WILLIAM JOHN GASCOYNE, 1856–1938

WILLIAM JOHN GASCOYNE

William John Gascoyne died of pneumonia at Johns Hopkins hospital December 27, 1938, after a short illness. He was the son of Elizabeth Wilson and William R. D. Gascoyne, an officer in the British Royal Artillery, and was born in Glasgow, Scotland, October 12, 1856.

His studies in chemistry were pursued at the University of Edinburgh, Scotland, where he received the degree of Doctor of Philosophy, and later he became an assistant to Stevenson MacAdam, F.R.S.E. He came to America and established himself as a representative of a laboratory apparatus supply house operating from New York. In 1883, he was appointed State Chemist of Virginia, which position he held until 1887, when he organized the firm of Gascoyne & Company, public analysts, with headquarters in Baltimore. His laboratory, library, and papers were destroyed in the great Baltimore fire of 1904.

He is survived by his wife, who was Miss Lucilla Clary, of Petersburg, Va.; three daughters, Mrs. H. E. Bucholz, Mrs. Kenneth F. Love, and Mrs. J. H. Wagner; and by one son, William J. Gascoyne, Jr.

Dr. Gascoyne was an active participant in the meeting of Agricultural Chemists held at Atlanta, Ga., May 15, 16, 1884, and in the Philadelphia Convention of September 8 and 9, 1884, where the Association of Official Agricultural Chemists was organized. At this meeting he was appointed a member of the Committee on Potash and of the Executive Committee. He also attended the second meeting, and at the third he was appointed on the Nominating Committee and on the Committee on Nitrogen. At the fourth meeting, held in 1887, he was elected Vice-President, but he was not elected President for 1889 because he had retired from official work.

However, he attended a majority of the meetings, including the last five, as he was much interested in the work of the Association, and enjoyed meeting his friends of the "Old Guard" and making new acquaintances. His life was given, primarily, to the study and analysis of agricultural materials.

Dr. Gascoyne was a member of the American Chemical Society, Society of Chemical Industry, American Institute of Fertilizer Chemists, American Oil Chemists Society, American Society for Testing Materials, and the National Fertilizer Association.

With the passing of Dr. Gascoyne, there remains only one of the charter members of the A.O.A.C., Dr. Charles W. Dabney.

H. B. McDONNELL

TUESDAY—MORNING SESSION

REPORT ON VITAMINS

By E. M. NELSON (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

As the work of the Associate Referees on Vitamins progresses it is desirable to take inventory of their accomplishments and also to review progress of other organizations in the development of methods so that the efforts of this Association may be coordinated and attention directed to the most urgent problems.

There is no doubt that the usefulness of the spectrophotometric method for the determination of vitamin A will be much greater in the near future. It is now being used extensively, but some fundamental studies are necessary before it can be used to the best advantage. Until very recently pure preparations of vitamin A have not been available and therefore the exact biological value of the pure compound has not been determined in International or U.S.P. units. Until this has been done it is impossible to translate directly spectrophotometric readings into vitamin A units.

The U.S.P. Vitamin Committee recognizes the value of the spectrophotometric method in establishing the vitamin A content of cod liver oil, but believes further collaborative studies are necessary before an official method is adopted. A subcommittee of that organization is now planning such a study. The American Drug Manufacturers Association has also appointed a committee, with Charles L. Barthen as chairman, which now has collaborative studies in progress.

The number of investigators in this field is rather limited and with the investigations just mentioned under way it seemed inadvisable for this Association to initiate studies this past year. For the present it seems that the Associate Referee, J. B. Wilkie, can serve as a clearing house for information on developments in the field. Also, until some decision is reached by the United States Pharmacopoeia regarding the acceptance of a spectrophotometric determination for vitamin A, it is not clear whether or not this Association should attempt to establish an official method.

Studies on vitamin B_1 have also become more closely related to the work of the U. S. Pharmacopoeia than had been anticipated. Last year O. L. Kline presented a method for the determination of vitamin B_1, *This Journal*, **21**, 305, and later he was appointed by this Association as Associate Referee on Biological Methods for the Vitamin B Complex. The method that Kline presented has been subjected to collaborative study by the U. S. Pharmacopoeia Vitamin Committee during the past year; very satisfactory results were obtained, and the method is now

being considered for adoption by the U. S. Pharmacopoeia. If it is adopted by that organization, there is no need for its duplication in A.O.A.C. methods. There will, however, be a need for some modification of this method or for some method better adapted to the assaying of foods of low vitamin B_1 potency. In his report Kline will present some data having immediate bearing on this subject (see p. 662).

Two methods that are now being studied appear to be wholly problems of this Association. Reference is made to the determination of vitamin D in milk, and to the same determination in products for poultry by the use of baby chicks. The Referee is indeed appreciative of the splendid cooperation received from a large number of collaborators on both of these methods. Considerable experience has shown that the method for determination of vitamin D in milk is fairly satisfactory. Certain alternatives, which do not appear to have an important bearing on the accuracy of the results, are permitted, but from the standpoint of enforcement procedures it would be well to eliminate them. Walter C. Russell, Associate Referee, is giving these matters his attention.

The Referee has given considerable attention to Biological Methods for Determination of Vitamin D Carriers because of the desirability of adequate control in this field and of developing this method to its greatest efficiency. There are at least five States using this method in controlling the quality of vitamin D carriers for poultry, and the Food and Drug Administration is also conducting an extensive survey of interstate shipments of these products. Since questions arose concerning the interpretation of certain sections of the text of this method of assay it has been entirely rewritten. Some minor changes have also been proposed both in the basal ration and in the procedure of assay.

It is with considerable regret that the Referee announces that W. B. Griem has tendered his resignation as Associate Referee. He has contributed a great deal of his time and effort to this project, and has been very successful in obtaining the cooperation of others in developing the method to its present status. The Association owes him appreciative thanks.

Recent developments indicate the desirability of developing a method for the determination of vitamin K. This term was proposed by Henrik Dam to refer to a substance which is necessary to prevent a type of nutritional deficiency in growing chicks. In the absence of vitamin K there is a delay in the clotting time of the blood, which condition is associated with low prothrombin content of the blood. Clinical studies have now led to the conclusion that post-operative hemorrhages, which so frequently occur in patients with obstructive jaundice, can be controlled to a marked degree by the proper use of vitamin K preparations. Patients with obstructive jaundice show a low prothrombin, just as chicks do when fed a vitamin-K-deficient ration. It is recommended that H. J. Almquist

of the University of California, who has contributed much to the knowledge of this vitamin, be appointed Associate Referee on Vitamin K to study the desirability of adopting a method.

The recommendations of the respective associate referees are approved.

REPORT ON VITAMIN A

By J. B. Wilkie (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

Several important problems relative to the spectrophotometric determination of vitamin A were indicated at this meeting last year. These problems include the investigation (a) of the destruction of vitamin A as a basis for its determination, (b) of separable fractions of fish liver oils relative to their respective spectrophotometric characteristics, and (c) of crystalline vitamin A to determine its correct E value with the hope of obtaining better correlation of all methods for determining vitamin A. The need for improvement and standardization of equipment specially suited for the purpose as well as for collaboratove work to establish the accuracy and reliability of spectrophotometric equipment by means of a stable inorganic solution was also emphasized.

Without in any way detracting from the importance of these problems, it now appears to the Associate Referee that immediate concerted action should be directed to the checking of instruments against a suitable standard with the hope of accounting for the spread in extinction coefficient values for the U. S. P. Reference Oil that has been reported from various laboratories. E values for this oil ranging from 1.4 to 1.79 have been more or less consistently obtained and reported.

It appears that Smith, Stern, and Young[1] may have the correct explanation for such discrepancies. They present data to show the effect on the absorption value of the different solvents used in vitamin A preparations, and state that with alcohol taken as 100, cyclohexane was 97.5, ether 107.5, and chloroform 89 in absorption value.

The differences between alcohol and cyclohexane are scarcely beyond experimental error, but certainly the chloroform value of 89 appears beyond experimental error. This was conclusively proved by the investigators above mentioned, who examined the same material first in a chloroform solution and in an ether solution after drawing off the chloroform in a vacuum. They found that chloroform lowered the absorption value, and that it was restored in large part in the ethereal solution. These same authors also mentioned the fact that the absorption value of an oil could be increased or decreased by 40 per cent through irradiation, but that it would practically return to normal after a period of dark storage.

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[1] *Nature*, 141, 551 (1938).

These phenomena were accounted for by the authors by assuming isomeric reversible changes presumably of a cis-trans type. It thus appears that the values 1.4 to 1.79 for the U. S. P. oil may likewise be attributed to such changes if the oil has been subjected to different storage conditions or if different solvents have been used.

In this laboratory the discrepancies in absorption values on raw oils having various storage periods and conditions could be explained by assuming isomerism. If such isomerism is the interfering factor, means should be sought to recognize it and avoid its effect upon the absorption. While nothing very pertinent to the solution of this problem can be offered at this time, it appears that by boiling with alcoholic potassium hydroxide as is usually done in obtaining the nonsaponifiable material for the determination, isomerism might be regulated to bring the vitamin A to some constant condition and thereby make more uniform results possible. However, this is contrary to available results, which have indicated more uniform absorption values from the raw oil than from the nonsaponifiable portion.[1] These more variable results may be caused by variations in the preparation of the nonsaponifiable fraction previous to the spectrophotometric examination. At the present time, in this laboratory, small volumes of water are being used for rinsing (each rinse amounting to only about 1/15 of the volume of the ether extract) in place of the more strongly alkaline rinses recommended previously. More vigorous agitation is also used. These rinses are diluted with about 75 cc. of water, and after very slight agitation they are discarded. This procedure sometimes necessitates the use of a drop or two of alcohol in the final rinsed extract to eliminate traces of turbidity. However, with this modification troublesome emulsions are avoided and higher absorption values are obtained than with the more strongly alkaline rinses previously recommended.

As a basis for checking over-all spectrophotometric performance, the use of a single inorganic solution was recommended last year. Conditions made collaborative work impossible, but some work pertinent to this matter was initiated. Several compounds were tried, but none seemed as promising as potassium chromate diluted with 0.05 *N* potassium hydroxide, which has been the subject of careful investigations by Von Halbon[2] and by Hogness.[3] Each of these investigators used a photoelectric system that admittedly is capable of greater precision and, with care, of greater accuracy than is the photographic method generally used for the vitamin A determination. Hogness reported 20 determinations of the molecular extinction coefficient of potassium chromate at 366 mu. Using his value for molar extinction coefficient obtained at a molar concentration of 2.96×10^{-5} and a 2 cm. cell, the Associate Referee calculated absorbency

[1] *J. Am. Pharm. Assoc.*, 26, 525–540 (1937).
[2] *Z. physik Chem.*, 100, 208 (1922).
[3] *J. Phys. Chem.*, 41, 379–415 (1937).

or density values over a range possible by the photographic method and the use of a 1 cm. cell. The table shows the experimental data obtained compared with those calculated from the coefficient published by Hogness. The experimental values at all of the concentrations are seen to be in substantial agreement with the calculated values.

The average experimental molecular extinction coefficient of the Associate Referee differs from the Hogness value by only 2.4 per cent, while the average deviation from the mean for all of the concentrations is only 4.8 per cent. When it is considered that some of these concentrations are not the optimum ones for the making of photographic spectrophotometric determinations the agreement is seen to be very good. These data thus substantiate a recommendation for the use of potassium chromate over

Dilution-absorption value for K_2CrC_4 at 366 mu
(0.05 *N* KOH used as solvent)

CONCENTRATION		MOLECULAR EXTINCTION COEFFICIENT AT 366 MU		ABSORBENCY OR DENSITY		TRANSMITTANCY		SPECIFIC ABSORPTIVE INDEX OR EXTINCTION COEFFICIENT $E^{1o/o}_{1cm.}$	
PER CENT	MOLS PER LITER	HOGNESS CELL—2 CM. CONC. $=2.96\times10^{-5}$	EXPTL. VALUES	CALCULATED	EXPTL.	CALCULATED	EXPTL.	CALCULATED	EXPTL.
3.88×10^{-3}	20×10^{-5}	4720 ± 10	5000	.945	1.00	12	10	244	258
3.10×10^{-3}	16×10^{-5}	4720 ± 10	5120	.755	0.82	17.5	15	244	264
2.33×10^{-3}	12×10^{-5}	4720 ± 10	5000	.566	0.60	27	25	244	258
1.55×10^{-3}	8×10^{-5}	4720 ± 10	4900	.378	0.39	42	40	244	250
$.077\times10^{-3}$	4×10^{-5}	4720 ± 10	4500	.189	0.18	64	65	244	234
$.038\times10^{-3}$	2×10^{-5}	4720 ± 10	4500	.0945	0.09	79	80	244	232
			Av. 4836						Av. 249

Experimental molecular extinction coefficient checks Hogness experimental value within 2.4%.
Extinction coefficient maximum deviation from mean =7%.
Extinction coefficient average deviation from mean =4.6%.

an entire range of concentration for checking over spectrophotometric performance. It is therefore recommended[1] that the instruments being used in collaborative work be checked against a suitable potassium chromate solution immediately preceding and after each vitamin A determination. The concentrations and absorption values of the standard test solutions should be reported with the data on the oils being studied.

Collaborative studies relating to the spectrophotometric determination of vitamin A are now being undertaken by the American Drug Manufacturers' Association as well as by the Vitamin Committee of the U. S. Pharmacopoeia. In order to avoid unnecessary duplication of effort it is

[1] For report of Subcommittee A and action by the Association, see *This Journal*, 22, 55 (1939).

therefore recommended that collaborative work directed by the A.O.A.C. on this phase of the problem be temporarily delayed.

Regardless of this temporary cessation of collaborative activity by the A.O.A.C., the several problems that were mentioned at the beginning of this report are very pertinent, but it now appears that such work may be carried out most effectively, at least in the preliminary stages, by individual effort.

REPORT ON VITAMIN D*

PRESENT STATUS OF THE USE OF THE TENTATIVE METHOD—FEEDING OF NON-VITAMIN D MILK WITH THE REFERENCE OIL

By WALTER C. RUSSELL (New Jersey Agricultural Experiment Station, New Brunswick, N. J.), *Associate Referee*

A tentative method for the assay of vitamin D milk was adopted at the 1936 meeting of the Association and was published in *This Journal,* **20**, 78. At the 1937 meeting a revision of the tentative method was approved and was published in *This Journal,* **21**, 90.

Since the principal features of the tentative method have been in force for over two years, and since several options are allowed, it seemed advisable to ascertain what options are being used and what experience collaborators have had with them.

Accordingly, a questionnaire was sent to 32 collaborators known to be concerned with the assay of vitamin D milk, and replies suitable for tabulation were received from 18. The portions of the replies that might be of interest to those concerned with the assay of vitamin D milk are summarized in the following paragraphs.

Depletion Period.—The age limits for starting the depletion period are given as 21 to 30 days. Ten assayers start animals on the depletion period at not less than 21 nor more than 26 days of age. The range of depletion periods was from 18 to 25 days, and 11 collaborators reported depletion periods of less than 21 days.

Assay Period.—Eleven assayers are using the 7-day and seven, the 10-day assay period. Supplements, both reference oil and milk, are fed for 1, 2, 3, 5, and 6 days, by those who employ the 7-day period and for 1, 3, and 8 days, by those who use the 10-day period. The results obtained with any of these procedures are apparently satisfactory if the oil and milk are fed in the same manner. Two of the 18 assayers mix the supplements with the feed. Those who use the 7-day period report the line test to be satisfactory. Some, however, find it necessary to increase the quantity of supplement ordinarily used for the 10-day period, in order to obtain a satisfactory response at 7 days.

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* Journal Series paper of the New Jersey Agricultural Experiment Station, Department of Agricultural Biochemistry.

Although more assayers are using the 7-day than the 10-day period there is no uniformity as to the number of feedings made for either period. It is possible that in some laboratories it will be difficult to feed enough irradiated milk in 6 days to obtain a satisfactory line at the end of a 7-day period, and it may be necessary to consider for adoption an official method which would permit a 7- to 10-day feeding period, with the stipulation that the reference substance and the sample are to be administered according to identical procedures.

TABLE 1.—*Effect of feeding skim milk or non-vitamin D whole milk with the reference oil*

		REFERENCE OIL		REFERENCE OIL PLUS MILK[1]	
		QUANTITY FED U.S.P. UNITS	AV. RESPONSE	QUANTITY	AV. RESPONSE[2]
				cc.	
Laboratory A 6-day feeding 7-day period		3.3	0.55	28.8 (reconstituted skim)	0.78
Laboratory B 5-day feeding 7-day period		4.0	0.52	29.1 (dry skim)	0.85
				29.1 (reconstituted skim)	0.69
Laboratory C 8-day feeding 10-day period	(Trial 1)	2.85	0.10	20.0[3]	0.60
		2.85	0.28	20.0	0.70
	(Trial 2)	4.28	0.35	30.0	1.03
		4.28	0.28	30.0	0.73
	(Trial 3)	5.70	0.68	40.0	1.20
		5.70	0.50	40.0	1.00
	(Trial 4)	5.70	0.77	40.0	1.50
	(Trial 5)	40 cc. of reconstituted non-vitamin D milk was fed without reference oil			0.06[4]

[1] The quantity of reference oil fed with the milk is the same as that used when the reference oil was fed alone.
[2] The value 1.0 means a narrow continuous line of calcification.
[3] Reconstituted non-vitamin D whole milk powder used by Laboratory C.
[4] Average of 25 animals.

To obtain a satisfactory line test, assayers report the use of 28.4–65 mg. of reference oil as the total for the assay period. Thirteen use 45 mg. or less. Some report a variation in the quantity in supplement required with the season of the year.

Rickets Resistance.—Seven assayers reported rickets resistance of various degrees of severity.

Feeding of Non-Vitamin D Skim or Whole Milk with the Reference Oil.—One assayer reported the feeding of skim milk and another the use of fresh, whole non-vitamin D milk with the reference oil.

The most important problem is that of finding a satisfactory reference substance other than cod liver oil or to ascertain whether the feeding of non-vitamin D skim or whole milk with the reference oil is a more satisfactory procedure than the use of the reference oil alone.

Table 1 shows the results obtained by three collaborators when various levels of reference oil were compared with the same quantity of reference oil plus the quantity of skim milk, or non-vitamin D whole milk, equal to the volume of irradiated milk (135 U. S. P. units per quart), which would have been fed in a routine assay. Groups of ten or more animals were used for each supplement in all of the nine trials. Trials 1, 2, and 3 of Laboratory C are in duplicate, so that for each trial two groups of ten animals each were fed the oil and two the oil plus milk during the same period of time. The radii were split, stained, photographed, and scored in the laboratory of the Associate Referee. A greater line test response was obtained when the milk was fed with the reference oil than when the reference oil alone was fed, and there was a greater response with non-vitamin D whole milk than with skim milk. However, only two comparisons were made with skim milk, whereas seven were made with the whole milk, and therefore more trials with skim milk will be necessary to determine definitely the relative effect of these two types of milk. In an earlier report, *This Journal,* **19**, 248, two of three trials showed enhancement of the line response when skim milk was fed with the reference oil, and for a third the responses of the two groups were essentially alike. Three assays were made on the reconstituted whole milk powder with 25 animals. All the animals gave a negative response except four, and these showed only a very slight degree of calcification. In view of the recent report by Morgareidge and O'Brien,[1] it will be necessary to give attention to the manner in which the oil and milk are administered, that is, as separate supplements or as mixed supplements.

RECOMMENDATIONS[2]

It is recommended that further studies be made of the feeding of skim milk or whole, non-vitamin D milk with the reference oil, in order to determine whether the reference oil and a quantity of milk, equal to that of the vitamin D milk being assayed, should be used as a reference standard instead of the reference oil alone.

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[1] *J. Nutrition*, 16, 395 (1938).
[2] For report of Subcommittee A and action by the Association, see *This Journal*, 22, 55 (1939).

REPORT ON PLANTS

By E. J. MILLER (Michigan State College of Agriculture
and Applied Science, East Lansing, Mich.), *Referee*

Less Common Metals.—From data obtained with a modification of the method for the determination of iodine in plant material, it is concluded that large samples of plant material can be completely burned and the iodine determined more satisfactorily and accurately than has been possible with apparatus previously used. Because of this and the fact that at least two other laboratories will be equipped with the apparatus, it is recommended that collaborative work be done on the method during the coming year.

Total Chlorine.—Collaborative work has been under way during the past year, and the associate referee will recommend the method for adoption as official, first action.

Carbohydrates.—The Referee approves the recommendations of the associate referee.

Inulin.—No results have been accomplished on the inulin problem but work is being initiated on the chemistry of timothy which will involve the determination of fructosans, and study will be extended to include fructosans in general.

Forms of Nitrogen.—No new analytical methods of significance in this field have been reported.

Hydrocyanic Acid in Plants.—No report will be presented. Because of pressure of other work, it was not possible for the associate referee to undertake the collaborative work intended. It is hoped that this work can be done during the coming year.

Sodium and Potassium.—No formal report will be presented, although some progress was made during the past year. The results with the Hicks method for potassium have been satisfactory and collaborative work is planned for next year. The recommendations of last year should stand for next year.

REPORT ON LESS COMMON ELEMENTS

MODIFICATION OF THE TENTATIVE METHOD FOR DETERMINATION OF IODINE IN PLANT MATERIAL

By J. S. MCHARGUE, *Associate Referee*, and E. B. OFFUTT
(Department of Chemistry, Kentucky Agricultural
Experiment Station, Lexington, Ky.)

During the past year an effort was made in the Department of Chemistry of the Kentucky Agricultural Experiment Station to improve the combustion method that has been used for the determination of iodine

in forage crops and vegetables. It was described at a previous meeting of this Association, *This Journal*, **16**, 207; *Methods of Analysis, A.O.A.C.*, 1935, 8.

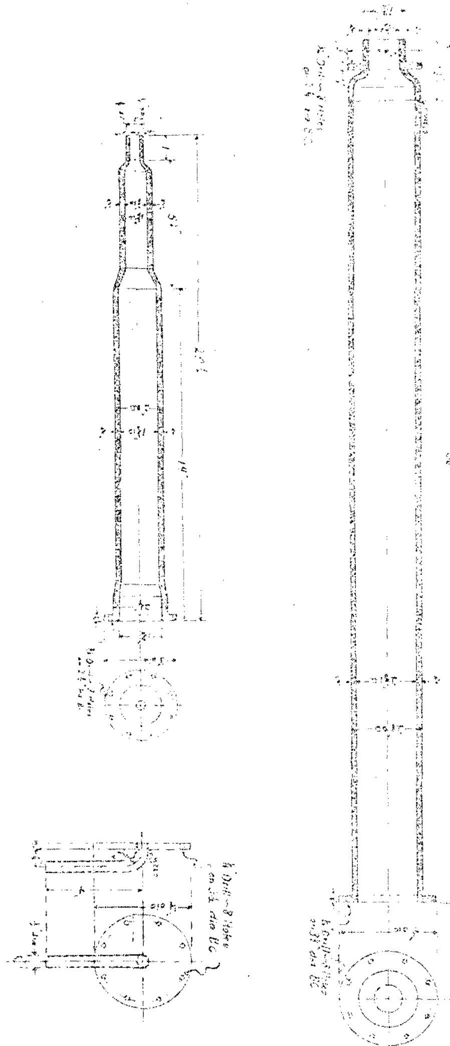

FIG. 1.—COMBUSTION TUBES.

The principal difficulties encountered with the method include: (1) maintaining an air-tight system in the silica tube during the combustion;

(2) the time required to make a complete extraction of the iodine from the potassium carbonate residue with alcohol; and (3) the complete destruction of all traces of organic matter in an electric furnace at 400° C. without loss of any iodine.

CHANGES PROPOSED IN THE METHOD

Figures 1 and 2 show the modifications that have been made in the combustion tubes and the absorption apparatus. The two sections of the combustion tube are made of Misco-B metal, which is an alloy of iron (64 per cent), chromium (24 per cent), and nickel (12 per cent), respectively. This alloy will withstand temperatures up to 1000° C. without any

FIG. 2.—ABSORPTION APPARATUS.

serious oxidation, corrosion, or deterioration in the physical structure of the alloy. However, for the combustion of plant material it is not desirable to maintain a temperature above 500°–600° C. in the tubes. Misco-B metal is manufactured by the Michigan Steel Casting Company, Detroit, Mich., the same company that made the combustion tube according to the specifications submitted by this laboratory. The two sections of the combustion tube and the cap at the right end of the larger tube are fast-

ened together and made air-tight by means of threaded bolts and nuts and asbestos gaskets between the joints.

The new absorber was designed and constructed from Pyrex glass tubing in this laboratory by the junior author. It is connected to the combustion tube by means of a ground joint, and one section of the joint is cemented to the combustion tube with gasket cement. By means of the new absorber the volume of the absorbing solution is held at the minimum.

A U-shaped boat about 30 inches long and made of sheet nickel replaces the alundum boats previously used. It has a capacity of as much as 100 grams of finely ground, dry plant material. The sample is spread on curved pieces of nichrome wire gauze placed in the boat. This arrangement allows a current of air to flow beneath and above the sample during combustion. The short section of the combustion tube is partly filled with 20-mesh copper wire gauze, which aids in the complete combustion of any smoke that is not burned in the larger section of the combustion tube. It requires 1–3 hours to make a complete combustion, depending upon the size and the nature of the plant material burned. Leguminous plants and tobacco burn more readily than do grasses and cereals. In case of the latter materials, finely pulverized copper oxide mixed with the sample aids in the combustion. The furnaces remain the same.

After the combustion is completed the absorbing solution is transferred to a modified 300 cc. Claissen flask having ground-glass stoppers and connections. The residue of ash is also washed into the same flask. From this point the method of Stimmel and McCullagh is followed.[1]

The contents of the flask are acidified with sulfuric acid (1+1), about 5 drops of a 10 per cent solution of ferric sulfate are added, and the contents of the flask are heated to boiling and distilled. During the distillation three 2 cc. portions of a 3 per cent hydrogen peroxide solution are added at intervals through a dropping funnel into the distillation flask. The distillate is received in a 50 cc. extraction flask, which contains 0.2 cc. of a 3 per cent solution of sulfuric acid and 0.2 cc. of a 10 per cent solution of sodium sulfite. The distillation is continued until the volume in the distilling flask is reduced to a few cc. The distillation flask is disconnected from the condenser, and the condenser tube is rinsed into the receiver with distilled water. The distillate is boiled gently for about 2 minutes to expel carbon dioxide and sulfur dioxide. The solution is made slightly alkaline with 10 per cent potassium hydroxide and concentrated by boiling until the volume is about 10 cc. The solution is then made acid with 3 per cent sulfuric acid; two drops in excess of the acid and 5–10 drops of a saturated solution of bromine water are added; and the mixture is boiled gently until all the bromine is expelled. At this point in the procedure the iodine is in the form of iodate. The contents of the flask

[1] *J. Biol. Chem.*, 116, 21 (1936).

are cooled as quickly as possible to about 15° C., two drops of a 1 per cent solution of potassium iodide and four drops of a 0.25 per cent starch solution are added, and the iodine is titrated with a 0.01 *N* sodium thiosulfate solution. The iodine is calculated in p.p.b.

The modified procedure was tested on a sample of kelp that contained a known amount of iodine (0.127 per cent) as determined with fusion, and colorimetrically in carbon disulfide with the use of a micro colorimeter.

Three portions consisting of 0.2 gram each of finely ground kelp were weighed into a nickel dish and fused with potassium hydroxide from 100°–400° C. until all the organic matter was destroyed. The residue was cooled, dissolved in water, and transferred to a Claissen flask, and the iodine was determined by the distillation and titration method described above. The following results for iodine were obtained on a sample of 0.2 gram of kelp.

	per cent
Iodine, colorimeter No. 1	0.127
Iodine, distillation No. 2	0.125
Iodine, distillation No. 3	0.129
Iodine, distillation No. 4	0.126
Average of 3 distillations	0.1266

For experiment 2, two 0.25 gram portions of the finely ground kelp were weighed and mixed with 25 grams of wheat straw, in which it was not possible to detect iodine. The two portions were burned separately in the modified combustion tube, and the iodine was determined according to the procedure described in this report. The following results were obtained on kelp, wheat, and tobacco.

			Iodine
		gram	*per cent*
Kelp	a	0.2500	0.1255
Kelp	b	0.2500	0.1260
			p.p.m.
Wheat grain	a	50	217
Wheat grain		75	220
Wheat grain	b	50	306
Wheat grain		100	366
Tobacco leaf	a	50	425
Tobacco leaf		50	530
Tobacco leaf	b	25	928
Tobacco leaf		25	980
Tobacco leaf	c	50	1,154
Tobacco leaf		50	1,052

From the results given and additional data not included in this report it was concluded that large samples of plant material could be completely burned and the iodine determined more satisfactorily and accurately

than was possible with the apparatus previously used. Since two other laboratories have recently submitted orders for combustion tubes made of Misco alloy metal, it is the recommendation[1] of the Associate Referee that cooperative work be sought on this subject during the next year.

