the outlet tube brought up the side of the tin and bent over in about an 180° arc. This again was found faulty in that the flow did not shut off sharply but continued siphoning after the water had reached the level of the outlet. This was remedied by grinding the outlet so that the upper edge of the opening is above the highest point of the lower interior surface of the bend.

* This method will be published in the 6th Edition of *Methods of Analysis, A.O.A.C.*, 1945.

Early experiments were made, using water as the liquid. Later kerosene was used since the ice cream or sherbet dissolved more slowly in this liquid. After details of the method were decided, 2 or 3 packages of commercial frozen desserts were obtained from each of several local manufacturers. The weight per gallon of these samples are tabulated in Table 1, Some of these, as indicated by footnote in table, were taken from one batch of mix; the others were random samples.

TABLE 1.—*Weight per unit volume*

SIZE	SHAPE	WT./GALLON (LBS.)	PRODUCT
†Pint	Truncated rect. pyramid	*4.16, 4.10, 4.08	Ice cream
†Pint	Truncated rect. pyramid	*5.41, 5.51, 5.52	Sherbet
Pint	Cups (truncated cone)	*4.61, 4.65, 4.54	Ice cream
Pint	Rect. tray	4.31, 4.36, 4.40	Ice cream
8 oz.	Cups	6.30, 6.19	Sherbet
†6 oz.	Cups	5.06, 5.17, 5.00	Ice cream (12% fat)
Pint	Trays	*4.34, 4.42, 4.34	Ice milk (4% fat)
Pint (approx.)	Novelty (hatchet)	4.57, 4.76, 4.66	Ice cream (16% fat)
†Pint	Octagon shaped tray	4.18, 4.32, 4.21	Ice cream (10% fat)
Quart	Football shape	4.62, 4.78	Ice cream, 2 flavors
?	Heart	5.62, 5.50	Sherbet
?	"Eskimo bars" (coating removed)	*4.20, 4.30, 4.14	Ice cream
Quart	Cylinder	*5.67, 5.69	Ice cream (18% fat)
†Pint	Truncated rect. pyramid	3.94, 3.79	Ice cream (15% fat)

* All packages made from one freezer batch, remaining were random sampling.
† Water used as liquid. Kerosene used in remaining determinations.

COLLABORATIVE RESULTS

1. P. H. Tracy, Agri. Experiment Station, Urbana, Ill.

A. Ice cream mix was frozen and filled into pint cartons and into 2½ gallon cans. Sample (a) is a pint carton, freezer-filled. (b) is a wedge cut from a 2½ gallon can, and (c) is a dipped pint from the same can.

	(a)	(b)	(c)
Pounds per gallon	3.03	3.33	4.20

B. This experiment was repeated on a second batch of ice cream, this time in duplicate.

	(a)	(b)	(c)
Pounds per gallon	3.25	3.23	5.01
	3.16	3.53	5.04

2. D. H. Tilden and M. G. Yakovitz, Food and Drug Administration, San Francisco, Calif.

2 bricks of mixed ice cream and sherbet were bought. Each brick was cut approximately in half, and each half run by a different analyst.

	D.H.T.	*M.G.Y.*
Brick No. 1	5.98 lbs./gal.	6.70 lbs./gal.
Brick No. 2	5.62	6.70

This experiment is valueless for comparison of results by 2 analysts, since the analysts state the bricks could not be divided so that the proportion of sherbet and ice cream in the two halves was equal. It is included merely to give results obtained on mixed sherbet-ice cream bricks.

3. Emma Mueller, Food and Drug Administration, Los Angeles, Calif.

2 quart bricks of ice cream were each cut approximately in halves, and the method applied on each half.

	Brick No. 1	*Brick No. 2*
lbs./gal.	4.51	4.52
	4.52	4.53

4. J. F. Armstrong and F. Y. Mendelsohn, Food and Drug Administration, Los Angeles, Calif.

3 bricks of sherbet, weighing the same amount and said by the manufacturer to be all from the same freezer batch were examined by these two analysts.

	J.F.A.	*F.Y.M.*	*F.Y.M.*
lbs./gal.	5.15	5.09	5.10

5. J. F. Armstrong and F. Leslie Hart, Food and Drug Administration, Los Angeles, Calif.

2 bricks of ice cream, weighing the same amount, and said by the manufacturer to be all from the same freezer batch, were examined by these analysts.

	J.F.A.	*F.L.H.*
lbs./gal.	3.93	4.04

CALCULATION OF "OVER-RUN"

Sommer defines "over-run" as the "increase in volume of the ice cream over the volume of the mix, expressed as percent of the volume of the mix." This definition, mathematically expressed, is—

$$\% \text{ over-run} = \left(\frac{\text{Wt. of mix—Wt. of same vol. of ice cream}}{\text{Wt. of same volume of ice cream}} \right) 100.$$

The usual commercial ice cream mix of 10–12½ percent fat content weighs about 9.2 lbs. per gallon. The richer ice cream mixes, containing 15–18 percent fat, weighs from 9.0 to 9.2 lbs. per gallon, the average being rather close to 9.1 lbs. per gallon. These figures are at cold-room temperatures, 40° to 45°F. A close approximation may therefore be calculated, using the above formula, and assuming that the mix weighs 9.1 or 9.2 lbs. per gallon.

CONCLUSIONS FROM COLLABORATIVE RESULTS

Collaborator's reports, and your Referee's experimental results on frozen desserts sampled in replicate from the same freezer batch, show that weight per gallon may be determined to a reproducibility of 2 or 3 percent. Referee's figures on frozen desserts sampled at random, given in Table 1, show a wider variation. This is to be expected. Collaborator's reports on packaged, bulk, and dipped ice cream from the same freezer show marked variations according to the method of packaging. Ice cream manufacturers endeavor to standardize the processing to give a fixed overrun, but even then, factors such as rate of flow from the freezer, rough

handling, r.p.m. variation of the dashers and scraper blades, etc., will affect the over-run.

DETECTION OF GELATIN IN ICE CREAM

This Association, as published in *Methods of Analysis*, Ch. **XXII**, has adopted official methods for detection of gelatin in milk (**29**), cream (**63**), evaporated milk (**74**) and cottage cheese (**131**). These methods all refer back to the procedure in **29**, and are based on the precipitate formed by picric acid and gelatin, after removal of proteins with mercuric nitrate

TABLE 2.—*Qualitative test for gelatin in ice cream*

Collaborator	M 1	M 1a	M 1b	M 1c	M 2	M 2a	M 2b	M 2c	AM 2a	AM 2b	AM 2c	PM 2
1	−[2]	−		−[1]	+		+		+			+
2	−	−		−	+			+		+		
3	−[1]	−			+	+			+			+
4	−[1]		−		+	+					+	
5	−[1]		−	−	+			+			+	
6	−[1]	−	−	−[2]	+	+	+	+	+	+	+	+

[1] Slight, or very slight haze or opalescence.
[2] Mix allowed to sour. Slight flocculent pres. corresponding to description in Note, XXII: 29, *Methods of Analysis*, 1940.

solution. This method has been applied to ice cream, in the presence, or absence of other stabilizers—gums or pectin—and found to give satisfactory results.

Two ice cream mixes were prepared containing 11 and 12 percent fat, and 30 and 38 percent total solids, respectively. Mixtures with and without gelatin, and with and without other stabilizers were prepared from these stock mixes. Tests by the writer on these two mixes demonstrated that the proposed method applied equally well to mixes with high or low milk solids. Therefore, varying amounts of gums and gelatin were added to portions of mix and submitted to collaborators. The collaborators were requested to apply the method in **XXII, 29**, using a 10g sample of mix. Their results are given in Table 2.

COLLABORATOR'S REPORTS

The following collaborator's participated in this work:

1. M. J. Gnagy, U. S. Food and Drug Administration, Los Angeles, Calif.
2. J. F. Armstrong, U. S. Food and Drug Administration, Los Angeles, Calif.
3. G. L. Cheney, Smith-Emery Laboratory, Los Angeles, Calif.
4. E. H. Miller, Griffin-Hasson Laboratory, Los Angeles, Calif.
5. M. Y. Longacre, Los Angeles Health Department, Los Angeles, Calif.
6. F. Leslie Hart, Food and Drug Administration, Los Angeles, Calif.

The various mixtures submitted were designated as follows:

M 1—11% fat and 30% total solids mix, no stabilizers.
M 1a, b, or c—M 1 plus 0.2% algin, agar, or locust bean gum, respectively.
M 2—M 1 plus 0.3% gelatin.
M 2a, b, or c—M 2 plus 0.2% algin, agar, or Locust bean gum, respectively.

AM 2a, b, or c—M 1 plus 0.1% gelatin and 0.2% algin, agar, or Locust bean gum, respectively.

PM 2—M 2 plus 0.2% pectin (200 grade).

SENSITIVITY OF METHOD

Sample AM 2a, containing 0.1 percent of gelatin and 0.2 percent of algin, was diluted with an equal quantity of milk and the test applied to the resultant mixture. A sample of this diluted mix was submitted to Collaborators 1 and 2, with no information as to its composition. Both reported definite positive tests for gelatin. The Associate Referee also obtained a definite positive test. This amount of gelatin will have little if any stabilizing effect on ice cream.

CONCLUSION

The official A.O.A.C. method for detection of gelatin in milk and cream will detect gelatin in ice cream in amounts as low as 0.05 percent. This is well below the amounts encountered in commercial ice cream. Other stabilizers, such as algin, Locust bean gum, agar, or pectin do not interfere. Since the dependability of this method has already been recognized on milk, cream, evaporated milk, and ice cream, through its adoption as an official method, it is believed this work justifies extension of the method to the detection of gelatin in ice cream.

RECOMMENDATIONS*

It is recommended—

(1) That *Methods of Analysis*, **XXII, 135,** 3rd sentence, be editorially, changed to read "Continue as directed under **31** and **105**; and under **XXI,** particularly **3** and **16b,** for detection of oil-soluble coal tar dyes and annatto," and the method be adopted as official.

(2) That the method for determination of weight per unit volume of frozen desserts described in this report be adopted as tentative.

(3) That the method now accepted as official for the detection of gelatin in various dairy products be extended to ice cream by the statement under Ice Cream—"Gelatin—Official—See **29,** using 10 gram sample."

(4) That the method for determination of solids in ice cream, *This Journal*, **25,** 616, be made official.

(5) That the method for preparation of sample of frozen desserts containing insoluble ingredients, *This Journal*, **25,** 614, be adopted as official, first action.

(6) That studies be undertaken on the quantitative determination of gelatin and other stabilizers in frozen desserts.

APPOINTMENTS

W. T. Mathis, Connecticut Agricultural Experiment Station, New Haven, Conn., has been appointed as General Referee on Spectrographic Methods.

* For report of Subcommittee C and action by the Association, see *This Journal*, **28,** 57 (1945).

CONTRIBUTED PAPERS

METHOD FOR DIFFERENTIATING BETWEEN EGG LECITHIN AND SOYBEAN LECITHIN IN MACARONI AND NOODLE PRODUCTS*

By JAMES J. WINSTON and BENJAMIN R. JACOBS (National Macaroni Manufacturers Association, New York, N. Y.)

Lecithin, as found in eggs and in soybeans, is very similar both in composition and properties. Investigators in the past have had great difficulty in differentiating between these two forms and in detecting one in the presence of the other (1). Attempts to detect soybean lecithin have been based largely on chemical constants such as the phosphorus and nitrogen percentages and the ratio between the two. (2)

Lecithin, a phospholipid, is a compound of phosphoric acid, glycerol, higher fatty acids, and choline. Winterstein and Hiestind (1) in 1906 found that plant phosphatides on hydrolysis yield not only the usual decomposition products of lecithin but sugar as well. Consequently, it was found that the phosphorus content of lecithin derived from eggs was higher than the phosphorus content of lecithin derived from plant life. The same was true of the other constituents, such as nitrogen and choline. Some analysts have made these facts the basis for distinguishing between the two types. However, these differences are too small to enable the analyst to definitely detect the presence of one type when mixed with the other.

Since soybean lecithin is finding its way into use in many food products, *e.g.*, candy, oleomargarine, shortening agents, and (experimentally) noodle products, it was deemed sufficiently important to investigate methods for its detection in order to prevent the simulation of eggs in certain products, principally noodles.

Prior to 1941, the method for the determination of egg solids in noodle products was based on the lipoid phosphoric acid content (3). The substitution of soybean lecithin, because of its high lipoid phosphoric acid, would therefore be confusing in this determination and would vitiate the analysis. In fact, the presence of soybean lecithin in an egg noodle makes it very difficult to arrive at the proper result for egg solids by any method now in use. The method of E. O. Haenni (4), which depended on the cholesterol content is also vitiated in the presence of soybean lecithin (5).

Soybean lecithin has the property of fluorescing blue and exhibits this very strongly when subjected to ultra-violet light (6), whereas lecithin derived from flour and egg products shows this property very feebly. The following method was built on this principal.

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* Presented at the 59th Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 25 and 26, 1944.

METHOD OF ANALYSIS

APPARATUS AND REAGENTS

Pfaltz and Bauer Fluorophotometer, Model B, with accessories. Filter set #630 consisting of one ultra-violet filter with a peak of 3700 Angstrom units. A double filter placed between cuvette and photo-cell with a peak of 4600 Angstrom units. These filters are identical to those used in the Thiochrome determination (7). The fluorophotometer was so standardized with quinine sulfate, one microgram per ml. in 0.1 *N* sulfuric acid, as to give a fluorescence of 50 divisions on the mirror galvanometer. This constituted the setting point in all the following experiments.

Wide stoppered glass bottles.* 95 percent ethyl alcohol, acetone C.P., naphtha, quinine sulfate U.S.P., anhydrous sodium sulfate C.P.

PROCEDURE FOR FLOURS, SEMOLINAS, MACARONI, AND NOODLE PRODUCTS

To a 5-gram sample, in a wide stoppered bottle, add 7 ml of 95 percent alcohol followed by addition of ½ gram of anhydrous sodium sulfate. After soaking ½ hour, add 93 ml of naphtha, shake the bottle well, and permit to stand in the dark overnight. Pipet the clear solution into a cuvette and examine for fluorescence; make a blank correction on all the readings.

PROCEDURE FOR YOLKS AND WHOLE EGGS

Place in wide-stoppered bottle suitable amount of egg product (previously weighed out on a strip of aluminum) and add 7 ml of acetone. Break up and disintegrate the egg by means of flattened end of stirring rod. Add ½ gram of anhydrous sodium sulfate and soak for ½ hour. Add 93 ml of naphtha, shake bottle well, and allow to stand in the dark overnight. Pipet the clear solution in a cuvette and determine its fluorescence, as above.

EXPERIMENTAL RESULTS

The soybean lecithin** used in this work contained about 60.0 percent phospholipids and 40.0 percent vegetable oil, which is typical of the average composition of commercial soybean lecithin. Different amounts of this product were dissolved in alcohol-naphtha solution, 7 ml of 95 percent ethyl alcohol and 93 ml of naphtha. The fluorescence of the different quantities of lecithin was obtained on the instrument and recorded in terms of scale divisions in order to determine whether there existed any relationship between the concentration and the fluorescence. Examination of a number of different commercial samples of soybean lecithin for fluorescence indicates that the variation that can be expected from these figures is about 8–10 percent, depending upon the amount of phospholipids present. (Commercial lecithin has a phospholipid content ranging from 55.0 to 65.0 percent which is mixed with a vegetable oil carrier to the extent of 35.0 to 45.0 percent.)

These results, plotted in Fig. 1, show that there exists a relationship between concentration and fluorescence, and that up to a concentration of 212 milligrams, the Lambert-Beer Law holds (8). Inasmuch as egg noodles

* Caution: Do not use rubber stoppers as this will impart fluorescence to the solution.
** Obtained from American Lecithin Company, Elmhurst, L. I., N. Y.

cannot be expected to contain more than 3 percent lecithin, owing to its shortening effect, this fluorescence-concentration relationship can be considered satisfactory for the determination of added soybean lecithin in noodle and macaroni products.

Samples of the usual farinaceous ingredients, flour, farina, and semolina, were examined for fluorescence by using the extraction method for carotenoids as advocated by Ferrari and Bailey (9), and Munsey (10).

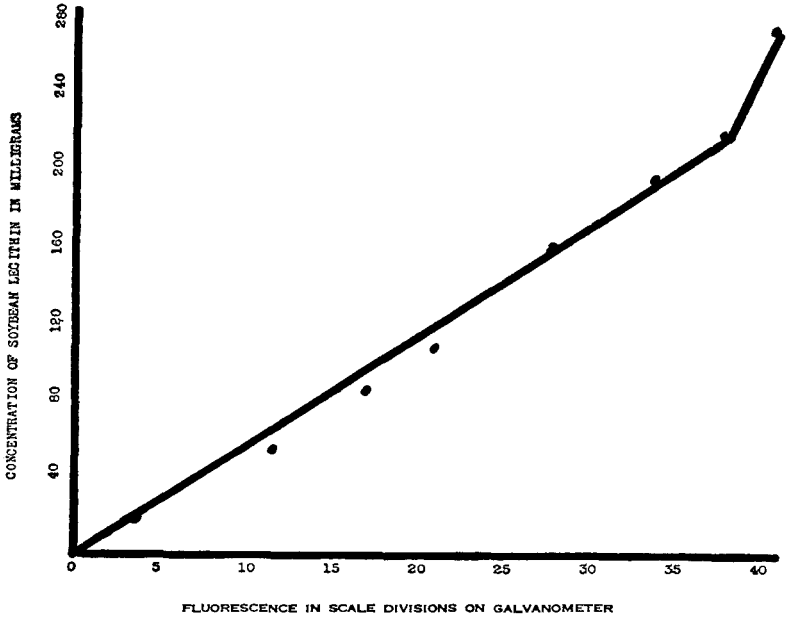

FIG. 1.—Concentration of soybean lecithin in milligrams.

A 5-gram sample was extracted with the alcohol naphtha reagent (7:93) for 24-hours in a wide stoppered bottle and the clear solution was pipetted into a cuvette and examined for fluorescence. The reagents used were examined for fluorescence and a blank correction was made on all readings. The results as indicated in Table 2 show that these ingredients exhibit a very small degree of fluorescence. This table shows typical examples taken from a much larger number. More than one hundred individual readings for fluorescence were made.

Egg products, consisting of frozen egg yolk and whole eggs, were likewise examined for fluorescence. Representative samples from a large number tested have been tabulated in Table 3. It will be noted that in this range the fluorescence of the yolks varied from a minimum of 1.0 to

TABLE 1.—*Concentrations of soybean lecithin*

CONCENTRATION IN MILLIGRAMS	FLUORESCENCE IN SCALE DIVISIONS
1.0	0.2
9.0	2.0
18.8	4.0
51.0	11.5
82.4	17.0
104.1	21.0
153.0	28.0
190.0	34.0
212.0	38.0
268.0	41.0

a maximum of 3.0 but it is interesting to see that the concentration of the yolk did not have any significant effect on the fluorescence. Whole eggs in general showed less fluorescence, with a maximum of 1.5 scale divisions, whereas an amount of soybean lecithin as small as 18.8 milligrams gave a scale deflection of 4.0 divisions.

The following procedure was used to determine which one of the components of soybean lecithin was the cause of the high fluorescence.

A sample of soybean lecithin was treated with acetone (11) and the filtrate, consisting of the vegetable oils, was filtered through a pledget of

TABLE 2.—*Typical examples of fluorescence in flour, etc.*

PRODUCT	WT. OF SAMPLE EXTRACTED IN GMS.	FLUORESCENCE IN SCALE DIVISIONS
Semolina	5.0	1.0
Semolina	5.0	0.5
Semolina	5.0	0.5
Semolina	5.0	1.0
Semolina	5.0	0.5
Farinas	5.0	0.5
Farinas	5.0	0.0
Farinas	5.0	0.0
Durum Flours	5.0	1.0
Durum Flours	5.0	1.0
Durum Flours	5.0	0.5
Durum Flours	5.0	0.5
Durum Flours	5.0	0.5
Durum Flours	5.0	0.5
Durum Flours	5.0	0.5
Durum Flours	5.0	1.0
Durum Flours	5.0	1.0
Durum Flours	5.0	1.0

TABLE 3.—*Samples of fluorescence in eggs*

PRODUCT	WT. OF SAMPLE EXTRACTED IN MGMS.	FLUORESCENCE IN SCALE DIVISIONS
Egg Yolks	853.6	1.5
Egg Yolks	807.2	2.5
Egg Yolks	729.6	3.0
Egg Yolks	534.0	3.0
Egg Yolks	558.8	2.5
Egg Yolks	637.8	3.0
Egg Yolks	800.0	1.0
Egg Yolks	654.4	1.5
Egg Yolks	601.8	1.5
Egg Yolks	843.0	1.5
Whole Eggs	915.8	0.5
Whole Eggs	883.0	1.0
Whole Eggs	843.0	1.5
Whole Eggs	599.0	1.0
Whole Eggs	980.0	1.5
Whole Eggs	670.0	1.0
Whole Eggs	779.4	1.5
Whole Eggs	436.5	0.5
Whole Eggs	500.0	1.0
Whole Eggs	779.5	1.0

cotton into a weighed aluminum dish. The residue on the filter was then treated with ethyl ether which dissolves the lecithin, and this extract was collected into another weighed aluminum dish. Both dishes were placed on the water bath and evaporated to dryness, dried in a water oven at 100°C. for one hour, and finally weighed. The two constituents of the above soybean lecithin were then examined for fluorescence in the usual manner. It will be noted from Table 4 that the phospholipid fraction is the cause of the fluorescence.

TABLE 4.—*Fluorescence of vegetable oil compared with lecithin*

WEIGHT OF SAMPLE	WEIGHT OF VEGETABLE OIL	PERCENT	WEIGHT OF LECITHIN	PERCENT	FLUORESCENCE, SCALE DIVISIONS	
					VEGETABLE OIL	LECITHIN
grams 0.7430	*grams* 0.2006	27.0	*grams* 0.5356	72.1	5.0	55.0

Several different samples of macaroni products were manufactured under strict supervision, and different quantities of soybean lecithin were incorporated. The same mixer, kneader, and press were used for each product, and they were all subjected to the same drying conditions.

These finished products were examined for fluorescence by extracting

a 5 gram, finely ground sample, with the usual alcohol naphtha reagent to make a total volume of 100 ml. It will be noted from Table 5 that the presence of a small amount of soybean lecithin was sufficient to give an abnormal degree of fluorescence. Plotting these values on Fig. 2 shows that the intensity of the fluorescence is a function of the concentration of the

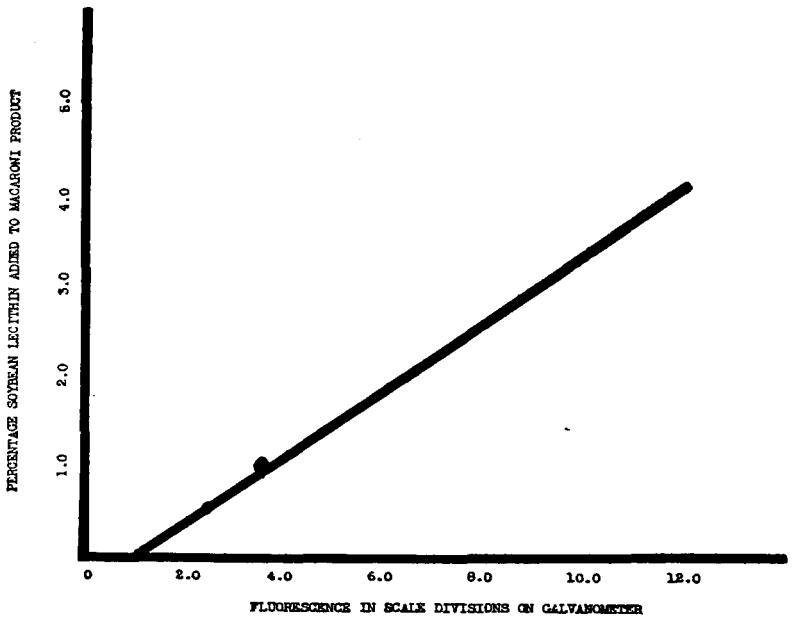

FIG. 2.—Percentage soybean lecithin added to macaroni product.

soybean lecithin and that the galvanometer deflection is generally in linear relationship to the concentration of the soybean lecithin.