REPORT ON TOTAL CHLORINE IN PLANTS

By HERBERT L. WILKINS (Bureau of Plant Industry, Beltsville, Md.), *Associate Referee*

This year's work consists entirely of collaborative studies on the tentative method presented by the Associate Referee, *Methods of Analysis, A.O.A.C.*, 1935, 131. Of the several chemists who were invited, four agreed to undertake the work.

Because the Associate Referee is recommending that the method be adopted as official, first action, and that the work be discontinued, a review of much of the material found in previous reports, *This Journal*, **17**, 268; **18**, 379; **19**, 72, 366; **20**, 335; **21**, 353, is included in this one.

Unfortunately the changes recommended in the 1937 report were not included in the published version. These had to do with the preparation of the iodine solution and the change in the weight of potassium iodide required to make the standard solution of this reagent. The instructions to the collaborators included all the changes that had been made in the procedure since it was published as a tentative method, *Ibid.*, **22**, 72.

The sample used was a portion of the one prepared for the 1935 report.

The collaborators who reported were T. A. Pickett of the Georgia Experiment Station, Experiment, Ga., and J. H. Mitchell and a class of his students at Clemson Agricultural College, Clemson, S. C. The Associate Referee takes this opportunity to congratulate these students on the excellence of their work, and to extend his thanks to all the collaborators for their help.

In Table 1 there is assembled all the available data on this sample. The grand average is 6.26 mg./gram of grass. The mean deviation of the 42 determinations from the grand average is ± 0.09 mg. Nineteen of these analyses were made by the nine students. The average of their analyses is 6.29 mg./gram, and their mean deviation (from 6.26) is ± 0.13 mg. The average of the 23 analyses by the four graduate chemists is 6.23 mg./gram and their mean deviation from 6.26 is ± 0.06 mg. In terms of the chlorine content of the sample these averages would be 0.629 per cent for the students and 0.623 per cent for the chemists.

The late O. B. Winter, formerly General Referee on Plants, prepared two samples on which both he and the Associate Referee made analyses

[1] For report of Subcommittee A and action by the Association, see *This Journal*, **22**, 54 (1939).

by the tentative method. The average difference in their reports in six cases is ±0.06 mg./gram.

If all the collaborative results from the fifty-seven analyses made by 14 people are considered, the deviations of the individual values from the appropriate means average ±0.08; they have been as large as

TABLE 1.—*Chlorine found by various collaborators in a sample of dried grass*

	mg./gram		mg./gram
Associate Referee	6.26	(Prof. Mitchell's class)	
	6.26		
Own average, 6.25 mg.	6.26	T. R. Bainbridge	6.15
Deviation, ±0.01 mg.	6.27		6.00
	6.27	T. J. Blalock	
	6.24		6.18
	6.26		6.19
	6.22		
	6.25	S. J. Boyd	6.66
	6.25		6.67
O. B. Winter	6.31	L. T. Garick	6.35
	6.32		6.30
Own average, 6.34 mg.	6.30		
Deviation, ±0.03 mg.	6.35	E. W. Griffin	6.14
	6.40		6.15
J. H. Mitchell	6.07	F. L. Moore	6.18
	6.08		6.35
Own average, 6.10 mg.	6.08		
Deviation, ±0.04 mg.	6.18	M. M. Nichols	6.30
			6.35
			6.40
T. A. Pickett	6.15		
	6.18	N. P. Page	6.20
Own average, 6.18 mg.	6.18		6.25
Deviation, ±0.01 mg.	6.20		
		H. A. Raysor	6.36
			6.35
Chemists' average	6.23	Students' average	6.29
Chemists' average deviation from general mean	±0.06	Students' average deviation from general mean	±0.13

Grand average 6.26 mg.
Average deviation from grand average (6.26) ±0.09 mg.

±0.41 mg. only once and have exceeded ±0.14 mg. only six times. Among the data obtained by the tentative method there are 39 analyses, of which each collaborator made at least four. When each collaborator's analyses are compared with his own mean, and the 39 resulting deviations are

averaged, ±0.02 mg. is shown as the over-all precision of the method itself.

Winter applied both the tentative and official methods to his dried beets and to the dried grass, and reported averages of 0.057 per cent (tentative) and 0.063 per cent (official) on the dried beets, and of 0.634 per cent (tentative) and 0.622 per cent (official) on the dried grass. Similarly Pickett reported for the grass averages of 0.618 per cent (tentative) and 0.636 per cent (official). The average difference shown by the three comparisons of the two methods is ±0.012 per cent or ±0.12 mg./gram.

In 1936, Winter wrote that he washed the filter paper thoroughly and obtained a faint blue color with starch on adding the iodine without adding any potassium iodide. Since he followed the Associate Referee's directions for filtering without difficulty, he did not try the Gooch as he had suggested in a previous letter. He was surprised to find the method so simple, rapid, and accurate.

Mitchell wrote that for the first determinations by his group of students he considers the results to be good. They may be too high or too low, but are quite consistent. He states that rather than have all nine students make the solutions separately, the permanganate, iodine indicator, and starch solutions were made as stock solutions (just one of each). The potassium iodide solutions were standardized by each student, and Mitchell set up three burets on a side desk for them to use. These were not calibrated burets. The time required was within two 3-hour periods. It is true that there are a great many reagents, but when once they are made the actual method is not long. There seems to be some doubt as to how much permanganate to use. If too much is used, the results will be high. Apparently the method is giving consistent results.

After obtaining low results earlier when using a different filtering arrangement and possibly also too little permanganate, Pickett wrote that he had received the duplicate samples of grass and had run several more analyses. He believes his low results are due to the fact that he did not add enough potassium permanganate. He ran a series of determinations, using exactly the same procedure except for the amount of potassium permanganate added. Dividing the analyses into three groups, he obtained 5.83, 5.87, and 5.87 mg./gram for the lower amount of potassium permanganate added; for the intermediate amount he obtained 5.95, 6.05, and 6.05 mg./gram; and for the highest amount added (which faded quite slowly) he obtained 6.15, 6.18, 6.18, and 6.20 mg./gram. He noticed that when he intentionally had an excessive amount of potassium permanganate in the blank, he obtained high results, 0.4–0.5 cc. He suggests that in the procedure more emphasis be put upon the direction "until the color fades slowly."

SUMMARY

The method presented is essentially a procedure for the determination of silver in a solution of sulfuric acid, which may vary from 3 to 40 cc. of

the concentrated acid in each 100 cc. of solution. Because it is a method for determining silver in such strongly acid solutions, it would seem to be useful whenever silver or a silver precipitable halogen can be used as a measure of the constituent sought.

The method has been shown to have a precision of ±0.02 mg. when each analyst's results are compared with his own mean, and one of ±0.09 mg. when 42 analyses by 14 analysts are compared with the general mean, including the work of a class of nine undergraduate students. The precision of the titration step in the procedure is shown by the results obtained when 10 successive equal aliquots of a solution of silver nitrate were titrated. In this experiment the average deviation from the mean (±0.015 mg.) was ±0.014 cc.

The accuracy of the method is independent of the amount of chlorine found in the range studied; it averaged ±0.03 mg. in the hands of the Associate Referee when using samples that contained 8, 13, 42, and 70 mg. of chlorine derived from a standard solution of hydrochloric acid prepared from the constant boiling acid. When average results by the tentative method were compared with results from the official or a modified official method, a value of ±0.12 mg. was obtained as the average of three such comparisons.

The method is applicable to a wide variety of substances because the modified open Carius digestion, which is used to disintegrate the sample, will decompose many organic materials and because the silver chloride and sample residue separated from the nitric acid digest are completely decomposed by the Kjeldahl digestion, thus providing a solution that is free of organic matter and of halides in which to titrate the resulting silver sulfate with potassium iodide.

The method has demonstrated its suitability for the use of the general technician by the success it gave in the hands of the collaborators, especially those of undergraduate standing. It requires no excessive amount of time as evidenced by the comments of the collaborators and by the fact that the students standardized their potassium iodide solutions and completed their analyses in two 3-hour laboratory periods.

It is not known to what extent the accuracy or precision of the method may be increased by the introduction of the elaborate precautions common to the atomic weight type of investigation, but such measures applied to this procedure should repay any worker who has need for such accuracy and precision. The method appears to be suitable for micro analysis because the end point is still very sharp when both the standard solution concentration and the titration volume are reduced to one-tenth of those given in the procedure.

RECOMMENDATIONS[1]

It is recommended—

(1) That the method discussed be adopted as official (first action).

[1] For report of Subcommittee A and action by the Association, see *This Journal*, 22, 54 (1939).

(2) That a statement of the limitations of the applicability of the present official (alkaline ignition) method be made a part of that method if it is retained.

(3) That the appointment of an Associate Referee on Total Chlorine in Plants be discontinued.

REPORT ON CARBOHYDRATES IN PLANTS

By J. T. SULLIVAN (Division of Forage Crops and Diseases, Bureau of Plant Industry, in cooperation with the Northeastern States, State College, Pa.),
Associate Referee

The last report of the Associate Referee, *This Journal*, **19**, 371, contained a recommendation for an improved procedure for the titration of reduced copper. The present report offers no new information but contains a brief discussion of the status of the methods and recommendations for new work.

REDUCING SUGARS

The reducing sugar methods that have been approved as tentative or official (first action) are the Munson and Walker and the Quisumbing and Thomas procedures, and for the determination of the reduced copper the gravimetric, the electrolytic, and the permanganate titration methods. Many other methods are in use, some having distinct manipulative advantages. Despite the need for standardization, the Associate Referee hesitates to place emphasis upon any of these other methods until more information about them is available. It has been pointed out a number of times that results by several methods vary with different plants. All oxidizing reagents are more or less susceptible to reaction with non-sugar reducing substances. Methods in which the excess cupric copper is determined after the reduction or in which the cuprous copper is determined without removing it from the reduction mixture are particular offenders in this respect, since an iodometric titration is usually involved. The potassium ferricyanide methods are subject to the same error. Some error is involved in the actual reduction but even more is due to the reactivity of the iodine to non-sugar substances. Those methods that do not involve an iodine titration in the presence of the reduction mixture should be studied further. At the present time no method can be recommended for indiscriminate use on all plant materials.

SUCROSE

The cold acid hydrolysis method and the invertase method are tentative. In the latter case no definite procedure has been recommended. Neither method is specific for sucrose, but the invertase method gives a

definite end point of hydrolysis with some plant materials but not with others, as shown in a former report, *This Journal*, 16, 471.

STARCH

The malt diastase method with subsequent acid hydrolysis is tentative. Takadiastase is also in wide use. Recently published methods are directed away from diastatic reagents and toward procedures that are more specific for starch; they involve solution and reprecipitation of the starch or of its iodine complex and often hydrolysis of a more or less purified starch. Such methods have shown that the vegetative parts of plants, especially the leaves, contain less starch than was previously supposed. These methods should be studied.

NEW STUDIES

It is recognized that many plants contain, in the absence of or in addition to starch, levulosans or fructosans, which on hydrolysis yield fructose. They are important physiologically and have a function similar to starch as carbohydrate reserves. Their analysis involves new problems of hydrolysis since fructose is less stable than glucose to acid-hydrolyzing reagents.

Since it has been shown that fructosans may yield a certain amount of glucose in addition to levulose, methods should be studied for the determination of glucose and fructose in mixtures. It is also of interest to determine them when they occur together and free in plants. The many different types of methods that have been proposed should be studied, especially Erb and Zerban's[1] recent combination of the Jackson and Mathews[2] method for fructose with the Munson and Walker method for reducing sugars.

RECOMMENDATIONS[3]

It is recommended—

(1) That methods for the determination of reducing sugars, sucrose, and starch be further studied,

(2) That studies be begun on methods for the determination of fructosans in plants,

(3) That studies be begun on methods for the determination of glucose and fructose in plants.

No report on inulin was given by the associate referee.

No report on forms of nitrogen was given by the associate referee.

No report on hydrocyanic acid was given by the associate referee.

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[1] *Ind. Eng. Chem. Anal. Ed.*, 10, 246 (1938).
[2] *Bur. Standards J. Research*, 8, 403 (1932).
[3] For report of Subcommittee D and action by the Association, see *This Journal*, 22, 64 (1939).

No report on sodium and potassium was given by the associate referee.

No report on lignin was given by the referee.

No report on enzymes was given by the referee.

No report on papain was given by the associate referee.

REPORT ON WATERS, BRINE, AND SALT

FLUORINE IN WATER

By ANNA E. MIX (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

An artificial mineral water solution was prepared for collaborative work; it contained 2193 p.p.m. of chlorides, 1465 p.p.m. of sulfates, 28 p.p.m. of phosphates, and 2.12 p.p.m. of fluorine.

The thorium nitrate titration method was suggested in view of the results obtained last year. The following collaborators reported:

COLLABORATORS

R. W. Bridges, Aluminum Co. of America
P. J. Buchanan, American Agricultural Chemical Co.
J. N. Carothers, Monsanto Chemical Co.
J. R. Davies, General Foods Corporation
W. K. Enos, Virginia-Carolina Chemical Corporation
J. B. Fullerton, The Upjohn Company
M. Given, Crystal Gelatine Co.
V. L. Harnack, United Chemical and Organic Products
H. B. Hodge, Lucidol Corp.
Simon Klosky, American Agricultural Chemical Company
H. V. Moss, Monsanto Chemical Company
W. C. Motz, Virginia Chemical Corporation
K. B. Peterson, North Dakota Regulatory Dept.
W. B. Sherry, General Chemical Company
W. E. Stokes, Standard Brands
O. I. Struve, Eastern States Cooperative Milling Corporation
J. J. Vollertson, Armour and Company

Because the collaborative results show that many of the analysts used variations of the suggested A.O.A.C. method the results are listed in two tables. Table 1 shows results obtained by the analysts that adhered strictly to the A.O.A.C. method, and Table 2 shows the results of those that deviated from the A.O.A.C. method.

The results of both tables were studied by the technic of analysis of variance.[1] The first findings are (1) that either by strict adherence to the

[1] Snedecor, Geo. W., Statistical Methods. Collegiate Press, Inc., Ames, Iowa.

TABLE 1.—*Results obtained by A.O.A.C. method*
(Fluorine present, 2.12 p.p.m.)

COLLABORATOR	NO. OF TRIALS	ACIDITY 0.02 *N* HCl	FLUORINE FOUND	
		aliquot	*p.p.m.*	
1	3	0.2 cc./25 cc.	2.0 2.0 1.9	Av. 1.97
2	2	0.95 cc./40 cc.	2.44 2.40	Av. 2.42
3	3	0.35 cc./40 cc. 0.20 cc./40 cc.	1.35 1.35 1.90	Av. 1.53
4	6	0.3 cc./40 cc. 0.3 cc./40 cc. 0.2 cc./40 cc. 0.2 cc./40 cc. 0.2 cc./40 cc. 0.2 cc./40 cc.	2.3 2.4 2.4 2.4 2.0 2.0	Av. 2.25
5	1	—	1.80	
6	2	0.2 cc./40 cc. 0.2 cc./40 cc.	1.50 1.50	Av. 1.50
7	7	1.05 cc./40 cc. 0.45 cc./40 cc.	1.8 1.9 1.8 2.1 2.0 2.1 2.3	Av. 2.0
8	8		1.87 1.80 1.82 1.80 1.77 1.80 2.10 2.07	Av. 1.88
	32		62.67	

Average recovery, 1.96 p.p.m.
Standard deviation, $\sigma_1 = 0.2871$.

given procedure, or by modifications of it, individual analysts can secure closer replication of measurements than agreement with other analysts, and (2) that a comparison of the variances within means for the two sets, arising from the errors of replication by the individual analysts, shows

TABLE 2.—*Results obtained by various modifications of A.O.A.C. method*
(Fluorine present, 2.12 p.p.m.)

COLLABORATOR	NO. OF TRIALS	FLUORINE FOUND (CALCULATED TO WEIGHTED AVERAGES)		VARIATION USED
		p.p.m.		
1	4	1.3 1.3 1.0 1.35	Av. 1.24	Own method of titrating
2	4	1.8 1.7 1.75 1.75	Av. 1.75	Not same number of cc. $Th(NO_3)_4$ in standard as in sample soln. Acidity, 2.09; in standard, 2.09
3	2	1.82 1.75	Av. 1.79	Acidity, 4.80—high
4	4	2.1 1.8 2.4 1.6	Av. 1.98	Acidity, 24.4, 6.5, 24.0 Own method of titration
5	6	1.4 4.0 1.4 1.4 1.9 1.2	Av. 1.89	No evaporation; H_3PO_4 instead of $HClO_4$ No constant aliquot
6	1	3.26		No evaporation; H_3PO_4 instead of $HClO_4$
7	1	2.50		Control of acidity before titration different
8	2	2.2 2.1	Av. 2.15	Own method of acidity control
9	2	0.8 0.8	Av. 0.80	Acidity, 3 cc. HCl
10	2	1.65 1.75	Av. 1.70	Acidity, 3.4 cc. Added 1.7 instead of 2 cc. to standard soln.
11	4	2.39 2.41 2.45 2.41	Av. 2.42	Acidity much too high
	32	59.46	Av. $\frac{59.46}{32}=1.86$	

Standard Deviation, $\sigma_2 = .6855$

$$\frac{\sigma_2}{\sigma_1}=\frac{.6855}{.2871}=2.39$$

that any one analyst, by strict adherence to the A.O.A.C. method, obtains a greater consistency in results than by favoring the particular modifications attempted in this study.

TABLE 3.—*Analysis of variance—collaborative study*

SOURCE OF SUM OF SQUARES	SUM OF SQUARES	DEGREES OF FREEDOM	VARIANCES	F
A.O.A.C. Method — mean value = 1.96 p.p.m.				
Total	2.5104	31		
Among Means	1.9867	7	.28381	9.208
Within Means	0.6237	24	.025988	
Modifications of A.O.A.C. method — Mean = 1.86 p.p.m.				
Total	15.0517	31		
Among Means	7.7351	10	.77351	2.22
Within Means	7.3166	21	.34841	

Further inspection suggests that the mean result of the collaborators following strictly the A.O.A.C. method (1.96 p.p.m.) is obviously low. Two causes for this are suggested: (1) The departure may be one of chance alone, arising from differences in operation by individual collaborators having slightly different interpretations of the wording of the method; (2) waters high in chlorides may not be amenable to the silver perchlorate treatment to control acidity. The method may be expected to produce more accurate results when used on samples containing moderate amounts of chlorides.

A survey of Table 2 indicates the manner in which the non-A.O.A.C. group departed from the standard procedure. Since these departures are accompanied by deviations in fluoride recoveries far too great to be explained by accident of errors of replication, a few general precautions seem to be in order.

1. Perchloric acid must be used for the distillation. Phosphoric acid is unsuitable.

2. The aliquot of the sample, made alkaline to phenolphthalein, must be evaporated to 20 cc. or less before the fluorine is liberated as hydrofluoric acid. Real importance is attached to this simple step.

3. Collaborative results indicate that the acidity of the solution for titration should contain 2 ml. of free hydrochloric acid (1+249) instead of some other amount. The standard and sample tubes must contain equal acid concentrations.

4. To maintain validity of color comparisons between sample and standard tubes, both must contain the same quantity of thorium nitrate, in the same volumes of liquid.

It is concluded from this study that the A.O.A.C. method for the

determination of fluorine, while in need of clarification, gives more accurate, more consistent, and more reproducible results than do the variations here attempted.

No report on effervescent salts was given by the associate referee.

No report on dairy products was given by the referee.

No report on butter—preparation of sample and fat—was given by the associate referee.

REPORT ON CHEESE

By IRA D. GARARD (New Jersey College for Women, Rutgers University, New Brunswick, N. J.), *Associate Referee*

The only project undertaken this year was a study of methods for the extraction of fat for examination. In addition to the two official methods, a third method, which has been used with some types of cheese,[1] was also given some consideration. This last method consists of melting the cheese at 45° to 50° C. on the wall of a beaker and allowing the fat to drain to the bottom of the beaker. To date the investigation has not advanced beyond the preliminary stages.

It is recommended[2] that a further study be made of the applicability and relative advantages of the different methods available for the extraction of fat from cheese.

REPORT ON MALTED MILK

By FRED HILLIG (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

Last year the unified method for determination of fat proposed by the Associate Referee, *This Journal*, **18**, 454, was submitted to collaborative tests. Certain precautions, as outlined in the report last year, were incorporated in the text of the method. The results obtained by five collaborators were not entirely satisfactory.

This year a chocolate flavored malted milk, prepared in the laboratory, was sent to collaborators with the request that the fat be determined by the method used last year. The results are given in Table 1.

The results are entirely satisfactory. Good correlation, both by individual analysts and between analysts, was obtained.

[1] Garard, Minsky, Baker, and Pascale, *Ind. Eng. Chem.*, **29**, 1167 (1937).
[2] For report of Subcommittee C and action by the Association, see *This Journal*, **22**, 60 (1939).

TABLE 1.—*Collaborative results on chocolate flavored malted milk*

ANALYST	FAT
	per cent
1	6.75
	6.72
2	6.85
	6.83
	6.84
3	6.74
4	6.84
	6.85
	6.90

Results of a collaborative study on two samples of malted milk submitted to eleven collaborators who used the tentative method are given in the report of the Associate Referee in 1936, *This Journal*, **19**, 382. They are unsatisfactory. In this report attention is called to three factors: (1) the small quantity of sample taken, (2) the occurrence of emulsions, and (3) difficulty in removal of non-fat material from the dried fat, which may be the cause of the poor agreement obtained by the collaborators.

The method for the determination of fat in dried milk is official. This method calls for the use of ammonia in preparing the sample for extraction. The procedure has become recognized as standard practice in analyzing for fat in milk products. The tentative method for fat in malted milk does not call for the use of ammonia, but otherwise it is similar to the method for dried milk. It is, therefore, recommended that a study be made on the application of this method to the determination of fat in malted milk. The unified method should also be compared to the tentative method for fat in malted milk and to the method for fat in dried milk.

Since the unified method is suitable for obtaining sufficient fat for the Reichert-Meissl determination, it is believed that it should be further studied with a view to its adoption for this purpose. It should be borne in mind also that the unified method is suitable for the determination of fat in food products other than milk products and also furnishes a convenient means for obtaining sufficient fat from most milk products for the determination of the Reichert-Meissel number.

Appreciation is expressed to Doris H. Tilden, Food and Drug Administration, San Francisco, and to Marie L. Offutt and J. H. Loughrey, Food and Drug Administration, New York, for their splendid cooperation.

RECOMMENDATIONS[1]

It is recommended—

(1) That the Associate Referee study the application to malted milk,

[1] For report of Subcommittee C and action by the Association, see *This Journal*, **22**, 60 (1939).

of the official method for the determination of fat in dried milk, with a view to final adoption of a single method for both products.

(2) That methods for the separation of fat from dairy products, except cheese, for the determination of fat constants be further studied.

(3) That studies of methods for the determination of casein be continued.

REPORT ON MALTED MILK—MICRO-ANALYTICAL METHODS

By B. G. HARTMANN (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

Several years ago the Association recommended that the glycerol-alcohol-water mixture (equal volumes) proposed by C. W. Ballard, New York Department of Health, be tried as a mounting medium in the microscopical examination of malted milk.

Under the supervision of G. L. Keenan, Microanalytical Division, Department of Agriculture, Washington, D. C., a number of commercial samples containing malted milk in varying amounts were examined, the mixture and mineral oil being used.

In all cases there was a pronounced tendency toward disintegration in the Ballard mixture, whereas the mineral oil left the material in suspension, so that the stippled appearance of malted milk remained plainly discernible.

While the Ballard mixture may be preferable to mineral oil in cases where clearing of certain ingredients is desired, its use in the identification of malted milk ingredients, particularly those that are soluble in aqueous vehicles, can not be recommended.

It is recommended that the study of the Ballard mixture be discontinued.

At the last meeting of the Association further collaborative study of the tentative method for the determination of citric acid in milk was recommended.

In this year's report on fruits and fruit products the Referee points out that the slow oxidation of a brominated citric acid solution yields materially more pentabromacetone than does the rapid oxidation procedure prescribed in the tentative method. Therefore, an investigation of this phase of the determination is recommended.[1]

Pending the outcome of the investigation it is recommended that further study of the present method for the determination of citric acid in milk be deferred.

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[1] For report of Subcommittee C and action by the Associatio , see *This Journal*, **22**, 61 (1939).

REPORT ON DRIED MILK

By FRED HILLIG (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

It was recommended last year that methods for the determination of lactic acid in dried milk be further studied. Accordingly, two samples of dried skim milk were submitted to collaborators with the request that lactic acid be determined by the colorimetric and the Troy and Sharpe aldehyde methods. The results are given in the table under Report on Neutralizers in Dairy Products (p. 495) and will not be repeated here. It is recommended that the work be continued.

No report on milk proteins was given by the associate referee.

No report on gelatin in milk and cream was given by the associate referee.

REPORT ON LACTOSE IN MILK

EFFECT OF VOLUME OF PRECIPITATE ON ACCURACY OF POLARIMETRIC DETERMINATION*

By E. R. GARRISON (Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Columbia, Mo.), *Associate Referee*

The procedure specified by the Association of Official Agricultural Chemists for the polarimetric determination of lactose in milk, *Methods of Analysis, A.O.A.C.*, 1935, 266, is based upon the method followed by Wiley (1884, 1885).[1] Wiley used 61.68 grams of milk in his work and found that the precipitated protein occupied a volume of 2.4 ml. He accordingly diluted his milk samples to 102.4 ml., after the addition of the clarifying agent, in order to prepare 100 ml. of solution. The present A.O.A.C. method specifies 65.8 grams of milk (twice the normal weight), which is diluted to 102.6 ml. after addition of the mercuric nitrate reagent. This dilution is proportional to that made by Wiley for a slightly smaller weight of milk. Wiley does not state whether he worked with whole or with skimmed milk but he failed to make any allowance in his dilution for any fat, which if present would be carried down with the protein and contribute to the volume of precipitate. The present A.O.A.C. method likewise does not make any allowance in the dilution for any variation in the volume of the precipitate, which would obviously vary with the fat and protein content of the milk.

Wiley and Ewell (1896)[2] noted that the volume of precipitate varied

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* Contribution from the Department of Dairy Husbandry, Mo. Agricultural Experiment Station, Journal Series No. *585*.

[1] *Am. Chem. J.*, 6, 289.

[2] *Analyst*, 21, 182.

with the composition of the milk and that it was necessary to dilute the sample according to the volume of precipitate if accurate results were to be obtained. They, therefore, proposed the method of double dilution for the accurate polarimetric determination of lactose in milk. Twice the normal weight of milk is placed in a 100 ml. and in a 200 ml. flask and the clarifying agent is added; the flasks are then filled to the mark with water and shaken, after which the contents are filtered and polarized. A mathematical formula was also devised by these individuals for calculating the true polarization from the results obtained. It may be summarized as follows: divide the product of the two readings made from the solutions in the 100 ml. and 200 ml. flasks by the difference in these readings. Woodman (1915)[1] proposed this same method, but used a slightly different procedure for calculating the percentage of lactose in the milk.

Scheibe (1901)[2] emphasized that the volume of precipitate depends upon both the protein and the fat content of the milk and that a serious error results in the optical determination of lactose if the sample is not diluted in accordance with the volume of precipitate. Milk samples containing 2.8–4.7 per cent fat had an average volume of 5.8 ml. of precipitate. He adopted the procedure of diluting the milk sample to 100 ml. after addition of the clarifying reagent, but multiplied the polarization results by 0.94 in order to correct for the volume of precipitate.

Ruffy (1932)[3] compared the polarimetric and gravimetric methods of determining lactose in milk. He attributed the lack of a consistent relationship in the differences in the results secured by the two methods to an error in the optical method because of failure to allow for variations in the volume of precipitate in the different samples of milk. He used a value of 6 ml. for the volume of precipitate in his samples.

Lactose determinations made on several samples of milk by the polarimetric and the Shaffer-Somogyi (1933)[4] copper-iodometric methods, in the Dairy Department at the University of Missouri, have shown consistently higher results by the former method. It was thought that inadequate allowance for the volume of precipitate in the polarimetric method might account in part at least for the higher results secured by this method. A study was therefore undertaken to determine the variation in the volume of precipitate in different samples of milk and to compare the lactose values obtained by use of the polariscope when these samples were diluted according to the A.O.A.C. specifications and when diluted according to the volume of precipitate.

PROCEDURE

In order to secure samples with a wide range of fat and protein content, the milk from 34 individual Holstein and Jersey cows was used in this

[1] *Food Analysis*. McGraw-Hill Book Co., New York.
[2] *Z. anal. Chem.*, 40, 1.
[3] *Lait*, 12, 95.
[4] *J. Biol. Chem.*, 100, 695.

study. The fat content of these samples was determined by means of the Babcock test. The fat (ml.) in the amount of milk used for the lactose determinations was computed on the basis that the specific gravity of the fat was 0.92 at 20° C. The specific gravity of the milk was determined at 20° C. with a standardized Quevenne lactometer.