TABLE 5.—*Percentage soybean lecithin added to macaroni products*

ADDED SOYBEAN LECITHIN	FLUORESCENCE IN SCALE DIVISIONS
percent	
0.0	1.0
0.5	2.5
1.0	3.5
2.0	6.5
3.5	10.5

A large number of macaroni products including spaghetti, macaroni, and elbows, manufactured in different macaroni factories, were subjected

TABLE 6.—*Fluorescence in macaroni products*

PRODUCT	FLUORESCENCE SCALE DIVISIONS
Macaroni	1.0
Macaroni	0.5
Macaroni	1.0
Macaroni	1.0
Macaroni	0.5
Spaghetti	0.5
Spaghetti	0.5
Spaghetti	1.0
Spaghetti	1.0
Spaghetti	0.5
Elbows	1.0
Elbows	1.0
Elbows	1.0
Elbows	0.5
Elbows	0.5

to the fluorescence test. As will be noted from Table 6, the fluorescence obtained in all cases was not over 1.0 scale division, whereas the presence of 0.5 percent soybean lecithin was sufficient to cause an abnormal fluorescence of 2.5 divisions.

Egg noodles containing 5.5 percent egg solids, as yolk, were likewise manufactured under supervision using the same mixer, kneader, and cutter, and the same drying process. Different quantities of soybean lecithin ranging from 0.5 to 2.0 percent, were added. The dried products were examined for fluorescence using a 5-gram, finely ground sample in each instance. The results, as indicated in Table 7 and Fig. 3, show that the fluorescence is practically proportional to the amount of lecithin present in the sample.

The addition of 0.5 percent soybean lecithin to an egg noodle can therefore be detected, owing to its significant fluorescence as compared to the feeble fluorescence exhibited by a normal egg noodle made solely from eggs.

TABLE 7.—*Percentage soybean lecithin added to egg noodles*

ADDED SOYBEAN LECITHIN	FLUORESCENCE IN SCALE DIVISIONS
percent	
0.0	1.0
0.5	4.5
1.2	11.5
2.0	19.5

Two hundred samples of egg noodles containing 5.5 percent egg solids have been examined in the past year. A statistical analysis indicates that the maximum fluorescence was 2.5 scale divisions, the minimum 1.0 scale divisions, the average 1.6 scale divisions, and the standard deviation 0.45 scale division. In Table 8 are the results of typical egg noodles, some made with yolk, some with whole egg, and some with dried eggs. The maximum

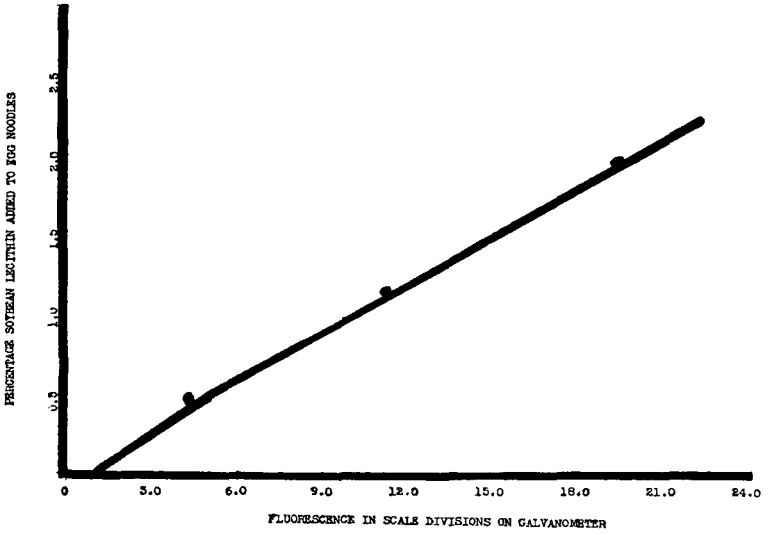

FIG. 3.—Percentage soybean lecithin added to egg noodles.

fluorescence in no case exceeded 2.5 scale divisions, whereas the addition of 0.5 percent soybean lecithin was sufficient to increase the fluorescence by 80–100 percent. The addition of 0.5 percent commercial soybean lecithin to a noodle product would correspond to 1.4 percent added egg solids as yolk, since this product has on the average 60 percent phospholipids.

TABLE 8.—*Maximum fluorescence in egg noodles*

NUMBER	FLUORESCENCE	NUMBER	FLUORESCENCE
1	1.0	11	2.5
2	1.0	12	2.0
3	1.5	13	1.0
4	1.0	14	1.5
5	1.2	15	1.0
6	1.4	16	1.5
7	1.0	17	1.0
8	1.2	18	1.0
9	1.6	19	1.6
10	1.0	20	1.0

The present Standards of Identity by the Food and Drug Administration include a macaroni product containing 12.5 percent soy flour. Experiments were therefore conducted to ascertain if the presence of this amount of soy flour, with its high lecithin content, would have any significant effect on the fluorescence of the finished product. Four different macaroni products were manufactured, each containing 85 percent durum flour and 15 percent soy flour. The finished products were examined in the usual manner using a 5-gram, finely ground sample.

TABLE 9.—*Maximum fluorescence in soy macaroni products*

TYPE SOY FLOUR USED	FLUORESCENCE IN SCALE DIVISIONS
1—Extracted Soy Flour, Fat content—1.0%	2.0
2—Extracted Soy Flour, Fat content—1.0%	2.0
3—Medium Fat—7.0%	2.0
4—High Fat—22.0%	2.5

It will therefore be noted, from Table 9, that the presence of 15 percent soy flour in a macaroni product will increase the fluorescence somewhat. However, a correction factor can be applied to a macaroni product, to yield the fluorescence due solely to the product exclusive of the soy flour used.

SUMMARY AND CONCLUSIONS

A need for a method of differentiating between soybean lecithin and egg lecithin has been demonstrated.

The strong fluorescence of soybean lecithin, when subjected to the ultra-violet light, has been proved to be an adequate basis for detecting the addition of soybean lecithin to macaroni and noodle products.

It has been demonstrated that the farinaceous ingredients and egg products show the property of fluorescence to only a very small extent, and that the addition of 15 percent soy flour will not tend to interfere with the detection of soybean lecithin.

The fluorescence obtained by the use of added soybean lecithin is practically proportional to the concentration used, and hence may serve as a means of estimating the amount added to a macaroni or noodle product.

A definite method for determining the addition to macaroni and noodle products of quantities of soybean lecithin as small as 0.5 percent (in most cases) has been presented and proposed.

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ERRORS IN THE SAMPLING AND ANALYSIS OF CANE MOLASSES*

By F. W. ZERBAN (New York Sugar Trade Laboratory, New York, N. Y.)

In the molasses trade it is customary to take samples by the continuous drip method, a small fraction of the flowing molasses being diverted into a bucket or other collecting vessel while the material is being pumped from or into a tank, tanker, or tank car. The sample is then thoroughly mixed by stirring, and filled into several sample containers, one or more each for the buyer and seller, and others kept in reserve for referee or other additional analyses.

During the past two years this laboratory has in many cases received samples, representing the same mix and bearing the same inscription on the label, from more than one of the parties interested in the transaction, or even from the same party for the purpose of verifying the analysis of the prior sample. A few samples were sent in as many as three times. Some of the duplicate samples arrived on the same day, others at varying intervals up to 72 days after the first sample. A comparison of the analytical results obtained gives interesting information on the reproducibility of the analyses and on the care with which the original bulk sample was mixed before being filled into the separate sample containers.

The first 100 of these duplicate samples received so far comprised 87 blackstraps, 11 high test molasses, and 2 mixed molasses consisting of part blackstrap and part high test molasses. In the case of triplicates the first and second sample are considered as one pair, the second and third sample as a separate pair. The detailed results have been tabulated, and a copy of this table will be gladly sent to anyone desiring it.

In this laboratory the total sugars are determined by the methods speci-

* Presented at the 59th Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 25 and 26, 1944.

fied in the U. S. Customs Regulations of 1931,[1] the sucrose content being ascertained by Jackson and Gillis method No. IV,[2] and the invert sugar by the gravimetric Munson and Walker method.[3] The sum of the sucrose and invert sugar gives the total sugars. The Brix determination is made by the double dilution method,[4] which is the usual commercial procedure. The Brix reading of the diluted solution is taken after all the air bubbles

TABLE 1.—*Summary of deviations of analysis of second sample from that of first sample*

CHECKING WITHIN	NUMBER OF CASES			
	SUCROSE	INVERT SUGAR	TOTAL SUGARS	BRIX
0.00	11	7	1	8
0.05	11	27	13	45
0.10	31	42	25	76
0.15	45	60	37	85
0.20	50	74	45	90
0.25	65	81	55	93
0.30	76	84	65	99
0.35	78	87	71	100
0.40	83	90	79	
0.45	85	92	86	
0.50	86	93	89	
0.55	95	94	91	
0.60	96	96	92	
0.65	98	98	94	
0.70	99	98	98	
0.75	99	99	98	
0.80	100	99	99	
0.85		99	99	
0.90		100	99	
1.42			100	
Av. deviation	±0.23	±0.17	±0.26	±0.08

have risen to the surface and the suspended matter has settled out completely. Each determination of sugars and the Brix determination are carried out in duplicate, by two different chemists where practicable, and the following maximum deviations between the two individual results have been adopted: Sucrose 0.3 per cent; reduced copper 2 mg., corresponding to about 0.2 per cent invert sugar on the average; total sugars 0.5 per cent; Brix 0.2 per cent. It has been found in practice that in most analyses the two results check within these limits; in this case they are

[1] *Natl. Bur. Standards*, Circ. C 440 (1942), pp. 792–797.
[2] *Bur. Standards, Sci. Paper* 375 (1920), pp. 187–189.
[3] *J. Amer. Chem. Soc.*, 28, 663 (1906); 29, 541 (1907); 34, 202 (1912).
[4] *Methods of Analysis, A.O.A.C.*, 5th edition (1940), p. 486.

averaged and the average is reported. Sometimes a third determination is necessary.

If the original bulk lot of a given molasses was thoroughly mixed and no subsequent change in the composition of the molasses samples has taken place, the analyses of the molasses filled into each of the different containers should also check within reasonable limits. The results actually obtained upon the 100 pairs of samples are summarized in Table 1.

The deviation in the result for sucrose was within 0.3 per cent in 76 cases, for invert sugar within 0.2 per cent in 74, for total sugars within 0.5 per cent in 89, and for Brix within 0.2 per cent in 90 out of the 100 cases.

Although the discrepancies exceeded 0.3 per cent sucrose in 24 cases, and 0.2 per cent invert sugar in 26, there were only 11 in which the difference in the total sugars was over 0.5 per cent. In the others the excess in either sucrose or invert sugar was compensated for by a deficiency in the other sugar. Since molasses is evaluated by the trade according to total sugar content the samples in which the total sugars checked within 0.5 per cent may be considered to be equally representative and it may be concluded that the original bulk sample was mixed in a satisfactory manner.

In 3 of the remaining 11 pairs of samples the discrepancies were 0.51, 0.53 and 0.56, only a few hundredths above the limit of 0.5 per cent total sugars. But in 6 others they varied from 0.64 to 0.77 per cent and may have been caused at least in part, by insufficient mixing of the original bulk sample.

The last two pairs of samples represented mixtures of blackstrap and high test molasses, and contained around 65 and 68 per cent total sugars, respectively. In one of them the second sample, analyzed only 7 days later than the first, gave 0.53 per cent less sucrose, 0.89 per cent less invert sugar, and 1.42 per cent less total sugars, although the Brix checked within 0.02 per cent, indicating that the Brix of the two types of molasses in the mixture was about the same, but that the original bulk sample was not stirred sufficiently to give a homogeneous mass. In the other pair of samples consisting of mixed blackstrap and high test molasses, analyzed on the same day, the deviations were 0.08 per cent sucrose, 0.60 per cent invert sugar, 0.68 per cent total sugars, and 0.35 Brix. Here the large difference in the Brix, in conjunction with that in the total sugars, again indicated insufficient mixing of the original bulk sample.

Another important factor affecting the composition of molasses is the time of storage. This subject has been studied exhaustively by Browne,[5] and also investigated by Kopfler.[6] They have found that the sucrose

[5] Proc. 5th Congr. Intern. Soc. Sugar Cane Tech., Brisbane, 1925, p. 2117.
[6] Proc. 10th Conf., Assn. of Sugar Tech. of Cuba, 1936, p. 69.

gradually diminishes through inversion, but that at the same time invert sugar is also destroyed. The invert sugar may decrease or increase, depending on whether the inversion or the destruction of invert sugar preponderates. The total sugars generally decrease during storage; but increases at certain periods have also been observed, when inversion is more rapid than destruction of invert sugar. The age at the time of analysis of the molasses samples considered here is unknown, but the time elapsing between the first and second analysis may conceivably have affected their composition. The analyses of the individual pairs of samples do not indicate any definite trend in this respect. But if the results are averaged it is found that in the course of an average period of 12.5 days between the analyses the sucrose decreased 0.056 per cent and that the invert sugar increased 0.021 per cent, showing that some inversion took place but that more invert sugar was destroyed than was formed by inversion. The Brix decreased 0.013 per cent. These changes are about what would be expected but they are small compared with the average deviations between the results of the first and second analyses and could not have any appreciable effect on the discrepancies found for any individual pair of samples. This is well illustrated by a molasses the second sample of which, analyzed 72 days after the first, showed 0.06 per cent more total sugars.

SUMMARY AND CONCLUSIONS

Different portions of the same original bulk sample of molasses are frequently received by this laboratory at varying intervals, sometimes on the same day, but occasionally more than two months apart. It was found that in 89 out of a total of 100 such cases the total sugars in the second sample checked within 0.5 per cent with those in the first sample, and that in 3 more cases the discrepancy exceeded this figure by a few hundredths only. On the basis of the deviations found in duplicate analyses of individual samples it is concluded that in these 92 cases the mixing of the original bulk sample was carried out in a satisfactory manner. In 6 other cases the discrepancies, amounting to as much as 0.77 per cent total sugars, may be due at least in part to insufficient mixing of the original bulk sample, and in the remaining 2 the large deviations found definitely indicate lack of care in the preparation of the original mixed sample. Deterioration of the molasses between analyses had only an insignificant effect on the results; in an average of 12.5 days the sucrose decreased only 0.056 per cent, the invert sugar increased 0.021 per cent, and the Brix was reduced 0.013 per cent.

DETERMINATION AND DETECTION OF DICHLORACETIC ACID IN FOOD PRODUCTS*

By CHARLES F. BRUENING (Food and Drug Administration, Federal Security Agency, Baltimore, Md.)

Dichloracetic acid has been suggested as a preservative for sugar syrups, beverages, and other food products. It has also been referred to as a stabilizer and inhibitor. This acid was first encountered in a commercial table sugar syrup which had an index of refraction equivalent to 60 percent of sucrose, and was apparently added for the purpose of preventing or inhibiting spoilage through fermentation or mold formation. An attempt was made to determine and identify dichloracetic acid in this preparation and the methods proposed in this report which were used successfully on this sample were developed. Briefly, the quantitative method involves the isolation of dichloracetic acid by an ether extraction using an efficient continuous extractor,[1] the subsequent conversion of the organic chloride in the extract to the ionizable form by igniting with sodium carbonate or by hydrolyzing with alcoholic potassium hydroxide solution, and finally, the determination of the chlorine by the Volhard Method. Identification methods are available in the literature for dichloracetic acid; and after some experimentation, one of these which involves the formation of the toluide derivative[2] was selected and, by modification, successfully adapted to the detection of this acid. No attempt will be made here to evaluate the efficacy or toxicity of dichloracetic acid when used as a preservative, but this report will be limited to the quantitative and qualitative determination of this compound in aqueous solutions.

When isolation methods were being studied, it appeared feasible to extract dichloracetic acid from dilute aqueous solutions by means of an immiscible solvent such as ether. Wilson,[3] in the determination of monochloracetic acid, a compound similar in physical properties and chemically related to dichloracetic acid, was successful in extracting this acid quantitatively from aqueous solutions with ether using a continuous extractor. With the type of extractors used, complete extraction was obtained in 2–3 hours. Because of the general similarities of the two acids, attention was directed to solubility relationships in the hope that this extraction method might also be used successfully for dichloracetic acid. Seidell[4] gives solubility data on the distribution of monochloracetic and dichloracetic acids between water and ether at various concentrations and the resulting dis-

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[1] *Methods of Analysis*, A.O.A.C., 1940, Fig. 57B, p. 594.

[2] Shriner and Fuson, "The Systematic Identification of Organic Compounds," Ed. 2, John Wiley and Sons, New York, 1940, pp. 130–133, 181.

[3] Wilson, J. B., *This Journal*, 25, 145 (1942).

[4] Seidell, "Solubilities of Organic Compounds," Ed. 3, D. Van Nostrand Co., Inc., New York, 1941, Vol. 2, pp. 78, 89.

tribution coefficients. Although there are variations in coefficients between the two acids in the concentrations covered, all coefficients are of approximately the same magnitude, and it was anticipated that dichloracetic acid could be extracted successfully by continuous extraction and with about the same ease as monochloracetic acid.

In order to determine the efficiency of extraction with ether using the Wilson procedure with continuous extractors,[1] experiments were conducted in which known amounts of pure dichloracetic acid contained in 100 ml. of aqueous solution were extracted for various periods of time. The results are shown in Table 1.

As chemically pure samples of dichloracetic acid were not commercially available, it was necessary to refine the purchased commercial acids by suitable methods which are described in this report under the heading, Purification of Dichloracetic Acid. The results shown in Table 1 were obtained using the preparation designated, Sample C.

The amount of dichloracetic acid extracted by the ether in each case was measured by determining total chlorine in the extract. This was accomplished by adding a sodium carbonate solution to the ether extract, evaporating off the ether and water on a steam bath, igniting the residue, and finally, determining the resulting chloride by the Volhard Method. Subsequently, this conversion of the organic chloride of the dichloracetic acid to the ionic form by the sodium carbonate ignition proved to be quantitative and was made part of the method of determination of dichloracetic acid and is described in detail under Method A.

In all the determinations the ether in the flask was kept boiling at a moderate rate by adjusting the hot plate temperature, and it was determined, under the experimental conditions used, that 7 to 9 cc of ether per minute was passing through the aqueous solution in the extractor. With this rate the results shown in Table 1 indicate that complete extraction was obtained in 3 hours.

TABLE 1.—*Efficiency of ether extraction of dichloracetic acid*

TIME	AMOUNT TAKEN	AMOUNT FOUND	RECOVERY
hrs.	*mg.*	*mg.*	*percent*
½	92.8	51.4	55.4
1	92.8	75.6	81.5
2	92.8	89.8	96.8
2	185.6	180.8	97.4
3	92.8	92.3	99.5
3	185.6	184.6	99.5

After it was determined that the above ether extraction was satisfactory several experiments were made in order to determine the amount of dichloracetic acid present in the ether extract by converting the organic

chlorine of the acid to the ionic form, so that the Volhard Method for chlorides could be used. Wilson[3] treated various amounts of monochloracetic acid up to 300 mg. with 40 ml. of 0.5 *N* sodium hydroxide, and after heating for 2 hours on the steam bath or boiling for one-half hour, was able to obtain quantitative conversion of the chlorine when using a modified Volhard Method for determining the amount of chlorine involved. This procedure was tried on 48.6 mg. of dichloracetic acid, boiling ½ hour with 50 cc. 1 *N* sodium hydroxide but only 15.5 mg., or 32 percent, of the acid was recovered. Although this procedure is satisfactory for monochloracetic acid, poor recovery for dichloracetic acid was not entirely unexpected because it is generally known that the accumulation of halogen

TABLE 2.—*Hydrolysis of dichloracetic acid by alcoholic potassium hydroxide solution*

CONCENTRATION OF KOH SOLUTION	TIME	ACID TAKEN	ACID FOUND	RECOVERY
	hrs.	*mg.*	*mg.*	*per cent*
1 Normal	½	191.5	47.5	24.8
1 Normal	1	191.5	78.9	41.2
2 Normal	½	191.5	150.4	78.5
2 Normal	1	191.5	182.0	95.0
3 Normal	½	191.5	189.8	99.1
3 Normal	1	191.5	190.9	99.7

atoms on the same carbon atom in simple aliphatic compounds generally increases the resistance to hydrolysis.

In an attempt to increase the conversion recovery, known amounts of dichloracetic acid (Sample C), were boiled under a reflux for various periods of time with alcoholic potassium hydroxide solution of various concentrations. The amount of ionic chloride formed was then determined by the Volhard Method.

Table 2 shows the actual recoveries obtained. In each case 30 cc. of alcoholic potassium hydroxide solution was used and the subsequent treatment is explained in detail under the proposed method (See Method B). The marked resistance of dichloracetic acid to hydrolysis is shown by the results, which indicate that at least 3 *N* alcoholic potassium hydroxide solution and one hour of refluxing are needed for quantitative recovery. At the same time, 191.5 mg. of the same sample was treated with 2 grams of sodium carbonate dissolved in 20 cc. of water, the solution evaporated to dryness, and the residue ignited at 500–550°C., as described in detail under the procedure, "Method A." The amount of organic chloride converted to the ionic form by this general method for chlorine was then determined by the Volhard Method. A recovery of 190.7 mg., or 99.6 per-

cent, was obtained, which is practically the same as that obtained with the 3 *N* alcoholic potassium hydroxide solution refluxed for 1 hour, and thus shows that either the sodium carbonate or the alcoholic potassium hydroxide method yields quantitative conversion.

The following procedure is proposed for the determination of dichloracetic acid in aqueous solutions and is based on the ether extraction and the two conversion methods, all of which have been studied and found to be individually satisfactory.

PROCEDURE

THE DETERMINATION OF DICHLORACETIC ACID

Place a suitable quantity of the aqueous soln cont. not more than 200 mg. of dichloracetic acid in the outer part of a continuous extractor.[1] Add 10 ml. of 50% by weight sulfuric acid, insert the inner tube of the extractor and add water until the level of the soln is about 1.0 cm. below the side arm of the extractor. Place 100–150 ml. ether in a 250-ml. Erlenmeyer or other suitable flask. Attach to the side arm of extractor, and extract for 3 hours. Disconnect condenser, remove inner tube, and transfer as much ether to the flask as possible by tilting the outer tube. Remove the flask and treat the contents by Method A or B.

Method A: Add to the contents of the flask 2 gm. of C. P. sodium carbonate dissolved in 20 ml. of water and heat on the steam bath until all ether has been removed. Transfer the aqueous soln to a flat-bottom platinum dish and evaporate soln to dryness on the steam bath. Dry the residue at 100°C for ca ½ hour and finally ignite at 500°–550°C for 15 min.

Dissolve the residue in 25–50 ml. of water, add 15 ml. HNO_3 and an excess of (N/10)$AgNO_3$. Filter off the precipitate of silver chloride and carbonized material and wash with water. To the filtrate add 1–3 ml. of a saturated soln of ferric alum indicator and titrate the excess $AgNO_3$ with N/10 NH_4CNS to the end point.

$$1 \text{ ml. } \frac{N}{10} AgNO_3 = .006447 \text{ gm. } CHCl_2COOH.$$

Method B: To contents of flask add 5 ml. of 3*N* alcoholic KOH soln and heat on steam bath until all the ether has been removed, *i.e.*, until the volume is ca 5 ml. Add 25 ml. more of the KOH soln to the flask and boil gently under a reflux for 1 hour. Add 75–100 ml. water, 15 ml. HNO_3, an excess of N/10 $AgNO_3$, and complete the determination as directed under Method A, beginning with "Filter off the precipitate," etc.

Method B is preferable when ether soluble substances other than dichloracetic acid are absent. In the presence of various ether soluble substances, this method yields dark colored solutions which obscure the final end point. With samples containing these interfering substances Method A is preferable.

To expedite the removal of the last portion of water during evaporation of the sodium carbonate solution in Method A, stir the sample with a pyrex glass rod when a crust forms on the surface. Allow the dish to remain on the steam bath and continue to stir until granules form which are more or less dry. The dish and contents are then heated at 100°C. as

directed. The pyrex rod need not be removed, but can be carried through the subsequent ignition. This procedure proved successful in overcoming spattering during the ignition.

PURIFICATION OF DICHLORACETIC ACID

Initial experimentation was done using a sample of Eastman Kodak Company dichloracetic acid which had been kept in the laboratory for a year or more. When assayed, using Method A directly on the sample, it was found to contain only 84 percent dichloracetic acid. A distillation was made using an efficient air jacketed reflux column, and the fraction boiling 194.4–195.0°C. at atmospheric pressure was collected and gave a product assaying 97.1 percent. This sample was used without further treatment and designated "Sample A."

Another sample of dichloracetic acid purchased from the same company assayed 95.4 percent. This sample was distilled as described above and, taking the fraction 194.8°–195.0°C. at 762 mm. pressure, gave a product assaying 98.0 percent. Subjecting this fraction to careful vacuum fractionation (23 mm.) failed to increase the purity. (Sample B.)

A third sample was purchased from the Dow Chemical Company and assayed 96.8 percent. Fractionation was performed and the fraction 194.3°–194.5°C. at 754 mm. pressure assayed 97.7 percent dichloracetic acid. Fractionation at 23 mm. also failed to yield any fraction that assayed higher than 97.7 percent. (Sample C.)