The protein (ml.) in the 65.8 grams of milk used for the lactose determination was computed from the total nitrogen determinations made in duplicate on 2 ml. of milk by the Kjeldahl method. The milk was pipetted at 20° C. and the weight of milk transferred was calculated from the specific gravity. A 5 per cent deduction from the total nitrogen value was made before the nitrogen (grams) was multiplied by 6.38 for conversion into grams of protein. Rowland (1938)[1] showed that approximately 5 per cent of the total nitrogen in normal milk is non-protein nitrogen. The protein (grams) was converted into protein (ml.) on the basis that the specific gravity was 1.35 at 20° C. The fat and protein were added together to determine the total volume of precipitate in each sample.

The milk samples, precipitating reagents, and dilution water were tempered in a water bath at 20° C. before being used for the lactose determinations. Twice the normal weight of milk (65.8 grams) was weighed into each of two 100 ml. flasks and into a 200 ml. flask. The samples were clarified by the addition of 15 ml. of acid mercuric nitrate solution (1:5 dilution) and 15 ml. of a 5 per cent phosphotungstic acid solution. One of the 100 ml. flasks and the 200 ml. flask were diluted to the mark with water, while the other 100 ml. flask was diluted to 102.6 ml. The flasks were well shaken, and the contents were filtered and polarized. The percentage of lactose was determined by the method of Wiley and Ewell (1896)[2] from the polariscope readings on the solutions from the 100 and 200 ml. flasks; the polariscope readings on the solution from the 102.6 ml. flask furnished the A.O.A.C. lactose values, while the solution from the 100 ml. flask supplied the data from which was calculated the lactose value if the sample had been diluted according to the volume of precipitate in the milk.

DISCUSSION OF RESULTS

The fat and protein in the samples of milk analyzed are shown in Table 1. The fat content of the 19 samples of Holstein milk ranged from 2.9 to 4.7 per cent; the protein and fat in 65.8 grams (2 N weight) of these samples varied from 3.37 to 5.10 ml., with an average volume of 4.17 ml. The percentage of fat in the 13 samples of Jersey milk varied from 4.1 to 8.7; the protein and fat in 65.8 grams of these samples ranged from 5.01 to 8.51 ml., with an average volume of 6.65 ml. The average volume of protein and fat in twice the normal weight of milk varied with the fat content as follows:

[1] *J. Dairy Research*, 9, 42.
[2] *Loc. cit.*

per cent fat	*ml.*
2.9–3.4	3.77
3.5–4.4	4.42
4.5–5.4	5.29
5.5–6.4	6.40
6.5–8.7	7.60

TABLE 1.—*Volume of precipitate in 65.8 grams of milk from individual cows*

COW	FAT	FAT[1] IN 65.8 GRAMS MILK	PROTEIN NITROGEN[2] IN MILK	PROTEIN[3] IN 65.8 GRAMS MILK	PROTEIN[4] IN 65.8 GRAMS MILK	PROTEIN AND FAT IN 65.8 GRAMS MILK
	per cent	*ml.*	*per cent*	*grams*	*ml.*	*ml.*
H726	2.9	2.10	.41	1.721	1.27	3.37
H741	3.0	2.15	.45	1.889	1.40	3.35
H750	3.0	2.15	.45	1.889	1.40	3.55
H685	3.3	2.35	.43	1.805	1.34	3.69
H740	3.3	2.35	.43	1.805	1.34	3.69
H743	3.2	2.29	.50	2.099	1.55	3.84
H722	3.5	2.50	.45	1.889	1.40	3.90
H744	3.6	2.57	.47	1.973	1.46	4.03
H616	3.4	2.43	.54	2.267	1.68	4.11
H720	3.8	2.71	.45	1.889	1.40	4.11
H724	3.7	2.66	.49	2.057	1.52	4.18
H729	4.0	2.85	.45	1.889	1.40	4.25
H714	3.4	2.43	.62	2.603	1.93	4.36
H727	4.0	2.85	.50	2.099	1.55	4.40
H725	4.2	3.00	.50	2.099	1.55	4.55
H751	4.4	3.15	.50	2.099	1.55	4.70
H611	4.3	3.07	.53	2.225	1.67	4.74
H709	4.3	3.07	.55	2.309	1.71	4.78
J880	4.1	2.93	.67	2.813	2.08	5.01
H707	4.7	3.36	.56	2.351	1.74	5.10
J889	4.8	3.43	.56	2.351	1.74	5.17
J856	5.4	3.85	.57	2.393	1.77	5.62
J901	5.6	4.00	.58	2.435	1.80	5.80
J883	5.7	4.07	.68	2.855	2.12	6.19
J893	5.8	4.15	.69	2.897	2.15	6.30
J853	6.0	4.29	.74	3.107	2.30	6.59
J877	6.6	4.71	.62	2.603	1.93	6.64
J882	6.4	4.57	.69	2.897	2.15	6.72
J839	6.3	4.51	.74	3.107	2.30	6.81
J893	7.3	5.21	.57	2.393	1.78	6.99
J884	6.7	4.80	.76	3.190	2.36	7.18
J838	8.3	5.93	.73	3.064	2.27	8.20
J887	8.7	6.21	.61	2.561	1.90	8.11
J864	8.0	5.71	.90	3.778	2.80	8.51

H = Holstein.
J = Jersey.

[1] Assuming sp. gr. of fat to be 0.92 at 20°C.
[2] 5% deduction made from total nitrogen for non-protein nitrogen.
[3] Grams nitrogen × 6.38.
[4] Assuming sp. gr. of protein to e 1.35 at 20°C.

These results (Table 1) seem particularly significant when it is recalled that the A.O.A.C. method only allows for a volume of 2.6 ml. of precipi-

TABLE 2.—*Lactose values for milk samples of different composition obtained by A.O.A.C. method and when diluted according to the volume of precipitate*

	LACTOSE (PER CENT)			DIFF. IN LACTOSE (PER CENT) BETWEEN—	
PROTEIN AND FAT IN 65.8 GRAMS MILK	A.O.A.C. METHOD	DOUBLE DILUTION METHOD	DILUTED ACCORDING TO ML. PPT.[1]	A.O.A.C. AND DOUBLE DILUTION METHOD	DOUBLE DILUTION METHOD AND DILUTED ACCORDING TO ML. PPT.
ml.					
3.37	4.90	4.65	4.79	.25	−.14
3.55	5.35	5.05	5.26	.30	−.21
3.55	5.00	4.78	4.95	.22	−.17
3.69	4.90	4.73	4.85	.17	−.12
3.69	5.48	5.28	5.38	.20	−.10
3.84	5.33	5.18	5.18	.15	.00
3.90	5.05	4.85	4.96	.20	−.11
4.03	5.20	5.13	5.07	.07	+.06
4.11	4.88	4.73	4.78	.15	−.05
4.11	4.95	4.73	4.88	.22	−.15
4.18	5.38	5.13	5.28	.25	−.15
4.25	5.00	4.85	4.89	.15	−.04
4.36	4.68	4.50	4.55	.18	−.05
4.40	5.30	5.20	5.17	.10	+.03
4.55	5.13	4.85	5.02	.28	−.17
4.70	5.00	4.80	4.87	.20	−.07
4.74	4.88	4.60	4.78	.28	−.18
4.78	4.78	4.58	4.66	.20	−.08
5.01	4.90	4.60	4.76	.30	−.16
5.10	5.05	4.88	4.88	.17	.00
5.17	5.25	4.95	5.14	.30	−.19
5.62	4.30	4.20	4.17	.10	+.03
5.80	5.38	5.15	5.20	.23	−.05
6.19	4.80	4.60	4.66	.20	−.06
6.30	5.03	4.80	4.80	.23	.00
6.59	4.43	4.18	4.25	.25	−.07
6.64	4.95	4.68	4.81	.27	−.13
6.72	5.03	4.83	4.85	.20	−.02
6.81	3.90	3.63	3.77	.27	−.14
6.99	4.55	4.40	4.28	.15	+.12
7.13	4.63	4.50	4.39	.13	+.11
8.11	4.25	4.00	4.07	.25	−.07
8.11	4.83	4.65	4.53	.18	+.12
8.51	4.23	4.03	3.94	.20	+.09

[1] Calculated from value obtained when diluted to 100 ml.

tate in the dilution of the milk. Whole milk of ordinary composition contains about twice as much precipitate as the present method makes allowance for.

The values found in this study are slightly lower than those reported by Scheibe (1901)[1] for milk of similar fat content. It would seem to be more consistent to vary the allowance for the volume of precipitate with the percentage of fat in the milk (since this is easily ascertained and since protein content is closely correlated with fat content) than to adopt an arbitrary standard for all milks regardless of their composition. The proper value to allow for the volume of precipitate could be ascertained by making a compilation from analyses reported in the literature of the amount of protein in a large number of milk samples of definite fat contents. The other alternative would be to follow the method of double dilution proposed by Wiley and Ewell.

The results of the lactose determinations on the samples of milk are given in Table 2. As would be expected the A.O.A.C. method gave higher values in all cases than did the Wiley and Ewell double dilution method and the calculated values based on full allowance for all protein and fat in the sample. The A.O.A.C. values ranged from a minimum of .07 to a maximum of .30, with an average value .21 per cent higher than the values obtained by the double dilution method. The differences in the results by the two methods, however, do not show any consistent variation with the amount of fat and protein in the milk. The amount of lactose in the milk would also be a factor affecting any differences in the results by the two methods.

The double dilution method gave values that were slightly lower in most cases than the calculated results based on the volume of precipitate but with seven samples the results were higher by the latter method.

CONCLUSIONS

From the data presented it is evident—

(1) That the volume of precipitate varies with the composition of the milk and is determined by the amount of both the fat and the protein in the milk.

(2) That the present A.O.A.C. allowance of 2.6 ml. for the volume of precipitate in 65.8 grams (2 *N* weight) of milk is too low for whole milk of average composition.

(3) That the polarimetric determination of lactose in whole milk by the A.O.A.C. method averages about 0.20 per cent higher than when the milk is diluted according to the volume of precipitate so that 100 ml. of solution is obtained from 65.8 grams of milk.

It is recommended that this study be continued by the referee with other collaborators in order to secure information on which to make recommendations to the Association for any changes considered desirable for the polarimetric determination of lactose in milk.

The Associate Referee wishes to acknowledge the collaboration on this

[1] *Loc. cit.*

study of L. D. Haigh of the Agricultural Chemistry Department, University of Missouri.

REPORT ON EXTRANEOUS MATTER IN DAIRY PRODUCTS

METHOD FOR ESTIMATION OF AMOUNT OF MOLD MYCELIA IN BUTTER

By J. D. WILDMAN (Microanalytical Division, U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

A method for estimating the amount of mold in butter was described in *This Journal*, **20**, 93. The method has been used since that time in the Microanalytical Laboratory of the Food and Drug Administration, for experimental work in the Chicago Station of the Food and Drug Administration, for experimental work at Purdue University, and for regulatory work by the North Dakota Regulatory Department. The method has received no adverse criticism from these sources.

Since the method is essentially the preparation of a butter sample in such a way as to render it adaptable to examination by the Howard method, *Methods of Analysis, A.O.A.C.*, 1935, 500, and since that method has been made official, it is considered that the butter method should be adopted as tentative. In this connection, as with the Howard method for tomato products, it should be borne in mind that adequate training under an experienced analyst is essential to proper use of the method. The modified method was published in *This Journal*, **22**, 76 (1939).

It is recommended[1] that the method of sample preparation of butter for examination for mold by the official method for mold in tomato products be made tentative.

No report on decomposition in dairy products was given by the Associate Referee.

REPORT ON NEUTRALIZERS IN DAIRY PRODUCTS

By FRED HILLIG (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

It was recommended last year that studies of methods for the detection of neutralizers in dairy products be continued. Accordingly, two samples of dried skim milk were sent to collaborators with the request that determination of lactic acid be made by both the colorimetric

[1] For report of Subcommittee C and action by the Association, see *This Journal*, 22, 60 (1939).

method and the Troy and Sharpe aldehyde method.[1] It was also requested that the titratable acidities and the alkalinities of the total ash and water-soluble ash be determined. The results are given in Table 1.

TABLE 1.—*Analysis of dried skim milks*

COLLABORATOR	ACIDITY cc. 0.1 *N* PER 100 GRAMS		ALKALINITY OF ASH cc. 0.1 *N* PER 100 GRAMS		ALKALINITY OF WATER-SOLUBLE ASH cc. 0.1 *N* PER 100 GRAMS		LACTIC ACID COLORIMETRIC METHOD PER CENT		LACTIC ACID TROY & SHARPE METHOD PER CENT	
	SAMPLE 1	SAMPLE 2	SAMPLE 1	SAMPLE 2	SAMPLE 1	SAMPLE 2	SAMPLE 1	SAMPLE 2	SAMPLE 1	SAMPLE 2
1	196	142	740	802	9.2	11.0	0.104	0.341	—	—
	200	142	736	798	9.8	11.6	0.108	0.341	—	—
2			701	785			0.039	0.278	—	—
	171	118	717	790	12.6	17.6	0.038	0.274	—	—
3	215	156	—	792	17.2	23.2	0.045	0.186	—	—
	211	157		794	17.2	25.2	0.054	0.190	—	—
4	186	130	730	794	20.0	34.0	0.150	0.350	—	—
5	202	133	692	792	12.4	25.8	—	—	0.116	0.295
	200	133	698	790	12.4	26.8			0.126	0.295
6	—	140	—	788	—	13.0	—	—	—	0.260
		140		784		12.6				0.260
7	—	128	—	790	—	22.0	—	—	—	0.272
8	—	124	—	772	—	33.0	—	—	—	0.290
		120		774		34.8				0.290

The results for the most part are disappointing. For the determination of acidity 5 grams of the milk was diluted with 100 cc. of water and titrated with 0.1 *N* sodium hydroxide, phenolphthalein being used as indicator. The results reported show that individual analysts agree very closely, but the checks obtained by the various collaborators are not satisfactory. During the coming year attention will be given to the Van Slyke and lead acetate methods for the determination of acidity. There are no procedures in *Methods of Analysis, A.O.A.C.*, for the determination of total alkalinity and alkalinity of water-soluble ash in milk. The collaborators were asked to make these determinations following the methods given in the wine chapter. It is very evident from the results given in the table that these methods are not applicable to milk. The Troy-Sharpe method gave fairly satisfactory results. The results obtained by the colori-

[1] Cornell University Agr. Expt. Sta. Memoir 179, June 1935.

metric method are not satisfactory, and it is believed that they are due to the failure of the analyst to prepare a good standard curve. Before the method is resubmitted for collaborative work, the instructions covering the preparation of the standard curve will be revised in order to eliminate any possibile ambiguities.

Appreciation is expressed to E. E. Mair of the H. J. Heinz Company; A. H. Robertson, State Food Laboratory, New York; A. H. Johnson, Sealtest, Inc., Baltimore, Md.; D. A. Magraw, American Dry Milk Institute, Chicago; F. B. Jones, Food and Drug Administration, New York; I. S. Shupe, Food and Drug Administration, Kansas City, Mo.; and Doris H. Tilden, Food and Drug Administration, San Francisco, for their splendid cooperation.

It is recommended that the work be continued.

REPORT ON TESTS FOR PASTEURIZATION OF DAIRY PRODUCTS

PHOSPHATASE TEST IN EXAMINATION OF MILK AND CREAM
II. A TECHNIC FOR USE IN THE FIELD

By F. W. GILCREAS (Division of Laboratories and Research, New York State Department of Health, Albany, N. Y.), *Associate Referee*

Two modifications of the phosphatase test for the detection of pasteurization of milk and cream developed in the chemical laboratories of the Department of Health of the City of New York have been described by Scharer.[1,2] One of these tests is a simple technic for use in the field, which the author reports as having an accuracy comparable to that of his laboratory test or of the Kay and Graham technic.[3] In both procedures the phenol liberated as a result of hydrolysis of disodium phenyl phosphate by the enzyme is estimated colorimetrically by the addition of an alcoholic solution of 2,6 dibromoquinonechloroimide.

Because of the importance of a field test in the control of pasteurization, a collaborative study similar to that made of the modified Kay and Graham test was undertaken by the Associate Referee, *This Journal*, **21**, 372–380 (1938), to determine its limitations when used by a number of different laboratory workers. Since the test had been revised subsequent to its publication, Mr. Scharer was requested to designate the technic to be followed. In order to eliminate as far as possible variations in the reagents used, he also supplied the disodium phenyl phosphate and the 2,6 dibromoquinonechloroimide in tablet form, together with color

[1] *J. Dairy Sci.*, 21, 21–34 (1938).
[2] *J. Milk Tech.*, 1, 35–38 (1938).
[3] *J. Dairy Research*, 6, 191–203 (1935).

standards corresponding to 0.2 per cent and 0.5 per cent of added raw milk, or the equivalent deficiencies in heat treatment.

PHOSPHATASE TEST FOR USE IN THE FIELD

REAGENTS

(a) *Buffer substrate.*—Dissolve 1.09 grams of disodium phenyl phosphate (washed with ether to remove free phenol and dried in a desiccator) in 900 cc. of distilled water previously saturated with $CHCl_3$. Add 50 cc. of borate buffer solution and dilute to 1 liter. Add 10 cc. of $CHCl_3$ and store in refrigerator. Or, dissolve in 50 cc. of distilled water one tablet containing disodium phenyl phosphate, $MgSO_4$, and $NaBO_2$ buffer to give 50 cc. of buffer substrate solution.

(b) *Borate buffer solution.*—Dissolve 28.427 grams of $Na_2B_4O_7 \cdot 10H_2O$ (analytical grade) in 900 cc. of warm distilled water. Stir vigorously while powder is being added to prevent lumping. Add 3.27 grams of NaOH in the form of a strong solution (2–5 N), cool, and dilute to 1 liter.

(c) *2,6 Dibromoquinonechloroimide (BQC) solution.*—Dissolve 0.04 gram of 2,6 dibromoquinonechloroimide in 10 cc. of 95% ethyl or methyl alcohol. *Do not use denatured alcohol.* Store in refrigerator. Or, dissolve in 5 cc. of 95% ethyl or methyl alcohol one tablet containing sufficient 2,6 dibromoquinonechloroimide to yield a solution of the correct concentration.

(d) *Normal butyl alcohol, neutral.*—To 100 cc. of normal butyl alcohol add 0.1–0.2 cc. of 0.1N NaOH or an amount sufficient to give a pale blue color when tested with bromothymol blue indicator. (Some normal butyl alcohol may not require neutralization.)

PURIFICATION OF BUFFER SUBSTRATE SOLUTION

Crush a buffered substrate tablet in test tube and dissolve in 5 cc. of distilled water. Add 2 drops of the BQC solution. Allow 5 minutes for color development, then extract the indophenol with 2–2.5 cc. of normal butyl alcohol. Allow to stand until alcohol layer has separated at top of tube. Remove alcohol layer with medicine dropper and discard. Dilute remainder of solution to 50 cc. This solution is then phenol-free.

PERMANENT COLOR STANDARDS

(a) *Red.*—Dissolve 59.59 grams of $CoCl_2 \cdot 6H_2O$ in and dilute to 1 liter with 1% of HCl W/V.

(b) *Blue.*—Dissolve 62.43 grams of $CuSO_4 \cdot 5H_2O$ in and dilute to 1 liter with1 % of HCl W/V.

(c) *Yellow.*—Dissolve 45.05 grams of $FeCl_3 \cdot 6H_2O$ in and dilute to 1 liter with 1% of HCl W/V.

Prepare permanent color standards corresponding to 0.2% and 0.5% raw milk added to pasteurized milk by combining the quantities of color solutions **a**, **b**, and **c**, indicated in the following table and diluting to 5 cc. with distilled water in each case.

Preparation of standards

Added Raw Milk	Color Solution		
	Red (a)	Blue (b)	Yellow (c)
per cent			
0.2	0.4	1.5	0.5
0.5	0.2	2.2	0.5

PROCEDURE

Add 0.5 cc. of sample to 5 cc. of buffered substrate. Shake briefly. Incubate 10 minutes in a water-bath at 98° F. (If no water-bath is available, incubate in vest pocket for somewhat longer period.) Remove from bath, and add 6 drops of the BQC solution. Shake well immediately.

Properly pasteurized milk should give a gray or brown color reaction; properly pasteurized cream, gray or white. Raw milk or cream reacts with an intense blue color. The appearance of any blue is indicative of improper pasteurization, the intensity of color being proportional to the degree of inadequate heat treatment.

After development of color as above, add 2 cc. of normal butyl alcohol (neutral). Invert the test tube *slowly* at least 10 times and allow to stand until separation of the alcohol layer is complete. (Rapid inversion will result in formation of an emulsion. The alcohol should separate clearly and should extract the indophenol formed by the reaction.) Compare the color in the alcohol layer with the permanent standards against an opaque milk-glass plate to diffuse light through standards and sample. The appearance of any blue or blue-green in the alcohol layer is indicative of improper pasteurization. In the absence of properly pasteurized milk to be used as a control, a boiled milk may be substituted.

PRECAUTIONS

Thoroughly wash and rinse all equipment before re-use. Avoid the use of phenolic resin bottle closures anywhere in the test; the BQC reagent is sufficiently sensitive to demonstrate the leaching of phenol from the resin by water.

The following laboratories were asked to examine, beginning June 27, 1938, 6 weekly series of 12 samples each. Some laboratories were unable to examine the complete series.

Bureau of Milk Sanitation, New York State Department of Health, Albany (Mobile Laboratories, Nos. 1 and 2), W. D. Tiedeman, Chief.

Bureau of Laboratories, Connecticut State Department of Health, Hartford, F. L. Mickle, Director.

South Dakota State Chemical Laboratory, Vermillion, Guy Frary, State Chemist.

Division of Laboratories and Sanitation, Jacksonville City Department of Health, Jacksonville, Fla., H. N. Parker, Director.

Division of Foods and Drugs, Massachusetts State Department of Health, Boston, Hermann Lythgoe, Director.

Division of Chemistry, Bureau of Laboratories, Baltimore City Health Department, Baltimore, Md., Emanuel Kaplan, Chief.

Sealtest Laboratories, Baltimore, Md., Jas. J. Johnson.

Best Foods, Inc., Bayonne, N. J., H. W. Vahlteich, Chemist.

Department of Dairy Technology, Ohio State University, Columbus, L. H. Burgwald, Associate Professor.

Geneva City Laboratory, Geneva, N. Y., R. S. Breed, Director.

Tompkins County Laboratory, Memorial Hospital, Ithaca, N. Y., B. F. Hauenstein, Director.*

Chemical Laboratory, New York City Department of Health, New York, Harry Scharer, Chemist.

Division of Public Health Methods, National Institute of Health, Washington, D. C., F. J. Moss, Sanitary Engineer.

* Dr. Hauenstein resigned as Director on July 1, but made arrangements for the examination of further series of samples.

TABLE 1.—*Observed readings of all samples by comparison with color standards*

SAMPLE	LABORATORY NUMBER																		TOTAL		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	a	b	c
M 30′/143°																					
701, 703, 709	bbb			aaa	aab	aaa	aab	aab	aaa		aaa	bab	aaa	aab	aaa	bbb	bbc		27	14	1
1201, 1203, 1208	cbc	aaa	aab	aaa	aaa	ccc	bab	aaa	aaa	abb	aaa	aaa	aab		aaa	bbb	aaa	aab	35	11	5
Grand total																			62	25	6
M 25′/143°																					
1103, 1108	ba	aa	aa	aa	ab	bb	aa	ab	ba	ba	aa	aa	aa	aa	aa	bb	aa	bb	25	11	0
M 20′/143°																					
702, 704, 711	bbb			baa	aaa	aaa	bbc	bbb	bab		aaa	bba	baa	bbb	aaa	bbc	bcb		18	21	3
909, 912	ac	ba	bb		—c	ba	bb	bb	aa	cc	aa	bb	aa	bb	aa	bb	aa	bb	13	16	4
1104, 1107, 1112	abb	baa	bba	aaa	bbb	bbb	aaa	abb	bbb	baa	bab	abc	baa	bbb	aaa	bbb	bbb	bbb	20	33	1
1204, 1207	cc	aa	bb	aa	aa	—c	bc	bb	aa	bc	aa	bb	ba		aa	bc	cc	bb	13	12	8
Grand total																			64	82	16
M 15′/143°																					
705, 712	cc			ba	bb	bb	cc	cc	bb		bb	ba	bb	cc	ba	cc	cc		3	13	12
1202	c	b	c	a		c	c	b	b	c	b	b	b		a	c	c	c	2	6	8
Grand total																			5	19	20
M 30′/143°+0.2% R																					
802, 805, 810	ccc	baa		aaa	bac	abb	ccc	ccc	bbb	cbb	bbb	aab	aaa	ccc	aba	ccc	cbb		14	16	18
902, 907, 911	acc	aaa	cca			bba	bbb	cbb	aaa	cbc	aaa	abb	aaa	bbb	aaa	cbb	aab	bbb	21	19	8
1102, 1105	cb	bb	aa	aa	ba	bb	aa	bb	bc	ba	bb	aa	ab	cc	aa	bc	cb	bb	13	17	6
Grand total																			48	52	32
M 30′/143° +0.5% R																					
903, 905, 910	ccc	bba	ccc			ccc	ccc	ccc	bbb	cbc	bbb	bbc	aaa	ccb	bba	ccc	bcb	ccc	5	16	27
M 30′/143°+2% R																					
706, 708, 710	ccc			ccc	ccc	ccc	ccc	ccc	ccc		ccc	ccc	ccc	ccc	cbb	ccc	ccc		0	2	40
M R																					
707	c			c	c	c	c	c	c		c	c	c	c	c	c	c		0	0	14
M 30′/142°																					
804, 809, 811	bcb	baa		aaa	cba	bbb	cbc	bbb	aaa	acb	aaa	abb	aaa	bbb	aaa	bcb	aaa		23	19	6
1003, 1011	ba	aa	aa	aa	aa	aa	cc		aa	bb	aa	ab	aa	bb	aa	bb	bb	bb	20	12	2
Grand total																			43	31	8

TABLE 1.—*Observed readings of all samples by comparison with color standards*—(*Continued*)

SAMPLE	LABORATORY NUMBER																		TOTAL		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	a	b	c
M 25′/142°																					
803, 812	cb	ba		aa	cb	ab	cc	bc	ab	cc	ab	ab	aa	bb	aa	cb	bb		11	13	8
1001, 1004, 1009	ccc	bbb	abb	baa	aaa	bbb	ccc		aab	bbc	abb	aab	abb	bbb	aaa	bbb	bcc	cbb	15	26	10
Grand total																			26	39	18
M 30′/142°+0.2% R																					
1005, 1007	ca	bb	bb	ca	aa	ba	cc		ab	bc	bb	ac	aa	cc	ba	bc	cc	cc	10	11	13
LC 30′/145°																					
806, 808	bb	aa		aa	ac	aa	cb	aa	aa	bb	aa	aa	aa	aa	aa	ab	aa		24	6	2
LC 30′/143°																					
1205, 1209, 1211	bca	aaa	a–a	aaa	aab	bab	aaa	aaa	aaa	baa	aaa	aaa	aaa		aaa	abb	aaa	aaa	42	7	1
LC 15′/143°																					
1210, 1212	cc	aa	cb	aa	aa	bb	aa	bb	aa	bb	aa	aa	bb		aa	cb	bb	bb	16	14	4
LC 25′/142°																					
1006, 1008, 1010	ccc	ccc	ccc	cbc	bba	ccc	ccc		ccc	ccc	ccc	bcc	ccc	ccc	cbb	ccc	ccc	ccc	1	6	44
LC 20′/142°																					
1002, 1012	cc	cc	cc	cc	cb	cc	cc		cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	0	1	33
LC 30′/143°+0.2% R																					
901, 904	bc	aa	cc			aa	aa	bb	aa	ac	ba	aa	aa	bb	aa	bb	ab	aa	19	9	4
1109, 1111	cb	aa	aa	aa	b	ab	aa	bb	bb	aa	ab	ab			aa	bb	ca	bb	16	13	2
Grand total																			35	22	6
LC 30′/143°+0.5% R																					
906, 908	cc	aa	cc			bb	ac	bc	ba	ab	bb	aa	aa	bb	aa	bb	bc	bc	11	13	8
HC 30′/145°																					
801, 807	aa	aa		aa	aa	aa	bb	aa	aa	aa	aa	aa	aa	aa	aa	aa	aa		30	2	0
HC 30′/143°																					
1101, 1106, 1110	aaa	aaa	aaa	aaa	aaa	aaa	aaa	aaa	aaa	aaa	aaa	aaa	aa	aaa	aaa	aab	aaa	aaa	52	1	0
HC 15′/143°																					
1206	b	a		a	b	a	a	a	a	b	a	a	a		a	a	a	a	13	3	0

M = Milk
LC = Light Cream
HC = Heavy Cream
R = Raw

a = <0.2
b = ≧0.2, <0.5
c = ≧0.5

TABLE 2.—*Interpretations of the examination of all samples*

SAMPLE	LABORATORY NUMBER																		TOTAL		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	P	S	U
M 30′/143°																					
701, 703, 709	SSS			PPP	PPP	PPP	PPP	PPP	PSS		PPP	SPP	PPP	PPP	PPP	PPP	SSP		34	8	0
1201, 1203, 1208	UUS	PPP		PPP	PPP	UUU	PPP	PPP	PPP	PSS	PPP	PPP	PPP		PPS	PPP	SSS	PPS	35	8	5
Grand total																			69	16	5
M 25′/143°																					
1103, 1108	UP	PP		PP	PS	UU	PP	PS	US	SS	PP	PP	PP	PP	SP	PP	SS		20	8	4
M 20′/143°																					
702, 704, 711	SSS			UPP	SSP	PPP	PPS	SSS	SPS		PPP	SSP	PPP	SSS	PPP	PPS	SUS		21	19	2
909, 912	PU	UP			−U	SP	PP	SS	PS	UU	PP	SS	PP	SS	SS	PP	SS	SS	12	14	5
1104, 1107, 1112	PPS	SPP		PPP	SUU	UUS	PPP	SSS	UUU	SSS	SPS	PSU	PPP	SSS	PPP	PPP	UUU	SSS	21	19	11
1204, 1207	UU	PS		PP	PP	−U	PS	SS	SS	SU	PP	SS	PP		SS	PS	UU	SS	11	14	6
Grand total																			65	66	24
M 15′/143°																					
705, 712	SS			UP	UU	SS	SS	SS	UU		SS	SP	SS	UU	US	SS	UU		2	16	10
1202	U	S		P		U	S	S	S	U	U	U	P		S	S	U	U	2	6	7
Grand total																			4	22	17
M 30′/143°+0.2%R																					
802, 805, 810	SSS	UPS		PSP	USU	PSS	UUU	SSS	UUU	USS	USS	PPS	PPP	UUU	SSP	SSS	USS		10	23	15
902, 907, 911	PUU	PPP				UUP	PPP	SSS	SPS	USU	PPP	PSS	PPP	SSS	SPS	SPP	SSU	SSS	19	19	7
1102, 1105	UU	UU		PP	SP	UU	PP	SS	UU	SS	US	PP	PP	SS	PS	PS	UU	SS	11	12	11
Grand total																			40	54	33
M 30′/143°+0.5% R																					
903, 905, 910	UUU	SSP				UUU	UUU	UUS	UUU	USU	UUU	SSU	PPP	UUU	UUS	SSS	UUU	USU	4	11	30
M 30′/143°+2% R																					
706, 708, 710	UUU			UUU	UUU	UUU	UUU	UUU	UUU		UUU	UUU	UUU	UUU	UUU	UUU	UUU		0	0	42
M R																					
707	U			U	U	U	U	U	U		U	U	U	U	U	U	U		0	0	14
M 30′/142°																					
804, 809, 811	SSS	SPP		PSS	USS	SSS	SPU	SSS	SSS	SUS	PPP	PSS	PPP	SSS	SPS	PSP	SSS		14	31	3
1003, 1011	SP	PP	PP	PS	PP	PP	UU		SP	SS	PP	PS	PP	PP	SS	PP	UU	SS	20	10	4
Grand total																			34	41	7

TABLE 2.—*Interpretations of the examination of all samples*—(*Continued*)

SAMPLE	LABORATORY NUMBER																		TOTAL		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	P	S	U
M 25′/142°																					
803, 812	SS	UP		PP	UU	PS	UU	SS	PU	UU	PS	PS	PP	UU	SP	SP	SU		11	10	11
1001, 1004, 1009	USS	SSS	PSS	UPS	PPP	USU	UUU		SSU	SSU	PSS	PPS	PSS	SSS	SSS	PPP	UUU	SSS	12	27	12
Grand total																			23	37	23
M 30′/142°+0.2% R																					
1003, 1007	SP	SU	SS	UP	PP	UP	UU		SU	SU	UU	PU	PP	UU	US	PS	UU	SU	9	9	16
LC 30′/145°																					
806, 808	SS	PP		PP	SU	PP	SP	SP	PP	SS	PP	PP	PP	PP	PP	PP	PP		24	7	1
LC 30′/143°																					
1205, 1209, 1211	USP	PPP		PPP	PPS	UPS	PPP	PPP	PPP	SPP	PPP	PPP	PPP		PPP	PPP	SSS	PPP	39	7	2
LC 15′/143°																					
1210, 1212	SS	PP		PP	PP	SS	PP	SS	SS	SS	PP	PP	PP		PP	SP	UU	SS	17	13	2
LC 25′/142°																					
1006, 1008, 1010	USU	UUU	UUU	UUU	SSP	UUU	UUU		UUU	UUU	UUU	SUU	UUU	UUU	UUU	UUU	UUU	UUU	1	4	46
LC 20′/142°																					
1002, 1012	UU	UU	UU	UU	UU	UU	UU		UU	UU	UU	UU	UU	UU	UU	UU	UU	UU	0	0	34
LC 30′/143°+0.2% R																					
901, 904	UU	PP				PP	PP	SS	SS	PU	PP	PP	PP	PP	PP	PP	SU	PP	21	5	4
1109, 1111	UU	PP		PP	S –	PS	PP	SS	UU	PP	PS	PS		SS	SS	PP	US	SS	13	13	5
Grand total																			34	18	9
LC 30′/143°+0.5% R																					
906, 908	UU	PP				UU	PU	SU	US	PS	UU	PP	PP	UU	PP	PP	UU	SU	12	4	14
HC 30′/145°																					
801, 807	PP	PP		PP	PS	PP	PP	PP	PP	PP	PP	PP	PP	PP	PP	PP	PP		31	1	0
HC 30′/143°																					
1101, 1106, 1110	PPP	PPP		PPP	PPP	PPP	PPP	PPP	PPP	SSP	PPP	PPP	PP –	PPP	PPP	PPP	SPP	PPP	47	3	0
HC 15′/143°																					
1206	U	P		P	S	P	P	P	P	S	P	P	P		P	P	P	P	13	2	1

M = Milk
LC = Light Cream
HC = Heavy Cream
R = Raw

P = Pasteurized
S = Slightly Underpasteurized
U = Underpasteurized

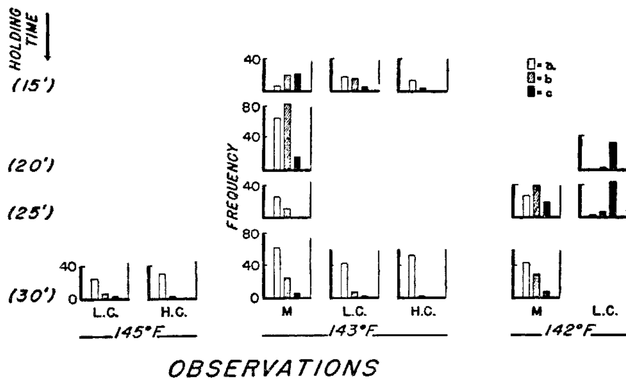

FIG. 1.—FREQUENCY DISTRIBUTION OF OBSERVED READINGS BY COMPARISON WITH COLOR STANDARDS. SAMPLES HEATED TO DESIGNATED TEMPERATURE AND TIME.

a = <0.2; b = $\geqq 0.2$ and <0.5; c = $\geqq 0.5$

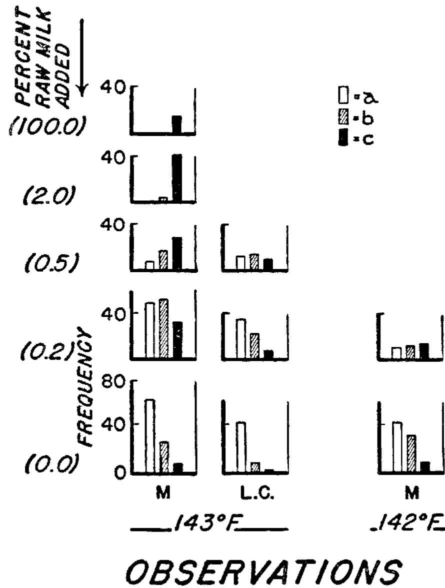

FIG. 2.—FREQUENCY DISTRIBUTION OF OBSERVED READINGS BY COMPARISON WITH COLOR STANDARDS. PASTEURIZED MILK AND CREAM WITH ADDED RAW MILK.

a = <0.2; b = $\geqq 0.2$ and <0.5; c = $\geqq 0.5$

Food Research Division, United States Department of Agriculture, Washington, D. C., Walter S. Hale, Associate Chemist.

City Health Department, Chicago, Ill., Herman N. Bundesen, President of the Board.

New Jersey Agricultural Experiment Station, New Brunswick, O. F. Garrett, Assistant in Dairy Manufacture.

The samples represented milk taken at various holding periods of commercial pasteurization at 143° F.; milk pasteurized at 142° F. in the Referee's laboratory; and also pasteurized milk or cream to which had been added definite quantities of raw milk. The samples were packed in sufficient dry ice to provide 6–10 hours of refrigeration without freezing and were shipped for delivery within 24 hours by parcel post, special delivery, or by air express to the more distant points. Each sample was identified by a serial number only.

The cooperating laboratories were requested to report their readings as equal to, less than, or greater than the standards and also to state their interpretation of the treatment of each sample as pasteurized, slightly underpasteurized, underpasteurized, or grossly underpasteurized. The results of the examinations by all laboratories reporting the readings in comparison with the color standards are given in Table 1 and the interpretation of results in Table 2. Figures 1 and 2 show graphically the frequency distribution of the observed results obtained by comparison with the color standards, for milk and cream heated to the given temperature and for the given period, and for pasteurized milk containing the stated percentage of added raw milk. Figures 3 and 4 show graphically the frequency distribution of the designated interpretation of the results of the examinations of the same samples.

Inspection of these results indicates that unheated milk and a pasteurized product containing 2 per cent of added raw milk were accurately determined in every case. In the examination of the 90 samples of properly pasteurized milk, 16 were reported as underpasteurized and 5 as grossly underpasteurized. Similarly, of the 93 samples of pasteurized milk examined by the extraction technic and compared with the color standards, 31 were reported as having a color >0.2 standard and thus as improperly treated. In the examination of 44 samples of milk heated for 15 minutes at 143° F., 5 were reported as properly pasteurized. Of 180 samples of pasteurized milk containing added raw milk in concentrations of less than 2 per cent, 53 were designated as pasteurized. Lowering the temperature of the heating of 165 samples was detected with an accuracy of 58 per cent.

From the results reported by the various cooperating laboratories, it is apparent that in the examination of cream the high percentage of butter fat interferes with the color reactions and thus renders the application of this test to such samples difficult. This probably accounts for the tendency to report a relatively large proportion of samples of underpasteurized cream as pasteurized.

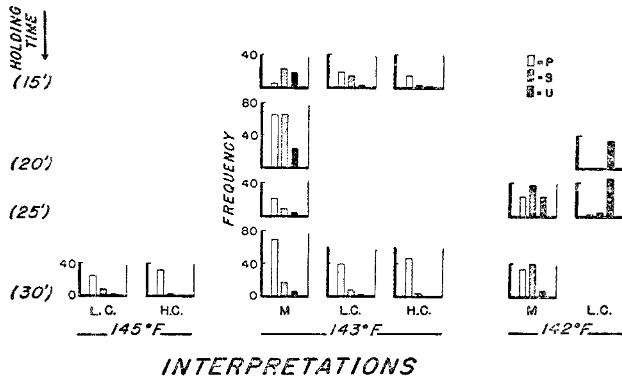

FIG. 3.—FREQUENCY DISTRIBUTION OF INTERPRETATIONS OF RESULTS. MILK AND CREAM HEATED TO DESIGNATED TEMPERATURE AND TIME.

P = pasteurized; S = slightly underpasteurized;

U = underpasteurized or grossly underpasteurized.

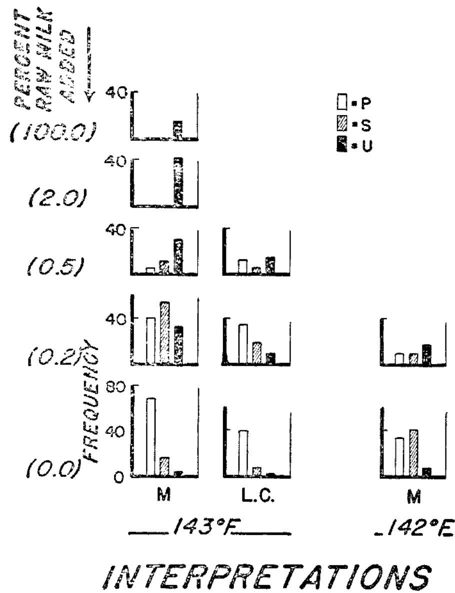

FIG. 4.—FREQUENCY DISTRIBUTION OF INTERPRETATIONS OF RESULTS. PASTEURIZED MILK AND CREAM WITH ADDED RAW MILK.

P = pasteurized; S = slightly underpasteurized;

U = underpasteurized or grossly underpasteurized

SUMMARY

The results of this collaborative study by 18 laboratories in examinations with identical samples, technic, and reagents, indicate that the phosphatase field test of Scharer should be subjected to further study for possible modifications toward the attainment of maximum accuracy.

Based on the data presented, the following recommendations[1] are made:

(1) That action be deferred on the adoption of the phosphatase field test as developed by Scharer.

(2). That further studies be made in order that a suitable, precise, and sensitive technic for field use in the control of pasteurization may be developed.

The assistance of Dr. William R. Thompson in the development of the graphic representation of the results was of great value in the preparation of this report and is appreciatively acknowledged.

No report on citric acid in milk was given by the Associate Referee. He stated that this project would be postponed until a method for the determination of citric acid in fruits and fruit products had been formulated.

REPORT ON DIFFERENCE BETWEEN DAIRY PRODUCTS MADE FROM COW'S MILK AND THOSE MADE FROM THE MILK OF OTHER ANIMALS

By IRA D. GARARD (New Jersey College for Women, Rutgers University), *Associate Referee*

Several dairy products made from the milk of sheep or goats are sold widely in the United States. It seems desirable, therefore, from the viewpoint of legal control and trade practice, that there be some recognized method for distinguishing these products from similar ones made from cow's milk.

There are few published analytical data on dairy products other than those made from cow's milk. However, Currie[2] in a study of Roquefort cheese reported Polenske values of 5.55, 6.25, 5.68, and 5.6, respectively, for the fat from four brands of this cheese. Except these results and a few other Polenske values of the same order, but widely scattered throughout the literature, almost no other data were available until Garard, Minsky, Baker, and Pascale[3] published the results of an investigation of the ap-

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[1] For report of Subcommittee C and action by the Association, see *This Journal*, **22**, 60 (1939).
[2] *J. Agr. Research*, **2**, 1–14, 429–34 (1914).
[3] *Ind. Eng. Chem.*, **29**, 1167–71 (1937).

Table 1.—*Collaborative results*

COLLABORATOR	A				B				D				E			
	REICHERT-MEISSL		POLENSKE		REICHERT-MEISSL		POLENSKE		REICHERT-MEISSL		POLENSKE		REICHERT-MEISSL		POLENSKE	
METHOD	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II
Burton Jordan	25.6	25.7	1.6	2.0	22.8	23.0	1.8	2.1	29.1	29.1	3.4	(2.65)*	30.8	30.3	4.6	3.2
State Chemical Lab.	25.7	26.1	1.5	1.9	23.1	22.9	2.0	1.8	29.2	29.6	3.5	4.2	30.8	30.7	4.4	5.1
Vermillion, S. D.	25.9	26.2	1.8	1.9	23.2	23.1	1.9	2.3	29.5	29.4	3.3	4.1	30.8	30.7	4.6	(1.10)*
					23.1		1.6			29.3		4.6		30.5		4.6
					23.4		1.8									
					22.9											
Ira D. Garard	26.2	25.5	1.9	2.4	23.1	23.0	2.1	2.7	27.2	27.5	3.0	4.4	29.9	29.6	4.3	5.2
	25.0	24.7	1.8	2.5	22.8	23.2	1.9	2.8	26.7	26.5	3.4	4.5	29.7	30.4	(6.4)*	4.5
K. R. Majors	27.1	26.6	2.1	2.1	22.8	23.4	1.8	2.7	29.0	28.8	3.7	4.6	30.3	29.6	5.4	5.0
U. S. Regional Soybean	27.0	26.3	2.0	2.4	22.6	23.2	1.8	3.0	29.0	29.3	3.2	3.9	29.3	30.2	4.7	5.0
Ind. Products Lab.	27.0		1.9							29.4		3.6	29.8		4.0	
Urbana, Ill.													29.6		4.6	
R. S. McKinney	25.3	25.6	1.4	1.7	22.7	22.0	1.9	1.8	28.1	27.4	(2.45)*	3.7	29.1	28.9	3.6	4.5
Bureau of Chemistry and Soils	25.1	25.7	1.4	1.8	22.5	22.3	1.9	1.9	28.3	27.4	3.1	3.7	29.3	29.1	3.6	4.5
Washington, D. C.		25.8		1.9		22.3		2.2	28.5		3.8		29.8		3.7	
						22.9										
J. L. Perlman	25.9	26.7	2.1	2.2	21.9	22.9	2.4	2.8	29.3	29.6	3.6	4.8	30.1	30.2	4.0	5.3
State Food Lab.	26.5	26.2	2.1	2.4	22.5	22.1	2.2	2.5	28.9	29.3	3.4	4.6	30.0	29.8	4.1	5.2
Albany, N. Y.																
M. L. Offutt	25.9	25.6	1.6	1.8	22.6	23.2	2.0	2.0	28.2	28.4	4.0	4.8	29.9	30.0	4.1	4.7
U. S. Food and Drug Adm. Lab.																
New York																
F. H. Lehlberg	26.2	26.3	2.5	2.6	23.8	23.4	2.4	2.6	30.1	30.2	5.1	5.1	30.5	30.2	4.2	5.1
Grain Research Lab.																
Winnipeg, Man.																
Mean	26.0	25.9	1.8	2.1	22.9	22.8	2.0	2.4	28.7	28.7	3.6	4.3	30.0	30.0	4.4	4.8

* Not included in averages.

plicability of the Polenske value to the distinction of Roquefort cheese from similar varieties made from cow's milk.

As Currie's work and the results on the percentage distribution of acids in the fats by Dhingra[1] and by Crowther and Hynd[2] indicate the usefulness of the Polenske value, and because of the amount of work involved, no other constant was investigated by these workers. The literature, however, was searched comprehensively and 28 loaves of Roquefort cheese of different years and brands were analyzed, together with over a hundred samples of other varieties of American and foreign cheese, in order to establish the variations to be expected from samples of known origin. This work appears to show that the Polenske value distinguished between Roquefort cheese that gave values from 3.6 to 5.95 and blue cheese from cow's milk that had not been found to give values in excess of 3.

All the results reported in this investigation were obtained by the four authors and most of them by two of the four. Consequently, the method of procedure in the determinations was fairly uniform. As the results found in the literature were obtained by Polenske methods of unknown detail, it was considered that collaboration would be desirable in order to ascertain the cause of the variability of results obtained by other analysts under the conditions of the official method.

Samples of fat were extracted from the cheese by the Associate Referee and sent to chemists who had consented to do collaborative work on these determinations. The work was a joint project with R. S. McKinney, who was making a study of the effect of pumice size on the results by the Polenske method. Although five samples were sent to collaborators, one was not a pure cheese fat and so the results from only four of them are included in this report. Each sample was analyzed by the official Polenske method (Method I) and also by the same method with 0.1 gram of powdered pumice (Method II) instead of the "few pieces" specified in the official method. Sample A was the fat from a loaf of Royal Beech, Danish Blue Cheese, and B was from Flora Danica, Danish Blue Cheese. Sample D was from Societé Bee brand and E from Puma brand, Roquefort cheese. All samples were extracted by the acid extraction method, *Methods of Analysis, A.O.A.C.*, 1935, 292, **99**(b), and washed free from acid. The results obtained by the collaborators are given in Table 1.

Table 1 contains all the results submitted, but the four values inclosed in parentheses are not included in the averages. Three of these are results obtained by Method II, with powdered pumice, and in each of the four cases the deviation of the discarded result was more than four times the mean deviation of the other results on the same sample. In one instance (Lehlberg) the results are the average of three or more determinations.

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[1] *Biochem. J.*, 27, 851–59 (1933).
[2] *Ibid.*, 11, 139–63 (1917).

DISCUSSION OF RESULTS

Sixty-one Polenske values on two samples of fat from blue cheese vary from 1.4 to 2.8 and give a mean value of 2.0; 59 Polenske values on two samples of fat from Roquefort cheese vary from 3.0 to 5.4 and give a mean value of 4.1.

The probable error of a single determination was calculated for each cheese separately, but the results of both methods of analysis are included. For example, the mean value for Sample A was calculated from all the Polenske values in Columns I and II in Table 1. These results are shown in Table 2.

TABLE 2.—*Probable error*

CHEESE FAT	NUMBER OF DETERMINATIONS	MAXIMUM AND MINIMUM	MEAN POLENSKE VALUE	PROBABLE ERROR
Royal Beech, Blue	30	2.6–1.4	2.0	0.22
Flora Danica, Blue	31	3.0–1.6	2.2	0.26
Societé Bee, Roquefort	29	5.1–3.0	4.0	0.42
Puma, Roquefort	30	5.4–3.2	4.5	0.38

In view of the bumping that occurred in several determinations with Method II, the probable errors are not large.

The Reichert-Meissl values have never been suggested as being significant in this problem, probably because values for the fat of cow's milk have been reported at least as high as 33. However, the literature has not been thoroughly searched for these values for cheese fat. The values obtained in this collaborative work are interesting and may be significant. Sixty-seven values on two samples of fat from the cow's milk cheese vary from 21.9 to 27.1, with an average value of 24.3; 67 values from two samples of fat from Roquefort cheese vary from 26.5 to 30.7, with an average value of 29.3. From these results it would seem that the usefulness of the Reichert-Meissl value deserves further investigation.

The Polenske values are in agreement with similar values found in the literature, and it appears to be well established that if two or more determinations give a mean Polenske value less than 3 for the fat of a cheese, the cheese has not been made from ewe's or goat's milk or a mixture of these two milks.

The Associate Referee wishes to express his appreciation of the thorough work of his collaborators and their excellent cooperation.

RECOMMENDATIONS[1]

It is recommended—

(1) That a study be made of the application of the Polenske method to the identification of cheese from goat's milk and to products from milk mixtures.

[1] For report of Subcommittee C and action by the Association, see *This Journal*, **22**, 60 (1939).

(2) That the other fat constants of milk and cheese fat, particularly the Reichert-Meissl and Kirschner values, be studied with reference to their application to these problems.

No report on naval stores was given by the Referee.

No report on rosin was given by the Associate Referee.

REPORT ON TURPENTINE

By V. E. Grotlisch (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

The report of the Associate Referee on Turpentine last year pointed out that there was possibility of the need of new official methods for detecting and determining adulteration of turpentine with certain new hydrogenated petroleum oil thinners, which behave very much like some of the coal tar oils. No evidence or indication, however, of adulteration of turpentine with such products has come to the attention of the Associate Referee during the year. On the other hand, there have been several cases of adulteration of gum spirits of turpentine with steam distilled wood turpentine and with sulfate wood turpentine.

Sulfate wood turpentine is recovered as a by-product in the manufacture of paper pulp from resinous wood by the sulfate process, in which the wood is subjected to the action of a solution containing sodium hydroxide, sodium sulfide, and sodium sulfate. The vapors from the digesters are condensed and yield a very foul-smelling oil, crude sulfate wood turpentine. The odor is due primarily to sulfur compounds in the nature of mercaptans. The crude is refined by a series of fractional distillations, the sulfur compounds being low boiling and concentrating in the "heads" of the distillation. However, it is practically impossible to remove all the sulfur. Some manufacturers of sulfate wood turpentine reflux the turpentine with sodium or calcium hypochlorite (chloride of lime), which removes most of the sulfur, so that ultimately the refined sulfate wood turpentine contains less than 0.03 per cent sulfur, but the hypochlorite treatment results in putting small quantities of combined chlorine in the turpentine instead of the sulfur. Aside from the distinctive odor of the sulfate wood turpentine, it is possible to prove its presence, therefore, by the detection or determination of sulfur and/or chlorine.

In a paper published several years ago, W. C. Smith[1] of the Bureau

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[1] *Ind. Eng. Chem. Anal. Ed.*, 3, 354 (1931).

of Chemistry and Soils, reported that the small quantities of these identifying elements present, ordinarily less than 0.03 per cent sulfur, and less than 0.3 per cent chlorine (the sulfur content ordinarily drops to around 0.01 per cent when the hypochlorite treatment is used) cannot be isolated or recovered in the ordinary combustion or oxidation methods. However, the sulfur and chlorine can be determined quantitatively by burning the turpentine in a carbureting device known as the Kennedy sulfur lamp,[1] in which a measured quantity (5 cc.) of the turpentine is slowly fed down into the lamp, where it is vaporized by heat from a resistance coil embedded in the lamp, and mixed with air that comes from an air line in the laboratory. The turpentine so vaporized is burned at the exit orifice or tip, and the products of combustion are absorbed in a volumetric solution of standardized sodium carbonate by placing a chimney over the flame and connecting the absorption apparatus to the laboratory vacuum line.

The sulfur burns to SO_2, and, strange to say, the chlorine in the turpentine is converted quantitatively in the combustion to hydrochloric acid.

Titration of the excess of sodium carbonate indicates the sulfur, or, if chlorine be present, the total sulfur equivalent. The presence and quantity of chlorine is determined, after titration of the excess alkali with standardized (usually less than 0.1 N) nitric acid, by washing the solution from the absorption chamber, warming, acidulating with several drops of nitric acid, and adding a solution of silver nitrate. The sodium chloride is precipitated as silver chloride which, on standing, settles, and can be quantitatively filtered and weighed. From the weight of the silver chloride, the weight and per cent of chlorine can be computed. From this, by proper allowance for the difference between the weight of chlorine and sulfur, the quantity of sulfur can be obtained by difference from the results of the first titration. The sulfur content can be checked by running another combustion and, after back titration and oxidation of the solution with bromine water, the sulfur is precipitated and weighed as barium sulfate.

Chadwick and Palkin[2] have shown that aside from its distinctive odor, steam distilled wood turpentine differs from gum spirits by the normal presence in the former of small quantities of benzaldehyde and fenchyl alcohol, neither of which has been found in gum spirits. The benzaldehyde can be extracted by repeated extractions of a sizable quantity of the turpentine with sodium bisulfite solution. After sodium carbonate has been added to liberate the aldehyde, the extracts are steam distilled, when if steam distilled wood turpentine is present in appreciable quantity in the sample, the odor of benzaldehyde should be noticeable. The

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[1] *Ind. Eng. Chem.*, 20, 201 (1928).
[2] Proc. A.S.T.M. 37, Part II, p. 574 (1937).

benzaldehyde may be extracted from the distillate with petroleum ether, and finally the benzaldehyde is identified by converting it into a crystalline addition product using 2,4 dinitrophenylhydrazine. The purified benzaldehyde, 2,4 dinitrophenylhydrazine melts at a temperature between 237° and 242° C.

Collaborative work on standard methods of detecting such sophistication of gum spirits of turpentine and determining the presence of the identifying constituents is planned.

A study is now being made, through the Sub-committee on Turpentine of the American Society for Testing Materials, of the possibility of developing a new partial-immersion sensitive thermometer for the turpentine distillation test, to replace the fully-immersed Anschutz type of thermometer now regularly used. Specifications have been developed, and several of the manufacturers have made up trial thermometers. These are now ready for testing to compare the distillation results obtained therewith with those obtained by the present standard thermometer. The initial results indicate that a sensitive partial-immersion thermometer can be manufactured at suitable cost to replace the fully immersed thermometer, which is rather difficult to read, especially at the start of a distillation test, when the turpentine contains a small quantity of dissolved water. As soon as this work is completed in the A.S.T.M., it will be brought to the attention of the A.O.A.C.

No recommendations are being made at this time, other than that the work on turpentine be continued.

REPORT ON PAINTS, VARNISHES, AND CONSTITUENT MATERIALS

By C. S. Ladd (Food Commissioner and Chemist, Bismarck, N. D.), *Referee*

It is recommended[1]—

(1) That the standard methods of the American Society for Testing Materials for testing skinning and alkali resistance of varnishes (D154-38) be adopted as tentative.

(2) That study of the methods of testing abrasion resistance and hardness of varnish films be continued.

(3) That study of a method for soap resistance of varnish be made and that a study be made with a view to revising the present method of testing elasticity or toughness of varnish films in order to make execution of the method less tedious.

[1] For report of Subcommittee A and action by the Association, see *This Journal*, 22, 54 (1939).

(4) That study of the accelerated weathering test of paints be continued.

No report on accelerating testing of paints was given by the associate referee.

REPORT ON VARNISHES

By F. ROBERTS (Paint and Varnish Laboratory, Bismarck, N. D.), *Associate Referee*

No significant developments in methods for varnish analysis and tests have been reported during the past year, but work along these lines has been planned for the coming year.

In the present method for determining elasticity or toughness of varnish films considerable time is involved due to the trial and error nature of the procedure in ascertaining the amount of run kauri solution (within 10 per cent), to add to the product to cause a break in the film when bent over a $\frac{1}{8}$ inch mandrel. It is planned to make investigations with a cone-shaped mandrel with which it may be possible to estimate the elasticity by determining the greatest diameter on the cone where cracking occurs under certain reductions. Considerable time may possibly be saved if a satisfactory procedure can be developed through the use of a conical mandrel in making this test.