The identity of all three samples of the acid was established by preparing derivatives, all of which had satisfactory melting points. The p-toluide derivative was prepared as directed in the latter part of this report, and the following melting points obtained: Sample A, 152.5–153.0; Sample B, 152.8°–153.3°C.; Sample C, 153.0–153.5°C. Mixed melting points caused no appreciable depressions with any of the samples, showing the absence of other interfering acids.

Stock solutions were made using these three samples and the amount of pure dichloracetic acid in each was calculated. In all determinations 10 or 20 ml. aliquots were used. Table 3 shows the results obtained on all three samples when using the proposed procedure.

After it was established that the proposed procedure gave satisfactory recoveries for dichloracetic acid when occurring alone in aqueous solutions with no interfering substances present, the method was tested for general applicability for the determination of this acid in various food products. Known amounts of dichloracetic acid were added to various food products and actual recoveries ascertained by using the proposed procedure.

Table 4 shows the recoveries obtained on the various food products tested.

Satisfactory recoveries were obtained in all cases, indicating general

TABLE 3.—*Recovery of dichloracetic acid by proposed procedure*

SAMPLE	PURE ACID TAKEN	FOUND	RECOVERY
	mg.	*mg.*	*percent*
A	60.7	60.2	99 2
A	60.7	60.2	99 2
A	60.7	60.3	99 3
A	60.7	60.5	99 7
A	60.7	60.9	100 3*
A	60.7	60.2	99.2*
B	60.1	60.0	99 8
B	60.1	60.1	100 0
B	120.3	120.0	99 8
B	120.3	119.8	99.6
B	60.1	60.0	99.8*
B	120.3	120.0	99.8*
C	61.9	61.8	99.8
C	61.9	61.7	99.7
C	123.7	123.1	99.5
C	123.7	123.2	99.6
C	61.9	61.7	99.7*
C	123.7	123.5	99.8*

* Hydrolyzed by 3*N* alcoholic potassium hydroxide; all others were ignited with sodium carbonate.

suitability of the method. The results indicate also that substances that may interfere with the method, *i.e.*, ether soluble halogen compounds, are normally absent in the products tested or occur in negligible amounts. Sodium chloride was present in the tomato juice but caused no difficulty because it was not extracted by the ether. It was found, however, that the tomato and orange juice beverages gave ether extracts that were so highly colored that the alcoholic potassium hydroxide method was inapplicable because the extracted color interfered with the determination of the end point. With these two products, the sodium carbonate ignition method

TABLE 4.—*Recovery of dichloracetic acid in food products*

PRODUCT	DICHLORACETIC ACID FOUND (MG.)			
	ADDED 0.0 MG.	25 MG.	50 MG.	100 MG.
Golden Crown Table Sirup (50 gm.)	0.0	24.8	49.4	99.5
Orange Beverage (75 ml.)	0.4	25.6	50.5	100.3*
Ginger Mint Julep Beverage (75 ml.)	0.0	24.8	50.0*	99.6
Root Beer Beverage (75 ml.)	0.0	24.8*	49.4	99.3
Canned Tomato Juice (65 ml.)	0.2	25.0	50.4	99.1
Canned Orange Juice (65 ml.)	0.0	24.3	48.7	99.0

* 3*N* alcoholic potassium hydroxide used for hydrolysis of acid.

was used, although the other conversion method was found to be suitable for the remaining four food products listed in Table 4.

IDENTIFICATION OF DICHLORACETIC ACID

Dichloracetic acid can be readily identified by the melting point of the p-toluide derivative. The toluides are excellent derivatives of acids because of the ease with which they may be made and purified. The derivative can be prepared from the free acid or an alkali salt of the acid, which can be readily isolated from solutions in sizeable amounts (100 mg. or more is suitable). The isolation of the sodium salt of dichloracetic acid involving an ether extraction and a subsequent extraction of the ether solution with sodium hydroxide solution is incorporated in the proposed method which is a modification of the method given by Shriner and Fuson.[2]

The p-toluide of dichloracetic acid Samples A, B, and C melted at 152.5–153°C.; 152.8°–153.3°C.; and 153.0°–153.5°C., respectively. Shriner and Fuson give the melting point at 153°C. The p-toluide of a pure sample of monochloracetic acid (also used in food products for the same purpose as dichloracetic acid) was found to melt at 162.8°–163.2°C. These melting points are all in good agreement with those reported in the literature.

The procedure was tested experimentally on an aqueous solution, a table sirup, and an orange beverage, to each of which was added 100 mg. of dichloracetic acid. In each case a derivative was obtained having a satisfactory melting point (153° ±0.5°C.). In using the 100 mg. size sample, it was found advisable to dissolve the benzene residue in the smallest amount of warm methyl alcohol (2 to 5 ml.), and then to add an equal quantity of boiling water. A good yield of crystals after cooling was obtained. In some cases a second recrystallization was feasible.

PROCEDURE

THE IDENTIFICATION OF DICHLORACETIC ACID

Extract sufficient sample, in one or more extractors by the method outlined under the quantitative procedure, to obtain preferably 100 mg. or more of the acid. Combine the ether extracts in a separatory funnel and extract successively with small amounts of N/10 NaOH until the combined aqueous extract is alkaline to litmus. Add N/10 H_2SO_4 to this extract until slightly acid to litmus. Evaporate the solution to dryness on the steam bath and dry at 100°C for approximately ½ hour. Transfer the dried salt to a test tube or small flask, add 2 ml. or more of thionyl chloride, and boil gently under a reflux for ½ hour. Cool the solution, add 1–2 gm. p-toluidine dissolved in 50 ml. of benzene, and heat on the steam bath for 2 min. Transfer the contents to a separatory funnel and extract successively with two separate portions of 30 ml. water, followed successively by identical extractions with 5% HCl, 5% NaOH, and finally water. Filter the benzene solution and evaporate the filtrate to dryness on the steam bath. Crystallize the residue, using small amounts of 50% methyl alcohol, and determine the melting point on the material after drying at 100°C.

SUMMARY

(1) A procedure is presented for the quantitative determination of dichloracetic acid in aqueous solution (as for example in food products). It involves the isolation of the acid by an ether extraction and the conversion of the chlorine of the acid to the ionic form by ignition with sodium carbonate or by hydrolysis with alcoholic potassium hydroxide solution. The chloride formed in either case is determined by the Volhard Method.

(2) Recoveries using this method on three purified samples of dichloracetic acid occurring in aqueous solution were satisfactory.

(3) When known amounts of dichloracetic acid were added to various food products, satisfactory recoveries were obtained, indicating general applicability of the procedure to food products.

(4) An identification test for dichloracetic acid is given which involves the isolation of the acid from aqueous solutions by an ether extraction, the formation of the sodium salt of the acid, and finally, the preparation of the p-toluide derivative of the acid which is characterized by a melting point determination.

PERSISTENCE OF MONOCHLORACETIC ACID IN WINE*

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

As in the case of fruit juices and carbonated beverages[1] it has been claimed that when monochloracetic acid is added to wine, the acid hydrolyzes and disappears within a short time; the experiments described herein were conducted to establish whether or not hydrolysis of monochloractic acid does occur in wine, and if so, to what extent.

A preliminary experiment consisted of adding known quantities of monochloracetic acid to wine which was known to have been bottled for several years. Monochloracetic acid was added to two types of such wine in three concentrations as follows:

Four 1-pint bottles of Muscatel wine were opened and mixed; 1.2 grams of monochloracetic acid was weighed, dissolved in some of the wine and made up to 100 ml. with wine in a volumetric flask. Three samples were prepared of which "A" contained 15 ml. of the preservative solution made to 600 ml. with the wine; "B" contained 25 ml. of the solution made to 600 ml.; and "C" contained 50 ml. of the solution made to 600 ml. The samples therefore contained 30, 50, and 100 mg. of monochloracetic acid per 100 ml. Each was divided among several 4-ounce bottles, stoppered tightly, and stored in the laboratory.

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* Presented at the 59th Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 25 and 26, 1944.

[1] *This Journal*, 27, 195 (1944).

Similarly, two 1.5-pint bottles of Burgundy wine were mixed, and three samples were prepared containing 30, 50, and 100 mg. monochloractic acid per 100 ml. and filled into four-fluid-ounce bottles for storage. These were designated "D," "E," and "F" respectively. Monochloracetic acid was determined by the procedure given below, on the day of preparation and after the various periods of storage.

METHOD

Place 100 ml of wine in a 0.75 to 1.0 L distilling flask and distil off into a conical flask at least 2 ml of distillate for each 1 ml of alcohol present in the sample. Add to the distillate sufficient 10 *N* NaOH to make mixture 1 *N* and digest on steam bath for two hours. Determine chloride ion as in Method II (*This Journal*, 27, 200 (1944)) beginning: "add 50 ml. of water, 15–20 ml. of HNO_3 and 1 ml. of ferric indicator. Etc."

Transfer the residue in the distillation flask to the continuous extractor and determine monochloracetic acid as described in Method I (*loc. cit.*, p. 199) beginning: "Dilute if necessary to 150 ml., etc." The sum of the quantities found in the two fractions equals the amount in the sample.

The monochloracetic acid found in the alcoholic distillate varied from 0.15 mg. to 2.45 mg. The greatest quantities were found when the highest concentration of the acid was present. Results are given in Table 1.

TABLE 1.—*Monochloracetic acid in stored wine*

VARIETY OF WINE	MUSCATEL			BURGUNDY		
DESIGNATION	A	B	C	D	E	F
ADDED G PER 100 ML	0.030	0.050	0.100	0.030	0.050	0.100
Found: Day of preparaion	0.028	0.048	0.095	0.028	0.047	0.098
Stored 7 days	0.029	0.047	0.092	0.028	0.048	0.096
Stored 28 days	0.028	0.046	0.094	0.028	0.046	0.095
Stored 70 days	0.024	0.045	0.092			
Stored 192 days	0.082	0.048	0.094	0.027	0.046	0.093

The results in Table 1 show that there is no essential change in monochloracetic acid content of the wine even after six months storage in the laboratory.

Experiments were then conducted on a semicommercial scale. The recommendation put forward at the time by the vendor of the chemical was that the monochloracetic acid should be added to the finished wine. However, it was deemed advisable to include experiments in which monochloracetic acid was added at various stages in the manufacture of wine, in anticipation of later suggestions that the chemical be added at intermediate points in the process of manufacture. As it was not practicable to follow a single batch of wine through the complete process of manufacture and add monochloracetic acid at various stages, the following procedure was employed:

At the California winery where the samples were obtained, the grapes were crushed in a rotary machine which removed the stems. The crushed grapes were pumped through a large iron pipe to the sumps, passing through a coarse screen. From the sumps the grapes were pumped to open fermentation vats where metabisulfite and starter yeast were added. After two days the fermenting liquid was racked off and pumped to closed fermentation vats where the sugar was fermented out in four or five days. The argols were precipitated by refrigeration and removed by filtration with diatomaceous earth. Finally, the wine was "polished" by filtration through a Seitz filter. Monochloracetic acid was added to each sample and it was then carried along in the laboratory to a later step in the process. The variety of grapes used was Columbard, a white grape.

At each stage of manufacture several gallons of sample were removed at the winery and subdivided into 1 gallon bottles, each containing 2 to 3 liters (measured in a graduate) to which the proper quantity of monochloracetic acid solution was added to give 10, 30, 50 or 100 mg. per 100 ml. as the case might be. Each bottle was provided with a bubbler, or water seal, so that any gas formed could escape without admitting air to the bottle. The bottles were placed on laboratory tables where they could be observed easily, and were observed daily during the first three weeks. When it was uncertain whether a particular bottle contained actively fermenting material, the bottle was shaken to see whether gas escaped through the bubbler. Each time a sample was removed for analysis, the contents of the bottle were thoroughly mixed and the necessary quantity poured into a 100 ml. graduated cylinder. Determinations of monochloracetic acid were made as already described. At the conclusion of the three-week period the material related to the early stages of wine manufacture was discarded, while that related to later stages was sealed and shipped from San Francisco, California, to Washington, D. C., for a continuation of the experiments. From this point on, samples were removed with pipets and monochloracetic acid was determined by the second method described in *This Journal*, **26**, 478 (1943), since this method had been found to facilitate the determination without loss of accuracy."[2]

The following methods of analysis were used in all experiments: Qualitative tests for monochloracetic acid were made by the indigo test following the directions of Mallory and Love.[3] Sulfur dioxide was determined by the Monier-Williams method as given in *Methods of Analysis, A.O.A.C.*, XXXII, **32** (p. 463). Alcohol and reducing sugars were determined by the official A.O.A.C. procedure for these constituents in wine.

The analyses reported in Table 2 pertain to the early stages of the manufacture of wine which extended over a period of about three weeks.

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[2] *This Journal*, **26**, 477 (1943).
[3] *Ind. & Eng. Chem. Anal. Ed.*, **15**, 207 (1943).

TABLE 2.—*Persistence of monochloracetic acid in fermenting grapes and grape juice*

EXP. NO.	SAMPLE	MONOCHLORACETIC ACID							TOTAL SUGARS AS INVERT[1]		ALCOHOL BY VOLUME AT 15° 56′	SULFUR DIOXIDE[2]	
		ADDED	FOUND					INDIGO TEST					
		10-13-'42	10-13-'42	10-44-'42	10-20-'42	10-21-'42	11-2-'42	11-2-'42	10-13-'42	11-2-'42	11-2-'42	10-15-'42	11-2-'42
		g/100 ml.	*g/100 ml.*	*g/100 ml.*	*g/100 ml.*	*g/100 ml.*	*g/100 ml.*		*g/100 ml.*	*g/100 ml.*	*per cent*	*p.p.m.*	*p.p.m.*
1	Crushed Grapes, taken at plant crusher, no culture added, natural fermentation; SO_2 added	0.010		0.009			0.008	+		1.65	10.28		126
		.030		.029		0.028	.028			1.81	10.78		75
		.050	.047				.049	+	21.72	7.76	7.27	108	90
		.100		.092			.094			11.56	4.89	104	90
2	Same as 1, except no SO_2 added	.010					.009	+		1.18	10.78		
		.030					.026			1.81	10.44		
		.050	.047				.046	+		5.14	8.14		
		.100					.091			5.05	8.79		
3	Free-run Juice, taken at plant crusher, no culture added, natural fermentation; SO_2 added	.010	.009				.009	+		2.86	10.86		172
		.030	.028		.027		.027			4.61	10.36		112
		.050	.049			.050	.047	+	23.40	17.10	3.33	130	118
		.100	.093				.092			16.60	3.63	131	120
4	Same as 3, except no SO_2 added	.010					.009	+		3.54	9.79		
		.030				.028	.026			6.62	8.31		
		.050	.046		.048		.047	+		7.29	5.73		
		.100				.090	.091			13.99	4.62		
5	Fermenting Juice, fermentation started 10-12-'42, no culture added, natural fermentation; SO_2 added	.010		.009		.009	.009	+		2.46	10.78		210
		.030		.029	.027	.029	.027			8.77	7.67		153
		.050	.049				.046	+	23.37	14.98	4.04	192	158
		.100		.098		.098	.094			21.46	1.20		151

[1] Determined by L. H. McRoberts, Chemist, U. S. Food and Drug Administration, San Francisco Station.
[2] Determined by D. A. Ballard, Chemist, U. S. Food and Drug Administration, San Francisco Station.

The following materials were treated with the proper quantity of monochloracetic acid solution in bottles as described above: (1) Crushed grapes, (2) free run juice (both grapes and juice taken at the plant crusher (a) with and (b) without the addition of sulfur dioxide), and (3) juice produced the previous day and just beginning to ferment (sulphur dioxide added). A sufficient number of analyses were made at various stages to give a picture of the effect of the monochloracetic acid upon the sugar and alcohol content, and the effect of the fermenting mixture upon the monochloracetic acid. The data show that there was no essential change in the monochloracetic acid content of any fermenting mixture throughout the experiment.

Table 3 contains the data from experiments conducted over a two-year period on samples taken at later stages in the manufacture of wine. The next stage beyond incipient fermentation (recorded in Table 2) was chosen 48 hours after the beginning of fermentation (expt. 6) at which time the juice may be considered heavily inoculated. When the monochloracetic acid was added, the density of the liquid was 6.8° Brix and the alcohol content was about 8.5 percent. The sample containing 0.01 percent monochloracetic acid in this group was used for other experiments, but the other data show that even with 0.03 percent and 0.05 percent, the alcohol gradually increased during the next year to a point near the maximum obtained in any sample. Two experiments (7 and 8) were started at the point where a spindle reading of 2.2° Brix was obtained upon the fermenting juice. In one, monochloracetic acid was added, followed by clarification with gelatin at the rate of 1 lb. for 1500 gal. of wine; while in the other, the clarification was conducted first, followed by the addition of the desired dose of monochloracetic acid. During the clarification process the wine was chilled in a refrigerator, then diatomaceous earth was added, and the mixture was filtered until clear. For the two remaining experiments (9 and 10) wine was removed from a tank containing 1941 vintage, and the chemical was added as described above. The wine in one set of bottles was not subjected to further treatment other than storage for the remainder of the experiment. The wine in the other set of bottles was sweetened by the addition of sufficient concentrated grape juice to increase the invert sugar content to 2 grams per 100 ml., and then inoculated with yeast. In the sweetened wine containing 0.01 percent of monochloracetic acid, a further increase in alcohol occurred, showing that this quantity of monochloracetic acid will not prevent fermentation under these conditions. These data show that 0.01 percent of monochloracetic acid is not sufficient to prevent secondary fermentation of sweet wine containing about 12 percent of alcohol. All of the wines in Table 3 contained substantially the same quantities of monochloracetic acid after two years of storage as were found at the beginning of the experiment.

TABLE 3.—*Persistence of monochloracetic acid in wine, when added at various stages in the process of manufacture*

EXP. NO.	SAMPLE	MONOCHLORACETIC ACID							TOTAL SUGARS AS INVERT.					ALCOHOL BY VOLUME AT 15.56°C.			
		ADDED	FOUND					INDIGO TEST									
			11-2-'42	1-23-'43	4-19-'43	11-8-'43	11-16'-44	7-21-'43	10-15-'42	10-30-'42	7-17-'43	11-8-'43	4-6-'45	10-15-'42	10-30-'42	7-14-'43	11-8-'43
		g/100 ml.	*g/100 ml.*	*g/100 ml.*	*g/100 ml.*	*g/100 ml.*	*g/100 ml.*		*g/100 ml.*	*g/100 ml.*	*g/100 ml.*	*g/100 ml.*	*g/100 ml.*	*per cent*	*per cent*	*per cent*	*per cent*
6	Free-run Juice[1] taken at plant crusher 10-13-'42, 15 ml. starter added to each bottle. Monochloracetic acid added 10-15-'42, after 48 hrs. of fermentation	0.010	0.009		0.009			+	6.40*	3.50*				8.84*	9.35*		
		.030	.024	0.027	.027	0.028	0.028	+	6.64*	7.15*	2.38	2.22		8.50*	9.21*	11.93	11.87
		.050	.046	.047	.046	.047	.044	+	6.62*	7.21*	5.04	2.47		8.47*	9.29*	10.72	12.08
		.100	.089	.095	.094	.093	.090	+	6.52*	6.96*	7.14	6.88		8.69*	9.27*	9.31	9.10
7	Same as 6, except: Monochloracetic acid added 10-18-'42 when Brix reached 2.2°. Clarified with gelatin	.010		.008	.009	.009	.009	+			0.69	0.16				12.60	12.55
		.030		.027	.027	.028	.028	+			0.59	0.61				12.87	12.90
		.050		.047	.044	.047	.047	+			0.69	0.68				13.12	12.73
		.100		.095	.096	.095	.093	+			0.77	0.74				12.95	12.85
8	Same as 6, except: Monochloracetic acid added 10-18-'42, after clarification with gelatin when Brix reached 2.2°	.010		.010	.009	.010	.010	+			0.14	0.14				12.17	12.47
		.030		.027	.029	.028	.028	+			0.67	0.68				12.13	12.29
		.050		.048	.048	.048	.048	+			0.67	0.67				12.51	12.12
		.100		.096	.098	.096	.092	+			0.78	0.76				12.06	12.42
9	Wine (1941 Vintage)[2] from same vineyard, taken from vat 10-13-'42. Monochloracetic acid added same day. No further additions	.010	.010		.010	.010	.009	+								12.38	
		.030	.028	.026	.028	.028	.028	+	0.18*							12.21	
		.050	.045	.044	.043	.045	.043	+	0.18*							12.90	
		.100	.093	.096	.095	.096	.092	+								12.81	
10	Wine, same as 9, except: Added grape concentrate to 2% sugar and starter culture 10-30-'42	.010		.010	.010	.010	.009	+		0.17*	0.92	0.82	0.21			13.60	13.43
		.030		.027	.027	.028	.028	+		0.18*	2.00	1.99	2.01			12.12	12.55
		.050	.044	.046	.046	.046	.045	+		0.18*	2.06	2.01	2.04			12.04	12.64
		.100		.091	.093	.092	.090	+		0.18*	2.00	1.96	1.97			12.64	12.65

[1] Analysis of Juice: Total Sugars as invert 23.4 g/100 ml.; Acidity as tartaric 0.8 g/100 ml.
[2] Analysis of Wine: Total Sugars as invert 0.18 g/100 ml.; Alcohol 12.9% by volume; SO_2 116 p.p.m.
* Determinations so marked made by L. H. McRoberts, Chemist, San Francisco Station.

CONCLUSIONS

1. Monochloracetic acid does not hydrolyze when added to wine at any point during the process of manufacture.

2. The quantities of monochloracetic acid added to wine during the process of manufacture remained unchanged even after two years of storage.

3. The addition of 0.05 percent or less of monochloracetic acid did not prevent secondary fermentation of wine containing less than 12 percent of alcohol by volume, when fermentable sugars were present.

4. The addition of 0.01 percent of monochloracetic acid did not prevent secondary fermentation of wine containing more than 12 percent of alcohol by volume, when fermentable sugars were present.

USE OF BUFFERS IN THE DETERMINATION OF COLOR BY MEANS OF TITANIUM TRICHLORIDE IV*

By O. L. EVENSON (Cosmetic Division, Food and Drug Administration, Federal Security Agency, Washington, D. C.)

The subject of this investigation has been dealt with in three previous articles[1,2,3] from this laboratory. In the first of the series, as well as in an article on electrometric titration of food colors,[4] it was shown that the pure dye in FD&C Red No. 2 (the trisodium salt of 1-(4-sulfo-1-naphthylazo)-2-naphthol-3,6-disulfonic acid; also known as amaranth) could not be correctly evaluated when sodium bitartrate was used as a buffer. It was also shown that sodium tartrate did not give correct results in all cases. The same discrepancies were noted in the case of FD&C Red No. 1 (the disodium salt of 1-pseudocumylazo-2-naphthol-3,6-disulfonic acid; also known as ponceau 3R). It was shown further that correct results could be obtained for these two colors if sodium citrate was used as a buffer, and that for all other water soluble food, drug, and cosmetic colors the tartrates were satisfactory as buffers.

The object of the present investigation was to develop further information on the efficacy of tartrate buffers, and on its correlation with *p*H. FD&C Red Nos. 1 and 2 were used for this purpose.

MATERIALS

The color samples used were of certified grade; buffer salts as well as other chemicals were of reagent grade. The titanium trichloride solution

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* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 25 and 26, 1944.

[1] Evenson, O. L., and McCutchen, D. T., *Ind. Eng. Chem.*, 20, 860 (1928).
[2] Evenson, O. L., and Nagel, R. H., *Ind. Eng. Chem., Anal. Ed.*, 3, 167–169 (1931).
[3] Forrest, S. S., and Evenson, O. L., *This Journal*, 25, 246–248 (1942).
[4] Evenson, O. L., and Nagel, R. H., *Ind. Eng. Chem., Anal. Ed.*, 4, 151–154 (1931).

was approximately 0.1 *N* as a reducing agent, and 1.45 *N* with respect to the hydrochloric acid present. The acidity of the titanium trichloride was determined as follows: A known excess of normal sodium hydroxide was added to 10 ml. of the titanium trichloride and permitted to react until the black titanous hydroxide changed to the white titanic compound. The excess sodium hydroxide was then determined by titration with standard hydrochloric acid, using phenolthalein as indicator.

The *p*H determinations were made with a Beckman *p*H Meter, before and after titration. Details of the method follow:

PROCEDURE

Dissolve the buffer salt, together with the color, in 130 ml. of water, by heating in a wide mouth 500 ml. Erlenmeyer flask. Then titrate the color solution while hot, agitating with a mechanical stirrer in an atmosphere of carbon dioxide. Add the titanium trichloride slowly in all cases. Determine the *p*H of the solutions before and after titration at about 25°C., the carbon dioxide being first eliminated by boiling.