It has been suggested that a soap resistance test would be of value in determining durability of interior finishes, especially floor varnishes. Work along this line will be done in an effort to develop a suitable practical method for making such a test.

Work on abrasion resistance will be continued with the end in view of developing a satisfactory method that may be standardized.

The following methods are presented for adoption as tentative:[1]

SKINNING TEST OF VARNISH IN CLOSED CONTAINERS[2]

Measure a 6 ounce sample of the varnish, by means of a graduate, into an 8 ounce wide-mouthed glass jar $4\frac{1}{2}$ inches in height and 2 inches in diameter. Screw the cover on tightly and invert the jar momentarily. Place the jar in an upright position in the dark (under a box or in a drawer is satisfactory) and examine the varnish for skinning after desired periods of time, such as each 24 hours for the first week and each week thereafter for a total period of four weeks.

ALKALI RESISTANCE TEST[2]

Thoroughly clean a 1×6 inch test tube in benzol. Dip the tube into the sample varnish, immediately invert the mouth of the tube, and allow the varnish to dry for 48 hours in an atmosphere free from dust, drafts, products of combustion, or laboratory fumes. Maintain a room temperature of 19°–25° during the drying

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[1] For report of Subcommittee A and action by the Association, see *This Journal*, 22, 54 (1939).
[2] Standard Method (D 154-38) of the American Society for Testing Materials, edited to conform in part to the A.O.A.C. style. 1938 supplement to Book of A.S.T.M. Standards, p. 132.

period. Suspend the varnish-coated tube in 3% NaOH solution maintained at 21°± 1.1°. Remove the tube after desired periods of time, such as 8, 16, 24, and 24 hours, rinse under a gentle stream of water, allow to air dry for 30 minutes, and examine the varnish, for whitening, blistering, or removal.

No report on leathers and tanning materials was given by the Associate Referee.

REPORT ON RADIOACTIVITY

SIMPLE AND INEXPENSIVE CHARGER FOR ELECTROSCOPES

By Anna E. Mix (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

Solid or liquid substances are tested for radioactivity with alpha, beta, or gamma ray electroscopes, and it is necessary to subject these instruments to an electric charge in order to place the leaf or fiber at a definite point on the scale that is set in the microscope of the instrument. The charging is usually done by rubbing a glass rod with a piece of silk cloth or by rubbing a hard rubber rod with rabbit fur.

A refined friction device of English manufacture used in connection with the work in this laboratory became inoperative, and the inability of the manufacturers to replace this charger prompted the acquisition of a substitute for this purpose.

The use of a thermionic device suggested by Alfred Christie of the Bureau of Agricultural Economics resulted in a small simple charger that may be built for less than $5.00 by anyone having some knowledge of thermionic tubes and their operation.

A half-wave rectifier tube, 25Z5, was selected, because it doubles the input voltage of the alternating current power line and gives a high voltage without the use of transformers. A line cord resistor of 280 ohms was used to reduce the voltage and heat of the tube filament. The two condensers used in the circuit are of 8 mfd. capacity and they should withstand a surge of 250 volts without a breakdown.

A six-prong socket, single-pole single-throw switch, a double-pole single-throw switch, and a pair of test leads with the necessary base and housing complete the parts necessary for the construction of the charger.

A bleeder resistance of approximately 60,000 ohms was used across the output.

This device may also be used for removing the static charge from finely powdered drugs and other powdered substances. The ampoule or capillary containing the sample is touched first with one point of the charger while the free lead is being grounded and then with the other. The container is supported by glass rods or on a beaker while being treated.

Operation

The charger is plugged into the alternating current power line, the single-pole power switch is turned on, and the double-pole charge switch is turned off. The tube is allowed to warm up for approximately 3 minutes, which gives the condensers time to become charged. The black or

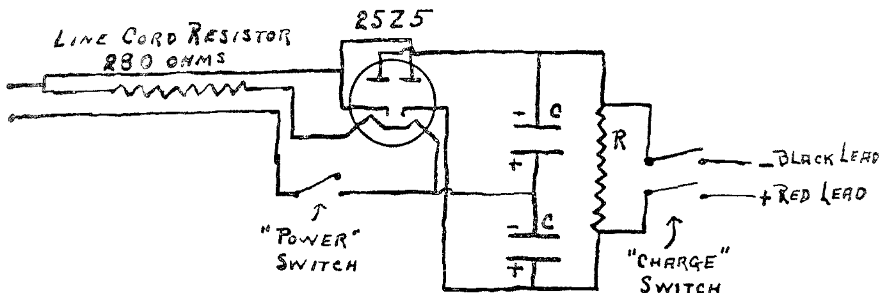

FIG. 1.—SHOWING CHARGER CIRCUIT.
R—60,000 ohm resistor.
C—8 mfd. electrolytic condenser.

negative lead is then attached to the frame of the electroscope, the charge switch is turned on, and the red or positive lead is repeatedly applied to the positive contact of the electroscope until it is fully charged. When the charger is not in constant use the charge switch is turned off and the power allowed to remain in the charger. When needed again it is then only necessary to turn the charge switch on and after completing the work with the electroscope, to turn the charger power switch off.

The Associate Referee wishes to thank Mr. Christie for his timely suggestions and for improving and reducing the cost of this instrument.

REPORT ON QUANTUM COUNTER

By ANNA E. MIX (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

Within the past few years much importance has been attached to the determination of radioactivity in foods, drugs, and mechanical devices. It was necessary, therefore, to acquire a sensitive device for its detection, as even a small amount of ingested radium causes undesirable and in some cases fatal results, while on the other hand mechanical devices, claimed to be radioactive, but containing none or such minute quantities of radium that they are of no value, are classed as cheats.

The alpha, beta, and gamma ray methods described in *Methods of Analysis, A.O.A.C.*, are satisfactory and give reliable results, but they are time-consuming.

Some time ago a quantum counter was developed by R. D. Evans of the Massachusetts Institute of Technology, and one of these instruments was purchased by the Food and Drug Administration. This instrument is more rapid and is claimed to be more sensitive than the gamma ray scope. Literature states that it is 10 to 100 times more sensitive to feeble gamma rays than is the best electroscope.

Briefly, the principle of operation is as follows: A glass cylinder containing gas at a low pressure, or evacuated, is grounded. A wire runs through this cylinder and is connected to the remainder of the circuit. The wire in the cylinder is charged positively. The cylinder therefore assumes a negative charge and the difference of potential between the wire and the cylinder is kept at about 1200 volts. The cylinder screens out alpha and beta particles. However, the gamma rays ionize the surrounding air and produce a discharge from the cylinder to the wire and thence to the recording device. Every gamma ray quantum producing one or more secondary electrons inside the cathode cylinder causes the instrument to discharge once.

The circuit in the quantum counter used in this laboratory is such as to permit three types of recording, one auditory and two visual. Of the two visual recording devices, one is an electric eye, the other a rate meter (volt meter type).

Neon glow lamps are used in the circuit for voltage stabilization. A detailed report of the circuit may be found in the paper: "Quantum Counter Amplifiers for Gamma Ray Detection and Applications to Studies in Radium Poisoning," by Robley D. Evans, *Proc. Amer. Phil. Soc.*, **78**, No. 1 (Oct. 1937).

While the Associate Referee has made over 1000 readings, no report as to method of calculation of sensitivity of this scope will be given this year for it is considered that additional work is needed to obtain more reliable results. Therefore it is recommended that work on the quantum counter be continued.

No report on the gamma ray scope was given by the Associate Referee.

REPORT ON COSMETICS

By Elmer W. Campbell (Department of Health and Welfare, Augusta, Maine), *Referee*

Owing to the fact that the collaborative study of cosmetics is a new project, it is rather difficult to decide where to start in this broad field to develop a worthwhile program.

Preliminary studies having shown or indicated that many lipsticks and rouges contain barium or arsenic, or possibly both, and that the probable

source of these ingredients is the dyes or coloring substances used in such preparations, the Referee suggested a study of coloring substances used in such cosmetics. Preliminary studies of a number of samples of dyes secured from various manufacturers were made.

Soluble barium salts in cosmetic colorings

LABORATORY	ACID-SOLUBLE Ba			WATER-SOLUBLE Ba			TOTAL SOLUBLE Ba		
	1	2	3	1	2	3	1	2	3
Sample	*per cent*			*per cent*			*per cent*		
1	0.2085	—	—	0.5065	—	—	0.715	—	4.57
2	0.0529	0.0145	—	0	—	—	0.0559	0.0145	2.65
3	0.2445	0.2646	—	0.3722	0.2881	—	0.6167	0.5527	4.30
4	0.1149	0.1146	—	0.2007	0.0017?	—	0.3157	0.1263	3.13
5	0.0557	0.1705	0.60	0.1515	0.1450	0.42	0.2072	0.3155	1.02
6	1.1656	0.6250	1.51	1.2922	0.9938	1.71	2.6240	1.6188	3.22
7	1.103	0.3163	1.42	0.8745	0.2375	1.44	1.9775	0.5538	2.86
8	2.585	0.1375	1.61	0	—	0.11	2.585	0.1375	1.72
9	0.4480	0.2675	1.06	0.1943	0.0823	0.19	0.6423	0.3498	1.25
10	7.88	3.9402	7.59	1.075	0.1805	1.32	8.955	4.1207	8.91

The method of analysis that was suggested follows. It may have been modified by the different collaborators.

BARIUM IN LIPSTICKS, CREAM ROUGES, AND LAKE DYES

Water-Soluble Barium

I. Weigh a sample in tared Pt dish and ash cautiously over a Bunsen burner.

(a) This precipitate, in case Ca was present in the original sample, is contaminated with $CaSO_4$, giving a high result in both water-soluble and HCl-soluble procedures. However, the percentage of these cases does not appear to warrant a separation by the chromate method. Possibly a spectrographic quantitation would solve this problem.

II. To the ash add 10 cc. of 3% H_2O_2, mix well, and evaporate at low temperature over a water bath to dryness. (b) To the ash add 25 cc. of water, heat to boiling, filter, wash well with hot water, and dilute the filtrate to about 200 cc. Use caution in the choice of filters. After filtering allow the filtrate to stand about 4 hours and then refilter through a double filter, as the $BaSO_4$ base used for the dyes is extremely fine and if carried through the filter would give high results.

(a) On ashing in a muffle furnace there was thought to be considerable reduction of $BaSO_4$ to BaS, thereby giving a higher percentage of water-soluble barium than was present before ashing.

(b) Even using a Bunsen burner and ashing cautiously there seemed to be some reduction. The H_2O_2 treatment is for the purpose of oxidizing the BaS back to the $BaSO_4$.

III. Add 2 cc. of dilute HCl and heat to boiling; add 2 cc. of dilute H_2SO_4 and let stand several hours, preferably overnight. If a precipitate settles out, collect on an ashless filter, place the filter in a tared ignition crucible, and ignite carefully to a white ash. Cool, and weigh as $BaSO_4$. From the weight calculate as Water-Soluble Barium (%). Check to see if free from calcium.

Dilute HCl-Soluble Barium

Ash the filter and residue from I over a Bunsen burner in the original Pt dish. Treat the ash with 50 cc. of water and 2 cc. of dilute HCl, heat to boiling, filter,

and wash the filter with hot water, using the same precautions as in II. Dilute the filtrate to 200 cc., add 2 cc. of dilute H_2SO_4 and allow the solution to stand several hours, preferably overnight.

If a precipitate settles out, collect it on an ashless filter and wash well. Place the filter in a tared crucible and ignite carefully to a white ash. Cool, and weigh as $BaSO_4$. (a) From this weight calculate as percentage of dilute HCl-Soluble Barium. Check to see if free from calcium.

Comment is solicited concerning the method or any modifications that might well be adopted.

COLLABORATORS

The following collaborators reported:

Henry C. Fuller, Washington, D. C.
Carl S. Ferguson, Division of Food and Drugs, Boston, Mass.
R. J. Hennessy, Office of State Food Commissioner and Chemist, Bismarck, N. D.
Elmer W. Campbell.

The results are shown in the table.

Work should be undertaken on the following subjects: Slenderizers, hair dyes, hair waves, lipsticks, rouges, eyebrows, eyelashes, shaving creams, tooth pastes and powders, face creams, face lotions, perfumes and toilet waters, bath salts, hair pomades, hair anticrinkle, hair removers, hair bleaches, B. O. preparations, hair tonics, and face powders.

It is recommended that study be continued on the soluble barium content of the colors used in cosmetics as well as in finished products and that the studies be enlarged to include arsenic determinations.

REPORT ON CEREALS

By V. E. Munsey (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

Methods of Analysis, A.O.A.C. contains a chapter on cereal foods that has numerous methods under the headings of wheat flour, bread, baked products other than bread, and macaroni products. With few exceptions, the methods under wheat flour were adopted after studies had been made on this product. Other cereals, such as whole wheat flour, gluten flour, corn meal, rye flour, buckwheat flour, malt and barley flour, rice flour, rolled oats, oat meal, and oat flour may be analyzed by similar procedures. However, before the appropriate methods under wheat flour are referred to as applicable to these other cereal products some confirmatory studies should be made. Likewise, under the heading "baked products other than bread" methods are given for total solids and crude fiber. It is probable that these methods would also apply to baked products other than bread, but this should be determined by experimental study on such products as crackers, cookies, and cakes.

It is recommended[1]—

(1) That an associate referee be appointed to study the application of the methods under wheat flour for the determination of water, ash, protein, fat, and crude fiber to corn meal, corn flour, and corn starch.

(2) Likewise for rolled oats, oat meal, and oat flour.

(3) Likewise for rye flour and buckwheat flour.

(4) Likewise for malt barley, barley flour, and rice flour.

(5) That an associate referee be appointed to study the application of methods under bread for the determination of ash, fat and, protein to such baked products as crackers, cookies, and cakes.

(6) That the tentative method for the preparation of sample of bread, *Methods of Analysis, A.O.A.C.,* 1935, 221, **50**, be made official (final action).

(7) That the method for collection and preparation of sample in macaroni products, *Ibid.,* 228, **68**, be made official (final action).

(8) That the method for the determination of apparent viscosity of flour, *This Journal,* **20**, 380, be adopted as official (final action). Through error last year the reference was given as *Methods of Analysis, A.O.A.C.,* 1935, 221.

(9) That the magnesium acetate method, *This Journal,* **20**, 69, be made official (final action).

(10) That an attempt be made to develop a method for determining sodium chloride-free ash.

(11) That the associate referee continue his studies on H-ion concentration of flour.

(12) That the search for a rapid and accurate method for starch be continued.

(13) That further study be given to the improvement of the polarimetric methods for starch.

(14) That the A.O.A.C. tentative method for the determination of acidity in flour, 208, **13**, be dropped.

(15) That the method proposed by the associate referee for determination of fat-acidity of flour be adopted as tentative.

(16) That further study be made of the proposed methods for acidity in flour and other cereal products.

(17) That the collaborative study of the proposed method for determination of reducing and non-reducing sugars in flour be continued.

(18) That the associate referee continue his studies on the baking test for soft wheat flour.

(19) That the study of methods for the determination of chlorine in bleached flour fat be continued.

(20) That the method for the measurement of carotenoid pigments in flour, *This Journal* 21, 339, be studied next year with a view to substituting

[1] For report of Subcommittee D and action by the Association, see *This Journal,* **22**, 68 (1939).

water-saturated normal butyl alcohol for the Varsol-alcohol mixture.

(21) That study of the method for the determination of benzoyl peroxide in flour be continued.

(22) That collaborative study on determination of the carbon dioxide content of self-rising flour be continued.

(23) That the citric acid procedure for calculation of milk solids be not further studied collaboratively for the present.

(24) That the lactose procedure be more extensively studied collaboratively.

(25) That the so-called fat method be not further studied collaboratively in the present form.

(26) That study be made of a method for estimation of butterfat content based on the direct saponification and distillation of bread samples without extraction of the fat.

(27) That the tentative method for extract soluble in cold water, 213, **30**, be adopted in its modified form, *This Journal*, **22**, 76, as official (first action).

(28) That collaborative study be continued on extract soluble in cold water on flour and other cereals.

(29) That the associate referee continue the study of ergot in flour.

(30) That the study on proteolytic enzymes in flour be continued.

(31) That further collaborative work on measurement of flour and bread color by the N. A. colorimeter be dropped.

(32) That study be continued on the sampling and preparation of flour and bread for color measurements by the photoelectric cell method based on reflectance.

(33) That work be continued on the determination of soybean flour in cereal products.

(34) That the study on macaroni be continued on the basis of the work of the present associate referee, but that no new associate referee be appointed this year to fill the vacancy caused by the inability of the present referee to serve any longer.

(35) That the associate referee continue his studies on the methods of determining cellulose as an index of the whole wheat content of cereal products.

(36) That further study be made of methods for determining the ash of the original flour in phosphated and self-rising flours, especially of old self-rising flours, with special reference to—

(a) Analysis for sodium chloride content of self-rising flours and the ash of the extracted flours.

(b) The study of means other than extraction with carbon tetrachloride for 100 per cent separation of the sodium chloride from old self-rising flours.

REPORT ON ASH IN FLOUR, MACARONI PRODUCTS, AND BAKED PRODUCTS

By L. H. BAILEY (Bureau of Chemistry and Soils, Washington, D. C.), *Associate Referee*

At the 1937 meeting of the A.O.A.C. the magnesium acetate method was made official (first action) as an alternative and rapid method of determining ash in flour, macaroni products, and baked products.

In order to obtain further collaborative work on this method, the Associate Referee submitted samples of whole wheat flour, corn meal, and bread to several analysts. They were furnished copies of the magnesium acetate method and were requested to determine ash on the samples submitted by the proposed and the present official A.O.A.C. methods. The following collaborators reported: V. E. Munsey, M. H. Neustadt, J. T. Keister, B. L. Kaspin, and L. H. Bailey.

The results are shown in the table.

Collaborative ash results (per cent)

COLLABORATOR	1		2		3		4		5	
	A.O.A.C.	Mg. Ac_2	A.O.A.C.	Mg. Ac_2	A.O.A.C.	Mg. Ac_2	A.O.A.C.	Mg. Ac_2	A.O.A.C.	Mg. Ac_2
Corn Meal	1.28	1.31	1.34	1.33	1.22	1.30	1.33	1.33	1.31	1.30
	1.27	1.31	1.34	1.35	1.23	1.35	1.34	1.33	1.33	1.28
	1.29	1.36		1.29	1.32		1.33	1.34	1.35	1.33
	1.31	1.36		1.32					1.32	1.32
Mean	1.29	1.34	1.34	1.32	1.26	1.33	1.33	1.33	1.33	1.31
Whole Wheat Flour	1.83	1.87	1.81	1.85	1.84	1.85	1.89	1.84	1.84	1.85
	1.82	1.87	1.82	1.84	1.92	1.92	1.83	1.82	1.85	1.84
	1.82	1.89		1.79			1.85	1.82	1.88	1.86
	1.83	1.90		1.82					1.88	1.88
Mean	1.83	1.88	1.82	1.83	1.88	1.88	1.86	1.83	1.86	1.86
Bread	1.81	1.75	1.79	1.66	1.56	1.60	1.91	1.75	1.82	1.75
	1.80	1.76	1.82	1.60	1.54	1.69	1.90	1.78	1.76	1.76
	1.86	1.85		1.62			1.91	1.76	1.73	1.66
	1.87	1.87		1.66					1.72	1.68
Mean	1.84	1.81	1.81	1.64	1.56	1.64	1.91	1.76	1.76	1.71

The collaborators, for the most part, obtained close agreement, both individually and with each other.

The values for corn meal and whole wheat flour by the magnesium acetate method agree well with those by the present A.O.A.C. method. With the bread there was more difficulty as was to be expected owing to

the added sodium chloride present and the greater losses that occur in ashing at 700° C., as required by the rapid method, than at 550° C. (A.O.A.C. method). This fact should be taken into account with any sample containing an appreciable quantity of sodium chloride.

There is need for a method for the determination of sodium chloride in cereal products.

It is recommended*—

(1) That the magnesium acetate method be made official (final action).

(2) That an attempt be made to develop a method for obtaining a sodium chloride-free ash.

No report on H-ion concentration of flour was given by the Associate Referee.

REPORT ON STARCH IN FLOUR

By C. Y. Hopkins (National Research Council, Ottawa, Canada), *Associate Referee*

It has been demonstrated by the earlier work of the Association that starch in flour may be determined conveniently by the modified Rask method, which is now tentative.

Last year's collaborative study indicated that the method was unsuited to the analysis of certain cooked products, and it was recommended that work should be directed towards the selection of a new method that would be applicable to all cereal products, cooked and uncooked.

A review of all available methods was made, including several appearing in the recent literature, and it seemed that none was sufficiently promising to submit for collaborative study without preliminary investigation.

CONSIDERATION OF AVAILABLE METHODS

A number of the methods found in the literature were developed for the analysis of plant material of low starch content and, for one reason or another, do not appear to be applicable to the problem at hand. These include the methods of Denny,[1] Hanes,[2] Pucher and Vickery,[3] and Sullivan.[4]

The use of diastase (Denny, Hanes) is open to question since Sullivan has shown that diastase preparations are not entirely specific for starch.[4]

The method of Pucher and Vickery is colorimetric and requires the use of a spectrophotometer, an instrument that is not available in all laboratories.

* For report of Subcommittee D and action by the Association, see *This Journal*, 22, 68 (1939).
[1] Contrib. Boyce Thompson Inst., 6, 129 (1934).
[2] *Biochem. J.*, 30, 168 (1936).
[3] *Ind. Eng. Chem. Anal. Ed.*, 8, 92 (1936).
[4] *This Journal*, 18, 621 (1935).

The Sullivan method has been carefully worked out and is capable of giving very accurate results. It was studied by the Associate Referee in 1936 and was found to be quite lengthy and to require extensive manipulation. These objections apply also to the other methods listed.

The Mannich and Lenz polarimetric method[1] was studied in 1936. It was found to give fairly good results with wheat flour but was subject to some manipulative difficulties.

Another polarimetric method was brought to the attention of the Associate Referee this year. It is a modification of the Lintner-Schwarz method[2] and was used with considerable success in determining starch in barley.

The method of Chinoy et al.,[3] in which the starch is weighed as starch iodide, was tried in 1935 with little success.

COLLABORATIVE WORK

Several of the proposed methods were tried out by apportioning them among the collaborators. The results are given in Table 1.

TABLE 1.—*Collaborative results*

ANALYST	MATERIAL	METHOD	PER CENT STARCH	MEAN
1	Cottonseed meal	Mannich-Lenz	negative rotation	
6	Cottonseed meal	Mannich-Lenz	negative rotation	
2	Cottonseed meal	Lintner-Schwarz		3.44
5	Cottonseed meal	Lintner-Schwarz	3.62, 3.75, 3.62	3.66
2	Cottonseed meal	Acid hydrolysis		3.25
4	Cottonseed meal	Rask	1.09, 1.28	1.18
6	Wheat starch	Mannich-Lenz		87.9
2	Wheat starch	Lintner-Schwarz	82.0, 81.9, 80.7	81.5
5	Wheat starch	Lintner-Schwarz	72.37, 72.50	
			72.25, 72.50	72.40
2	Wheat starch	Acid hydrolysis	83.22, 82.68	82.95
6	Wheat starch	Chinoy	would not filter	
2	Food product	Lintner-Schwarz		11.44
2	Food product	Acid hydrolysis		11.22

The cottonseed meal was ground to minus 100 mesh and when boiled with aqueous calcium chloride the solution gave a faint color with iodine, showing that it contained a mere trace of starch.

The wheat starch contained 10.8 per cent moisture and the sum of the impurities (protein, sugars, ash, fat, fiber) was 1.0 per cent. The starch content is therefore assumed to be 88.2 per cent.

Analysts 1 and 6 reported a negative rotation when analyzing cottonseed meal by the Mannich-Lenz polarimetric method. This is probably due to protein material that was not eliminated in the procedure. Re-

[1] *Can. J. Research*, 11, 751 (1934).
[2] *Z. Brauweisen*, 1913, No. 7 and 8.
[3] *Analyst*, 59, 673 (1934).

peating the work and adding phosphotungstic acid to precipitate proteins, Analyst 6 was able to reduce the observed rotation from $-0.10°$ to $-0.01°$. The latter figure is within experimental error.

In addition to the collaborative work, analyses of potato starch, potato flour, and barley kernels were carried out by two other chemists. They used the Mannich-Lenz method with fair success, although some difficulty was reported due to frothing. Some of the results with barley are shown in Table 2.

TABLE 2.—*Starch in barley*

MATERIAL	MANNICH-LENZ METHOD	LINTNER-SCHWARZ METHOD
	per cent	*per cent*
High protein barley (1)	53.7, 53.1	52.6, 52.8
High protein barley (2)	50.2, 50.8	50.1, 51.5
Low protein barley (3)	58.4, 58.9	60.0, 59.8
Low protein barley (4)	58.2, 58.2	58.9, 59.0

DISCUSSION

The results with cottonseed meal indicate that those methods using acid to disperse the starch tend to give high results owing to the interference of hemicelluloses. The Mannich-Lenz method (using calcium chloride) gave a low result due to the interference of proteins. A modification of the method is suggested.

In the analysis of wheat starch, Analyst 2 obtained fairly good agreement between the Lintner-Schwarz method and direct acid hydrolysis. The Mannich-Lenz method gave a higher figure. Analyst 5 obtained excellent checks using the Lintner-Schwarz method but was not in agreement with Analyst 2. The Chinoy method was again found to be unsatisfactory.

The following comments were made:

Mannich-Lenz method.—Less tedious than other methods. Frothing is difficult to control.

Lintner-Schwarz method.—This method has some very good features. Ten to twelve analyses can be done in one day.

Pucher-Vickery method.—Undoubtedly lengthy. May not be applicable to samples high in starch. The blue color with iodine is not always specific for starch.

The possibility of determining starch by fermentation to alcohol was considered by one collaborator. He reported that the results would not be sufficiently quantitative, even under carefully controlled conditions.

MODIFIED LINTNER-SCHWARZ METHOD

Weigh the sample (2.0–2.5 grams) into a 50 cc. round-bottomed centrifuge tube with lip. Wash by adding 10 cc. of aqueous alcohol (D_{20}, 0.88) and stirring thoroly with a glass rod. Place the rod carefully aside while the mixture is centrifuged and the liquid poured off. Repeat the washing until 60 cc. of wash liquid has been used,

stirring each time with the same rod. (For samples containing more than 2–3% of fat, a preliminary wash with ether is advised.)

Wash the residue into a 100 cc. volumetric flask by adding 50 cc. of H_2SO_4, sp. gr. 1.40, in portions. Place the mixture in a thermostat at 20° C. for 1 hour and shake occasionally. Add 10 cc. of freshly prepared 2% phosphotungstic acid solution and fill the flask to the mark.

A quantity of the liquid is filtered, the first 10 cc. is discarded, and the filtrate is polarized. $\alpha D = 200$.

RECOMMENDATIONS*

It is recommended—

(1) That the search for a rapid and accurate method for starch be continued.

(2) That further study be given to improvement of the polarimetric methods.

REPORT ON ACIDITY IN FLOUR

By LAWRENCE ZELENY (Agricultural Marketing Service, Washington, D. C.), *Associate Referee*

Titratable acidity has frequently been used as a measure of the age or "condition" of a flour, because the acidity increases with age under normal storage conditions and in general increases more rapidly under storage conditions that favor deterioration.

The methods most commonly used for determining titratable acidity in flour may be summarized as follows:

(1) *A.O.A.C. tentative method.*[1]—This method specifies digesting of the flour for one hour at 40° C., titrating of the filtered extract with standard alkali, and the use of phenolphthalein as an indicator. Acidity is reported as per cent lactic acid.

(2) *Balland or Greek method.*[2]—The Greek government has adopted as its official method for determining the acidity of flour a method by which the flour is extracted with 85 per cent alcohol and filtered and the filtrate is titrated with alcoholic potash, curcuma being used as an indicator. Results are expressed as per cent sulfuric acid.

(3) *Schulerud's method.*[3]—The flour is digested with 67 per cent alcohol, and the filtrate is titrated with standard alkali, phenolphthalein being used as an indicator. Results are expressed as the number of milliliters of normal alkali required to neutralize the acid from 100 grams of flour.

All acidities determined by the above methods are expressed in different terms, and even when these values are converted to the same

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* For report of Subcommittee D and action by the Association, see *This Journal*, 22, 68 (1939).
[1] *Methods of Analysis, A.O.A.C.*, 1935, 208.
[2] Am. Assoc. Cereal Chemists, Cereal Laboratory Methods, 1935, 41–42.
[3] *Ibid.*, 42.

terms, wide differences are shown. A high acidity by one method is generally associated with high acidities by the other methods, but no mathematical relationship exists between values obtained by the different methods, indicating in each case a different type of acid fraction.

SEPARATION OF ACIDS INTO CLASSES

The acidic substances present in flour consist principally of three classes of compounds: (1) acid phosphates, (2) amino acids, and (3) free fatty acids. These three types of acids are present as normal constituents of all wheat and flour, and under certain conditions they may be increased in quantity by the enzymatic hydrolysis of phytin, protein, and fat, respectively.