A summary of the results obtained is shown in Table 1. The figures for percent pure dye are the averages of two or more closely agreeing results. The percent pure dye obtained with varying amounts of sodium citrate is given for comparison. It was shown in a previous article[1] that the percent pure dye obtained with the sodium citrate buffer agrees closely with the "color by difference." It may be seen that the use of varying amounts of sodium citrate does not materially affect the pure dye obtained, even though the *p*H at the end of the titration decreases from 5.5 to 2.5. Unfortunately, however, as was shown previously,[2] this citrate buffer was not suitable for some of the other water soluble food, drug, and cosmetic colors.

Sodium bitartrate gives low results in all cases. The *p*H is maintained within a somewhat narrow range beginning at 3.4. A *p*H of 4 or above cannot be obtained with this buffer.

With sodium tartrate, results comparable to those shown for sodium citrate may be obtained only if enough of the buffer salt is used to keep the *p*H at 4 or above at the end of the titration. This acidity cannot be obtained with sodium bitartrate. The amount of sodium tartrate necessary will depend upon the acidity of the titanium trichloride and the quantity used for the reduction, which in turn varies with the weight of dye being reduced. For 0.25 grams of FD&C Red No. 1 and 0.3 to 0.4 grams of FD&C Red No. 2 (90 and 86 percent of pure dye, respectively) and approximately 0.1 *N* titanium trichloride (acidity 1.45 *N* or less), 15 grams of sodium tartrate is sufficient to keep the *p*H at about 4 and give correct results. Sodium tartrate may therefore be used under these conditions for all the water soluble food, drug and cosmetic colors and all mixtures of

[1] *Loc. cit.*
[2] *Loc. cit.*

TABLE 1.—*Varying amounts of different buffers in the titration of FD&C Red Nos. 1 and 2*

COLOR	BUFFER		DYE	pH		PURE DYE
				BEGINNING	END	
		grams	*grams*			*per cent*
FD&C Red No. 2	Sodium Citrate	20	0.3	7.4	5.5	86.2
		15	0.3	7.4	5.4	86.6
		7.5	0.3	7.4	4.9	86.1
		3	0.3	7.4	2.5	86.1
						Av. 86.25
	Sodium Bitartrate	25	0.3	3.4	2.8	82.3
		15	0.3	3.4	2.8	81.9
		7.5	0.3	3.4	2.4	80.8
	Sodium Tartrate	25	0.3	7.4	4.3	86.4
		20	0.3	7.4	4.2	86.4
		15	0.3	7.4	4.1	86.1
		7.5	0.3	7.4	3.5	85.3
		3	0.3	7.4	1.8	84.0
		25	0.4	7.4	4.3	86.5
		20	0.4	7.4	4.2	86.1
		15	0.4	7.4	4.0	86.0
		10	0.4	7.4	3.6	85.1
		5	0.4	7.4	2.5	83.7
		30	0.5	7.4	4.2	86.6
		25	0.5	7.4	4.1	86.0
		15	0.5	7.4	3.7	85.0
		7.5	0.5	7.4	2.8	83.3
FD&C Red No. 1	Sodium Citrate	15	0.25	7.4	5.5	90.5
		7.5	0.25	7.4	4.9	90.5
		3	0.25	7.4	2.5	90.5
	Sodium Bitartrate	15	0.25	3.4	2.8	85.5
		7.5	0.25	3.4	2.4	84.5
	Sodium Tartrate	20	0.25	7.4	4.2	89.7
		15	0.25	7.4	4.1	89.7
		7.5	0.25	7.4	3.5	88.1
		3	0.25	7.4	1.8	86.7

these. It seems probable also that it may be used for most other certified dyes, since it gives a final *p*H intermediate between that given by sodium citrate and sodium bitartrate.

SUMMARY

Sodium tartrate buffer in the titration of FD&C Red Nos. 1 and 2 with titanium trichloride gives correct results if a sufficient amount is used to keep the *p*H of the titrated solution at or above 4. The amount necessary depends upon several factors but under normal conditions 15 grams is sufficient.

Sodium citrate gives equally correct results although the final *p*H of the titrated solution varies from 5.5 to 2.5.

ESTIMATION OF FD&C YELLOW NOS. 3 AND 4 IN COTTONSEED AND OTHER VEGETABLE OILS*

By S. H. NEWBURGER (Cosmetic Division, Food and Drug Administration, Federal Security Agency, Washington, D. C.)

Among the colors submitted to the Food and Drug Administration for certification in accordance with the Food, Drug, and Cosmetic Act are solutions of FD&C Yellow Nos. 3 and 4 in oils such as cottonseed, peanut, soybean, corn, and castor oil. The pure dye content of these solutions varies from 2 to 8 percent.

Although the solutions can readily be analyzed spectrophometrically there is no satisfactory chemical method of analysis. The difficulties encountered in the reduction with titanium trichloride are twofold: The oils are not soluble in the usual solvents, and the end points are obscured by the yellow color of the oils. An attempt was therefore made to separate the colors from the oils, and a procedure based on the saponification of the oil and extraction of the dye with ether was developed which gives accurate and reproducible results with cottonseed oil.

METHOD

Weigh a sample not exceeding 3 grams and containing from 0.05 gram to 0.10 gram of color into a 300 ml. round bottom flask equipped with a ground glass joint. Add 25 ml. of alcohol and 1 gram KOH, insert a water condenser into the flask, and reflux vigorously for one-half hour over a burner. Cool and transfer the mixture to a 300 ml.-separatory funnel with 80 ml. of water, and extract with four 50 ml.-portions of ether. Combine the ether extracts and wash with two 25 ml. portions of water by slowly inverting separatory funnel several times. Wash a third time by shaking vigorously with another 25 ml. of water. Drain washed ether extracts into a 500 ml. titration flask, rinse separatory funnel with several ml. of ether, and add the rinsings to titration flask. Evaporate the ether on steam bath, take up residue in 100 ml. of alcohol, add 120 ml. of hot water cont. 15 grams of $NaHC_4H_4O_6$, and heat the solution to boiling. Add 1 ml. of FD&C Green No. 2, indicator solution (aqueous, 1 ml. = 0.2 ml. of 0.1 *N* $TiCl_3$) and titrate the hot solution with 0.1 *N* $TiCl_3$ in an

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* Presented at the Annual Meeting of the Association of Official Agricultural Chemists held at Washington, D. C., October 25, 1944.

atmosphere of CO_2. The end point is a change from blue to a pale reddish orange. Subtract the indicator blank and calculate the pure dye content.

1 ml. of 0.1 *N* $TiCl_3$ = 0.00618 gram of FD&C Yellow No. 3
= 0.00653 gram of FD&C Yellow No. 4

Known solutions of cottonseed oil and dye were analyzed by the above procedure. The results are presented in Table 1.

It is apparent from Table 1 that the method is accurate to within 1 percent. In the analysis of the solutions containing both dyes the theoreti-

TABLE 1.—*Recovery of FD&C Yellow Nos. 3 and 4 from cottonseed oil*

EXP. NO.	CONTAINED	WT. OF SAMPLE	TOTAL DYE PRESENT	FOUND	RECOVERY
	per cent	*grams*	*gram*	*gram*	*per cent*
1.	FD&C Y. #3....10 C.S.O.....90	1.000	0.1000	0.1000	100.0
2.	FD&C Y. #4....10 C.S.O.....90	1.000	0.1000	0.1003	100.3
3.	FD&C Y. #3.....5 FD&C Y. #4.....5 C.S.O.....90	1.000	0.1000	0.1000	100.0
4.	FD&C Y. #3.....3.50 FD&C Y. #3.....3.50 C.S.O.....93	1.430	0.1001	0.1001	100.0
5.	FD&C Y. #3.....1.67 FD&C Y. #4.....1.67 C.S.O.....96.66	3.000	0.1002	0.0995	99.3
6.	FD&C Y. #3.....0.83 FD&C Y. #4.....0.83 C.S.O.....98.34	3.000	0.0498	0.0495	99.4

cal titration value was computed and compared with the one obtained. No effort was made to separate the dyes.

This analytical procedure was then applied to color samples submitted for certification and containing soybean, peanut, castor, and corn oils. The solutions were assayed both chemically and spectrophotometrically and the results compared (Table 2).

The analytical results obtained by the two procedures are in good agreement. The time required for a single analysis by the proposed method is two hours.

It is possible to determine accurately by chemical means either FD&C

Yellow No. 3 or FD&C Yellow No. 4 in vegetable oils. Where the solution contains both dyes, it is necessary to know the ratio in which the dyes are present in order to calculate the theoretical titration value. However, if a titration value based on a 1:1 ratio of the dyes in the mixture is used for calculation (1 ml. of 0.1 *N* $TiCl_3$=0.00635 gram of "dye mixture") the

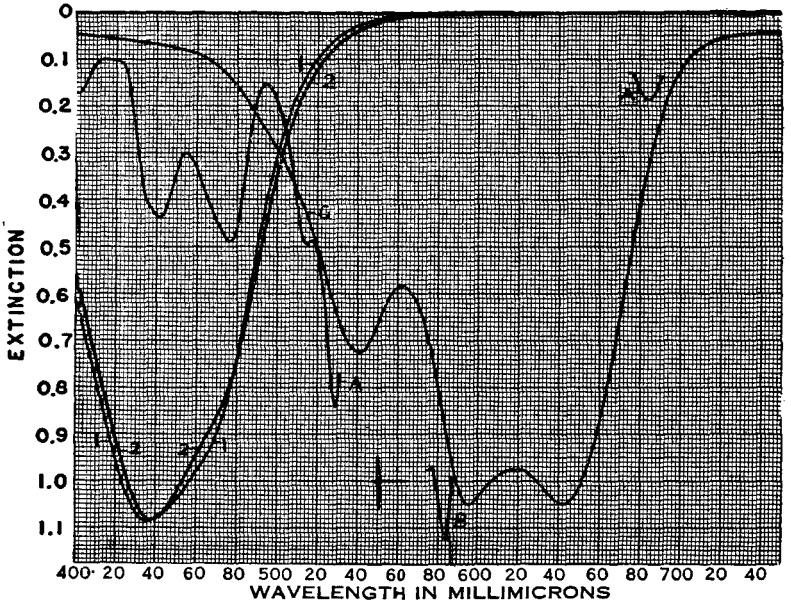

FIG. 1.—Extinction curves of FD&C Yellow Nos. 3 and 4 in chloroform
Curve 1 = FD&C Yellow No. 3
Curve 2 = FD&C Yellow No. 4
Concentration—20 mg./liter
Cells—1 cm.
A. = Corning Didymium Glass 512, 6.0 mm.
(Absorption peaks at 400.4, 441.4, 477.1, 529.0, and 684.8 mμ)
B. = Corning Didymium Glass 592, 4.02 mm.
(Absorption peak at 583.7 mμ)
C. = Signal Lunar White Glass—H-6946236

error will not be excessive. If the true dye content is set at 100 per cent the found values would theoretically lie between 100 ± 2.8 percent. Analytical errors would have to be added to this.

Spectrophotometrically, these two colors have very similar absorption curves and can therefore not be individually determined in solutions containing both of them. However, their extinction values at 437 mμ for equal concentrations are the same. This makes it possible to analyze ac-

TABLE 2.—*Recovery of FD&C Yellow Nos. 3 and 4 from various vegetable oils*

EXP. NO.	CLAIMED BY CERTIFIER (PER CENT)		PER CENT OF DYE FOUND	
			PROPOSED METHOD	SPECTROPHOTOMETRICALLY*
1.	Total dye Soybean oil	3.11	3.06	3.08
2.	Total dye Peanut oil	3.15	3.23	3.23
3.	Total dye Castor oil Corn oil	3.0	3.04	3.03

* For the spectrophotometric determinations chloroform solutions containing about 20 milligrams of color per liter were prepared and the extinction curves in the spectral region 400–750 mμ obtained with a General Electric recording spectrophotometer having slit adjustments for an 8 mμ wave length band. One-centimeter cells were used. These curves were compared with those of standard solutions of known dye concentration. If but one extinction value reading is desired the wave length 437 mμ (Fig. 1) should be chosen.

curately the total dye content irrespective of the ratio in which the two components are present (Fig. 1).

SUMMARY

A method has been developed for the estimation of FD&C Yellow Nos. 3 and 4 in vegetable oil solutions.

A spectrophotometric procedure for the same type of analysis has been outlined.

The results of the two methods are in good agreement.

LOW PRESSURE-LOW TEMPERATURE SYSTEM FOR DECOMPOSITION STUDIES IN FOOD

By HENRY FISCHBACH (Food and Drug Administration, Federal Security Agency, Washington, D. C.*)

Past methods with few exceptions depend on distillation (or less frequently on aeration or diffusion) at atmopsheric pressure as a means of isolating the "criteria" of decomposition from a relatively enormous mass of food. The criteria are thereby restricted to the relatively few constituents of comparatively high volatility such as the lower members of the fatty acid series, ammonia and the amines, indole, hydrogen sulfide, and the mercaptans. Further, the food is necessarily subjected to considerable "rough treatment" during such isolation procedures.

There is thus an urgent need to so extend the isolative technique as to

* Contributed from the Food Division, W. B. White, Chief.

embrace compounds with vapor pressures less than that of water, and at the same time to treat the food more gently during the isolation. Such an extension should increase the chances that the criteria will represent some components at least of the decomposed odor or flavor, which is, by definition, the primary criterion of decomposition. A measure of decomposition that nearly always accompanies the sensory evidence, and never runs counter to it, is good; but one which actually is the thing smelt or tasted (or at least is an important component) would be perfect.

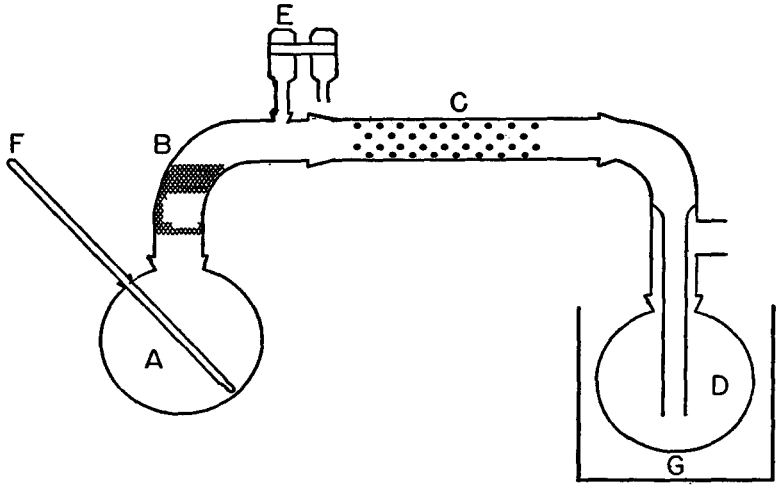

FIG. 1.—Low-pressure distillation apparatus.
A—Reaction bulb
B—Anti-spray tube (loosely filled with glass wool)
C—Chemical trap containing glass beads coated with appropriate chemical—effective length 20 cm.
D—Cold trap
E—Pirani gauge
F—Thermometer
G—Dewar flask containing dry ice and acetone.

That some of the most evil-smelling substances are not highly volatile will be evident by referring to the volatility tables for food flavors given by Crocker.[1,2] If some method were available for isolating compounds having a vapor pressure at 20°C. as low as 3.0 mm. (or even lower), and perhaps for collecting them in small volume from a rather large mass of food, the chances of identifying them, and therefore of measuring them, should be very good.

The advent of low pressure equipment seems to offer a new approach to

[1] Crocker, E. C., *Am. Perfumer*, 32, Oct. 1935.
[2] Crocker, E. C., *Ind. Eng. Chem.*, 37, 214, 1945.

an old problem, and one which is free of some of the limitations of the older methods.

In the hope that it will be useful to other workers, a general method of this character is given in broad outline, together with some of the data thus far obtained.

METHOD FOR VOLATILE BASES

A representative sample of the food is placed in the reaction chamber A (Figure 1) with or without adjusting the *p*H to the desired alkalinity. The reaction chamber A, the anti-spray tube B (filled loosely with glass wool), the chemical trap C (glass beads coated with 85% H_3PO_4), and the cold trap (immersed in a freezing bath of dry ice and acetone) are evacuated with a suitable pump.* Glass joints (29/42 standard taper) are used thruout. For greater capacity a booster pump can be used in tandem with the mechanical fore-pump. As the material degasses and the temperature begins to fall, the reaction chamber is immersed in a suitable bath for maintaining the desired internal temperature in the distillation vessel. Distillation is continued until the desired pressure is attained.

TABLE 1

COMPOUND	MILLIGRAMS IN AQUEOUS MEDIUM	RECOVERY		DISSOCIATION CONSTANT	ANALYTICAL METHODS
		IN H_3PO_4	IN COLD TRAP		
	mg.	*per cent*	*per cent*		
Trimethylamine	13	99.6	0	7.4×10^{-5}	Kjeldahl-Gunning-Arnold Method, *Methods of Analysis, A.O.A.C.*, 5th Ed., p. 26.
Aniline	100	99.6	0	4.6×10^{-10}	
Pyridine	100	99.2	0	2.3×10^{-9}	
Butyronitrile	100	0	60	1.8×10^{-15}	Clarke *et al.* (with slight modifications), *This Journal*, 20, 475 (1937).
Indole	25	0	100	(unknown)	

Those bases which are volatile under the distilling conditions are concentrated as the phosphates in the chemical trap. Neutral volatile constituents, condensible at −78.0°C. are entrapped, together with water, in the cold trap (D).

In studying the recovery of volatile basic substances from an aqueous medium by the new technique, a useful phenomenon was observed. Ammonia and trimethylamine were caught quantitatively in the H_3PO_4 trap. Indole, on the other hand, passed through the H_3PO_4 trap and was recovered quantitatively in the cold trap. In fact, complete separation of indole and trimethylamine (present in equal amounts) was effected by distilling an aqueous solution through an H_3PO_4 trap connected in series to a cold trap. This suggested that the degree of dissociation might serve to predict which basic compounds would react with the H_3PO_4. To test this theory, aqueous solutions of suitable organic bases of varying degrees of dissociation were subjected to the proposed technique (Table 1).

It appears that those volatile bases which have a dissociation constant of 10^{-10} or more are trapped quantitatively by orthophosphoric acid, those

* A "Megavac" was used in this work.

with a dissociation constant of 10^{-15} or less are not affected by the acid, and pass into the cold trap. Only 60 percent of the butyronitrile was held back by the cold trap but a liquid air trap might have completely retained this highly volatile compound.

METHOD FOR ACIDS

The procedure is identical with that for volatile bases except that the food is made slightly acid with H_3PO_4, and the chemical trap consists of glass beads coated with sat. KOH. The volatile acids are thus concentrated as the potassium salts in the chemical trap. Complete recovery of formic, acetic, propionic, and butyric acids from aqueous media was accomplished.

METHOD FOR ESTERS

To observe the effect of the potassium hydroxide trap and the orthophosphoric acid trap on a low boiling ester, a 50% aqueous solution of ethyl acetate was distilled by the above procedure through a H_3PO_4 trap, a cold trap, and a KOH trap in the order named (Table 2).

TABLE 2.—*Ethyl acetate distillation*

	QUANTITY OF ESTER DIST.	ACIDITY IN CHEMICAL TRAP	ACIDITY IN COLD TRAP
	ml	*per cent*	*per cent*
KOH trap	10	0.1	0.0
H_3PO_4	10	0.01	0.0

The steam distillation method for volatile acids[3] was used for determining the acidity in the chemical traps. The acidity in the cold trap was determined by transferring the contents to a separatory funnel and washing with three 25 ml. portions of distilled water, drawing off and combining the washings, evaporating them on a steam bath until no odor of ethyl acetate was detectable, and finally titrating against 0.01 *N* barium hydroxide.

Since the amount of ester used in the above experiment is far in excess of what one might encounter from a natural food source, the slight hydrolysis of the ester in the chemical traps is considered negligible.

DISCUSSION

Water is of course the main constituent of many foods and when such foods are subjected to the proposed technique the temperature in the reaction chamber drops rapidly during the first few minutes of active distillation. Ordinarily this would soon result in the freezing of the entire contents. However, a larger pressure gradient between the reaction bulb and the vacuum pump, and therefore a faster rate of distillation is obtained if ice is not permitted to form. This is accomplished by imparting

[3] Hillig and Knudsen, *This Journal*, 25, 176, 1942.

to the system enough heat to offset the heat of evaporation. The volatile constituents may then be distilled at 0°C. (or somewhat lower, depending on the concentration of ionic material in the aqueous phase) at a reasonably slow rate. It obviously requires a greater heat input to distill from a solid-vapor system (heat of sublimation = heat of fusion + heat of vaporization) than from a liquid-vapor system (where the supplied heat is offset only by the heat of vaporization.)

As the more volatile components disappear from the reaction chamber the pressure slowly falls; and the most advantageous end conditions for each product and constituent must therefore be explored. In the preliminary study of some canned foods, using trimethylamine and indole as indices of decomposition, 0.05 mm. and 20°C. were adopted as the most desirable end point, although complete recovery of added known amounts of the above constituents was also obtained at 0.75 mm. and 10°C.

If subzero temperatures are necessary for the particular problem, distillation can be continued from the frozen mass while maintaining a sufficient heat input by means of the constant temperature bath to expedite the distillation. (Products which distill with excessive foaming can be subjected initially to this subzero treatment to avoid complications.)

The proposed method exposes the food material to a minimum of rough handling, for as the temperature of the reaction chamber approaches the finishing temperature the oxidizing atmosphere approaches exhaustion.

Preliminary experiments with such foodstuffs as canned oysters, canned tuna, butter, and frozen cod fillets, have shown real promise with this technique.

Higher temperatures of distillation, under exceedingly high vacuum and high frequency heating, remain to be explored as devices for distilling low vapor pressure compounds and for more closely controlling the desired heat input.

SUMMARY

A new technique is proposed for the study of decomposed foods, based on a low temperature–low pressure system with suitable chemical and cold traps. Separation of very weakly dissociated volatile bases (K as low as 10^{-15}) from those more strongly dissociated, together with separation of neutral volatiles, is possible in one operation. In like manner volatile acids can be separated from non-acidic material.

ACKNOWLEDGMENT

The author expresses grateful appreciation to W. I. Patterson for his helpful suggestions and interest in this work.

SEPARATION AND IDENTIFICATION OF THE VOLATILE SATURATED FATTY ACIDS (C_1 to C_4)

By L. L. RAMSEY and W. I. PATTERSON (Food Division,* Food and Drug Administration, Federal Security Agency, Washington, D. C.)

Quantitative analytical methods for the determination of individual volatile acids in a mixture have been usually based upon such differences as rate of distillation or partition between immiscible solvents. Since there was no available specific chemical test for each component acid, other than formic, in a mixture, nor any satisfactory procedure to achieve an essentially quantitative separation of small smounts of the individual acids, every method involved mathematical equations and these often magnified the errors, unavoidable under practical conditions, especially with micro quantities. In those cases where the component acids of the mixture were known with reasonable certainty, satisfactory results could be obtained; however, the presence of small amounts of one or more unrecognized acids might conceivably lead to erroneous conclusions, both qualitatively and quantitatively.

In natural products, especially where microbiological action may have taken place, a great variety of volatile acids may theoretically occur, even excluding the volatile acids of certain fats, particularly when the degradation products of proteins and their constituent amino acids are involved. That five or more acids might occur simultaneously is not at all improbable. Lack of any practical method for analysis of such complex mixtures has prevented their thorough study. Thus the need is obvious for a procedure for completely separating the lower volatile fatty acids from one another and then identifying each with a simple confirmatory test.

Although no available publication has described such a method of separation in detail, Smith has outlined one for this purpose (1); and Elsden has a paper in press on this subject (2). The method is an adaptation of a similar procedure for the separation of amino acids called "partition chromatography" (3). It is an amplification of the earlier principle—distribution of the volatile acids between two immiscible solvents—but is so much more efficient that complete separation (within the limits of confirmatory tests) of the lower fatty acids, formic, acetic, propionic, and butyric, is readily and quickly achieved, even when one or more may be present to the extent of only a few percent of the total acids. Furthermore, the method of separation, and qualitative confirmatory tests by microscopic examination of crystalline salts, can be applied to a mixture of volatile acids containing a total of 5 to 10 mg., if there is at least 1 mg. of each acid present. In its present state of development, the separation is limited to acids differing by at least 1 carbon atom; *i.e.*, a mixture of

* W. B. White, Chief.

propionic and n-butyric acids is separated, but a mixture of n-butyric and isobutyric acids is not separated, these two behaving in the separation as if they were a single acid.

Silica gel, which has been saturated with water containing a suitable indicator in its alkaline form, is put into a column as in ordinary chromatography. The chloroform solution of the volatile acids is added to the column which is then developed by washing with chloroform containing suitable amounts of n-butanol. The position of each acid in the column is indicated by the change in color of the indicator. The different bands, each containing 1 acid (or a mixture of isomeric acids in the case of butyric), are washed through the column and collected separately, the indicator color being used as a basis for changing receivers.

The range of the method here described is limited to those saturated fatty acids which contain from 1 to 4 carbon atoms. Since the acids with 1, 2, or 3 carbons have no isomers, these are obtained pure. The two four-carbon acids, n-butyric and isobutyric, will not be separated on the column; however, in a mixture of these, isobutyric acid can be detected qualitatively by its conversion, with acidic potassium permanganate (4) and silver sulphate to acetone, which can be detected by its reaction with salicylaldehyde (5). With silver ion a much higher yield of acetone is obtained. n-Butyric acid gives only a trace of acetone under the same conditions, and even this may be due to an impurity of an iso acid. n-Butyric acid can be detected when present with isobutyric acid by complete oxidation of the isobutyric acid with concentrated sulfuric acid at 200° (4). A portion of the n-butyric acid is lost at the same time, but 2 or 3 mg. in the original mixture should be ample for identification after oxidation and redistillation of the residual acid.

A great advantage of this method is its ability to separate micro amounts of the acids. A few mg. of total acids are sufficient for separation; 1 mg. of butyric acid will give a readily detectable band on a column 1 cm. in diameter. For confirmatory identification several mg. of some acids may be necessary. With still smaller diameter columns and more refined micro techniques, less than a mg. of each acid would probably suffice for both separation and identification. To detect traces of other acids in a mixture containing a major acid constituent, 100 mg. or more of total acids should be put into the column, which may necessitate a larger column than the one here described. As was explained in the original outline of the method (1), 1 percent of a lower member in the series of fatty acids, say 1 part of acetic in 100 parts of propionic, can be separated. Only about 5 parts of butyric in 100 parts of propionic acid will separate completely; however, one part of butyric acid gives a clearly detectable band which does not completely separate from that of propionic under the conditions used herein.

Fermented products usually contain lactic acid in addition to the

saturated fatty acids. The small amounts of this acid which are present in steam distillates do not interfere with the separation of the acids above formic; and formic can readily be detected in its presence by the reduction of mercuric chloride.

In certain foods sulfur dioxide or benzoic acid (as sodium benzoate) may be present as a preservative. Both interfere with the separation of the volatile acids. Benzoic acid does not separate completely from butyric acid when the former is present in greater amount, and when present at all benzoic acid is usually found in relatively large amounts. Sulfur dioxide changes the indicator, over the whole column, to the acid color, and this is unchanged by washing with more solvent. Thus when sulfur dioxide is present it must be removed before the remaining acids can be separated; this can be done with the Monier-Williams method for the determination of sulfur dioxide (6).

Pyruvic acid behaves like sulfur dioxide on the column; however, it seldom occurs in significant quantity. If present it may be readily detected and separated with 2,4-dinitrophenylhydrazine (7), or destroyed by refluxing the volatile acids in the presence of mercuric sulphate (8). The latter treatment will also destroy lactic and formic acids.

In a conclusive identification of the individual acids, separation is only the first step. A tentative identification can be gained by comparing the behavior of the column containing the unknown acids with that of a column containing a mixture of known acids. However, proof of what acid or acids are present in each band must be obtained with a confirmatory test. The small amount of each acid which is available makes most of the usual methods of qualitative organic analysis inapplicable. Resort to microscopic methods of crystal examination is therefore advisable. This technique for volatile fatty acids has been thoroughly studied by Klein and Wenzl (9) and their method for the preparation and examination of metal salts of the organic acids has been adopted here. For satisfactory conclusions from these tests, pure solutions of each acid (not a mixture of isomeric acids) are very important. Where only one isomer is present in a given sample this is readily achieved by redistillation, and evaporation of the neutralized distillate either to dryness or to a volume containing the required concentration of sodium salt for the confirmatory test. Where a mixture of isomers contains only a small proportion of one isomer, then identification of the major constituent by means of crystalline salts may still be possible.

Although the scope of this paper is limited to acids with not more than 4 carbons, a sample of unknown compostion may contain higher molecular weight volatile fatty acids. These do not interefere, however, since they will form one or more bands on the column below the butyric acid. In practice, any band below propionic acid must be assumed to contain butyric acid, until an examination of the crystalline salts has been made.

The butyric acids can thus be differentiated from the valeric acids; *i.e.*, if a column has a band which may contain a mixture of isomers of either butyric or valeric acids, the appearance of the crystalline salts will tell which is present.

For qualitative purposes, the acids in natural products may be prepared for the separation by distillation, ether extraction, or any other procedure which will not produce any of the acids concerned through decomposition of the sample. If there is any possibility that esters of the acids (other than the glycerides) are present, and if it is desired to include these in the analysis, then a hydrolysis step must be included.

Since a quantitative recovery of the acids is not essential for the purposes of this method, direct steam distillation of the acidified sample is sufficient to get the acids away from the non-volatile constituents. If a volume of distillate equal to twice the volume in the distilling flask is collected, all the acids above acetic are almost quantitatively distilled, and the ratio of acetic (usually present in greatest amount) to other acids is decreased. This is an advantage in that any higher acids which may be present will be more readily discerned on the separation column.

When refinements such as quantitative distillation of the acids and subsequent extraction into chloroform are made, then this qualitative procedure may become a satisfactory quantitative method for the determination of the volatile acids.

METHOD

REAGENTS

A. *Separation reagents*

(1) Silicic acid—
- **(a)** Commercial silicic acid;* or
- **(b)** Laboratory silica gel prepared from commercial water glass as described below.

(2) Solvents—
- **(a)** 1% butanol in chloroform by volume: Remove ethyl alcohol from U.S.P. chloroform by washing it three times with distilled water, using a quantity of water each time approximately equal to one-half the volume of chloroform. To 1 liter of washed chloroform in separatory funnel add 10 ml of n-butanol (acid free) and shake vigorously. Add 25 ml of water and shake again. Allow chloroform to stand until clear, draw it off, and discard water.
- **(b)** 10% butanol in chloroform by volume: Using 900 ml U.S.P. chloroform, 100 ml n-butanol, and 25 ml of water, proceed as in **(a)** with this exception: Do not wash chloroform to remove ethyl alcohol.

(3) Indicators—
- **(a)** Ammonium salt of 3,6-disulfo-beta-naphthalene-azo-N-phenyl-alpha-naphthylamine ($R\text{-}NH_4$ indicator). Prepare according to Liddell & Rydon (10). Dissolve 50 mg of dye in 25 ml. of water.
- **(b)** Bromocresol green: Dissolve 100 mg of dye in 25 ml of water +1.5 ml of ca N/10 NH_4OH.
- **(c)** Thymol blue: Dissolve 100 mg of dye in 100 ml of water +2 ml of ca N/10 NaOH.
- **(d)** Phenolphthalein: 1% solution in 95% ethyl alcohol.

* Mallinckrodt's AR precipitated powder is satisfactory.

(4) Alkali solutions—
  (a) NaOH, ca N/10.
  (b) NaOH, ca N/100, silica free.
  (c) NH_4OH, ca Normal.
(5) HCl, ca N/5.
(6) H_2SO_4, ca Normal.

B. *Identification reagents*
(1) Mercurous nitrate, $HgNO_3 \cdot H_2O$.
(2) Cerous nitrate (hydrated).
(3) Potassium permanganate solution, 4%: Dissolve 20 g in 480 ml of water.
(4) Sulfuric acid (1+1).
(5) Sodium hydroxide, 40%: Dissolve 40 g of the alkali in 60 ml of water. Remove insoluble sodium carbonate by centrifuging, and decanting or siphoning off supernatant liquid.
(6) HCl, ca Normal.
(7) $HgCl_2$, 5% solution W/V.
(8) Sodium acetate-sodium chloride solution: Dissolve 25 g of sodium acetate+12 g of sodium chloride in water and make to 500 ml.
(9) Formic, acetic, propionic, n-butyric and isobutyric acids, and one of the valeric acid isomers.
  (a) Prepare N/10 solutions of these acids in the 1% butanol-chloroform solvent.
  (b) Prepare aqueous solutions of the following salts of the fatty acids, by neutralization of the acids: N/10 sodium formate, sodium acetate, sodium propionate, sodium butyrate, and sodium isobutyrate.
(10) Salicylaldehyde solution: Pipet 1.6 ml of salicylaldehyde* into 25 ml volumetric flask, dilute to volume with absolute alcohol, and mix well.

PREPARATION OF SILICA GEL (11)

To one volume of commercial water glass (sodium silicate), add two volumes of water and enough thymol blue indicator solution to give strong blue color. While stirring mechanically, add dropwise conc. hydrochloric acid until mixture is definitely and permanently acid (pink) to thymol blue. Continue stirring at least 15 min. after mixture becomes permanently acid. If the gel solidifies during addition of acid, add more water and break up mass with a heavy stirring rod, after which continue process of adding acid while stirring. Allow mixture to stand at room temp. 3 hours. Siphon off supernatant liquid and filter on Büchner funnel, using a rapid filter paper. After most of the water has drained from the gel, transfer it to a large beaker, add sufficient water to wash gel thoroughly, and filter again. Wash a total of 3 times in this manner. The filtration is slow and the washing process may require several hours. However, if a basket centrifuge is available the time required for filtration and washing may be reduced. After third washing, transfer to a large beaker, cover the gel with N/5 HCl, and allow to stand (age) 2 days at room temp. At the end of this period filter on a Büchner funnel and wash 5 times in manner described above. After the final washing, transfer gel to dish with a large surface and dry in an oven at 110°C. When the gel is dry, remove from the oven and pulverize with a large mortar and pestle.

TESTING A SILICA GEL FOR ITS SUITABILITY

Using 3–5 g of the sample gel, prepare a small partition column as described below. Check the recovery of a mixture of three acids—acetic, propionic, and butyric, adding 0.5 ml of N/10 solutions (in 1% butanol-chloroform) of each. If the

* Eastman grade is satisfactory.

bands are not clearly differentiated or recovery falls below 90%, the gel should be rejected.

PROCEDURE

Preparation of Acids for Separation:

(a) *Distillation–Suitable for high protein materials—*

(1) Use the neutralized distillate obtained in the Hillig method (12) and continue as in (2); or put 50 g of comminuted sample into a 500 ml distilling flask equipped for steam distillation, add water to a total volume of ca 150 ml, make acid to Congo red paper with H_2SO_4 (1+1), and steam distil until ca 300 ml of distillate are collected. Prevent excessive increase in volume by heating the flask with a burner. Neutralize with N/10 NaOH.

(2) Evaporate to less than 5 ml and transfer to a test tube (18×150 mm is satisfactory). Immerse test tube in steam bath and evaporate to dryness with current of air. Add to cooled residue a few glass beads and enough H_2SO_4 (1+1) to convert all the sodium salts to free acids; avoid a large excess of H_2SO_4. Make certain that all of the solid salts have been wetted with the H_2SO_4 by rotating the glass beads in the tube. Add enough solid anhydrous Na_2SO_4 to make contents at least semi-solid. Add 1 ml of 1% butanol in chloroform, and shake by rotation so that all the solid is thoroughly wetted. Transfer the chloroform with an eye dropper pipet directly to the silicic acid column described below; exercise care to avoid loosening the upper surface of the column. Wash test tube twice more with 1 ml of the 1% butanol in chloroform, shaking vigorously the last time before transferring to column.

(b) *Extraction–Suitable for high carbohydrate materials—*

For samples containing insoluble solids put 250 g into a 500 ml graduated cylinder, make to 500 ml with water, shake and comminute in a Waring blendor. Centrifuge and transfer 200 ml of the supernatant liquid to a large continuous extractor similar in design to the lactic acid extractor of Hillig (13). Add 2 ml H_2SO_4 (1+1) to make the solution acid to Congo red paper and extract with ether for about 2 hours. A direct extraction can be made with the aqueous solution of samples containing no insoluble solids. Wash the ether extract with ca 30 ml of water containing enough NaOH to neutralize all the acids. Transfer the aqueous layer to a 100 ml round bottom flask with a standard taper joint, add 1 or 2 glass beads, and acidify to Congo red paper with H_2SO_4 (1+1). Connect with a condenser by means of a trap with all glass connections and distil rapidly to 5 ml or less of residual liquid. Neutralize the distillate and continue as in (a-2).

Separation on the Column:

For amounts of acid up to 2 ml N/10 use a glass tube 11–13 mm O.D. and ca 30 cm long, one end of which is drawn out to 5 or 6 mm O.D. or to which a short glass tube of that size is sealed. For amounts of acid from 2 to 6 ml N/10 use a glass tube 22–25 mm O.D. and 15–20 cm long, one end of which is prepared as above. (A test tube, 25×150 mm or 25×200 mm, with a 2″ long glass tube 6±1 mm O.D. sealed to the bottom is satisfactory.)

The Small Partition Column:

To 5 g of commercial silicic acid (or 3 g of the laboratory prepared gel) add 1 ml of the R-NH_4 or bromcresol green indicator solution and sufficient *N* NH_4OH to give the alkaline color of the dye; .05–.10 ml is usually sufficient for the commercial product. Add some H_2O; the amount for best results must be determined for each batch of silicic acid. (For one lot of silicic acid, 1.5 ml was sufficient.) Mix well in a mortar, and first add a few ml of the 1% butanol-chloroform solvent to make a slurry, then ca 25–30 ml until the slurry will pour readily.

Plug the constricted end of the 11–13 mm tube with a small piece of cotton or

glass wool. Tilt the tube slightly to avoid airpockets and pour the gel suspension slowly into the tube. Connect the top of the tube to compressed air line in which is inserted a T-tube fastened to a stopcock which is adjusted to control the pressure. Adjust the pressure so that the excess solvent is forced through the column dropwise and rotate the column from time to time to obtain a level surface. During the removal of the excess solvent, the gel will pack down. When the gel suspension becomes so viscous that it will no longer pour, the column is ready for use. Care must be exercised to avoid drying out the column below the surface of the gel. Such drying out renders a column useless.

After the third butanol-chloroform extract (see "Preparation of Acids for Separation," above) has drained into the gel (pressure may be used here also), fill the tube above the gel with solvent, and connect to compressed air. If desired a small separatory funnel fitted with a rubber stopper may be placed in the column to serve as a reservoir, in which case the compressed air may be connected to the top of the funnel.

The Large Partition Column:

To 15 g of commercial silica gel or 10 g of the laboratory prepared product, add 3 ml of the R-NH_4 or bromcresol green indicator solution, sufficient *N* NH_4OH to give the alkaline color of the dye, and the appropriate amount of water as determined by experiment. Proceed with the 22–25 mm tube as outlined above for the small column, using the proportional amount of 1% butanol-chloroform. However, instead of a plug of cotton or glass wool, use two perforated porcelain or glass disks, between which is a thin layer of cotton or glass wool to support the silica gel in the column. The disks should fit snugly against the wall of the tube.

As the solvent percolates through the column it carries the fatty acids with it, the higher members of the series moving faster. The column changes from its alkaline color to its acid color in the sections containing acid. A mixture of formic, acetic, propionic, and butyric acids first forms one blue band (R-NH_4 indicator) at the top. Gradually this band separates into 4 distinct bands representing each of the 4 acids in the order given above reading from top to bottom on the partition column.

Collect each band as it moves through the bottom of the column, using a different receiver for each one. After the band, tentatively identified as butyric acid by comparison with a known, has moved through, change the wash solvent to 10% butanol-choloroform in order to increase the rate of movement of the propionic and acetic acid bands. Formic acid, however, is not readily removed from the column with this organic solvent. After removal of all the other bands, push the gel out of the tube and place the top portion containing the formic acid band in a small Erlenmeyer flask. Add a few ml of water and excess alkali, stir the mixture thoroughly, transfer to a small separatory funnel, draw off the chloroform, and filter the residue through a small, rapid, folded filter paper. Wash the gel with two small portions of water and aerate the filtrate to remove traces of chloroform. Transfer the filtrate to a 100 ml round bottom flask with a standard taper joint, add 1 or 2 glass beads, and make acid to Congo red paper with *N* H_2SO_4. Connect with a condenser by means of a trap with all glass connections, and distil rapidly almost to dryness (ca 1 ml of residue.) Titrate with standard alkali N/10 or N/100.

Preparation of the Separated Acids for Identification:

For identification of the fatty acids by the formation of characteristic crystalline salts, the sodium salts of the acids must be reasonably free of soluble impurities, such as indicator or excess alkali.

Add a small amount of water (10–25 ml) to the percolates of each band, and

titrate the acids with standard alkali (N/10 or N/100 as appropriate), using phenolphthalein as the indicator. At the end point a faint tinge of pink should remain in the aqueous layer after vigorously shaking the mixture. Correct the titration for a blank determined on the same amount of solvent and water. Separate each of the titrated percolates from the solvent in a separatory funnel and remove traces of the chloroform by aeration.

Transfer each of the titrated solutions, including the formic acid, to a 100 ml round-bottom flask, acidify to Congo red paper with N H_2SO_4, and distil almost to dryness (ca 1 ml residue) in the all glass apparatus used above for formic acid.

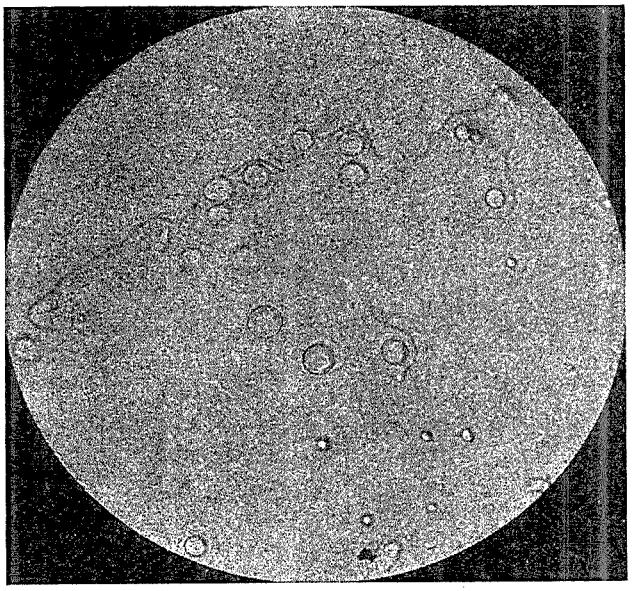

FIG. 1.—Cerous formate (100×).

To each of the distillates add ca 90% as much silica-free sodium hydroxide as was required in the titrations. This amount of alkali obviates over-neutralization, which might occur were the previously titrated amount of alkali to be added. Use no indicator in this final neutralization. Evaporate the neutralized solutions to dryness on the steam bath. Add sufficient water to the residue to give the strength of salt solution required for the qualitative tests below.

Identification of the Volatile Fatty Acids:

Compare the number, relative position, and relative rate of movement of the bands of the unknown mixture with a known mixture in order to identify tentatively the individual fatty acids. Make a confirmatory identification as directed below. The accompanying microphotographs (Figs. 1–5) illustrate the crystalline salts.

1. *Formic Acid*

(a) *Cerous formate test.*—Crush a small crystal of cerous nitrate and place a few tiny fragments on a microscope slide. To these add a drop of the solution of the salt

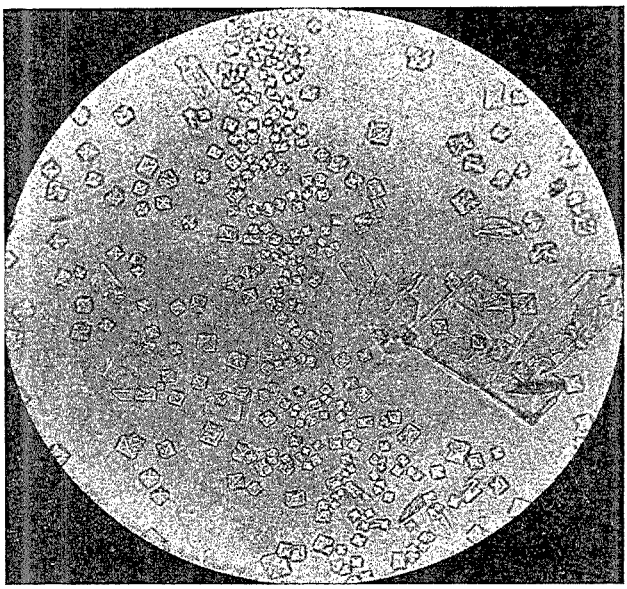

FIG. 2.—Mercurous acetate (100×).

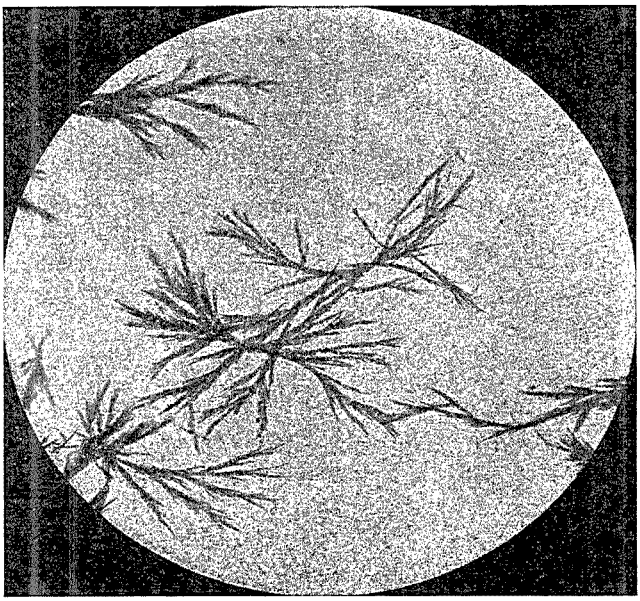

FIG. 3.—Mercurous propionate (50×).

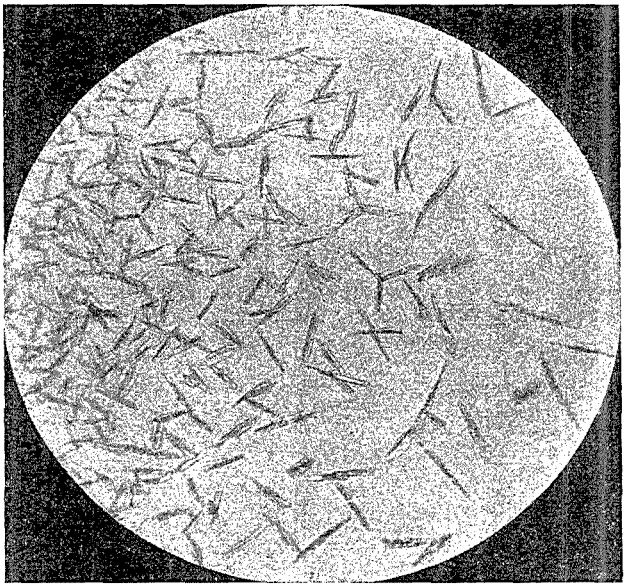

FIG. 4.—Mercurous butyrate (100×).

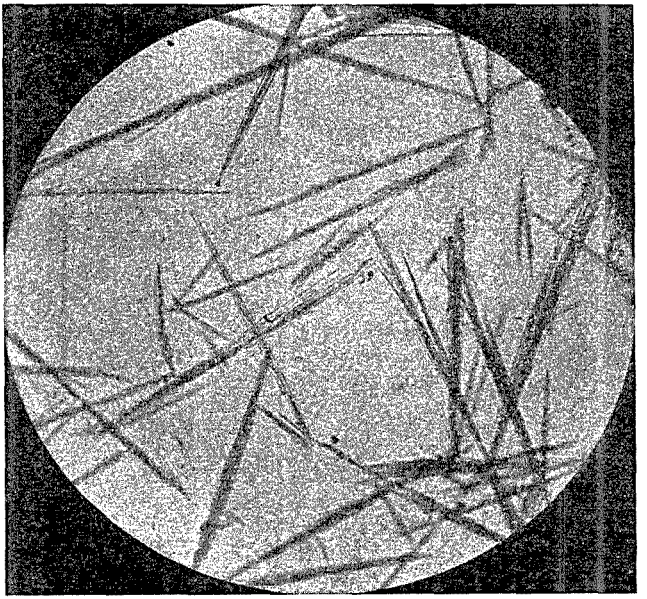

FIG. 5.—Mercurous isobutyrate (50×).

(ca N/10), tentatively identified as sodium formate, and allow to dry. Examine under a microscope with a magnification of 50–100 and compare with known cerous formate prepared in the same way.