The acid phosphates and amino acids in flour are soluble in water and dilute ethyl alcohol, but relatively insoluble in strong alcohol. Most of the amino acids, however, are neutral in reaction in water and dilute alcohol and therefore do not contribute to the titratable acidity of water extracts even though present. In 85 per cent alcohol, on the other hand, the carboxyl groups of most of the amino acids may be titrated quantitatively.

The free fatty acids in flour are essentially insoluble in water but are soluble in fat solvents or in strong alcohol.

In order to determine the relative amounts of the various types of acidic substances extracted by different concentrations of alcohol, the following experiment was performed:

Eleven 10 gram portions of a commercial hard red winter wheat flour were shaken for 30 minutes with 100 cc. portions of neutral alcohol-water mixtures of different concentrations. The suspensions were then centrifuged, and two 25 cc. aliquots of each centrifugate were taken. To one of these aliquots was added sufficient neutral 95 per cent alcohol to bring the concentration of alcohol in the mixture to 85 per cent. To the other aliquot was added sufficient CO_2-free water to reduce the concentration of alcohol 5 per cent. The titration value of each solution was determined with phenolphthalein as an indicator.

A second series of eleven 10 gram portions of the same flour was extracted for 16 hours with petroleum ether in Soxhlet extractors. The free fatty-acid content of the extracts was determined, the residues were extracted with various concentrations of alcohol, and the acidities of the extracts were determined in the same manner as in the case of the original flour samples.

From the data obtained it is possible to divide the acidity extracted from the original flour by each of the 11 concentrations of alcohol into three distinct fractions, the amino-acid acidity, the acid phosphate acidity, and the fat acidity (Table 1). This may be understood more clearly by referring to the curves in Figure 1. Curve I represents the total titratable

acidity of each extract as determined by titrating the extracts of the original flour in 85 per cent alcohol. Curve II represents similar values for the alcoholic extracts of the fat-free flour, and therefore represents total acidity minus fat acidity. Obviously the differences between the values in Curve I and Curve II represent the fat acidity, which is plotted as

TABLE 1.—*Different acid fractions extracted from a commercial hard red winter wheat flour by different concentrations of alcohol*

(Acidity values are in terms of mg. of KOH required to neutralize acid extracted from 100 grams of flour, dry-matter basis.)

CONCENTRATION OF ALCOHOL	ORIGINAL FLOUR		PETROLEUM ETHER EXTRACTED FLOUR		AMINO-ACID ACIDITY C−D	FAT ACIDITY* A−C
	A ACIDITY IN 85% ALCOHOL. TOTAL ACIDITY	B ACIDITY IN 5% ALCOHOL	C ACIDITY IN 85% ALCOHOL	D ACIDITY IN 5% ALCOHOL-"PHOSPHATE" ACIDITY		
per cent						
95	47.6	29.6	28.2	12.6	15.6	19.4
90	57.0	34.1	39.1	18.9	20.2	17.9
80	109.5	57.5	91.6	41.8	49.8	17.9
70	135.6	65.5	117.6	54.3	63.3	18.0
65	144.1	66.9	126.6	60.6	66.0	17.5
60	143.7	68.7	129.7	60.2	69.5	13.5
50	140.1	66.4	131.5	60.6	70.9	8.6
40	134.7	67.3	131.1	61.5	69.6	3.6
30	132.4	58.8	129.7	55.7	74.0	2.7
20	127.0	56.6	126.2	55.2	71.0	0.8
10	141.0	65.1	140.5	65.1	75.4	0.5
0	153.1	73.2	154.4	70.9	83.5	−1.3

* Fat acidity as determined by petroleum ether extraction: 19.0.

Curve VI. Curve V represents the acidity of the alcoholic extracts of the fat-free flour as determined by titration in 5 per cent alcohol and may be considered to be essentially the curve for the acid phosphates. The difference between Curve II and Curve V represents the amino acid acidity, which is plotted separately as Curve IV.*

The high values for amino-acid acidity, phosphate acidity, and total acidity in the water (0 per cent alcohol) and 10 per cent alcohol extracts are probably due to the presence of acids formed by enzymatic action during the extraction process, in addition to the acids already present in the flour. That this is the case is evidenced by the fact that in water solu-

* It should be pointed out that the acid fraction which is designated "phosphate" acidity and which is represented by Curve V in Figure 1 consists of certain other acid-reacting substances in addition to the acid phosphates. These additional acidic factors consist of one of the carboxyl groups of any dicarboxylic amino acids present, the acid-binding power of any peptized protein present, and possible traces of organic acids not soluble in petroleum ether. Acid phosphates, however, undoubtedly account for the major part of this fraction.

It should further be pointed out that the amino-acid fraction as represented by Curve V, since it is based on the Foreman titration, does not account quantitatively for any proline or dibasic amino acids that may be present.

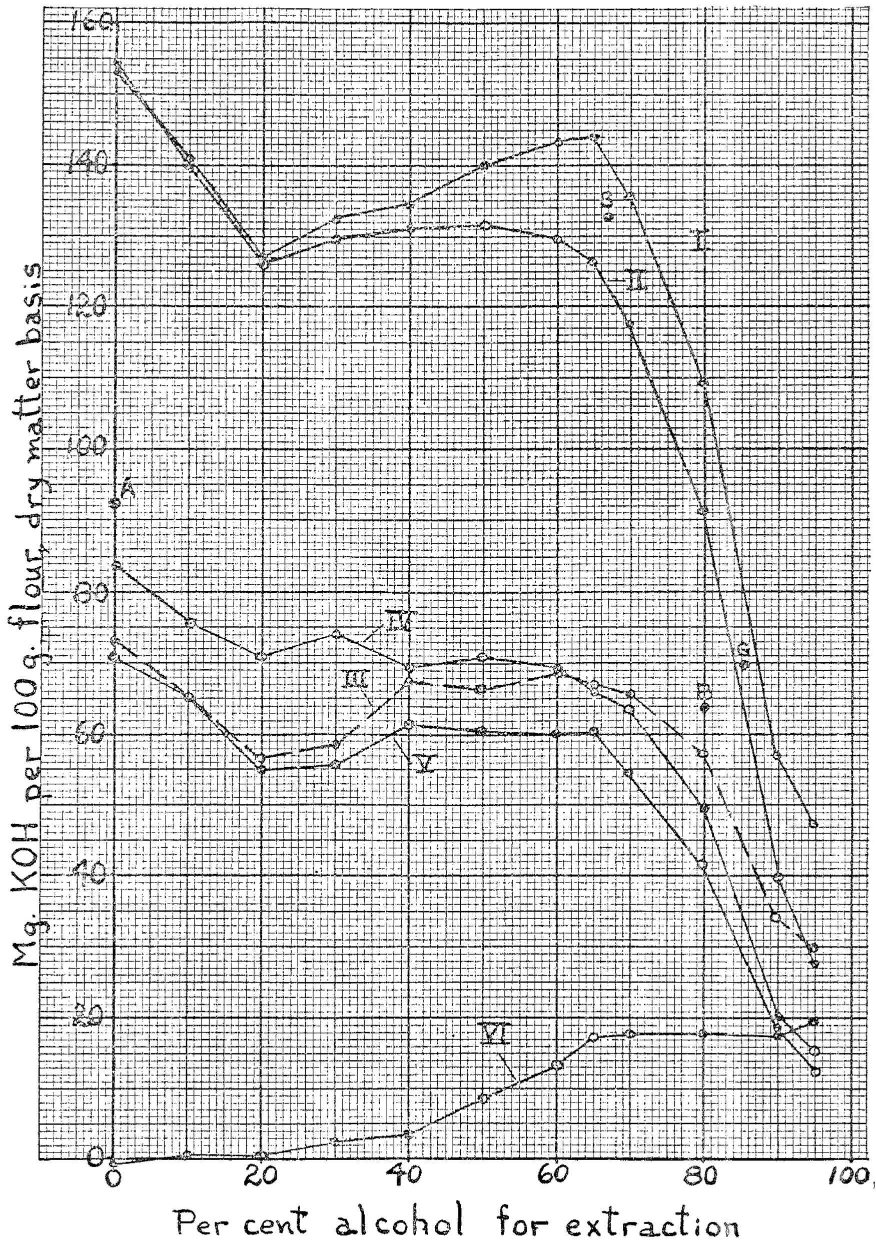

FIG. 1.—ACID FRACTIONS EXTRACTED FROM A COMMERCIAL HARD RED WINTER WHEAT FLOUR BY DIFFERENT CONCENTRATIONS OF ETHYL ALCOHOL.

Curve I. Total acidity. Titration in 85 per cent alcoholic solution. Curve II. Total acidity of fat-free sample. Titration in 85 per cent alcoholic solution. Curve III. Acidity by titration in 5 per cent alcoholic solution. Curve IV. Amino acidity. II minus V. Curve V. "Phosphate" acidity. Titration of extract of fat-free sample in 5 per cent alcoholic solution. Curve VI. Fat acidity. I minus II. Point A. Acidity by the A.O.A.C. tentative method. Point G. Acidity by the Greek or Balland method. Point S. Acidity by Schulerud's method.

tion the quantity of acid extracted is a function of the time and temperature of extraction, whereas in alcoholic solutions stronger than 20 per cent no appreciable increase in acidity occurs after the first 15 minutes of digestion. Thus the present A. O. A. C. tentative method must be considered empirical, as the acid determined is the sum of the acid extracted plus the acid formed by enzymatic action under the conditions of extraction specified by the method.

If the extracts made with alcohol concentrations of 20 per cent or over, in which enzymatic activity appears to be inhibited, are considered, it will be observed that a maximum total acidity is obtained at an alcohol concentration of about 65 per cent, thus essentially confirming Schulerud's observation that 67 per cent alcohol extracts the maximum quantity of acid from flour. It should be noted, however, that whereas Schulerud's 67 per cent alcohol extract may contain the maximum total acidity, this concentration of alcohol is too low to determine the maximum acidity. The strength of alcohol in such an extract should be increased to 85 per cent before titration in order to account for the maximum possible amino acid content.

It will also be observed that the amino-acid content is essentially constant at a maximum value in extracts containing between 20 per cent and 60 per cent alcohol, and "phosphate" acidity between 20 per cent and 65 per cent alcohol. Fat acidity values are nearly constant at concentrations of alcohol greater than 65 per cent and agree very well with the value determined by petroleum ether extraction. Thus no single concentration of alcohol is capable of extracting quantitatively all three types of acidic products, when the ratio of solvent to flour commonly employed in acidity determinations is used. Alcohol concentrations between 60 per cent and 70 per cent will in general extract most of the acids of all three types from flours containing less than 1 per cent of fat.

Curves similar to those shown in Figure 1 are obtained for both wheat and corn when maximum total acidity values in all samples tested are extracted with alcohol of between 60 per cent and 70 per cent.

The following procedure, based on the foregoing experiments, has been used for determining the three classes of acid-reacting compounds in flour:

METHOD

Extract 10 gram portions of the flour for 16 hours with petroleum ether in Soxhlet extractors. After evaporation of the solvent, dissolve the extracts in 50 cc. of 1:1 benzene-ethyl alcohol mixture containing 0.02% phenolphthalein, and determine fat acidity by titration with standard alkali. Subtract a blank titration on the benzene-alcohol mixture from the titration values of the extracts.

Suspend the extracted flours in 100 cc. of 60% ethyl alcohol neutral to phenolphthalein in stoppered flasks. Shake the suspensions at frequent intervals for 30 minutes and then filter. Dilute one 25 cc. aliquot of the filtrate with neutral 95% alcohol to a concentration of 85% alcohol and titrate. Dilute a second 25 cc. aliquot with CO_2-free water to a concentration of 5% alcohol and titrate. (The titration in

5% alcohol corresponds to the "phosphate" acidity and the difference between the two titrations corresponds to the amino-acid acidity.)* Calculate the results as the number of mg. of KOH required to neutralize the acids extracted from 100 grams of the flour on a dry-matter basis.

SIGNIFICANCE OF DIFFERENT TYPES OF ACIDITY

Zeleny and Coleman[1] showed that as wheat and corn deteriorate in storage the fat acidity increases rapidly even during the early incipient stages of deterioration, whereas the phosphate acidity and amino acid acidity increase only during relatively advanced stages of deterioration.

TABLE 2.—*Acidity of flour at intervals during storage at 32° C.*

SAMPLE NUMBER	CLASS OF WHEAT	FAT ACIDITY				PHOSPHATE ACIDITY				AMINO ACID ACIDITY			
		FLOUR, DAYS AT 32° C.				FLOUR, DAYS AT 32° C.				FLOUR, DAYS AT 32° C.			
		0	20	112	203	0	20	112	203	0	20	112	203
1	Soft Red Winter	17	24	46	60	40	42	43	48	69	72	67	70
2	Soft Red Winter	16	25	50	61	42	44	45	51	63	72	67	67
3	Soft Red Winter	18	24	49	61	42	48	46	47	69	71	67	68
4	Soft Red Winter	18	25	52	65	38	40	45	48	68	72	65	64
5	Soft Red Winter	18	26	46	58	40	43	43	47	63	69	65	62
6	Soft Red Winter	17	27	50	61	49	46	46	49	68	74	72	67
7	Soft Red Winter	19	26	49	61	44	48	45	54	69	73	70	67
8	Soft Red Winter	20	31	60	75	40	45	46	49	74	75	73	69
9	Soft Red Winter	24	30	54	65	39	41	45	48	66	73	65	67
10	Soft Red Winter	22	33	60	74	37	48	46	50	68	73	74	69
11	Soft Red Winter	26	36	55	72	43	56	55	59	69	76	75	68
12	Soft Red Winter	25	39	74	87	50	55	57	59	81	81	80	77
Av.	Soft Red Winter	20	29	54	67	42	46	47	51	69	73	70	68
13	Hard Red Spring	17	21	40	51	47	56	59	64	89	113	89	87
14	Hard Red Spring	22	44	90	100	48	58	65	81	117	116	120	115
15	Hard Red Spring	19	31	69	89	56	64	68	70	103	100	105	108
Av.	Hard Red Spring	19	32	66	80	50	59	64	72	103	110	105	103
16	Hard Red Winter	20	34	71	100	46	50	50	57	72	78	80	76
17	Hard Red Winter	17	27	58	71	41	47	50	52	82	85	79	79
Av.	Hard Red Winter	18	30	64	85	43	48	50	54	77	81	79	77
Av.	All Samples	20	30	57	71	44	49	50	55	76	81	77	75

The same type of study was applied to flour in storage. Seventeen samples of freshly milled straight grade unbleached flour were stored at 32° C. at moisture contents between 12.0 and 12.3 per cent, and the different types of acidity were determined at intervals over a period of 203

* See footnote on p. 528.
[1] *Cereal Chem.*, 15, 580–95 (1938).

days. The results are given in Table 2, and the average percentages of increase in the different types of acidity are shown graphically in Figure 2. Here again the rate of increase in fat acidity is much greater than that of the other types of acidity, and fat acidity alone showed a large and easily measurable increase during the first 20 days of storage.

Kozmin[1] and more recently Barton-Wright[2] showed that deterioration of gluten quality during storage of flour is due largely to the accumulation of free fatty acids, particularly the unsaturated fatty acids. Since the extent of deterioration is roughly proportional to the concentration of

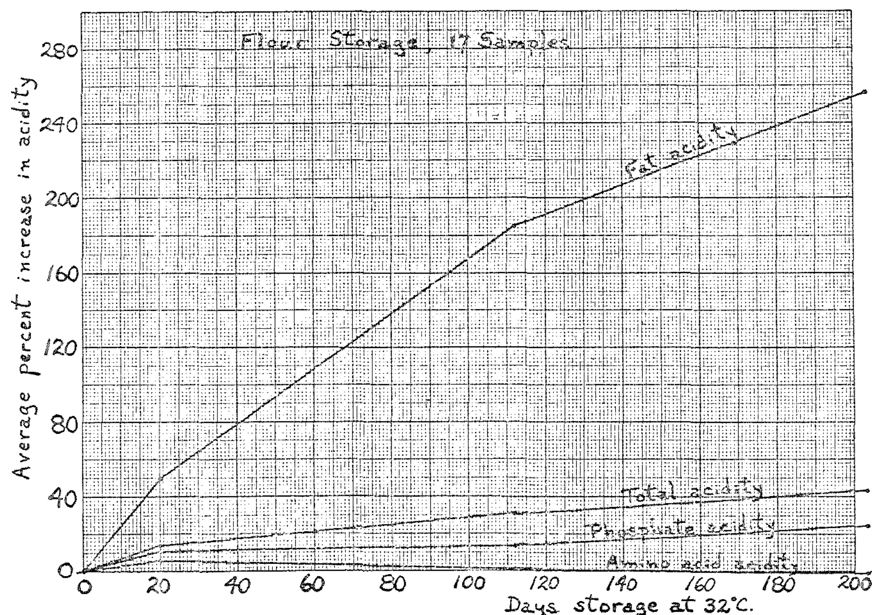

Fig. 2.—Average percentage increase in acidity of different types in 17 samples of flour stored at 32°C. and at moisture contents between 12.0 and 12.3 per cent.

free fatty acids in the flour, the "fat acidity" value as herein defined should be a better measure of such deterioration than would the acid value of the flour fat.

Baking tests with a basic formula were made at intervals on three samples of Hard Red Spring flour and two samples of Hard Red Winter flour during storage, and loaf volumes were compared with fat acidity values. Figure 3 shows graphically the decrease in loaf volume associated with increase in fat acidity for each of these flours. The winter wheat

[1] *Cereal Chem.*, 12, 165–171 (1935).
[2] *Ibid.*, 15, 521–540 (1938).

flour appears to be somewhat more resistant to the effects of high fat acidity than do the spring wheat flours, but in all cases high fat acidity was associated with significant decreases in loaf volume.

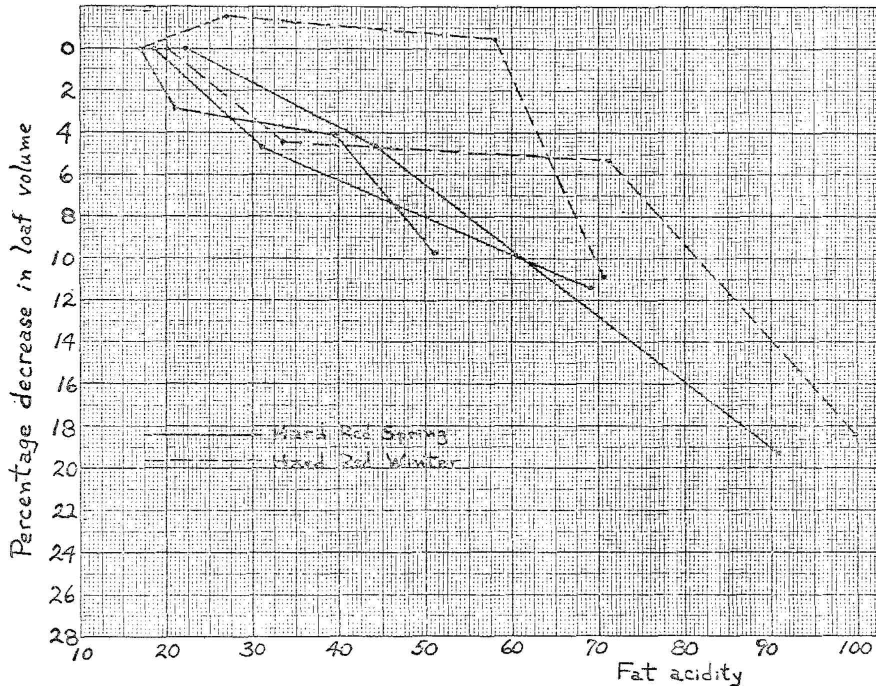

FIG. 3.—PERCENTAGE DECREASE IN LOAF VOLUME OF BREAD ASSOCIATED WITH INCREASE IN FAT ACIDITY OF FLOUR.

DISCUSSION

None of the three principal methods used for determining titratable acidity in flour appears to be based on a fundamental concept of the nature of the acids being determined.

The A.O.A.C. tentative method determines acidity due primarily to acid phosphates. The amino acids are present in the extract but are not determined by the titration procedure used. Fatty acids are neither extracted nor determined. The principal weakness of the method appears to be that it determines not only the acid phosphates originally present in the flour but also acid phosphates formed by enzymatic action during the extraction process.

The Greek or Balland method extracts and determines essentially all the free fatty acids plus varying fractions (generally less than half) of the acid phosphates and amino acids.

Schulerud's 67 per cent alcohol method extracts the major part of all three types of acids but fails to determine quantitatively the amino acids extracted.

Fat acidity appears to be a better index of the aging of flour than are either of the other principal types of acidity present or than any combination of the various types. A certain amount of evidence exists indicating that the accumulation of free fatty acids in flour is largely responsible for the deterioration of its baking quality. Whether or not this view can be fully substantiated, the fact remains that deterioration in storage is accompanied by a large and easily determined increase in fat acidity.

COLLABORATIVE STUDY

Four samples of flour of different ages were analyzed for fat acidity by six collaborators using the following method:

Extract duplicate 10 gram samples with petroleum ether for approximately 16 hours, using a Soxhlet, or similar extraction apparatus, and double thickness paper thimbles or Alundum R.A. 360 thimbles.

Completely remove the solvent from the extract by evaporation on the steam bath and dissolve the extract in the extraction flask with 50 cc. of a mixture of equal parts by volume of ethyl alcohol and benzene, containing approximately 0.02% of phenolphthalein.

Titrate the dissolved extract with carbonate-free standard alkali to a distinct pink color. For convenience use exactly 0.0178 *N* alkali for the titration in order to simplify calculations, 1 cc. of this solution being equivalent to 1 mg. of KOH. Make a blank titration on 50 cc. of the benzene-alcohol mixture and subtract the value obtained from the titration value of the sample.

Report fat acidity as the number of mg. of KOH required to neutralize the free fatty acids from 100 grams of flour on a dry-matter basis.

The results obtained by the various collaborators are listed in Table 3.

TABLE 3.—*Fat acidity values obtained by seven collaborators* on four samples of flour* (Results are averages of duplicate determinations and are expressed in terms of mg. of KOH required to neutralize free fatty acids in 100 grams of flour on a dry-matter basis.)

SAMPLE NUMBER	COLLABORATORS							AVERAGE	MEAN DEVIATION
	A	B	C	D	E	F	G		
1	29.4	26.0	28.9	28.2	24.3	28.7	27.2	27.5	1.5
2	69.0	66.3	75.7	61.5	64.2	68.2	58.6	66.2	4.1
3	65.8	64.5	73.5	62.5	65.1	62.1	60.8	64.9	2.8
4	20.8	18.3	26.9	18.1	28.9	21.5	18.2	21.8	3.5

* The collaborators participating in this work are listed as follows: L. H. Bailey, Bureau of Chemistry and Soils, Washington, D. C. R. A. Barackman, Victor Chemical Works, Chicago Heights, Ill. Leo Krenowitz, Bureau of Agricultural Economics, Washington, D. C. M. H. Neustadt, Bureau of Agricultural Economics, Washington, D. C. H. K. Parker, Wallace & Tiernan Co., Inc., Newark, N. J. Julius Siebenberg, Schwartz Laboratories, Inc., New York, N.Y. The Associate Referee. These names are not listed in the order used in Table 3.

RECOMMENDATIONS*

It is recommended—

(1) That the A.O.A.C. tentative method for acidity in flour be dropped.

(2) That the proposed method for determining the fat acidity of flour be adopted as tentative.

(3) That further study be made of the proposed methods for acidity in flour and other cereals.

REPORT ON SUGAR IN FLOUR

By R. M. Sandstedt (University of Nebraska, Lincoln, Neb.), *Associate Referee*

The identity of the reducing and non-reducing sugars of flour is still questionable. Genevois and Pavloff,[1] Geoffroy,[2] Colin and Belval[3,4,5,6,7] and Guillement[8] believe that the reducing sugars are hexoses and that much of the non-reducing sugar is levosin. Levosin is a levulosan that is fermentable in dough or in the presence of the fermentation activators that occur naturally in flour.[2,5,7,8] This levulosan is probably much more abundant in flours produced from European wheat than in those produced from American hard wheats.[1] Accordingly, until the identity of these sugars is more completely known, it is advisable to use the terms reducing sugars and non-reducing sugars rather than the specific terms maltose and sucrose. Since levosin is non-reducing, soluble in water, easily hydrolyzed, and is fermentable in dough, for practical purposes it may be included with the non-reducing sugars and calculated as sucrose.

The tentative method for reducing sugars and sucrose in flour as given in *Methods of Analysis, A.O.A.C.*, 1935, is the same as that given for the determination of sugars in feeds. This method involves the extraction of the sugars with 50 per cent alcohol and the subsequent clarification with lead acetate. This procedure is not satisfactory with flour owing to the solubility of gliadin in 50 per cent alcohol and to the poor clarification obtained with lead acetate on flour extract.[9] Since water extraction with subsequent acid tungstate clarification has proved satisfactory for determination of the maltose value[10,11] of flour, it would seem to be the logical method for the extraction and clarification of the other sugars.

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* For report of Subcommittee D and action by the Association, see *This Journal* 22, 68 (1939).
[1] *Comp. rend.*, 200, 690 (1935).
[2] *Bull. soc. chim. biol.*, 17, 848 (1935).
[3] *Comp. rend.*, 200, 2032 (1935).
[4] 14 me. Congr. chim. ind., Paris. Oct. 1934.
[5] *Bull. soc. chim. biol.*, 17, 1040 (1935).
[6] *Bull. soc. chim.*, 2, 1907 (1935).
[7] *Bull. soc. chim. biol.*, 19, 65 (1937).
[8] *Comp. rend.*, 201, 1517 (1936).
[9] Blish, *J. Biol. Chem.*, 33, 551 (1918).
[10] Rumsey, *Am. Inst. Baking Bull.*, 8 (1922).
[11] Blish, *This Journal*, 16, 497 (1933); Blish and Sandstedt, *Cereal Chem.*, 10, 189 (1933).

Sandstedt[1] has proposed a method for the determination of sucrose directly from the extract that is obtained for the determination of maltose value. He utilizes the acid added for clarification for the sucrose hydrolysis. It would seem that this method for sucrose could also be used on the extract made for the determination of the original reducing sugars before diastasis.[11] Thus the non-reducing sugars could be determined in conjunction with either the maltose value or the reducing sugars.

Accordingly, a method for reducing and non-reducing sugars (based on the ferricyanide method for non-reducing sugars[11]) was submitted to collaborative study. The collaborative results are far from satisfactory. The values reported for reducing sugars are exceedingly variable. In checking over the method for the possible sources of error, the Associate Referee found that differences in the technic of putting the flour in suspension could cause almost as much variation as was shown in the collaborative results. To eliminate this source of error it was found necessary to keep the liquid (while being pipetted into the flask) from coming in contact with the flour. The flour and liquid could then be shaken together to form a suspension instantaneously.

The method as now recommended for further collaborative study is as follows.

REDUCING AND NON-REDUCING SUGARS IN FLOUR

REAGENTS

(a) *Ethyl alcohol.*—95% (by volume).

(b) *Acid buffer solution.*—Make 3 cc. of glacial acetic acid, 4.1 grams of anhydrous Na acetate, and 4.8 cc. of H_2SO_4 (sp. g. 1.84) to 1 liter with water.

(c) *Sodium tungstate.*—12%. Make 12.0 grams of Na tungstate to 100 cc. with water.

(d) *Ferricyanide solution.*—Alkaline 0.10 *N*. 33.0 grams of pure dry $K_3Fe(CN)_6$ and 44.0 grams anhydrous Na_2CO_3 per liter.

(e) *Thiosulfate solution.*—0.10 *N*. 24.82 grams of $Na_2S_2O_3 \cdot 5H_2O$ and 3.8 grams of borax per liter.

(f) *Acetic acid-salt mixture.*—200 cc. of glacial acetic acid, 70 grams of KCL, and 40 grams of $ZnSO_4 \cdot 7H_2O$ per liter.

(g) *Combined 2% soluble starch-50% potassium iodide solution.*—Suspend soluble starch in a small quantity of cold water and pour slowly into boiling water with constant stirring. Cool (cooling must be thorough or the resulting mixture will be dark colored), add KI, make to volume, and add one drop of saturated NaOH solution per 100 cc. Use 1 cc.

DETERMINATION

(1) *Maltose.*—Introduce 5 grams of flour into a 100 or 125 cc. Erlenmeyer flask. Tip the flask so that all the flour is at one side and wet the flour with 5 cc. of 95% alcohol. Then tip the flask so that the wet flour is at the upper side and add 43.4 cc. of the acid buffer solution, keeping the solution from coming in contact with the flour until it has all been added to the flask. Then shake the flask to bring the flour into suspension.

Add immediately 2 cc. of the Na tungstate solution and again mix thoroly.

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[1] *Cereal Chem.*, 14, 767 (1937).
[11] See footnote on preceding page.

Filter at once (Whatman No. 4 or equivalent), discarding the first 8–10 drops of filtrate. Pipet 5 cc. into a test tube of approximately 75 cc. capacity (Pyrex 1″×8″). Add to the test tube with a pipet exactly 10 cc. of the alkaline $Fe(CN)_6$ solution, mix, and immerse the test tube in a vigorously boiling water bath. (The surface of the liquid in the test tube should be 3–4 cm. below the surface of the boiling water.)