(b) *Reduction of mercuric chloride.*—Test for formic acid on the solution used in (a) or on an aliquot of the original neutralized distillate from the food sample. Add 1 ml of *N* HCl (solution should be distinctly acid), dilute to ca 30 ml, mix, and filter through a small paper into an Erlenmeyer flask. Add 10 ml of sodium acetate-sodium chloride solution and 10 ml of 5% mercuric chloride solution and place on the steam bath for 1 hour. A precipitate indicates the presence of formic acid.

2. *Acetic Acid*

Crush, but do not grind to a powder, a small crystal of mercurous nitrate and place a few tiny fragments on a microscope slide. Add a drop of solution (ca N/10) of the salt tentatively identified as sodium acetate, being careful that initially only the outer edge of the drop makes contact with the mercurous nitrate. Examine immediately under the microscope and compare with known mercurious acetate prepared in a similar manner.

3. *Propionic Acid*

Proceed as for acetic acid.

4. *n-Butyric and Isobutyric Acids*

(a) Proceed as for acetic acid. Since examination of the mercurous salts of these two acids in a mixture usually fails to identify both acids, further tests are necessary.

(b) *Oxidation with H_2SO_4.*—Make the solution remaining from (a) up to a small volume (5 or 10 ml) with water. Transfer half of the solution to a 100 ml beaker and evaporate to dryness on the steam bath. Add 5 ml of concentrated sulfuric acid and place in a muffle furnace at 200° for 1 hour. Cool, transfer the acid to a 100 ml round bottom flask, bring the volume to about 60 ml, and distil in an all glass apparatus. Collect about 50 ml of distillate and titrate with standard alkali using phenolphthalein as indicator. If the titration is less than 2 ml of N/100 positive identification may not be possible. If the titration is more than 2 ml of N/100, return the titrated distillate to the distillation flask, acidify with *N* H_2SO_4 making the solution acid to Congo red paper, and redistill almost to dryness (ca 1 ml residue). Add ca 90% as much silica-free sodium hydroxide as was required in the titration. Evaporate to dryness on the steam bath and prepare and examine the mercurous salt as in (a). The salt, if any at this stage, should be that of pure n-butyric acid.

(c) *Oxidation with acid potassium permanganate.*—Transfer the remaining half of the solution (from b) to a 100 ml round bottom flask with standard taper joint. Add 0.5 ml of H_2SO_4 (1+1), 5 ml of 4% $KMnO_4$ solution, a few mg of Ag_2SO_4, 1 or 2 glass beads, and enough water to bring the volume to ca 20 ml. Reflux gently under a condenser (at least 2 feet long) ca 10 minutes. Cool, rinse the condenser with distilled water, and connect for distillation in an all glass apparatus. Collect the distillate in a receiver containing ca 10 ml of water. Distil slowly with the end of the condenser dipped below the surface of the liquid. Collect 5–10 ml of distillate and dilute with water to a volume of 25 ml.

Pipet an aliquot of 8 ml into a 25 ml glass stoppered cylinder, add 8 ml of 40% sodium hydroxide, mix, add 1 ml of the salicylaldehyde solution, and mix well. Place in a hot water bath at 55°C for 30 minutes.

Remove and compare the red color developed with that produced by the same amount of n-butyric acid, oxidized and otherwise treated in the same way. When

the amount of red color produced by the unknown is definitely greater than that produced by the same amount of n-butyric acid, isobutyric acid is present.

ANALYTICAL RESULTS

Several samples of commercial fatty acids were analyzed by the proposed method. Propionic acid was found to contain a small amount of acetic acid as an impurity; n-butyric acid contained acetic and propionic acids as impurities; and isobutyric acid contained propionic acid as an impurity. However, none of the homologs was found in formic and acetic acids.

In one sample of decomposed mackerel, formic, acetic, and propionic acids were found; and in a sample of decomposed dried eggs, formic and acetic acids were found.

Nine mixtures of the volatile fatty acids were analyzed to test the proposed method. The composition of the mixtures is shown in the table below; correct results were obtained for the first 5 acids in all cases. n-Valeric or isovaleric acid was added to some samples to show that these acids do not interfere.

TABLE 1.—*Ml of aqueous solution of N/10 acids*

SAMPLE NO.	FORMIC	ACETIC	PROPIONIC	N-BUTYRIC	ISOBUTYRIC	N-VALERIC	ISOVALERIC
1	1.0	2.2	0.4	0.4	0	0	0
2	0.5	3.7	0.4	2.0	0	0	0
3	1.5	3.5	0.4	0	1.5	0	0
4	0.5	2.0	0.5	1.0	0.4	0	0.4
5	0.5	2.0	0.3	0	0.4	0.4	0
6	0	2.0	0.4	0	0.4	0.4	0
7	0	2.0	0.5	1.0	0.4	0	0.4
8	0	3.5	0.4	0	1.5	0	0
9	1.0	2.0	0.4	0.4	0	0	0

Grateful acknowledgment is made to Frank R. Smith of Microanalytical Division for making the microphotographs.

SUMMARY

A method for the separation and identification of micro-amounts of the saturated volatile fatty acids (C_1–C_4) is proposed; it incorporates a number of previously published procedures with some modifications and extensions.

The volatile acids are separated on a chromatographic partition column of silicic acid, saturated with an aqueous solution of a suitable indicator, using butanol-chloroform as the second solvent. Formic, acetic, and propionic acids are separated completely from one another, but n-butyric and isobutyric acids are obtained together, free of their homologs, however.

Positive identification of all the acids except isobutyric is based upon the microscopic examination of a characteristic crystalline salt. Identification of isobutyric acid is based upon its oxidation to acetone by acid potassium permanganate.

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DEVELOPMENT OF A PHOSPHATASE TEST APPLICABLE TO CHEDDAR CHEESE

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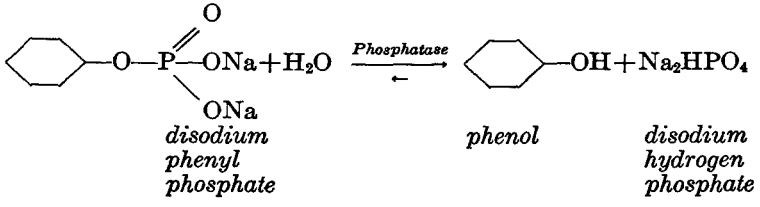
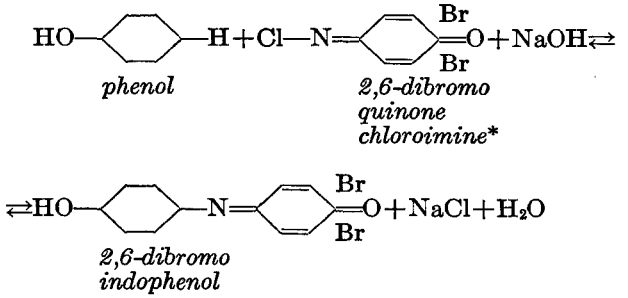
The phosphatase test for detecting under-pasteurization of milk has been modified in these laboratories so that it may be applied to Cheddar cheese to determine whether the milk used in its manufacture was adequately pasteurized. A brief description of this modified method has been published (12). This paper describes details of the development of the method and gives results of its application to a large number of cheeses of which the milk-pasteurization treatments and the ages were known.

The phosphatase test involves two reactions. The first is hydrolysis of disodium phenyl phosphate, by the phosphatase, which produces phenol.



[1]

The second is the chemical combination of phenol with 2,6-dibromo (or -dichloro) quinone chloroimine (BQC), forming blue indophenol, the quantity of which can be estimated colorimetrically.



[2]

The determination of phosphatase activity as a test for pasteurization was introduced by Kay and Graham (9), following the discovery by others (4) that milk contains a phosphate-splitting enzyme. Their test was based on a procedure developed by King and Armstrong (11), who, working with serum and bile, suggested the use of disodium phenyl phosphate to replace sodium β-glycerophosphate (used in an earlier test by Kay and Graham), and who also suggested the use of the Folin-Ciocalteu reagent to estimate phenol rather than the use of a test for inorganic phosphate. Later, following descriptions by Gibbs (6, 7) of the use of 2,6-dibromo and -dichloro quinone chloroimine as reagents for producing blue indophenol in a test for phenol, Scharer (13) introduced the use of the Gibbs reagent to simplify and sensitize the phosphatase test for milk.

Although the Scharer test has been applied widely to detect underpasteurization of milk, and has been recommended for cheese (14), definite data as to the possibility of using the test successfully for all samples of cheese, of different ages, have been lacking.

Caulfield and Martin (2), using a modification of the milk test, found less phosphatase activity in cheese than in the milk from which it was made. They found it necessary to reduce the buffering effect in the cheese by diluting the cheese before testing, and the results showed that apparently the phosphatase decreased in amount, and sometimes disappeared, during a 6-month ripening period.

Difficulties were encountered in these laboratories in attempting to determine whether or not cheese can be tested successfully to detect underpasteurization. Because the buffer capacity (proteins, salts, and acids) is greater in cheese than in milk, because cheese samples differ considerably in composition, and because titratable acidity increases with the age of the cheese, the buffers used for milk did not always produce optimal *p*H conditions, and tests on under-pasteurized—and even on raw

* Sometimes called "chloroimide."

—samples were in many instances negative for phosphatase. When the sodium compounds available as buffers were used, and when an additional reagent was added to precipitate the protein, it was necessary either to increase the quantity of buffer to such an extent as to cause interference, or to use such a small sample that underpasteurized samples yielded false negative results. Other serious difficulties were: The distinct decrease in *p*H that occurred upon addition of various protein precipitants—includ- compounds of lead, zinc, and copper; the uncertainty and extra work involved in readjusting the *p*H accurately for different samples; the blue tint in filtrates resulting from precipitation by copper; and the interfering effect of sodium pyrophosphate, used to clarify turbid filtrates.

It was therefore deemed necessary to attempt to find a reagent that would, in one operation and with all samples, establish optimal *p*H conditions and also precipitate interfering proteins and salts.

The procedure for conducting the test with a combined buffer-precipitant, barium borate-hydroxide, follows below. A few additional details, not mentioned in the previous description of the method (12), are included.

METHODS

REAGENTS

Buffer and precipitant.—Mix 18.0 g. of C. P. barium hydroxide with 8.0 g. of C. P. boric acid, add distilled water to bring the volume to 1 liter, warm and stir until as much as possible of the material has dissolved, and filter. The *p*H of the barium borate-hydroxide filtrate is approximately 10.2, and that of a mixture of 10 ml. of it with 0.5 g. of Cheddar cheese is approximately 9.6.

For adjusting the *p*H in preparing the standards and in extracting free phenol from the disodium phenyl phosphate, a buffer with a *p*H value of approximately 9.8 is prepared as follows: Mix 13.0 g. of $Ba(OH)_2 \cdot 8\ H_2O$ with 6.5 g. of H_3BO_3, make up to 1 liter, warm, stir, and filter as described above.

A mixture of 9 ml. of this buffer with 1 ml. of milk has a *p*H value of approximately 9.6, and it is suitable for tests on milk.

Butyl alcohol.—Specify n-butyl alcohol, B.P. 116°–118°C., for making standards and for quantitative work. The cheaper grade of the alcohol can be used for routine testing. Add 5 ml. of 0.1 *N* NaOH per liter, to adjust the *p*H within the proper range.

2,6-dibromoquinonechloroimine (BQC) solution (Gibbs reagent).—Dissolve a BQC tablet (Scharer test), or 20 mg. of the pure substance, in 5 ml. of methyl alcohol, transfer to a dark-colored dropper bottle, and keep in a refrigerator. Prepare a fresh solution every few days, or when it begins to turn brown.

Disodium phenyl phosphate substrate.—Specify phenol-free, crystalline disodium phenyl phosphate. Keep it in a refrigerator. Even when it is kept under these conditions a trace of phenol will be liberated. The reagent may be partially purified at this point, by washing several times with ethyl ether, filtering each time, and drying in a desiccator. Prepare a stock solution by dissolving 1 g. in 8.0 ml. of H_2O, adjusting the *p*H by adding 1.0 ml. of buffer, developing the color by adding 2 drops of BQC, incubating for 30 min. at 37°–38°C. (99–100°F.), and extracting the color with 5 ml. of n-butyl alcohol (Scharer extraction method). The extraction may need to be repeated by using 2 drops of BQC and extracting with 5 ml. of the alcohol, it being essential to extract until the alcohol layer is free of blue color. This stock solution should be kept in a refrigerator and re-extracted daily before use. Each day, for

use, prepare fresh buffer substrate by adding 1 ml. of this stock solution to 100 ml. of buffer.

Phenol standards.—Weigh exactly 1 g. of warmed phenol and make up to about 900 ml. with distilled water in a liter flask. Add 4 ml. of *N* sodium hydroxide to form sodium phenolate, which is more stable than phenol. Add 3 ml. of chloroform and water to the 1000-ml. graduation, and mix. One ml. of this stock solution contains 1 mg. (0.001 g.) of phenol. Put 1 ml. of the stock solution in a liter flask, add water to the 1000-ml. graduation, and mix. One ml. of this standard solution contains 1 mmg. (0.000001 g., or 1 unit) of phenol. Prepare additional standards containing 2, 5, 10, 20, and 40 mmg. of phenol per ml. by diluting 2 ml. of the stock solution to 1000, 5 to 1000, 5 to 500, 10 to 500, and 20 to 500 ml., respectively. From these, measure appropriate quantities into a series of about 12 tubes (graduated at 5, 10, and 15 ml.) to provide a suitable range of phenol standards containing from 0.5 to 40 units. Add distilled water to the 5 ml. graduation, 2.5 ml. of buffer to each standard containing from 0 to 25 mmg. of phenol, or 3 ml. of buffer to each standard containing more than 25 mmg. of phenol, and then water to the 10-ml. graduation. (It was previously recommended (12) that 0.25 ml. of buffer be added in each standard. Further work, however, has shown that, because of a decrease in *p*H that occurs following the addition of BQC, *p*H values nearer to the optimal value are established by adding 2.5 or 3 ml. of buffer as stated above. It was also recommended that it was preferable to use buffered filtrate from boiled cheese, instead of water, in which case it was not necessary to add additional buffer. However, phenol standards prepared with water and extracted in butyl alcohol have been found more stable than those prepared with cheese filtrate and extracted in butyl alcohol; the intensities of the colors in either case are practically the same. The butyl alcohol extracts of standards have been found sufficiently stable, when stored in a refrigerator, for use for at least 2 months.) Develop the color and extract with butyl alcohol in the manner described for the laboratory test.

SAMPLING

Take a sample, by means of a *clean* Roquefort trier, from the interior of the cheese, place it in a small tube, stopper the tube, and keep it in a refrigerator.

LABORATORY TEST

Weigh, on a *clean* balance pan or watch glass, 0.5 g. of cheese and place in a culture tube 16 or 18×150 mm. Macerate sample by means of a glass rod about 8×180 mm., add 1 ml. of buffer substrate, complete the maceration, add 9 ml. more of buffer substrate, mix contents thoroughly by means of the rod, and stopper the tube. Incubate in water bath at 37°–38°C. (99–100°F.) for 1 hour, shaking tube occasionally. Place the tube in boiling water and allow to boil until proteins are seen to separate and bubbles begin to appear, then turn off heat and allow to remain for about 5 min. Place in a rack in cold, running water and cool to the temperature of the water. Filter through 9-cm. analytical filter paper in a 5-cm. funnel, into a tube graduated at 5, 10, and 15 ml. (bottom of meniscus). Draw off filtrate to the 5-ml. graduation, add distilled water to the 10-ml. graduation, add 6 drops (0.12 ml.) of BQC reagent, mix thoroughly, and incubate at 99–100°F. for 30 minutes.

At this point, the blue color in tests with phosphatase values of about 10 or more units can be detected easily in the aqueous solution.

For samples with lower values, and for quantitative results, add n-butyl alcohol (Scharer extraction method) to the 15 ml. graduation and invert the tube slowly several times. Compare the blue color in the alcohol layer with colors of a set of standards prepared similarly, or measure the color by means of a photometer.

Since 0.5 g. of cheese is used in the test and the quantity of filtrate collected is

equal to one-half the quantity of buffer substrate used, the result is recorded as units of color or phenol equivalents (mmg.) per 0.25 g. of cheese.

It is highly desirable to conduct a blank test daily, using a 0.5-g. sample of cheese, macerating and boiling it in the tube before adding the buffer substrate.

For samples that are observed during the color development to be strongly positive (*e.g.*, between approximately 40 and 500 units), in which the quantity of BQC specified is not sufficient to combine with all of the phenol, make dilutions as follows: While the color is developing, draw off and discard exactly half the contents by means of a suction tube attached by a rubber tube to a vacuum flask, or by means of pipette with a rubber bulb on the top. Dilute with distilled water to the original volume, add 3 drops more BQC, and replace in the bath. [It was previously recommended (12) that 4 drops of the alcoholic BQC solution be added to each test, and also that, in making dilutions, 2 additional drops be added to each dilution. This quantity is adequate for all so-called border-line instances, since the quantity in 4 drops has been found sufficient to combine with approximately 20 mmg. of phenol in the test. For quantitative work in tests that develop blue color very rapidly, it is now recommended that 2 additional drops (total, 6 drops) be added before diluting and that 3 drops (as stated above) be added to each dilution.] Repeat this one-half dilution procedure as many times as necessary to reduce the color at the end of the development period to conform with the range of the standards or of the photometer. As many as 4 dilutions (factor, $\times 16$) may be required with raw-milk cheese.

FIELD TEST

The field test is conducted in the same manner as the laboratory test, except that the sample is incubated for 30 minutes, the color is developed for 15 min. (one-half the time intervals of the laboratory test), and the mixture is not filtered.

It is desirable, when the test is completed in the original tube, to have additional graduations on the tube at points 0.5 ml. above those mentioned, so that provision is made for the volume of the sample.

For roughly quantitative results, the tube is shaken thoroughly and one-half of the contents is removed by means of a suction tube when the incubation is completed, and the amount removed is replaced with distilled water before the BQC is added.

PHOTOMETRIC DETERMINATION

After adding butyl alcohol and extracting the color, centrifuge the sample for 5 minutes to break the emulsion and to remove the moisture suspended in the alcohol layer. A Babcock centrifuge can be adapted for this purpose by making special tube holders as follows: Slice a section ¼-inch thick from a rubber stopper of suitable diameter to fit in the bottom of the centrifuge cup. Glue together two cork stoppers of appropriate diameter, bore through the center a hole of proper size to hold the tube snugly, and insert the double cork section in the cup. After centrifuging, remove nearly all of the butyl alcohol by means of a pipette with a rubber bulb on the top end. Filter the alcohol into the photometer cell and read with filters having a wave length of 650 millimicrons.

If more than approximately 4 ml. is required for the particular photometer used, the test is conducted in the larger culture tube (18 mm. diameter), the tube is graduated at 5, 10, and 20 ml., and the color is extracted with 10, instead of 5, ml. of the alcohol.

PRECAUTIONS

An important cause for erroneous values is the presence of free phenol in the buffer substrate. The stock solution of disodium phenyl phosphate should be extracted daily before use, more than once if necessary, until the butyl alcohol layer is perfectly colorless.

Because of the danger of contamination from samples taken previously, the trier and the sample-weighing surface must be cleaned and wiped thoroughly before each sampling and weighing.

The pipets, tubes, and stoppers should be scrupulously clean, and it is desirable also to soak them in hot running water after cleaning.

Small plugs of cotton may be inserted in the upper ends of the pipets to prevent the possibility of saliva contaminating the tests.

The solid barium hydroxide and the buffer must be kept stoppered tightly to prevent absorption of carbon dioxide.

RESULTS

The optimal pH range.—In the preliminary experiments, with the buffers then available and with samples of various ages, the tests were negative in most instances even though the cheese was made from raw milk. Dilutions of the samples resulted usually in more or less positive tests on raw-milk cheese samples, but the values were considerably lower than those found in tests on raw milk. In these false negative tests, it was found that the *p*H values decreased during incubation and color development, and were lower than has been recommended for optimal enzyme activity and color development.

Gibbs (6) showed that the indophenol reaction is about 8 times more rapid at *p*H 10 than at 8.5, and recommended a *p*H of 9.4. Folley and Kay (5) found the optimal *p*H for mammary-gland phosphatase activity to be between about 9.4 and 10, the optimum varying slightly with different concentrations of substrate. Optimal *p*H values of about 8.8 (1) and 8.9 (9) have been reported.

Determinations were made of the optimal *p*H ranges for both reactions. For the hydrolysis reaction (reaction 1), tests were prepared with uniform concentrations of sodium tetraborate buffer and with definite, varying quantities of added acid or alkali. Milk and substrate in the usual quantities were added to each test, and the tests were made up with water to uniform volumes, incubated, and boiled. Aliquots were readjusted to *p*H 9.3 to 9.5, made up to constant volume with water, and determinations were completed in the usual manner. Similar tests were also conducted on samples of cheese. The intensities of the colors were determined by means of the photometer developed by Clifford & Brice (3), with filters at a wave length of 600 millimicrons. The readings were converted to indicate phenol equivalents by comparison with a standard curve, with appropriate corrections for the interfering color in control tests made with a corresponding quantity of BQC.

The optimal *p*H for the activity of the enzyme was approximately 9.6, as illustrated in Figure 1. A range of about 9.4 to 9.8 was found to be satisfactory.

To determine the optimal *p*H for color development (reaction 2), 1 ml. aliquots of a phenol solution containing 15 mmg. of phenol per ml. were added to aliquots of a boiled cheese filtrate, each was buffered at a differ-

ent *p*H value, and the samples were made up with water to constant volume. BQC was added and the intensities of the colors were measured after the elapse of various time intervals.

The results shown in Figure 2 indicate that, during a period of one-half hour, the greatest color intensities occurred at *p*H about 9.4 to 9.6, although tests at *p*H values as low as 9.2 and as high as 9.8 were found to be

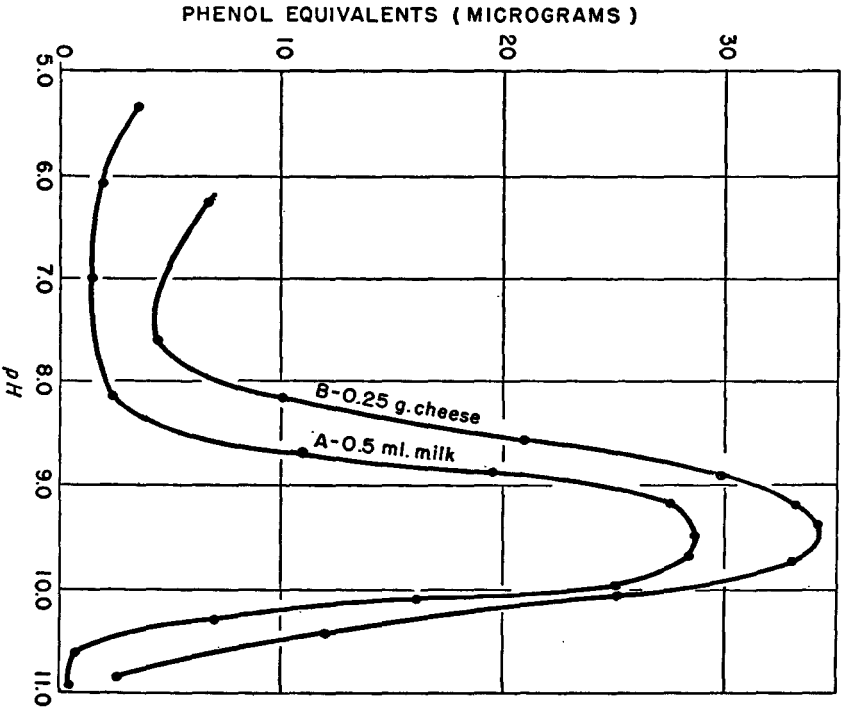


FIG. 1.—Effect of *p*H on the quantity of phenol liberated from disodium phenyl phosphate by the phosphatase enzyme in milk and in the Cheddar cheese made from it (pasteurized milk with 5 per cent raw milk added; incubation time, 1 hour; temperature, 99.5°F.).

almost equally satisfactory. At slightly higher *p*H values, the color development was relatively rapid but the blue color faded quickly and the solutions became pink. In the *p*H range of optimal activity, the color continued to increase slightly for 24 hours. The maximum peaks of the curves tended to shift toward a *p*H 8.6 to 8.8 range as time elapsed, and there was correspondingly less of the off color in the lower *p*H range.

The data in Figures 1 and 2 show that uniformly excellent results can be obtained by establishing such conditions as to insure that the *p*H

is between about 9.4 and 9.8 in all tests; that lower values, tending toward false negative tests, can be expected if the *p*H varies much above or below this range; that so-called border-line conditions can be expected to yield false negative tests if the *p*H decreases to a point as low as 8.75; and that

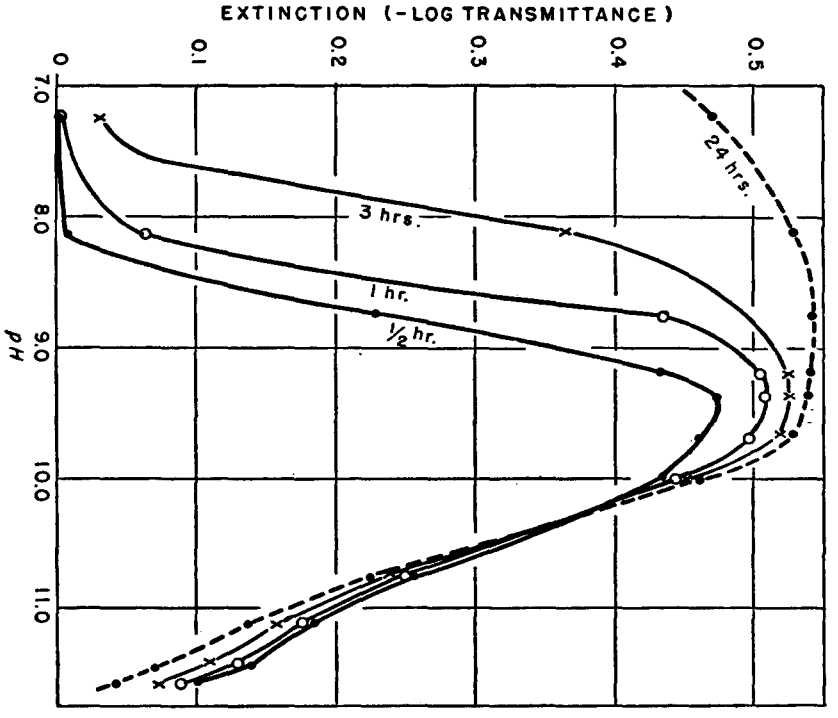


FIG. 2.—Effect of *p*H and reaction time on the development of indophenol blue color in the phosphatase test (15 mmg. phenol per 10 ml. buffered, aqueous solutions, with 6 drops BQC reagent; temperature 68°F.; measured with the Clifford and Brice photometer with 600-millimicron filters).

the amount of blue color becomes progressively less at high *p*H values, beyond approximately 10.2.

There is some evidence, based on data showing slight decreases in *p*H during both reactions, that the acidity increases during enzymic hydrolysis and also during the formation of indophenol, and that these shifts are greater in raw than in pasteurized samples. The acidity of the BQC reagent causes a slight decrease in *p*H.

Readjustment of the *p*H with the aid of a colorimetric indicator, following the addition of an acid-forming precipitant, constitutes an additional step, and further has yielded erratic results.

The *p*H value of the cheese was found in many instances to be a poor indication of the *p*H value required in a buffer to yield tests in the proper *p*H range. Samples with high percentages of solids, and those obtained from aged cheese, have relatively larger buffer capacities, and tend to yield false negative tests more often than do current, high-moisture cheeses.

Buffers and precipitants.—The effects produced by each of several dif-

TABLE 1.—*Effects of different buffers on the color intensity produced in phosphatase tests on Cheddar cheese*

(Cheese made from pasteurized milk with 2.5% raw milk added; 10 ml. buffer substrate added to 0.5 g. cheese)

BUFFER			*p*H		PHENOL EQUIVALENT
TYPE	QUANTITIES OF REAGENTS PER LITER		DURING HYDROLYSIS	DURING COLOR FORMATION	
	grams				*Mmg. per 0.25 g. cheese*
1. Barium borate, cheese buffer	18 8	barium hydroxide, boric acid	9.64	9.40	30
2. Barium borate, milk buffer	13 6.5	barium hydroxide, boric acid	9.21	9.12	26
3. Sodium borate	18 2.8	borax, sodium hydroxide	9.66	9.56	15
4. Sodium carbonate	18 15	borax, sodium carbonate	9.63	9.60	12
5. Sodium carbonate	3.75	sodium carbonate	9.80	9.52	15
6. Sodium phosphate	18 15	borax, trisodium phosphate	9.52	9.45	3
7. Sodium citrate	18 10 2	borax, sodium citrate, sodium hydroxide	9.50	9.35	9

ferent buffers were investigated. Michaelis' veronal (sodium diethyl barbiturate) buffer (10) yielded higher *p*H values than were obtained with sodium tetraborate, but the buffer capacity of veronal was found to be relatively slight between *p*H 9 and 10, and it was therefore not used.

Sodium tetraborate is used generally in tests for phosphatase. A 1.8 percent solution in water has a *p*H value of about 9.2, and it is necessary to add hydroxide to secure the desired *p*H. Jenner and Kay (8) stated

that the borate ion inhibits phosphatase activity. Our results indicated that a relatively large concentration of borate in the test decreased the amount of blue color formed (Table 1), and therefore the quantity of borate used in the test should be as small as possible.

Sodium carbonate was found to have a much greater buffer capacity than sodium hydroxide at *p*H values between 9 and 10, but in the presence of carbonates, as in the presence of phosphates, citrates, and proteins, more interfering color was produced when the BQC solution was added. These interfering compounds apparently undergo side reactions with BQC, diverting it from the coupling reaction (reaction 2) and decreasing the quantity of indophenol formed.

The use of a phosphate buffer resulted in more interference than was caused by any other compound used. It is apparent that, by precipitating phosphate, the equilibrium in the first reaction is shifted toward a greater production of phenol, in accordance with the law of mass action. The precipitation of phosphate also acts to prevent the reversal of the reaction and the possible synthesizing effect of the enzyme.

Results obtained in the test with the buffers mentioned above were compared with the results obtained with barium borate buffers, as indicated in Table 1. The phenol-equivalent values were determined by comparing the colors of the tests with those of a set of standards prepared with barium borate buffer. The tests with barium borate yielded higher values than those with any of the other buffers studied. The tabulated results show that the values obtained with barium borate were considerably higher than those obtained with the buffers containing phosphate, carbonate, or citrate.

The effects of various protein precipitants were investigated. To several series of tests prepared in the proper *p*H range with a tetraborate buffer, various precipitants were added in equivalent quantities, sufficient in all instances to precipitate the protein upon boiling, and the resulting *p*H changes were determined. The average decreases in *p*H units produced by each of several precipitants were: barium chloride, 0.24; strontium chloride, 0.23; calcium chloride, 0.34; silver nitrate, 0.69; copper sulfate, 0.99; lead acetate, 1.30; and zinc sulfate, 1.36. It was found that the use of strontium resulted in the formation of a trace of precipitate when BQC in alcohol was added. The use of barium in the test was then investigated further.

In the *p*H range of 9 to 10, experiments showed that the solubility of the barium borate-hydroxide buffer decreases rapidly as the alkalinity of the solution is decreased by the addition of the buffer to the sample. Saturated solutions containing various proportions of barium hydroxide ($Ba(OH)_2 \cdot 8\ H_2O$) and boric acid (H_3BO_3), were prepared and filtered. Aliquots of the filtrates were then dried to constant weight at 212°F. (100°C.) under partial vacuum, with CO_2-free air admitted slowly. The

respective dry weights per liter, of the solids in the solutions at different *p*H values, were: *p*H 11.1, 19.3 g.; *p*H 10.8, 18.0 g.; *p*H 10.6, 17.0 g.; *p*H 10.4, 15.4 g.; *p*H 10.2, 13.0 g.; *p*H 10.0, 11.4 g.; *p*H 9.8, 10.2 g.; *p*H 9.6, 9.1 g.; *p*H 9.4, 8.4 g.; *p*H 9.2, 7.8 g.; *p*H 9.0, 7.4 g.; and *p*H 8.9, 7.2 g.

The fact that precipitation buffering occurs in the test, in addition to ordinary homogeneous buffering, is an important property of this buffer, insuring that the *p*H is in the proper range and also that there is a minimum of solutes in the filtrate that would interfere with the subsequent reaction between phenol and BQC (reaction 2).

Apparent advantages of the barium borate-hydroxide buffer are: (1) slight solubility, reducing the interference caused by the presence of borate; (2) adequate buffer capacity, giving *p*H values that are in all instances within the proper range; (3) precipitation of proteins without causing an excessive decrease in *p*H; (4) absence of added phosphate and carbonate; (5) almost complete precipitation of the phosphates, carbonates, and citrates present, thus reducing considerable interference in the coupling reaction; and (6) combination of precipitation with buffering, in one solution, thus eliminating the extra steps of adding a precipitant and having to readjust the *p*H.

Quantity of sample.—To determine an appropriate quantity of sample, several lots of cheese were made from milks containing varying proportions of raw milk added to pasteurized milk, to represent an effect similar to that of under-pasteurization. Samples of various weights, and also dilutions of 1-g. samples, were tested. Samples recorded as 0.2 g. or less were prepared by grinding 1-g. samples in a mortar, diluting with an appropriate quantity of buffer while grinding, and using 1 ml. of the emulsion for the test. The emulsions were tested with 9 ml. of barium borate buffer substrate and, after filtration, the results were recorded as phenol equivalents per 5 ml. of filtrate, or one-half of the sample.

Some typical data are given in Table 2. The tests with 1 g. of cheese yielded somewhat higher values than with 0.5 g. The larger sample required the addition of a buffer of unusually high *p*H value, and resulted in rather large variations in *p*H values among tests on different samples.

The tests with 0.5 g. of cheese yielded values comparable with, or in some instances very slightly greater than, those obtained with 1-ml. samples of the corresponding milk. As Scharer (14) has indicated, the enzymic activity in the cheese is apparently greater than that in an equal quantity of the milk. Using 0.5-g. cheese samples, as little as 0.1 percent of raw milk in admixture with pasteurized milk could be detected as readily in the cheese itself as in the original milk mix, using a 1-ml. sample (Tables 2 and 4). It was found convenient to mix 0.5 g. of cheese in a tube and add 10 ml. of the buffer substrate specified for cheese (Table 1), with a *p*H value of approximately 10.2, yielding tests with *p*H values

usually about 9.6 and in all instances within the desired range.

In the tests on samples ground in a mortar and diluted (0.1 and 0.2 g. of cheese, dilutions of $\frac{1}{10}$ and $\frac{1}{5}$, Table 2), the phenol values were much smaller than those on 1.0-ml. samples of the corresponding milks. The

TABLE 2.—*Effects of quantity of sample on the phosphatase test, with barium borate buffer substrate, on milk and on the Cheddar cheese made from it*

SAMPLE	QUANTITY OF SAMPLE	pH OF BUFFER	pH OF TEST	PHENOL EQUIVALENT
				Mmg.
A. Milk: pasteurized (160°F., about 15 sec.), with 0.1% raw milk added	1.0 ml.	9.96	9.48	4
Resulting cheese:	1.0 g.	10.9	9.55	6
	0.5 g.	10.2	9.6	5.5
	0.2 g.	10.0	9.52	2
	0.1 g.	9.96	9.55	1.5
B. Milk: pasteurized (162°F., about 15 sec.), with 0.1% raw milk added	1.0 ml.	9.96	9.5	3.5
Resulting cheese:	1.0 g.	10.9	9.36	5
	0.5 g.	10.2	9.48	4
	0.2 g.	10.0	9.5	1
	0.1 g.	9.96	9.53	0.5
C. Milk: pasteurized (160°F., about 15 sec.), with 2.5% raw milk added	1.0 ml.	9.96	9.46	18
Resulting cheese:	1.0 g.	10.9	9.40	26.5
	0.5 g.	10.2	9.52	21
	0.2 g.	10.0	9.5	11
	0.1 g.	9.96	9.52	8.5
D. Milk: pasteurized (162°F., about 15 sec.), with 2.5% raw milk added	1.0 ml.	9.96	9.5	24
Resulting cheese:	1.0 g.	10.9	9.70	33
	0.5 g.	10.2	9.55	26
	0.2 g.	10.0	9.53	12
	0.1 g.	9.96	9.6	10

values were not considered sufficiently great to detect as little as 0.1 percent raw milk added to pasteurized milk for cheese, and were considered inadequate for detecting border-line instances.

Because of insufficient sensitivity, and also because of the extra work involved in grinding and making dilutions in a mortar or blendor, the dilution method for preparing samples was not adopted for regular testing.

In tests conducted on the filtrates from dilutions of cheese macerated with buffer, the results were still lower than those obtained on the unfiltered macerations. This fact, together with the observation that the results on whey were lower than on the corresponding milk, is additional evidence that the enzyme tends to be associated with the solids somewhat more than with the serum. For this reason, it is believed the cheese itself should be used, rather than the aqueous portion of a maceration that has settled or the filtrate from a macerated sample.

Sensitivity of the test.—Since it has been found that phosphatase values on cheese can be obtained that are of about the same magnitude as those obtained on the milk, it seems possible, by testing milks heated under exactly specified temperature-time conditions, to gain some idea of the effects of different heat treatments on the enzymic activity in the cheese.

Preliminary experiments have been begun to determine the temperature-time conditions required to inactivate the enzyme in milk. A coil-type tubular Mallory heater is used, with which the milk is heated from room temperature to a specified pasteurizing temperature in less than 1 second, and the samples are collected at the outlet in 16-mm. tubes immersed in ice water.

Whole milks were held at specified temperatures for 15 seconds, with a variation of not more than 1 second, and samples of each, subjected to different temperatures between 155° and 165°F., were tested with two different buffers and for two different time periods. The results, shown in Table 3, indicate that the enzyme was not completely inactivated at 160° in 15 seconds, but was completely inactivated at 162° in that time. The marked difference between the phosphatase activity of milk held at 157° for 15 seconds and that of milk held at 159° for the same period shows the extreme sensitivity of the test in detecting underpasteurization.

The results in Table 3, checked with four variations of the test, seem to give a clear-cut indication concerning thermal inactivation during the specified time period. These experiments are considered as preliminary only, and are to be re-checked and also enlarged to include a range, within practical limits, of temperatures and holding-time periods.

The results show that the test described can be used effectively as a field test, for routine control in plants, with an incubation period as short as 10 minutes at about 99.5°F. and a development period of 10 minutes at room temperature. The test designed for laboratory conditions is much more sensitive, however, and the results indicate also that the test with barium borate buffer substrate yields more color than the test with PHOSPHAX* buffer substrate.

The tests described in Table 3 were not heated to boiling temperature at the end of incubation, and the protein was not removed. The *p*H values

* The trade name given by the Applied Research Institute to their prepared, ready-to-use buffer substrate tablet.

were: 20-minute period, with PHOS-PHAX buffer, about 9.55 during incubation and 8.75 to 8.9 during color development; barium borate buffer, about 9.6 during incubation and 9.3 to 9.4 during color development.

Heating the tests to the boiling temperature after incubation reduced the *p*H very markedly, when the PHOS-PHAX buffer was used, with the result that under-pasteurized samples did not yield any blue color with BQC.

Several series of Cheddar cheese were made from 400-lb. lots of pasteurized milk to which different proportions of raw milk had been added, and the phosphatase values were determined on samples of the raw milk,

TABLE 3.—*Effects of various pasteurizing temperatures on the results of milk phosphatase tests conducted in two different time periods and with different buffers*

TEMPERATURE AT WHICH MILK WAS HELD FOR 15 SECONDS	PHENOL EQUIVALENTS IN TESTS WITH DIFFERENT BUFFERS			
	TEST: 10 MIN. INCUBATION AT 99.5°F., 10 MIN. COLOR DEVELOPMENT AT ROOM TEMPERATURE (85°F.)		TEST: 1 HR. INCUBATION, AND 30 MIN. COLOR DEVELOPMENT, AT 99.5°F.	
	BUFFER: PHOS-PHAX	BUFFER: BARIUM BORATE	BUFFER: PHOS-PHAX	BUFFER: BARIUM BORATE
°F.	*Mmg. per 0.5 ml.*	*Mmg. per 0.5 ml.*	*Mmg. per 0.5 ml.*	*Mmg. per 0.5 ml.*
Raw milk	70	80	256	385
155	18	36	100	110
157	14	32	80	85
159	2.5	4	8	10
160	2	2.5	6	6.5
161	0.4	0.5	2	2.5
162	0	0	0	0
163	0	0	0	0

the pasteurized milk, the mixed milk in the vat, and the resulting cheese and whey, by the laboratory method with barium borate buffer substrate.

Results obtained in these tests are shown in Table 4. The quantities of samples were 1.0 ml. of milk, 0.5 g. of cheese, and 1.0 ml. of whey. Since the quantity of filtrate used was approximately one-half the quantity of liquid in the sample-plus-buffer substrate mixture, the results were recorded per 0.5 ml. of milk, 0.25 g. of cheese, and 0.5 ml. of whey, respectively.

The results showed that as little as 0.1 percent of raw milk added to pasteurized milk can be detected in the cheese. The tests with 0.5-g. samples of cheese were about as sensitive in the low-value range as those with 1.0-ml. samples of milk. These results showed also that the enzymic activity of the milk is concentrated in the cheese at the expense of the whey.

Advantages of extracting the color with butyl alcohol.—It was found to be

practically impossible to estimate accurately, in aqueous solutions, the amounts of blue color in tests on border-line samples. Therefore the butyl alcohol method proposed by Scharer (13, 14) was studied. Gibbs (6) has pointed out that spectrophotometry is by far the most precise method of evaluating the indophenol blue color, and that the proper wave length for readings in aqueous solutions is about 600 millimicrons. Curves showing results of spectrophotometric analyses are presented in Figure 3. The chloroimine reagent used in this series was a methyl alcohol solution of BQC tablets, procured under the trade name of INDO-PHAX. The results show that, in using a photometer, the readings in butyl alcohol ex-

TABLE 4.—*Results of phosphatase tests on pasteurized (162° F., about 15 seconds) milk containing various proportions of raw milk, and on the resulting cheese and whey*

MILK LOT NO.	PERCENT RAW MILK ADDED TO PASTEURIZED MILK	PHENOL EQUIVALENTS		
		VAT MILK	CHEESE	WHEY
		Mmg. per 0.5 ml.	*Mmg. per 0.25 g.*	*Mmg. per 0.5 ml.*
1	0	1	1	<1
2	0.1	4	5.5	3
	0.25	5	6	4.5
3	2.5	18	21	15
	5.0	28	34	22
4	100	480	550	400
5	0	1.5	2	1
6	0.1	4	4	3
	2.5	24	26	15
7	100	460	500	410

tracts should be made with filters transmitting light with a wave length of approximately 650 millimicrons, and in water with filters of approximately 600 millimicrons. They show also that the extinction (intensity of blue color) is considerably greater in a butyl alcohol extract than in aqueous solution, and that the interference is much less at 650 millimicrons than at 600, and much less in butyl alcohol than in water.

Butyl alcohol intensifies the blue color and extracts nearly all of it, leaving most of the interfering color in the aqueous layer. It is apparent that the butyl alcohol extraction method is much to be preferred, especially for estimating small amounts of blue color. Since the blue color is more intense in the alcohol than in water, we have avoided increasing the intensity, which would cause border-line cases to appear more positive,

and recommended extracting with 5 ml. of the alcohol rather than with a smaller quantity.

Curves made on tests containing different quantities of BQC, included in Figure 3, show the marked interference present when an excess quantity of BQC is used. It is necessary, in order to measure the intensity of the blue color accurately, to conduct control tests on phosphatase-free samples without phenol present, but with the corresponding quantity of BQC, and make appropriate corrections for the interfering color.

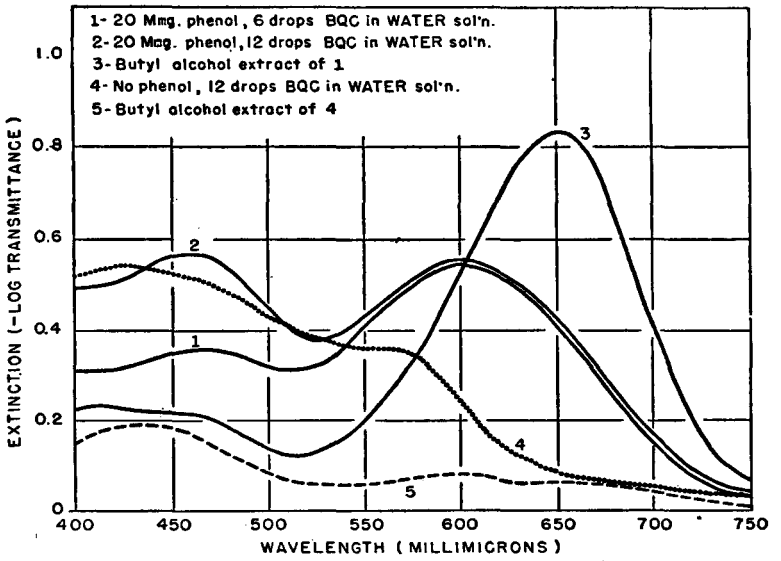


FIG. 3.—Spectrophotometric analyses of color produced in the phosphatase test on Cheddar cheese (boiled cheese samples with 20 mmg. of phenol added and also without phenol, per 10 ml. of filtrate at *p*H 9.5; measured with General Electric recording spectrophotometer).

Standard curves for photometric analyses were prepared by determining the color intensities in tests made with different quantities of phenol from 0 to 30 mmg., and with different quantities of BQC from 0 to 12 drops for each quantity of phenol. The percentage transmission readings, compared with the transmission in water, were then converted to extinction (−log transmittance) values, and it was found that the resulting data followed a straight-line pattern, showing that the intensity of blue color is a linear function of the quantity of phenol present in the test.

Spectrophotometric analyses were made of tests in which the indophenol color was developed at various *p*H levels. It was found that the intensity of the interfering color increased progressively as the *p*H

increased, especially above 10. Apparently, high alkalinity is responsible for the fact that false negative tests are obtained when the *p*H is above the proper range (Figure 2).

Gibbs (7) showed that, in the presence of excess hydroxyl ions, BQC is reacted upon to form the quinone monoxime, in which the chlorine atom is replaced by a hydroxyl group, and which has a reddish tint. This side reaction, like the reactions produced by proteins and other interfering substances mentioned (Table 1), uses BQC to yield an off color and diverts it away from the indophenol reaction (reaction 2).

Results of applying the test to cheese.—Data obtained in tests on more than 350 samples of Cheddar cheese, of which records of the milk treatment are available, using the barium borate buffer-precipitant substrate, are summarized in Table 5. All samples of cheese made from raw milk gave very strongly positive values; some of these were more than 1 year old, and one was a canned cheese more than 5 years old. All samples of cheese made from underpasteurized milk gave results that were positive in varying degree, and none of the cheeses made from milk pasteurized at 143°F. for 30 minutes, or at 160° or higher for about 15 seconds, gave values higher than 4 units, regardless of the age of the cheese. It was found that a decrease of 2° in the pasteurizing temperature for 30 minutes, or the addition of as little as 0.1 percent of raw milk to pasteurized milk, could be detected in the cheese. Data for cheese of different ages indicate that there was some decrease of enzymic activity in the cheese as it aged for a long period, but such decrease was so slight as not to impair the usefulness of the test.

A fact of importance in connection with the records and data in Table 5 is that the pasteurizing process was not under absolutely accurate control at first. The pasteurizer used was a vertical, continuous-flow heater with a revolving agitator, the milk flowing in at the bottom and out through a vertical pipe at the top. The duration of heating was calculated as 15 seconds, on the basis of the average rate of flow and the volume of the heating chamber, but the rate of flow varied slightly and there was no assurance that some particles of the milk did not pass through the heater in more or less than 15 seconds. The temperature of the milk in the pasteurizer varied as much as 2°F.; the lowest temperature noted for each lot was recorded as the pasteurizing temperature. The milk in the first vat, and the cheese made from it, yielded more positive tests, and higher values, than that in the second vat. It was found eventually that a small quantity of under-pasteurized milk was being forced upward into the outlet pipe when the pasteurizer was being started, before the full flow of milk was turned on. The maximum values of 0.5 unit for cheese from milk recorded as pasteurized at 164°, 0.5 to 1 for that at 163°, and 1 to 4 for that at 162°, shown in Table 5, were all obtained on cheese made under the conditions mentioned.