After the test tube has been in the boiling water bath exactly 20 minutes, cool the tube and contents under running water, and pour at once into a 100 or 125 cc.

TABLE 1.—*0.10 N ferricyanide maltose conversion table*[1]

0.10 *N* THIOSULFATE	MALTOSE PER 10 G. FLOUR	0.10 *N* THIOSULFATE	MALTOSE PER 10 G. FLOUR	0.10 *N* THIOSULFATE	MALTOSE PER 10 G. FLOUR
cc.	*mg.*	*cc.*	*mg.*	*cc.*	*mg.*
0.10	618	3.40	373	6.70	166
0.20	608	3.50	367	6.80	161
0.30	598	3.60	360	6.90	156
0.40	588	3.70	353	7.00	151
0.50	578	3.80	347	7.10	145
0.60	568	3.90	341	7.20	140
0.70	558	4.00	334	7.30	135
0.80	550	4.10	328	7.40	130
0.90	542	4.20	322	7.50	126
1.00	534	4.30	315	7.60	121
1.10	527	4.40	308	7.70	116
1.20	519	4.50	302	7.80	111
1.30	512	4.60	295	7.90	106
1.40	505	4.70	288	8.00	101
1.50	499	4.80	282	8.10	96
1.60	492	4.90	276	8.20	90
1.70	485	5.00	270	8.30	85
1.80	478	5.10	264	8.40	80
1.90	472	5.20	257	8.50	76
2.00	465	5.30	251	8.60	71
2.10	458	5.40	244	8.70	65
2.20	451	5.50	237	8.80	60
2.30	445	5.60	231	8.90	56
2.40	438	5.70	225	9.00	51
2.50	431	5.80	218	9.10	46
2.60	425	5.90	213	9.20	41
2.70	418	6.00	207	9.30	36
2.80	412	6.10	201	9.40	31
2.90	406	6.20	195	9.50	25
3.00	398	6.30	188	9.60	20
3.10	392	6.40	182	9.70	15
3.20	385	6.50	176	9.80	10
3.30	379	6.60	171	9.90	5

[1] *Cereal Chem.*, 14, 603 (1937).

Erlenmeyer flask. Rinse out the test tube with 25 cc. of the acetic acid-salt solution, add to contents of Erlenmeyer flask, and mix thoroly. Then add 1 cc. of the 2% soluble starch-50% KI solution. Titrate with 0.10 *N* $Na_2S_2O_3$ solution to the complete disappearance of the blue color. A 10 cc. micro-buret is recommended for the titration. This titration value represents a definite quantity of reducing sugar per

10 grams of flour, which may be ascertained (as maltose) by consulting the 0.10 *N* Ferricyanide Maltose Conversion Table (Table 1).

Non-Reducing Sugars.—Pipet 5 cc. of the filtered, clarified flour extract (made according to the above specifications) into an 8″ test tube and immerse in a vigorously boiling water bath. After 15 minutes' boiling, cool the test tube and contents under running water and add 10 cc. of the alkaline 0.10 *N* $Fe(CN)_6$. Carry out the reduction of the $Fe(CN)_6$ (immersion in boiling water bath for 20 minutes) and the subsequent titration exactly as directed for the determination of maltose (1). Subtract the $Fe(CN)_6$ reduced by the maltose in the flour from the $Fe(CN)_6$ reduced after hydrolysis and obtain the non-reducing sugars represented by the difference (calculated as sucrose) from the Ferricyanide Sucrose Table (Table 2).

TABLE 2.—*0.10 N ferricyanide sucrose conversion table*[1]

0.10 *N* FERRICYANIDE REDUCED	SUCROSE PER 10 G. FLOUR	0.10 *N* FERRICYANIDE REDUCED	SUCROSE PER 10 G. FLOUR	0.10 *N* FERRICYANIDE REDUCED	SUCROSE PER 10 G. FLOUR
cc.	*mg.*	*cc.*	*mg.*	*cc.*	*mg.*
0.10	5	3.00	143	5.90	280
0.20	10	3.10	148	6.00	285
0.30	15	3.20	152	6.10	290
0.40	19	3.30	157	6.20	294
0.50	24	3.40	161	6.30	299
0.60	29	3.50	166	6.40	304
0.70	34	3.60	171	6.50	309
0.80	38	3.70	176	6.60	313
0.90	43	3.80	181	6.70	318
1.00	48	3.90	185	6.80	323
1.10	52	4.00	190	6.90	328
1.20	57	4.10	195	7.00	333
1.30	62	4.20	200	7.10	337
1.40	67	4.30	204	7.20	342
1.50	71	4.40	209	7.30	347
1.60	76	4.50	214	7.40	352
1.70	81	4.60	218	7.50	357
1.80	86	4.70	223	7.60	362
1.90	91	4.80	228	7.70	367
2.00	95	4.90	233	7.80	372
2.10	100	5.00	238	7.90	377
2.20	104	5.10	242	8.00	382
2.30	109	5.20	247	8.10	387
2.40	114	5.30	251	8.20	392
2.50	119	5.40	256	8.30	397
2.60	123	5.50	261	8.40	402
2.70	128	5.60	266	8.50	407
2.80	133	5.70	270	—	—
2.90	138	5.80	275	—	—

[1] Cereal Chem., 14, 767 (1937).

No report on baking test for soft wheat flour was given by the associate referee.

REPORT ON FLOUR-BLEACHING CHEMICALS

By DOROTHY B. SCOTT (U. S. Food and Drug Administration, New York, N. Y.), *Associate Referee*

Samples for a collaborative study of Munsey's modification of the Kent-Jones and Herd method, *This Journal*, **18**, 497, for the determination of small quantities of chlorine in Agene-bleached flour, were sent to nine analysts, in accordance with the recommendation of Subcommittee D.

TABLE 1.—*Chlorine* (*moisture-free basis*)—*p.p.m.*

COLLABORATOR	SAMPLE A 5 GRAMS AGENE PER BBL.	SAMPLE B 1 GRAM AGENE PER BBL.	SAMPLE C UNBLEACHED	SAMPLE D BETA-CHLORA BLEACH
R. A. Barackman Victor Chemical Works Chicago, Ill.	7.2	3.1	2.7	154
Robert D'Orazio Schwarz Labs., Inc. New York, N. Y.	6.0	2.3	3.7	83.1
G. M. Johnson Food & Drug Adm. Minneapolis, Minn.	4.0 3.7 } 3.9	1.7 2.1 } 1.9	0.6 1.6 } 1.1	173 149 } 161
H. V. Moss Monsanto Chemical Co. St. Louis, Mo.	5.03	1.67 1.56 } 1.61	1.20 1.08 } 1.14	127.1 119.7 } 123.4
V. E. Munsey* Food & Drug Adm. Washington, D. C.	4.5 4.3 } 4.4	1.9 1.7 } 1.8	1.1 0.9 } 1.0	131 125 } 128
H. K. Parker* Wallace and Tiernan Co., Inc. Newark, N. J.	4.11 3.64 } 3.87	1.69 1.52 } 1.6	0.55 0.31 } 0.43	112.3 123.14 } 117.7
T. H. Riggs* Food & Drug Adm. New York, N. Y.	5.5	1.7	1.2	135
Manuel Tubis Food & Drug Adm. Philadelphia, Pa.	6.29	3.02	2.0	102.4*
J. H. Watkins Food & Drug Adm. New Orleans, La.	6.93 6.95 } 6.94	4.50 4.26 } 4.38	3.90 3.96 } 3.93	146 144 } 145
Average	5.46	2.38	1.91	127.7

* Aviation gas used for extraction. Parker used high boiling petroleum ether, 60°–100° C.

The method was changed in several minor respects. The analyst was requested to use petroleum ether as a fat solvent because of the difficulty of obtaining lead-free aviation gas, to use 475 cc. of the extract in the determination, to use 475 cc. of the petroleum ether in the blank, and to add 5 cc. more silver nitrate than was specified in the method.

Two samples bleached with known quantities of Agene (nitrogen trichloride) and the unbleached flour were submitted to the analysts. A fourth commercial flour bleached with Beta-chlora was also submitted for a collaborative study of Munsey's method for the determination of large quantities of chlorine in flour, *This Journal,* **18**, 500. The results are shown in Table 1.

COMMENTS OF COLLABORATORS

R. A. Barackman.—Please note that the volumetric titration method was used for determining chlorine in Sample D rather than the gravimetric method, which was suggested. Volume of the filtrate: A—277.5 cc. B—437.5 cc. C—437.5 cc. D—100 cc.

G. M. Johnson.—When filtering the petroleum ether extracts, I noticed considerable evaporation of the solvent, particularly because the Büchner funnel I was using (the only one available) did not make a good fit for the filter paper, which made it impossible to get a filtrate free from flour particles regardless of the number of times that it was passed through the paper. I would suggest that aviation gasoline, if obtainable, is superior to petroleum ether. The chloride blank on the reagents was high, amounting to 1.22 cc. of 0.01 $AgNO_3$.

V. E. Munsey.—Your instructions were to use 475 cc. of gasoline extract in this procedure. You will note that my average for 65 samples was only 477 cc. Therefore, you would expect many of the samples to yield less volume than 475 cc. On these particular samples the following results were obtained: 370, 415, 430, 435, 485, 520. With the use of petroleum ether these volumes would be even less. Therefore, in only two cases was it possible to use as much as 475 cc.

H. K. Parker.—Following the method as read, I determined the blank by using 475 cc. of the high boiling petroleum ether (Viking Brand) and evaporating "nearly to dryness" before adding sodium ethylate. A zero blank was obtained. However, when the flour extract was evaporated nearly to dryness, heavy chloride precipitates were observed on Flour B, which was apparently lightly bleached, so the blank determination was repeated except that instead of evaporation "nearly to dryness" the sodium ethylate was added to about the last 7–10 cc. of concentrate. A blank equivalent to 6.75 p.p.m. was obtained. This showed the presence of volatile chlorides, which might have been held back in the fatty extract, depending upon one's judgment as to arrival at the stage of "nearly to dryness," so for the remainder of the determinations the petroleum ether was purified by refluxing over a mixture of sodium-sodium methylate and silver oxide. A blank of 0.3 p.p.m. was obtained when the sodium ethylate was added to the last 10 cc. of the petroleum ether concentrate. I suggest that the blank be determined upon either the gasoline or petroleum ether concentrate or by use of a known chloride-free fat. The latter procedure might be preferable to lessen attack of caustic upon platinum. The Volhard method was used in determination of chlorides in Flour D.

T. H. Riggs.—The Agene method was followed. Aviation gas, lead-free, and petroleum ether, were used, but the petroleum ether results were discarded due to the extremely high blank of the petroleum ether used.

Manuel Tubis.—In the case of Sample D analyzed for Beta-chlora bleach, I used aviation gasoline of doubtful purity and it yielded a high blank. In the Agene method, the values of the filtrates were A—380 cc.; B—430 cc.; C—370 cc. and 475 cc. in the blank. A second set of determinations was made, but owing to the poor conditions and unknown history of the aviation gasoline used the blank was very high and the final results were much lower than those with petroleum ether.

J. H. Watkins.—On account of the volatility of petroleum ether, it seemed advisable to cool the extracts somewhat just before filtering, which was carried out as quickly as possible so as to avoid undue evaporation. Sample D was run according to your directions, but on another sample I avoided filtration and exposure to evaporation of a large surface of flour wet with petroleum ether by withdrawing 100 cc. of extract with a 100 cc. pipet loosely plugged with cotton. The sample had been allowed to stand overnight and the 100 cc. was withdrawn without disturbing the flour layer so that a clear extract was obtained.

DISCUSSION

From the experiences of the analysts, several suggestions for the improvement of the methods should be studied.

Riggs and Watkins have suggested that the flour and gasoline mixture be made up to a definite volume, the flour allowed to settle overnight, and a definite volume syphoned off in the morning without disturbing the flour layer.

This procedure would eliminate the excessive evaporation of the solvent, which is dependent on temperature, rapidity of filtration, and the extent of the surface of the flour exposed in the large Büchner funnel when filtering. There would be no flour particles in the extract and it would also answer the question of the correct quantity of gasoline to be used in the blank. The solvent must be known to be very low in chlorides before it is used.

The collaborators were also asked to determine carotenoid pigments in the flour samples, using Munsey's method, *This Journal,* **21**, 339, if a neutral wedge photometer was available, and also the gasoline color values as given in *Methods of Analysis, A.O.A.C.*, 1935, 218. The results are given in Table 2.

TABLE 2.—*Carotenoid pigments as carotene—p.p.m.*

COLLABORATOR	SAMPLE A	SAMPLE B	SAMPLE C	SAMPLE D
G. M. Johnson	0.45 0.61 } 0.53	1.23 1.45 } 1.34	1.65 1.84 } 1.75	0.10 0.20 } 0.15
V. E. Munsey	0.8	1.7	2.0	0.3
H. K. Parker	0.924 0.825 } 0.874	2.37 2.025 } 2.197	2.65 2.635 } 2.64	0.250 0.225 } 0.237
T. H. Riggs	0.8	1.8	2.2	0.3
M. Tubis	0.53	1.78	2.03	0.40
J. J. Winston	0.81	1.76	2.01	0.27
Nat. Macaroni Mfrs. Assoc. New York, N. Y.				
J. H. Watkins	0.68 0.71 } 0.70	1.40 1.49 } 1.47 1.52	1.83 1.84 } 1.84	0.18 0.19 } 0.19
Average	0.72	1.72	2.07	0.27

Parker used the neutral wedge photometer at the New York Station with the 4 inch cell, filter No. 46, and a calibration curve made by J. L. Hogan a year ago.

The agreement of the collaborators' results is very good, and the collaborators report satisfaction with the method.

TABLE 3.—*Gasoline color value*

COLLABORATOR	SAMPLE A	SAMPLE B	SAMPLE C	SAMPLE D
R. A. Barackman	0.77	1.11	1.25	0.60
Robert D'Orazio	0.35	0.97	1.17	0.09
H. V. Moss	0.44, 0.45 } 0.445	1.12, 1.08 } 1.10	1.42, 1.48 } 1.45	0.087, 0.105 } 0.096
V. E. Munsey	0.7	1.9	2.2	0.3
H. K. Parker	0.81, 0.63 } 0.71	1.47, 1.39 } 1.43	1.92, 1.82 } 1.87	0.16, 0.19 } 0.175
Manuel Tubis	0.33	0.93	1.30	0.08
J. H. Watkins	0.45	1.23	1.66	0.05
Average	0.54	1.25	1.16	0.20

COMMENTS OF COLLABORATORS

R. A. Barackman.—We had considerable difficulty in the use of the Duboscq colorimeter on petroleum ether extracts of the flours so that results were entirely unreliable.

H. V. Moss.—We call your attention to the fact that the gasoline color values were determined on a Duboscq colorimeter.

H. K. Parker.—Solvent was high boiling petroleum ether 60°–100° C. Gasoline color was measured in Hellige-Klett colorimeter at 50 mm. depth. Blue glass light filter. Considerable difficulty was experienced in matching the standard chromate to the higher flour color concentrations due to brown shade. The match is approximated upon intensity rather than shade of color. The most difficult to match seemed to be flour B, which we assume to be the light bleach, and which results are farthest out of line with the Munsey method.

RECOMMENDATIONS

It is recommended[1]—

(1) That the study of methods for the determination of chlorine in flour be continued.

(2) That the method for the measurement of carotenoid pigments in flour given in *This Journal*, **21**, 339, be studied next year with the view to substituting water-saturated n-butyl alcohol for the Varsol-alcohol mixture.

(3) That study of the method for the determination of benzoyl peroxide in flour be continued.

[1] For report of Subcommittee D and action by the Association, see *This Journal*, **22**, 68 (1939).

REPORT ON CARBON DIOXIDE IN SELF-RISING FLOURS

By R. A. BARACKMAN (Victor Chemical Works, Chicago, Ill.), *Associate Referee*

Last year, *This Journal*, **21**, 388–403, it was demonstrated that the gasometric method for the determination of carbon dioxide in self-rising flour is subject to errors that result in recovery lower than theoretical. No single factor was found to be responsible. The gasometric procedure was considered to be satisfactory for all practical purposes. Both Whiting, *This Journal*, **15**, 588–591, and Adler, *Ibid.*, **20**, 365–369, suggested that

Carbon dioxide in self-rising flour

DATE OF TESTS (1938)	MIXING DATE 9/2	1 WEEK 9/7	TEST DAYS			AVERAGE CO_2	RECOVERY
			9/12	9/14	9/16		
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
L. H. Bailey U. S. Bur. Chemistry and Soils, Washington			—	.682	.672	.677	90.6
R. A. Barackman Victor Chemical Works Chicago	(.692)	(.691)	.656	.677	.675	.669	89.6
Wm. Bruton Kroger Food Foundation Cincinnati			.647	.644	.675	.654	87.6
F. A. Collatz General Mills, Inc. Minneapolis			.660	.655	.663	.659	88.2
J. R. Davies General Foods Corp. Chicago			—	.665	.656	.661	88.4
E. McKim Monsanto Chemical Co. St. Louis			.673	.681	.680	.678	90.7
H. W. Putnam Igleheart Bros. Inc. Evansville			.685	.682	.673	.680	91.0
W. Tholstrup Pillsbury Flour Mills Minneapolis			.676	.672	.685	.678	90.7
L. D. Whiting Ballard and Ballard Co. Louisville			.700	.689	.686	.692	92.6
L. Zeleny U. S. Bur. Agr. Economics Washington			.667	.670	.684	.674	90.2
Average			.670	.671	.675	.672	90.0
Maximum						.692	92.6
Minimum						.654	87.6

a factor be established for the conversion of carbon dioxide recovered to carbon dioxide present. Adler implied that this factor might be dependent on laboratory conditions in various geographical locations. An attempt was made this year to develop such a factor or factors.

A single sample of self-rising flour was prepared having a theoretical carbon dioxide content of .7474 per cent. A soft wheat patent flour having a moisture content of 12.9 per cent was used. The sample was submitted to ten collaborators with the request that determinations of carbon dioxide be made in duplicate on the same calendar days and that modified Method A, *This Journal*, 20, 365–369, be used. Thus the age of the sample and widely varying climatic conditions were eliminated as possible sources of error.

The results from individual collaborators show about the same differences from the average percentage of carbon dioxide recovered as was reported by Adler and by Whiting. There was an indication that a laboratory reporting a result lower than the average with one sample also reports a lower figure with another.

The average percentage recovery of carbon dioxide, 90 per cent of theoretical, is lower than that reported by Adler and by Whiting. Some loss of carbon dioxide occurred between the time of mixing the sample and of testing by collaborators, and because of this no attempt was made this year to establish a factor for converting carbon dioxide found to carbon dioxide present.

It is suggested that next year the Associate Referee submit the ingredients of self-rising flour to collaborators with instructions for mixing immediately before determining the carbon dioxide content. This procedure will eliminate aging effects. The results should show a constant loss of carbon dioxide, characteristic of the gasometric method. A factor may then be established.

There remains to be determined a conversion factor as previously suggested, the influence of flour grade and type, and the percentage recovery of carbon dioxide when variable amounts of sodium bicarbonate (and monocalcium phosphate) are present in self-rising flour.

It is recommended[1] that collaborative testing of the carbon dioxide in self-rising flour be continued along the lines suggested in this report.

REPORT ON MILK SOLIDS IN BREAD

By V. E. MUNSEY (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

The study this year was in accordance with the recommendation of the committee, which included further collaborative work on the citric

[1] For report of Subcommittee D and action by the Association, see *This Journal*, 22, 68 (1939).

acid method, the so-called fat method, and the lactose method for the determination of milk solids in bread.

The bread samples studied in previous years by this Referee had been made with known amounts of fresh liquid milk. This year three samples of bread were prepared from known amounts of powdered whole milk. These breads contained 2.6, 5.1, and 7.7 per cent whole milk solids and were numbered, respectively, 1, 2, and 3. They were analyzed for milk solids by four collaborators according to the citric acid procedure on page 224, *Methods of Analysis, A.O.A.C.*, 1935. The results are shown in Table 1.

TABLE 1.—*Milk solids (m.f.b.) by the citric acid procedure (per cent)*

COLLABORATOR	SAMPLE 1		SAMPLE 2		SAMPLE 3	
1	2.9 2.6	Av. 2.8	4.5 5.4	Av. 5.0	10.3	
2	2.6		4.8		5.9	
3	3.2 3.5	Av. 3.4	6.5 6.7	Av. 6.6	9.3 9.1	Av. 9.2
4	3.3 3.5	Av. 3.4	6.4 6.5	Av. 6.5	9.4 9.3	Av. 9.3

The results of Collaborators 3 and 4 are much too high for milk solids on all samples. In view of the satisfactory results from a majority of collaborators in previous years the cause of these high results was investigated. The powdered whole milk used in these breads contained by analysis 1.54 per cent citric acid on the moisture-free basis. This represents a citric acid content on the basis of liquid milk of 0.184 per cent, which is considerably higher than the average value, 0.16 per cent, in liquid milk on which the method of calculation is based. A recalculation of results for Collaborators 3 and 4 based on the actual citric acid content of the milk actually used indicates 2.8 and 3.0 per cent milk solids for Sample 1, 5.5 and 5.5 per cent milk solids for Sample 2, and 8.0 and 7.9 per cent milk solids for Sample 3. Results by Collaborators 1 and 2 would be lowered by about 15 per cent and would be lower than the amount of milk solids present in all except Sample 3, Collaborator 1.

The results in Table 1, together with those of previous years, indicate the variation to be expected among analysts with no significant amount of experience with the methods, and also the possibility that results may not be in close agreement with the actual amount of milk present. The results this year also illustrate the variation based on the natural citric acid content of the milk. It should be emphasized that while the results by this procedure are calculated as whole milk solids, the method actually indicates only the non-fat milk solids unless there is further evidence to establish the corresponding amount of butterfat.

Whole milk solids may also be calculated by determination of the non-fat milk solids by application of the lactose procedure published in *Cereal Chem.* 13, 541 (1936). The crumbs of the loaves of the same composition as those used for the citric acid procedure were analyzed by three collaborators for milk solids by the same lactose procedure (Table 2).

TABLE 2.—*Milk solids* (m.f.b.) by the lactose procedure (per cent)*

COLLABORATOR	SAMPLE 1		SAMPLE 2		SAMPLE 3	
	2.1		5.6		8.3	
1	2.3	Av. 2.4	5.9	Av. 5.6	7.7	Av. 8.0
	2.7		5.2		7.9	
2	3.0		5.3		8.1	
3	2.2	Av. 2.2	5.5	Av. 5.5	8.0	Av. 7.9
	2.2		5.4		7.9	

* Calculated from non-fat milk solids by Referee.

All the results by the lactose procedure are in good agreement with the amount of milk solids actually present. Too much confidence should not be placed in these limited data since a previous study was not so satisfactory and some collaborators have not had success with this long procedure of a biological nature. It may be desirable to include in each batch of samples analyzed by this procedure a sample with a known amount of milk solids to serve as an indication of the proper biological action in the different steps throughout the procedure.

A method based on determination of calcium may be worthy of consideration for judging compliance of the non-fat component of the milk solids in milk bread. The German literature indicates the value of such a method, particularly a paper in *Z. Untersuch. Lebensm.*, 75, 150 (1938). On the dry basis the results show 40–70 mg. of calcium oxide per 100 grams for water bread and 100–150 mg. per 100 grams for milk bread.

The three samples of bread used in this collaborative study were analyzed by this procedure, which represents a one-third, two-thirds, and all-milk bread with the following results, respectively: 75 mg. per 100 grams, 112 mg. per 100 grams, and 143 mg. per 100 grams. Water breads have analyzed from 28 to 70 mg. per 100 grams, depending on the presence or absence of calcium salts used in so-called dough improvers. The calcium contents of wheat flour and milk are fairly constant, but a variation due to addition of materials containing calcium must be borne in mind in the application of this procedure. It has the advantage of being simple, short, and highly accurate.

The so-called fat method studied is published on page 222, *Methods of Analysis, A.O.A.C.*, 1935. The collaborators were specifically instructed to dry completely the filter pad containing the absorbed fat, to use

(1+4) sulfuric acid instead of (1+1), which is an error in the book, maintain a constant rate of distillation, use carbon dioxide-free distilled water and carbon dioxide-free sodium hydroxide, and to run a blank on reagents to be used as a correction. Since there is some evidence that the sodium hydroxide saponification may not be complete, some of the collaborators were instructed to saponify with the 4 cc. of soda-glycerol mixture under the official Reichert-Meissl method instead of with 1 cc. sodium hydroxide (1+1) specified in the method. The breads submitted were of the same composition and identity as those analyzed in Tables 1 and 2.

TABLE 3.—*Results on % total fat and milk solids (m.f.b.) by so-called fat method*

COLLABORATOR		SAMPLE 1	SAMPLE 2	SAMPLE 3
			per cent	
1	Fat	5.1, 5.0, Av. 5.1	5.7, 5.7, Av. 5.7	6.4, 6.4, Av. 6.4
	Milk Solids	2.2, 2.1, Av. 2.2	4.4, 5.5, Av. 5.0	6.5, 4.6, Av. 5.6
2	Fat	5.0–5.0, Av. 5.0	5.7–5.7, Av. 5.7	6.2–6.3, Av. 6.3
	Milk Solids	2.5–2.7, Av. 2.6	5.0–4.6, Av. 4.8	7.0–6.8, Av. 6.9
3	Fat	—	5.7–5.0, Av. 5.4	5.6–5.8, Av. 5.7
	Milk Solids	—	6.4–6.9, Av. 6.7	9.3–9.2, Av. 9.3
4	Fat	4.8–4.9, Av. 4.9	5.6–5.6, Av. 5.6	6.3–6.3, Av. 6.3
	Milk Solids	2.9–2.9, Av. 2.9	5.2–5.3, Av. 5.3	7.8–7.9, Av. 7.9
5	Fat	5.1–4.9, Av. 5.0	5.7–5.7, Av. 5.7	6.3–6.3, Av. 6.3
	Milk Solids	2.3–2.3, Av. 2.3	4.0–4.2, Av. 4.1	7.0–6.5, Av. 6.8
6	Fat	4.9–4.9, Av. 4.9	5.6–5.7, Av. 5.7	6.2–6.2, Av. 6.2
	Milk Solids	2.0–2.2, Av. 2.1	3.9–4.3–3.7–3.6 Av. 3.9	6.7–6.0–6.9–8.0 Av. 6.9

These results (Table 3) show good agreement on total fat and confirm previous studies. The results for milk solids on Sample 1, and some of the results on Samples 2 and 3 are good. In general, the results indicate variations from the actual amount of milk solids present similar to those reported in previous studies. The results of Collaborators 2 and 3 by saponification of the fat by the glycerol-soda procedure indicate no ma-

terial improvement. However, as the result of recent work by the Associate Referee it is recommended that the glycerol-soda saponification be used in place of the sodium hydroxide on future analysis with this method.

Results by the so-called fat method are necessary to establish the butterfat equivalent to the non-fat component. While the results are not in all cases close to the actual amount of milk solids present, they do indicate during the past three years' study the probable variation in results expected and a fair estimation of the amount of butterfat present.

By using the average of three closely agreeing so-called fat numbers a closer estimation of milk solids based on butterfat present may be expected.

The Associate Referee expresses appreciation of the cooperation of the following collaborators who took part in this study: A. H. Wells, Los Angeles Station, Food and Drug Administration; C. E. Hyndsy, Department of Agriculture and Markets. Albany, New York; L. W. Ferris, Buffalo Station, Food and Drug Administration; J. H. Watkins, New Orleans Station, Food and Drug Administration; R. Carson, Department of Agriculture, Ottawa, Canada; D. A. Magraw, American Dry Milk Institute, Inc., Chicago, Ill.; Charles Hoffman, Ward Baking Company, New York City; George H. Marsh, Division of Agricultural Chemistry, Montgomery, Ala.

RECOMMENDATIONS[1]

It is recommended—

(1) That the citric acid procedure for calculating milk solids be not further studied collaboratively for the present.

(2) That the lactose procedure be more extensively studied collaboratively.

(3) That the so-called fat method be not further studied collaboratively in the present form.

(4) That study be made of a method based on the direct saponification and distillation of the bread samples without extraction of the fat for estimation of butterfat content.

REPORT ON COLD WATER EXTRACT IN FLOUR

By H. C. Fellows (Agricultural Marketing Service, Washington, D. C.), *Associate Referee*

Collaborative studies on cold water extract in flour were continued this year, and special attention was given to the following points:

[1] For report of Subcommittee D and action by the Association, see *This Journal*, 22, 68 (1939).

(1) Grade of filter paper—XX 588 folded filter paper, 18½ cm. diameter, Carl Schleicher & Schüll.

(2) Time of extraction—40 minutes.

(3) Extraction temperature—ice bath (32° F.).

(4) Drying temperature—Approximately 100° C. in vacuum oven to constant weight.

A soft wheat "patent" flour, a soft wheat "straight grade" flour, and a soft wheat "first clear" flour were used in these studies. The collaborative results are given in the table.