TABLE 5.—*Results of phosphatase tests, with barium borate buffer substrate, on samples of Cheddar cheese of various ages and made from milks subjected to various pasteurization treatments*

AGE OF CHEESE	PASTEURIZATION RECORD	NUMBER OF CHEESES TESTED	PHENOL EQUIVALENTS PER 0.25 G. CHEESE		
			MAX.	MIN.	AV.
			Mmg.	*Mmg.*	*Mmg.*
6 yrs.[1]	raw	1			135
4½ to 5 yrs.[1]	141° F., 30 min.	1			5
4 yrs.[1]	160°, about 15 sec.	1			0
3 to 4 yrs.	158°, about 15 sec.	1			1
	raw	2	140	105	122
2 to 3 yrs.	159°, about 15 sec.	4	1	0	0.5
	160°, about 15 sec.	11	1	0	0.6
	161°, about 15 sec.	2	1	0	0.5
	162°, about 15 sec.	7	1	0	0.4
	163°, about 15 sec.	24	0.5	0	0.1
	165°, about 15 sec.	15			0
1 to 2 yrs.	150°, 15 sec.	2	68	77	72
	160°, about 15 sec.	6	3	0	2
	161°, 15 sec.	1			0.5
	162°, about 15 sec.	66	4	0	0.4
	163°, about 15 sec.	25	0.5	0	0.06
	164°, about 15 sec.	16			0
	170°, about 15 sec.	1			0
	135°, 30 min.	2	40	95	67
	143°, 30 min.	2			0
	raw	7	450	70	118
6 mos. to 1 yr.	162°, about 15 sec.	20	2	0	1
	163°, about 15 sec.	6	1	0	0.4
	raw	7	540	380	410
3 to 6 mos.	162°, about 15 sec.	51	4	0	1.5
	164°, about 15 sec.	22	0.5	0	0.1
	raw	2	400	380	390
less than 3 mos.	162°, about 15 sec.	47	4	0	1.7
	raw	3	490	470	485

[1] Canned cheese.

Approximately 40 samples have been tested, which were obtained from cheese made from milks pasteurized at a temperature recorded as 162°, and made after steps were taken to correct the conditions described. Among these, none has yielded a test of greater than 0.5 unit.

The cheese tested was made from milks to which were added several commercial starters, and different organisms, isolated from raw- and from pasteurized-milk cheese. None of the starters or cheese-isolated organisms used has caused the test on the cheese to become positive.

DISCUSSION

The test with a combined buffer-precipitant substrate, regulating the

*p*H correctly and precipitating interfering substances in one operation, was designed to eliminate interfering conditions and make the results depend more directly upon the amount of phosphatase activity.

The quantities of barium hydroxide and boric acid specified (Table 1) may be weighed on a cream test balance, if an analytical balance is not available, without appreciably affecting the results. The barium buffers specified for cheese and for milk may be used interchangeably if desired, but for laboratory testing it is preferable to prepare both buffers and use each for the purpose specified.

It should not be inferred from the above description of laboratory work that photometric equipment and a wide range of standards are needed for routine control in the plant. When it is necessary only to detect underpasteurization, a few standards, *e.g.*, 1, 2, and 5 units, may be sufficient. Also, under such conditions, boiling and filtering the tests can be omitted, and extraction with butyl alcohol is necessary for only those tests in which the results are doubtful.

The later results with more accurate pasteurization control, described under "results of applying the test to cheese," indicate that a suggested standard of slightly higher than 5 units for detecting underpasteurization, as mentioned in the earlier description (12), could properly be revised to a somewhat lower number of units. It appears, however, from the data given under "sensitivity of the test," that setting a standard of 0 for adequate pasteurization would be equivalent, in effect, to raising the present temperature standard for "flash" pasteurization to some value above 160°F. Also, such a standard would allow no tolerance for any trace of color that may develop because of insufficient purity of the reagents.

SUMMARY

In experiments designed to determine whether or not a phosphatase test could be applied successfully to detect the use of raw milk or underpasteurized milk in Cheddar cheese, it was found necessary to investigate the reasons for failures in the test and to make an intensive study of the conditions necessary for accurate determinations of the activity of the phosphatase enzyme in the cheese.

Investigations were made of the optimal *p*H range for the test, the suitability of different buffers and precipitants, the effects of various quantities of cheese samples, the sensitivity of the test, and the advantages of using butyl alcohol for extracting the blue color and evaluating the results.

The optimal *p*H was found to be approximately 9.6, and a *p*H not lower than approximately 9.4 or not higher than approximately 9.8 was almost equally satisfactory. The use of a barium borate-hydroxide buffer is proposed for establishing the *p*H uniformly within the proper range and for precipitating proteins and certain other interfering substances.

Application of this test to more than 350 samples of Cheddar cheese, of

which records of the milk treatment are available, showed that a very slight lowering below the proper pasteurizing temperature, and contamination with as little as 0.1 percent of raw milk in pasteurized milk, could be detected in the cheese test. The activity of the phosphatase enzyme in raw-milk cheese was detected very readily in all instances, regardless of the age of the cheese. No instances were found in which phosphatase activity was produced in the cheese by starter organisms or by other organisms that occur commonly in the interior of Cheddar cheese.

ACKNOWLEDGMENT

We acknowledge the technical assistance of P. A. Clifford of the Food Division, and G. R. Clark and Miss Rachel Sclar of the Cosmetic Division, Food and Drug Administration, in conducting the spectrophotometric analyses described herein.

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ERRATA—MAY JOURNAL

In the paper on "Determination of Thiocyanate Nitrogen in Organic Thiocyanates and Mixtures," by John W. Elmore, published in the preceding number of *This Journal*, **28**, p. 364, first paragraph, 7th line, "inorganic groups" should read "organic groups."

NOTES

Determination of Salt in Butter by a Mercurimetric Method*

A mercurimetric titration method for determining chlorine in biological fluids has been suggested by Schales and Schales.[1] It is stated that this method has been found to be free from errors frequently encountered in other chloride determination procedures. Investigations based upon these findings have been made by this laboratory on the use of the mercurimetric method for determining the salt content of butter. The results indicate that the mercurimetric titration method can be successfully adapted to this use. The method is somewhat more delicate, but gives results comparable in accuracy to titrating with standard silver nitrate using potassium dichromate as an indicator. It also has the advantage of giving a sharp and permanent end point; an intense violet-blue color develops upon the addition of one drop of mercuric nitrate in excess.

METHOD

REAGENTS

Mercuric nitrate solution—0.1711 *N*. Dissolve 29.31 g. of mercuric nitrate C.P. in a few hundred ml. of water, with the addition of exactly 40 ml. of 2 *N* nitric acid, and make the solution to one liter volume with water.

s-Diphenylcarbazone indicator solution. Dissolve 100 mg. of s-diphenylcarbazone (Eastman 4459) in 100 ml. of neutral alcohol and store in a dark place, preferably in a refrigerator.

Standard sodium chloride solution—0.1 *N*. Dissolve 5.845 g. of sodium chloride C.P., dried at 120°, in water and make to one liter. Use the standard sodium chloride to standardize each new solution of mercuric nitrate.

PROCEDURE

Weigh a 10 g. sample of butter into a counterpoised beaker. Secure 250 ml. of washings from sample as directed in the Official Method[2] for the determination of salt in butter. (Or, for plant laboratory procedure rinse dry residue, from Kohman method of fat and moisture determinations, into 250 ml. volumetric flask with 3 separate 30 ml. portions of hot distilled water. Cool the rinsings; dilute to 250 ml. graduation mark on flask, and mix thoroly.)

Pipet a 25 ml. portion of solution into 125 ml. Erlenmeyer flask. Add exactly 0.6 ml. of s-diphenylcarbazone indicator. Titrate slowly with standard mercuric nitrate solution using a buret graduated in 0.1 ml. divisions. Single drops should be approximately 0.05 ml. The clear and colorless solution turns a pale violet color on the addition of 1 drop of mercuric nitrate at end point, and an intense violet-blue color on addition of 1 drop of mercuric nitrate in excess. The mls of mercuric nitrate used in titration equal percentage of salt in sample.

The exact amount of nitric acid must be used in preparing the standard mercuric nitrate solution, since if more or less acid is used end point will not be as sharp. The specified amount of indicator should always be used, and best results are secured if indicator is stored not longer than 1 month.

The table shows agreement obtained in comparing mercurimetric method and the Official Method[2] in recovery tests on samples of butter containing known added amounts of salt.

Recovery Tests by the Mercurimetric and Official Methods on Butter of Known Salt Content

SAMPLE	KNOWN ADDED AMOUNT OF NaCl	MERCURIMETRIC METHOD NaCl	OFFICIAL METHOD NaCl
	Percentage	*Percentage*	*Percentage*
(1)	0.994	0.99	1.02
(2)	1.987	1.98	2.04
(3)	2.981	2.95	3.06
(4)	3.974	3.98	3.99
(5)	5.026	5.00	5.06

* By W. S. ARBUCKLE (Division of Dairy Husbandry, Texas Agricultural Experiment Station, Agricultural and Mechanical College of Texas).

[1] *J. Biol. Chem.*, 140 879 (1941).

BOOK REVIEWS

Scientific Societies in the United States. By RALPH S. BATES, Ph.D. A publication of The Technology Press, Massachusetts Institute of Technology, New York—John Wiley and Sons, Inc.; London—Chapman and Hall, Ltd. (1945). v+246 pp. Price $3.50.

As stated by Dr. Bates in his Preface: "The history and the influence of the scientific societies of the United States are the themes dealt with in this book. . . . As hitherto no extensive account of the history and work of American scientific societies has appeared, perhaps this book on the subject will help to fill a gap in the literature dealing with the intellectual history of our country." In the opinion of the reviewer the task of preparing a readable work of this kind that is concise, and at the same time fairly inclusive, has been exceedingly well performed. The book will not only be helpful to the student of the history of science in America but it will also serve a useful purpose in pointing out the mutual relationships of various scientific societies, and in indicating why some of them have succeeded and why others have failed.

Members of so specialized a scientific body as the Association of Official Agricultural Chemists, whose chief concern is with methods of chemical analyses, may possibly wish to qualify somewhat the advertising notice on the wrapper of the book that it is "a full-scale account of the evolution of American scientific organizations, including the main national scientific societies, and the state, local, specialized and technological societies." It is stated, for example, on pg. 114 (not pg. 144 as given in the Index) that "the Association of Official Agricultural Chemists was founded in 1884," but no reference is made to the fact that this Association (the A.O.A.C.) was the direct outgrowth of a preexisting society called "The Convention of Agricultural Chemists," which was organized at Washington in July 1880, and published proceedings of this and of subsequent meetings at Boston, Cincinnati, Atlanta, and Philadelphia. Similarly, no reference is made to the formation of more highly specialized agricultural chemical societies, such as those of Soil Chemists, Leather Chemists, Cereal Chemists, Oil Chemists, Feed Control Chemists, etc., whose work is closely related to various activities of the A.O.A.C., but whose members felt the need of organizations with more highly specialized fields of activity.

Another oversight that will be noted by the older members of the A.O.A.C. is the omission of any reference to the International Congresses of Pure and Applied Chemistry which owed their origin to an international meeting of chemists under the leadership of Dr. H. W. Wiley at the Chicago Exposition of 1893, and in whose activities members of the A.O.A.C. and of other American chemical societies took a leading part until this important organization went out of existence as a result of World War I. The work of the International Congresses of Chemistry, frequently held in connection with expositions, was partially revived with the organization of the International Union of Chemistry in 1919 (mentioned on page 164), some of whose meetings have also coincided with important expositions. The influence of national and international expositions upon the development and growth of scientific societies is an interesting phase of the subject that might be considered in future editions of the book.

Agricultural chemists of the Southern States will regret that Dr. Bates did not include in his list of agricultural societies the important Louisiana Sugar Planters' Association, whose early members, many of them educated in the technical schools of France, were among the most highly trained agriculturists of the United States. Their monthly meetings dealt with problems pertaining to all the branches of agricultural science; and their weekly publication, The Louisiana Planter and Sugar Manufacturer reported the proceedings of their meetings and exerted a wide influ-

ence, two to six decades ago, among all who were interested in the production not only of sugar, but of rice, cotton, and other crops of the South. The scientific activities of the Louisiana Sugar Planters' Association were later absorbed by the International Society of Sugar Cane Technologists which, though highly specialized in focusing attention on a single crop, is broadly organized, and is establishing cooperation between chemists, agronomists, entomologists, pathologists, geneticists, and workers in other fields of science in all the cane-producing countries of the world. This important organization, established in 1924 as an offshoot of the Pan-Pacific Food Conservation Conference, might well have been mentioned in Dr. Bates' list of international scientific societies.

It must be recognized, however, that a discussion of all the specialized scientific societies (which, as Dr. Bates correctly states, "have multiplied *ad infinitum*") is not possible in a general work, and that this limitation, so far as it goes, should not cause one to overlook the many praiseworthy features of the present volume. Its first four chapters give an excellent account of the historic development of scientific societies in America for a period of over two hundred and fifty years, from the founding of Increase Mather's Boston Philosophical Society in 1683 to the establishment of the various emergency research organizations that were called into existence by World War II.

In the concluding (fifth) chapter of the book the various influences now at work in promoting "the increase and diffusion of knowledge" are ably discussed. In addition to such influences as scientific conventions, abstract journals, popular scientific magazines, scientific museums, intersociety cooperation, etc., discussed by Dr. Bates, mention might also have been made of the growing employment of microfilms. From the experience gained during the present war, these seem destined to offer almost limitless opportunities to the scientific societies of the future.

A well-classified 28-page Bibliography of the principal guides to American Scientific Societies, and a full 26-page Index, contribute to the usefulness of a volume which to a high degree fulfills the hope expressed by its author that it will help "to fill a gap in the literature dealing with the intellectual history of our country."

C. A. BROWNE

Analysis of Foods. By ANDREW L. WINTON and KATE BARBER WINTON. John Wiley & Sons, Inc., New York; Chapman & Hall, Ltd., London (1945). 6×9 inches, 999 pages, 208 illustrations, cloth. Price $12.00.

A book by the Wintons is distinctly an event in the lives of regulatory chemists; indeed one might well call it a "blessed event." The successive volumes of "The Structure and Composition of Foods," issued between 1932 and 1939, have now been supplemented by this equally encyclopedic compendium of analytical methods.

After a few preliminary pages on apparatus, reagents, microscopic and physical methods, the main subject of chemical methods takes the center of the stage and holds it for nearly 1,000 closely packed pages, covering about that number of methods in ample detail, and well illustrated by excellent cuts. The spectrum extends from ultimate analysis through proximate analysis and on to analytical methods for specific inorganic constituents and for a score of classes of organic constituents including amino acids, alcohols, acids, vitamins, colors, and preservatives. The first half of the book is devoted to such methods in general; the second to those, and other useful methods, as they apply to the various twelve broad classes of food, not forgetting such minority groups as alkaloidal products (tea, coffee, and cacao products to you), spices, flavors, leaven, and salt (a mere pinch of half a page).

By an ingenious use of key letters, numerals, and topic heads on the left hand pages, and subheads on the right hand ones, the usefulness of the text as a reference book is greatly enhanced. Another excellent device is the italicizing of the key re-

agents, pieces of apparatus, and the like, which enables the reader to glance over a method and suck the meat out of it, so to speak, without reading it in detail. Since the book quite wisely makes no pretense of being a critical compilation of methods; and since, further, certain groups of methods (sucrose and reducing sugars; carotenoids and vitamin A, for example) run to 20 or 30 pages, the italics device is a very great time saver indeed.

The question of which of alternate methods is "best" for any given purpose, and the still thornier one of interpretation of results, has been cannily left to the specialists, where such questions belong. One suspects that even "the experts" may many times disagree on such matters. Such compilations are after all for the "general practitioner" in food chemistry, and not for the man who specializes in a narrow field. Here, and here only, can one find under one cover a representative selection of the most useful methods for solving the riddles of normal and abnormal food composition, together with an exhaustive but carefully sifted bibliography which takes the reader back to the original sources.

In the opinion of this reviewer, the book is a "must" for every director of a regulatory laboratory, and for every professor who is giving instruction in any of the numerous subdivisions of food analysis.

To call attention to a few minor shortcomings is to run the risk of seeming captious. For no author can hope to bring such a monumental compilation strictly up-to-date. There comes a time when he must say to himself, "This is as good and as complete and as up-to-date as I can make it and ever get it published." There has never been a time when one has had to run so fast in order to stay in the same place as in the present era of almost feverish change in food technology, organic analysis, vitamin chemistry, and in the other fields which form a part of the intricate mosaic of food analysis. The authors do not labor such an obvious point, being no doubt mindful of the French proverb, "Il s'excuse, s'accuse." But it can be seen between the lines. For example (page 787), they say of the Waterhouse test " . . . this test distinguishes with some degree of certainty normal and imitation butter from oleomargarine, at least of the type on the American market forty years ago."

A glance at the bibliography at the chapter ends discloses few references later than 1943, and most of them are not later than 1941. Perhaps this is inevitable but one cannot but regret that even our own *Journal* was not often consulted later than 1941, since a method which has run the gauntlet of A.O.A.C. collaborative study has much to recommend it. Hartmann's general method for the polybasic fruit acids is quoted, and that was published in August, 1943. But we find no reference to the work of Fitelson on squalene as a measure of olive oil, to Clifford's painstaking work on fluorine determination, or to Wichmann's fundamental studies on ash—to name only a few. The only data cited on the composition of hens' eggs (page 843) are from a German text published in 1914, while the figures of Mitchel *et al.*, published in *This Journal* in 1932 and 1933, are not even referred to, although they were given in Volume III on "The Structure and Composition of Foods."

A few trifles are noted in passing, with no other purpose than the hope of making a fine book still better when the authors revise it a few years hence.

The reader is at times given "the run-around." For example, on page 202 under "I, C6a" (Starch) he is referred to "II, A2," which however returns him to the place from whence he came without further enlightment. Again on page 309 (Carotenoids) one is referred to "II, A1 and B2" but again he returns, sad and empty handed, to "I, C10" where he started. It is only because the general scheme of indexing and cross reference is so helpful and intelligent that one notices such little things.

On page xi, in a very useful little outline for a short course in food analysis, the student is directed to determine nitrogen in cereal foods and "calculate the protein by the factor 6.25." The conventional factor of 5.7 is given elsewhere, however.

The composition tables, usually at or near the chapter beginnings, are of course somewhat subsidiary; but most readers would still like to know the source of the data, which was quite uniformly given in the "Structure and Composition" volumes. Such citations are infrequent in the book under review.

While the average composition of milk (page 707) is perhaps as mythical a concept as that of the average man, it is useful if the calculation is made on a national, and not on a regional basis. Then too there have been many changes in milk composition since 1914. One could not be expected to spot Government Exhibit No. 3 in the transcript of the hearing to establish a standard of identity for evaporated milk under the Food, Drug and Cosmetic Act, and yet the weighted averages for doorstep milk in the entire U. S. which appear there are nevertheless interesting and valuable (fat 3.806%, solids not fat 8.659%). But "Market Milk" by Kelly and Clement of the U. S. Bureau of Dairy Industry is readily accessible and the averages there given are also broad gauge figures tallying well with those just mentioned.

It may be doubted, especially in the light of Wichmann's work on ash already mentioned, whether (page 710) one can correct the ash of milk for the amount of inorganic preservative added.

An example of the utter impossibility of keeping an encyclopedic reference work up-to-date is the citation on page 482 of cocoanut oil as an important ingredient of "lard and butter substitutes." What with the tariff and the war, the domestic oils now rule the roost.

But no review of a Winton book would be complete, or tolerable, if it did not pay tribute to the shrewd wisdom and dry humor which permeate the whole volume. This is an elusive thing (and unfortunately not a common thing in such books), but an example or two might be given for the benefit of "the generation which knows not Joseph." The authors coin the word "nifext" in place of the time-honored and cumbersome "nitrogen-free extract," and then say with perfect poker faces:—"It is not a capital offense such as NFX would be."

Again the selection of illustrations may or not be a deliberate hint that, in our modern age of gadgetry, there is nothing new under the sun in many fundamental pieces of apparatus. Figures 32 and 33 (Kjeldahl racks), Figure 40 (Johnson fat extractor), and Figure 44 ("Bunsen burner with wire gauze cap") will seem "quaint" to many; and yet a few doors away (Figures 15 and 16) one finds excellent cuts of the GE recording photoelectric spectrophotometer and the B&L quartz spectrograph in all their ultra-modern splendor.

A quotation from page 55 will perhaps furnish an appropriate close, since it has the authentic Winton flavor. It has to do with the big six "proximate" constituents, of which they say, "All are crude figures, fiber being most often so branded." And a little later: "The indictment that the six constituents are crude is not overstated; nevertheless the results on this century-old plan, employing methods that are (and should remain) much as first proposed, are for many purposes quite as valuable as if every constituent, known or unknown, were determined with impeccable accuracy. They are not mathematically exact, but are universally useful. They appeal to one's common sense."—W. B. WHITE.

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METHODS OF ANALYSIS, 6th edition, 1945

It is expected that the 6th edition of "Official and Tentative Methods of Analysis, A.O.A.C." will be available for distribution by the fall of this year. The list price of the new edition will be $6.25, domestic postpaid.

BENJAMIN WESLEY KILGORE, 1867–1943

BENJAMIN WESLEY KILGORE

1867–1943

Dr. Benjamin Wesley Kilgore, State Chemist of North Carolina, 1899–1919 and 1937–1943, died December 27, 1943, at the age of 76. He is survived by one daughter, Mrs. Robert H. Gibbs, of Washington, D. C., and two sons, Benjamin Wesley Kilgore, of Franklin, Ky., and James Dinwiddie Kilgore, of Raleigh, N. C.

Dr. Kilgore, son of Benjamin Moon and Susan Bruce Kilgore, was born in Lafayette County, Miss., on March 27, 1867. On August 10, 1898, he was married to Bettie Dinwiddie, of Raleigh, N. C., who died in 1909.

He attended local public schools and the Mississippi Agricultural College from which he was graduated with a B.S. degree in 1888 and an M.S. degree in 1891. He later did special work in chemistry at Johns Hopkins University.

Dr. Kilgore was assistant professor of chemistry at the Mississippi State College from 1888 to 1889, resigning to become assistant chemist of the North Carolina Agricultural Experiment Station, which position he filled from 1889 to 1897. At that time his native State called him back as State Chemist and as professor of chemistry at the Agricultural College of Mississippi, where he remained until 1899, when again he returned to North Carolina as State Chemist, to make this State his permanent home.

During his long and useful life he ably held many positions of responsibility and honor, among them:—twice Director of the North Carolina Agricultural Experiment Station; the first Director of the North Carolina Extension Service; Dean of Agriculture of North Carolina College of Agriculture and Engineering; President of the North Carolina Cotton Growers' Cooperative Association; President of American Cotton Exchange; President of Association of Official Agricultural Chemists of the United States; Editor of the *Progressive Farmer;* Chairman of Wake County Farm Debt Adjustment Committee; President of North Carolina Dairymen's Association; and President of the North Carolina Dairy Products Association. In 1937 he was (a second time) appointed State Chemist of North Carolina, which position he held until his death.

In 1918 Davidson College, Davidson, N. C., conferred upon him the honorary degree of Doctor of Science; and in 1943 State College of the University of North Carolina gave him a similar degree. In 1939 agriculture's highest award, the American Farm Bureau Federation's medal for distinguished services to American agriculture, was presented to him; and in 1943 the North Carolina State Grange gave him an award for distinguished services to the agricultural life of the State.

Dr. Kilgore was not merely a trained chemist confining his efforts to the routine work in hand; he also entered the field of research and developed a new method for testing fertilizer for phosphoric acid content, a method now in general use throughout America. His long-time record of service in the fields of agricultural education, cooperative marketing, and agricultural journalism is outstanding.

He was a scientific agriculturist with a broad, clear vision of rural needs, and with the quiet courage to fight for the things he thought would improve rural life. To him credit is due for the active part he took in sponsoring the establishment of test farms in North Carolina, and of cooperative marketing in the South; for passage of legislation by the Congress giving the same protection to agriculture as that provided for industry; and, as dean of agriculture at State College, for the building up of the long-neglected department of animal husbandry. He was also a practical farmer, owning a large herd of pure bred cattle. He was also interested in the manu-

facture of dairy products, being, at the time of his death, president and principal owner of the Pine State Creamery, one of the largest dairy establishments in the State.

The men and women associated with him admired and respected him for his fairness in his dealings with them. He never spoke harshly or in anger. He was always courteous and considerate. His decisions were never reached hurriedly; and no problem was ever too small for his careful attention. The quiet strength of his mind, his wisdom, gentleness of manner, kindness of heart, and spirit of helpfulness inspired those who worked with him with a desire to justify the confidence he placed in them.

Indicative of his deep interest in the religious, educational, and civic life of the community, he was an elder in the First Presbyterian Church of Raleigh for twenty-five years; a member of the board of trustees of Peace Junior College, Raleigh, N. C.; a member of the Raleigh Rotary Club, and of the Young Men's Christian Association. He was generous in his support of all civic improvements and was a liberal contributor to many charitable institutions.

The keynote of his success in life was his unselfishness, his spirit of helpfulness, his confidence in people, and his love for his fellowman.

SARAH G. ALLEN
E. W. CONSTABLE