Cold water extract, per cent

COLLABORATOR	PATENT FLOUR	STRAIGHT FLOUR	FIRST CLEAR FLOUR
1	4.14	4.06	4.07
2	4.51	4.48	4.63
3	4.22	4.13	4.17
4	4.47	5.04	4.47
5	4.15	4.31	4.37
6	4.17	4.13	4.09
7	3.98	3.98	3.87
8*	4.20	4.02	4.01
8A†	4.17	4.13	4.03

* Filtered as recommended in the method.
† Centrifuged for 10 minutes at high speed and then filtered.

The results obtained by the various collaborators are on the whole in close agreement. The centrifuging process does not have any material effect on the final results but it does speed up filtration.

One collaborator reported his results as dried at 100° C. in the air oven, and the data obtained by him are in line with the findings of the other collaborators, who used the vacuum oven at approximately 100° C.

The collaborators were the following:

1. F. A. Collatz, General Mills, Inc., Minneapolis, Minn.
2. M. H. Neustadt, Agricultural Marketing Service, Washington, D. C.
3. L. W. Haas, The W. E. Long Company, Chicago, Ill.
4. Rae H. Harris, Agr. Expt. Station, Fargo, N. D.
5. M. J. Blish, Agr. Expt. Station, Lincoln, Nebr.
6. Howard M. Simmons, Mid-West Laboratories Co., Inc., Columbus, Ohio.
7. C. G. Harrel, Pillsbury Flour Mills Co., Minneapolis, Minn.
8. H. C. Fellows.

RECOMMENDATIONS[1]

It is recommended—

(1) That the method be rewritten [revised method was published in *This Journal*, **22**, 76 (1939)].

[1] For report of Subcommittee D and action by the Association, see *This Journal*, 22, 68 (1939).

(2) That the method then be adopted as official (first action).
(3) That further studies be carried on with other cereals.

No report on ergot in flour was given by the associate referee.

REPORT ON PROTEOLYTIC ENZYMES

By QUICK LANDIS (The Fleischmann Laboratories,
New York City), *Associate Referee*

Both chemical and physical methods have been devised for the measurement of proteolytic activity. Generally speaking, the former determine directly an increase in split peptid linkages, and the latter a change in the colloidal properties of the substrate, which may or may not be a direct consequence of peptid link hydrolysis. Marked changes in physical properties frequently appear long before chemical methods can detect any appreciable change.

The numerous ways in which complex protein molecules can be decomposed is in full harmony with the experimental recognition of various types of specific proteolytic enzymes.[1] Thus, although the methods proposed for study may give only a measure of over-all conversion one should not fail to remember that concurrent specific processes, including "activation," contribute to the final observation. A single method can seldom if ever give a complete picture of the activity of any food sample.

Methods may be further classified as autolytic or analytic. Each class has its own function in enlarging knowledge of the product under consideration. In some cases, of which flour is representative, the results of an autolytic method are perhaps more susceptible of translation to certain practical applications, but for concentrates autolytic methods are usually of little value.

It is therefore proposed to submit three separate classes of methods to simultaneous investigation. The extremely low activity exhibited by flours is a great handicap, but if improvements can be devised a limited collaborative effort may be made. Among the chemical methods developed, the Willstätter alcoholic titration of soluble free carboxyl groups is probably most sensitive and it is proposed to continue the investigation of Balls and Hale, *This Journal*, **18**, 135–40, on this method. Probably the most promising autolytic physical method for flour, involving measurement of the softening of a flour paste, was also reported by Balls and Hale, *Ibid.*, **19**, 372–3.

If the conditions can be adequately standardized this method may be of practical application. The Associate Referee has also been engaged in the development of a highly sensitive analytic physical method, utilizing

[1] Bergmann and Rosa, *J. Am. Chem. Soc.*, **58**, 1503 (1936).

the *change* produced in gelation capacity of a gelatin substrate by proteolytic enzymes.[1] As detailed at present the method is somewhat cumbersome, although readily detecting differences among samples with activities still less than those exhibited by flour. It is hoped that simplification can be achieved without loss of sensitivity.

It is recommended[2] that these three types of methods be investigated.

REPORT ON COLOR IN FLOUR

By H. K. Parker (Wallace & Tiernan Co., Newark, N. J.), *Associate Referee*

In a short review of the previous collaborative work to develop a method and a machine to measure the color of flour and bread by reflectance, perhaps it should be pointed out that a colorimeter[3] has been developed whereby the color of the sample can be matched by means of color cards mounted upon a rapidly rotating disk (Maxwell Disk). Nine machines were lent to different laboratories where flour and bread were examined and judged almost daily. It is significant to note that with one exception neither the machine nor the method proved satisfactory enough for the laboratory to wish to purchase the machine. The one purchaser apparently finds the machine more useful for colored materials other than flour or bread.

The difficulties encountered seem to be with the preparation of the sample and more particularly with the variation in eye reaction of the various observers. Besides these objections most operators complain that the measurement is time consuming and causes fatigue due to eye strain. Apparently quite concordant results can be obtained by the same observer upon the same sample of flour, and in practice it was expected that one observer could be used in each laboratory to establish a standard flour that could be prepared each week and used to Pekarize against the various unknowns. This procedure has not worked out too well.

Moreover, in practice two different firms with laboratories situated at different cities attempted to check the same sample on various occasions and were unable to do so satisfactorily. This work was done in addition to the A.O.A.C. collaborative tests, *This Journal*, **18**, 593; **19**, 569. In speculation that the preparation of the sample had much to do with discrepancies, since it has been shown that the rate of drying affects the color and quite likely a difference in humidities at different cities occurs, an attempt was made to obviate this difficulty as proposed in the oral report given at the 1937 A.O.A.C. meeting, *Ibid.*, **20**, 382. Upon the

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[1] Landis and Frey, *Cereal Chem.*, **15**, 91–101 (1938).
[2] For report of Subcommittee D and action by the Association, see *This Journal*, **22**, 68 (1939).
[3] *Cereal Chem.*, **10**, 437 (1933).

suggestion of Miss Nickerson, Messrs. Rouse and Shearer of Trenton, N. J., who have prepared sub-standards for the silk industry, were contacted. Enamelled steel slabs were prepared by them to match wet flour color. Before sending these slabs out to various collaborators it was found in this laboratory that not much better checks could be obtained on the same slab by different observers than previously and for this reason the sub-standard was not sent out. Hence it appears that the various observers' eyes differ so greatly in this sort of comparison work that further effort with the N-A colorimeter for flour and bread color measurement has been suspended and the collaborative machines have been called in.

The measurement of flour color by reflectance has much merit, since it closely follows what the miller or baker sees on the Pekar test or finished bread. The problem of evaluation of the various factors is somewhat difficult because of the ever-changing values during aging and drying. It is probable that samples held at the same moisture content (assuming no color change due to enzymatic action) could be measured reproducibly.

Some work was done to study the use of the photoelectric cell upon dry flours but to date differences found have been too small to record accurately, for while the brightness as a whole may be measurable, there seems to be no simple means of evaluating the various shades of color other than by the use of light filters.

RECOMMENDATIONS[1]

It is recommended—

(1) That further collaborative work on measurement of flour and bread color by the N-A colorimeter be dropped.

(2) That study be continued on the sampling and preparation of flour and bread for color measurements by the photoelectric cell method based on reflectance.

REPORT ON SOYBEAN FLOUR IN FOODS

By J. W. HAYWARD (Archer-Daniels-Midland Co.,
Minneapolis, Minn.), *Associate Referee*

Nitrogen-Free Extract Method.—In this laboratory several successful attempts have been made to determine the quantity of soybean flour in sausage by a nitrogen-free extract method, which consists of analyzing the sausage for moisture, protein, fat, fiber, and ash; calculating the nitrogen-free extract by difference; and multiplying the result by a factor to give the soybean flour content of the sausage.

The underlying principle necessary to the success of this proposed procedure is based on the assumption that there is little or no nitrogen-free

[1] For report of Subcommittee D and action by the Association, see *This Journal*, 22, 68 (1939).

extract present in meat (liver products being the exception), while the nitrogen-free extract of soybean flour is quite constant, running about 30 per cent.

NITROGEN-FREE EXTRACT METHOD

(All references given are to *Methods of Analysis, A.O.A.C.*, 1935.)
Amount of sample.........................500 grams preferred
Preparation of sample.....................Chap. XXVIII, sec. **1(c)**, p. 353.
Analysis of ground fresh sample:
  Moisture..............................Chap. XXVII, sec. **2**, p. 353.
  Protein...............................Chap. XXVIII, sec. **3(b)**, p. 353.
  Fat...................................Chap. XXVIII, sec. **6**, p. 354.
    Regrind for complete extraction.
Analysis of dried extracted sample, Chap. XXVIII, sec. **1(c)**, p. 353:
  Moisture..............................Chap. XXVII, sec. **2'** p. 335.
  Protein...............................Chap. II, sec. **19**, p. 23.
  Fat...................................Chap. XXVIII, sec. **6**, p. 354.
  Fiber (crude).........................Chap. XXVII, sec. **25**, p. 340.
  Ash...................................Chap. XXVII, sec. **8**, p. 336.

Calculate the fiber and ash found in dried extracted material to the moisture and fat basis of the freshly ground sausage, using the following formula:

$$\frac{100-(\%\text{ moisture in meat }+\ \%\text{ fat in meat})}{100-(\%\text{ moisture in extract }+\ \%\text{ fat in extract})}\times\%\text{ fiber in extract or }\%\text{ ash in}$$

extract = per cent fiber or ash in original material.

Calculate nitrogen-free extract, using actual and calculated values for freshly ground sample.

Express the nitrogen-free extract in terms of soybean flour by using the factor 100/30 as follows:

Nitrogen-free extract $\times$100/30 = amount of soybean flour added to sausage.

It is not necessary to make a protein determination on the extracted residue except to have a check on the analysis of the original meat. The calculated result should check the analysis of the original material by 0.2 per cent.

Immunological Method by John H. Glynn, M. D., of Armour Laboratories, Chicago, Ill.—The following account is taken from a preliminary report submitted by the Armour laboratories.

We have recently obtained accurate quantitative results by the use of an immunological method that is both simple and rapid. The method is based on a quantitative precipitin test, the "optimal proportions" reaction, first described by Dean and Webb[1] and subsequently proved by Taylor, Adair, and Adair[2] to be well within the limits of accuracy of the best known chemical methods.

The test depends on the fact that in any antigen-antibody titration system the velocity of the reaction is related to the proportion of antigen to antibody. Thus, for a given antibody, precipitation is most rapid when the ratio of antigen to antibody is at an optimum that can be readily

[1] *J. Path. Bact.*, **29**, 473 (1926).
[2] *J. Hyg.*, **32**, 340 (1932).

determined. This optimum ratio is a constant for each antibody solution and is independent of the concentrations of either antigen or antibody in any specific test.

For example, suppose a given antigen reacts most rapidly with a given antibody at a ratio of 1 to 50; that is, one part of antigen forms a precipitate with 50 parts of antibody at a faster rate than with 45 or 55 parts of antibody. In fact, any ratio other than 1 to 50 will be slower than this optimum. Then, since this optimum ratio is a constant, the actual concentration of reagents may vary within fairly wide limits. It may be 3 to 150, or 10 to 500, or 25 to 1250.

It is a simple matter to standardize any particular antibody against a known antigen in terms of optimal ratio. If this ratio is used the concentration of antigen in any unknown mixture can be determined.

In the specific instance of quantitative assay of soybean protein in sausage, the test is performed as follows:

Rabbits are immunized against a 5 per cent sodium chloride extract of soybean flour. For practical purposes, it is unnecessary to use purified glycinin. Several courses of injections over three or four months are usually necessary to produce a serum of satisfactory potency. The serum is collected and standardized against known soybean flour extract. Its optimal ratio is determined as accurately as possible. This ratio is then a constant for that particular serum.

Sausage containing soybean flour is extracted with 5 per cent sodium chloride. This unknown extract is then titrated against the standard serum and its ratio determined. By dividing the test ratio by the standard ratio the percentage of soybean protein in sausage is given.

For example, a standardized serum had a ratio of 1:30 against pure soybean flour extract. An extract of sausage gave a ratio of 1:3 with this serum. Therefore the sausage contained 10 per cent of soybean flour. The accuracy of the method is limited only by the care with which the test is performed; that is, the ability of the operator to distinguish the most rapidly precipitating tube in a rack of ten or twelve tubes. The specificity of the method is limited only by the phylogenetic relationship of the protein mixture under test, a well established immunological fact.

Details of the test will appear in a subsequent publication.

RECOMMENDATIONS[1]

It is recommended that work be continued on the determination of soybean flour in sausage and other foods following the proposed nitrogen-free extract and immunological methods.

No report on macaroni was given by the associate referee.

[1] For report of Subcommittee D and action by the Association, see *This Journal*, 22, 68 (1939).

REPORT ON WHOLE WHEAT FLOUR

By C. S. LADD (State Food Commissioner and Chemist, Bismarck, N. D.), *Associate Referee*

The determination of the whole wheat content of baked products is regularly made by calculation from the crude fiber content of the product. Such calculation is theoretically sound, but in the experience of the Associate Referee the results ordinarily obtained often show percentages not compatible with the actual formulas used in the manufacture of the products. It appears that the difficulties inherent in the crude fiber determination as applied to commercial feeding stuffs are multiplied, or at least are of more significance, in dealing with a product such as whole wheat flour.

The official crude fiber determination has always been open to several serious objections when applied to the determination of the actual fiber content of plant materials, *This Journal*, **2**, 32. Recent investigations indicate that although reproducible results on the same sort of materials are obtained with the standardized technic, they have but little significance when applied to different sorts of plant materials.[1,2] This is explained by the fact that this determination accounts for a variable proportion of the cellulose and lignin present, depending somewhat upon their mutual interference in the digestion.

Another factor influencing the fiber determination that is of recognized importance is the degree of fineness of the samples, *This Journal*, **5**, 56. According to Korfhage[3] fineness of more than 60 mesh gives inconsistent results. This may be of little importance in the analysis of feeding stuffs where the particle size can be selected in grinding the sample, but in the case of flours the results are open to this objection, and it is evident that a proportion of the sample is lost through the linen filter. Pickel[4] found that in the case of cacao products the loss is as high as 0.46 per cent of solids. Losses due to colloidal suspensions in the sodium hydroxide digesting solution have not been measured, and perhaps are insignificant. Various attempts to avoid loss of sample through the filter, such as the modification of Sweeny,[5] et al.,[6,7] (use of a 3.5 per cent sodium hydroxide solution for the caustic digestion and omission of the filtration at the midpoint) were moderately successful. However, this method consistently gives high results compared with those obtained by the regular A.O.A.C. procedure, *This Journal*, **2**, 132; **3**, 256; **4**, 39, with one exception[8] and for this reason has been discounted.

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[1] *Science Progress*, **30**, 702 (1936).
[2] *J. Agri. Science*, **25**, 529–40 (1935).
[3] Minn. State Dairy and Food Com., Div., Feed Insp. Ann. Rpt., **4**, 32–8 (1922).
[4] *J. Ind. Eng. Chem.*, **2**, 280 (1910).
[5] U. S. Dept. Agr. Bur. Chem. Bull., **137**, p. 157.
[6] *J. Ind. Eng. Chem.*, **4**, 600 (1912).
[7] Landw. Jahrb. Schweiz., **48**, 909 (1934).
[8] *J. Am. Assoc. Cereal Chem.*, **7**, 208 (1922).

Although the regular official procedure is recommended by the Association for the investigation of bread and baked products, *This Journal*, **16**, 518; **17**, 404, in cases where the percentage of whole wheat flour in a product is calculated from the crude fiber content a slight variation in the result may give rise to a large error in the estimation of whole wheat content.

To obviate the difficulties inherent in the present method some of the newer methods proposed for actual cellulose determination were investigated.

The method of Kurschner and Hanak[9] was selected for the first experimentation.

EXPERIMENTAL

Samples were obtained of two grades of whole wheat flour, designated here as "Whole Wheat Medium Grind" and "Whole Wheat Fine Grind." Bread containing some whole wheat flour of known composition was also obtained, as well as samples of the flours used in its manufacture. For purposes of comparison regular analyses by the official methods were made first. The results, expressed as per cent, follow:

	Moisture	Protein	Fat	Crude Fiber	Ash
1. Whole Wheat Flour Medium	11.92	16.68	1.87	2.38	1.65
2. Whole Wheat Flour Fine	12.52	16.78	1.93	2.29	1.60
3. Bread (⅓ whole wheat)	36.57	10.84	1.37	0.84	2.06
4. Whole Wheat Flour	9.94	17.78	1.85	2.48	1.61
5. Straight Flour	11.62	14.66	0.96	0.31	0.49

Calculated to a dry basis the crude fibers found above are: (1) 2.70, (2) 2.62, (3) 1.32, (4) 2.75, and (5) 0.35 per cent.

When the Sweeney modification of the crude fiber method was used, the results obtained compared with those obtained by the official method as follows:

			per cent		
1. Whole Wheat Flour Medium	2.40	2.35			Av. 2.38[1]
	2.49	3.27	2.83	2.84	
	3.06	2.90	2.93	3.32	
	3.42	2.84	2.70	3.69	Av. 3.02[2]
2. Whole Wheat Flour Fine	2.32	2.25			Av. 2.29
	2.51	3.00	2.87	2.60	
	2.64	2.55	2.62	2.54	
	2.73	3.02	2.83	2.76	Av. 2.72

[1] A.O.A.C. method.
[2] Sweeney modification and moisture and fat extracted by official short method.

These results support the findings of the investigators mentioned previously in that they average considerably higher than do those by the official method and also lack consistency.

[9] *Z. Untersuch. Lebensm.*, 59, 484 (1930).

The same samples were used with the Kurschner-Hanak[9] method, which follows:

The defatted sample is intimately mixed with 60 cc. of 80% AcOH and 1.5 cc. of HNO_3 (d. 1.4) in a flask fitted with a ground-in air condenser. The mixture is gently refluxed for 25 minutes and then filtered through a tared Gooch or alundum crucible previously wetted with the acid mixture. The residue is washed successively with 7–10 cc. of the acid mixture, hot water, alcohol, ether, 1–2 cc. of the acid mixture, and finally with hot water sufficient to remove all traces of the acid, the flask being rinsed with each washing. The residue is dried at 105° and weighed.

According to the authors this method gives most consistent results and extracts pure cellulose.

The following results were obtained:

	Cellulose (*per cent*)				
1. Whole Wheat Flour Medium					
Moisture and fat extracted by short official method	2.08	2.34	2.20	2.38	Av. 2.25
Fat not extracted	2.19	2.21	2.23	Av. 2.21	
2. Whole Wheat Flour Fine					
Moisture and fat extracted by short official method	1.81	2.21	2.10	Av. 2.04	
Fat not extracted	2.16	2.28		Av. 2.22	
3. Bread (½ whole wheat, moisture reduced to 6.62%)					
Moisture and fat extracted by short official method	1.64	1.55	1.52		
	1.46	1.49	1.55		
	1.55			Av. 1.54	
		dry basis		Av. 1.65	
Moisture and fat extracted by regular A.O.A.C. method	1.29	1.22	1.85		
	1.55	1.81	1.71		
	1.81	1.68	2.06		
	2.15	2.15	1.65		
	1.50	1.74	1.50		
	1.88	1.65		Av. 1.75	
		dry basis		Av. 1.87	
4. Whole Wheat Flour					
Moisture and fat extracted by short official method	2.14	2.45	2.13		
	2.15			Av. 2.13	
		dry basis		Av. 2.37	
Moisture and fat extracted by regular A.O.A.C. method	2.28	2.25	2.56		
	2.56	2.65	2.27		
	2.52	2.43	2.67		
	2.45	2.53	2.77		
	2.88	2.67	2.71	Av. 2.59	
		dry basis		Av. 2.88	
5. Straight Flour					
Moisture and fat extracted by short official method	0.32	0.54	0.30	Av. 0.39	
		dry basis		Av. 0.44	
Moisture and fat extracted by regular A.O.A.C. method	0.24	0.34	0.47		
	0.50	0.65	0.49		
	0.16	0.23	0.55		
	0.70	0.92	0.73	Av. 0.58	
		dry basis		Av. 0.66	

When the determinations were made without extraction of moisture and fat the following results were obtained:

			per cent	
Sample 3 (Bread)	2.15	2.06	Av. 2.11	dry basis 2.26
Sample 4 (Whole Wheat Flour)	2.24	2.84	Av. 2.59	dry basis 2.88
Sample 5 (Straight Flour)	0.85	0.83	Av. 0.84	dry basis 0.95

It was naturally expected that the samples that had not been defatted would not give reasonable results.

It is apparent that too wide a spread obtains between determinations. However, all results are reported, and undoubtedly lack of experience with the technic of the determination was responsible for some of the differences. It also seems possible that some high results may be due to insufficient washing of the residue in the alundum crucible or to the fact that that part of the material soluble in the acid mixture was being precipitated in the pores of the crucible. To avoid such difficulty the cellulose was filtered on an asbestos pad in a Gooch crucible. The results follow:

			per cent	
Sample 3 (Bread)	0.96	1.09	Av. 1.02	dry basis Av. 1.09
	0.96	1.13		
Sample 4 (Whole Wheat Flour)	2.12	2.05	Av. 2.07	dry basis Av. 2.30
	2.05	2.07		
Sample 5 (Straight Flour)	0.09	0.12	Av. 0.10	dry basis Av. 0.11
	0.10	0.10		

Mixtures of the two flours gave the following results:

	per cent			per cent	
¼ Whole Wheat Flour	0.61	0.62	¾ Whole Wheat Flour	1.63	1.64
¾ Straight Flour	0.61	0.61	¼ Straight Flour	1.64	
Av.		0.61	Av.		1.64
dry basis		0.69	dry basis		1.83

These percentages are in agreement with values calculated from the percentages immediately preceding. The agreement between results also seems satisfactory although the total number of analyses made by the Kurschner-Hanak method is not large enough to justify definite conclusions.

CONCLUSION

The results obtained indicate that it may be possible to substitute a cellulose determination for the official crude fiber method in the investigation of the whole wheat content of whole wheat products and thereby save considerable time. Determinations made with the Gooch filter gave more consistent results than those made with an alundum crucible. Further work will be necessary to establish reliability of the method although it does show promise at present.

It is recommended[1] that the Associate Referee continue his studies on

[1] For report of Subcommittee D and action by the Association, see *This Journal*, 22, 68 (1939).

methods of determining cellulose as an index of the whole wheat flour content of cereal foods.

ACKNOWLEDGMENT

Acknowledgment is made by the Associate Referee of the analytical work contained in this report by E. J. Hennessy and O. Maercklein, Assistant Chemists in the Associate Referee's laboratories.

REPORT ON PHOSPHATED FLOUR

By J. R. DAVIES (Calumet Baking Powder Division, General Foods Corp.), *Associate Referee*

The results of the work on phosphated flours carried on during 1938 confirm those of Gustafson,[1] Shuey,[2] and Barackman and Vaupel.[3] The method of Gustafson for the determination of the ash of the original flour is based upon removal of added inorganic ingredients by digestion in and sedimentation from carbon tetrachloride.

The Associate Referee found the Gustafson method to be excellent for the removal of added salts (monocalcium phosphate, sodium bicarbonate, and sodium chloride) from flours, except in the case of self-rising flours that had been exposed to humid conditions. In this case sufficient moisture is absorbed to cause formation of "shot-falls" of high sodium chloride content. These balls collect in the flour mass on the surface of the carbon tetrachloride and give high ash values. The difference between fresh and aged self-rising flours is shown in the percentage of ash due to the sodium chloride content. Analyses for the sodium chloride content of the fresh and aged self-rising flours show differences in the same range as found in the ashes from the same. Grinding the flour to pass several different sized screens before extraction with carbon tetrachloride does not eliminate the salt effect. In fact, grinding the flour in a mortar and pestle gives slightly higher values; namely, 0.732 per cent ash in the unground, and 0.839 per cent ash in the ground extracted flour. Analyses of these ashes for sodium chloride content show substantially this difference.

The ashes of flour extracted with carbon tetrachloride are generally slightly lower than those of the control flour. This error was reported by Gustafson,[1] Shuey,[2] and Barackman and Vaupel.[3] The Associate Referee's results show 0.358 per cent ash on the control flour and 0.342 per cent ash on the extracted control flour.

Results of experiments indicate that two extractions of the phosphated and the self-rising flours with 50 cc. portions of carbon tetrachloride in a

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[1] *Cereal Chem.*, **8**, 475–81 (1931).
[2] *Ibid.*, **12**, 289–93 (1935).
[3] *Ibid.*, 486–93.

250 cc. Squibb separator give separations of the added salts that are in close agreement with results obtained by centrifuging as in the Gustafson method. A comparison of the results of analyses for ash after extraction by (a) centrifuging, and (b) separator are shown as follows:

Ash of original flour after extraction by carbon tetrachloride—Reported on 15% moisture basis

	Collaborator A Centrifuge	Associate Referee Sedimentation
	per cent	*per cent*
Plain flour	0.343	0.343
Phosphated flour	0.356	0.353
Self-rising flour	0.354	0.351
Phosphated flour—aged	0.353	0.357
Self-rising flour—aged	0.724	0.732
Control flour (not extracted)	0.364	0.361

It is recommended[1] that further study be made of methods for determining the ash of the original flour in phosphated and self-rising flours, and especially of old self-rising flours, with special attention given to (a) analyses for sodium chloride content of self-rising flours and the ash of the extracted flours; and (b) the means other than extraction with carbon tetrachloride for 100 per cent separation of the sodium chloride from old self-rising flour.

[1] For report of Subcommittee D and action by the Association, see *This Journal*, 22, 68 (1939).

REPORT ON STANDARD SOLUTIONS

By R. L. VANDAVEER (U. S. Food and Drug Administration, New Orleans, La.), *Referee*

There are two methods by which the chemist determines the effective strength of a standard solution: (1) A substance of known strength and purity can be diluted to a definite volume; (2) the solution may also be titrated against a definite weight of a pure chemical compound (usually called a primary standard). To eliminate the possibility of dilution error, the determination of normality after preparation of the solution is preferable. Obviously, the standardization of a solution's concentration by both of the above general methods or by more than one primary standard substance should yield a result that is closer to the solution's absolute strength than if only a single method of determining the normality is used.

It is logical that in determining the strength of any one solution, the method chosen should only measure (1) the molecule or (2) the active part of the molecule which is involved in the chemical reaction during the determination. For example: In the standardization of sulfuric acid solution, aside from the analytical difficulties involved, many chemists prefer to measure the effective acid strength rather than determine the relatively inert SO_4 radical.

Thus it is believed desirable—whenever there is available more than one equally accurate direct method for determining the strength of the standard solution under study—not to give preference to one procedure, but to submit at least two to this Association for consideration.

RÉSUMÉ OF COLLABORATIVE EFFORT

The reported studies on standardization of acid solutions this year deal principally with methods for standardization of hydrochloric acid solutions by precipitation with silver, and by direct titration with both recrystallized borax and sodium carbonate as primary standards. Collaborative results reported indicate that the borax and sodium carbonate methods have considerable merit. Normalities obtained by the silver chloride method were disappointing, but rather expected, since no provision is made in this procedure for solubility.

Studies were made on iodine and thiosulfate solutions for the first time by an associate referee. By the two methods proposed for standardization of iodine solution, the associate referee obtained results that agree to within 1 part in 2000. The associate referee suggests further study on methods for thiosulfate, which suggestion is approved by the Referee.

Last year no recommendation was made for any official action on the tentative indirect method for standardization of hydrochloric acid solution, *Methods of Analysis, A.O.A.C.*, 1935, 682, since it was considered to be desirable to have a direct method available before this recommendation was submitted. The collaborative results on this hydrochloric acid solution were obtained by titration against sodium hydroxide solution. At the same time, the collaborators standardized the sodium hydroxide solution against pure acid potassium phthalate. The data obtained are reported in *This Journal*, **21**, 411. These results on both methods are in such good agreement as to warrant both being recommended as official.

In addition to the methods that have been studied, there is throughout the present book of methods a host of other standard solutions in general use. All these methods and many others, should be considered by the Referee on Standard Solutions. Naturally, those of most importance deserve early consideration. Next year, in addition to acidimetry and iodometry, the Referee proposes to have studies conducted in the field of argentometry and permanganometry.

RECOMMENDATIONS[1]

It is recommended—

(1) That the methods for the preparation and standardization of solutions of sodium hydroxide, *Methods of Analysis, A.O.A.C.*, 1935, 681, be adopted as official (first action).

(2) That the method for the preparation and standardization of hydrochloric acid solutions, *Ibid.*, 682, be adopted as official (first action).

(3) That the method submitted by the Referee for the standardization of acid solutions with sodium carbonate be adopted as tentative.

(4) That the method submitted by the Referee for the standardization of acid solutions with borax be adopted as tentative.

(5) That studies on the standardization of hydrochloric acid solutions by the silver chloride procedure, *Ibid.*, 23, be discontinued.

(6) That the preparation and standardization of sulfuric acid solutions be studied.

(7) That the methods submitted by the associate referee for the standardization of iodine solutions be made tentative.

(8) That studies on methods for the standardization of thiosulfate be continued.

(9) That the preparation and standardization of potassium permanganate solutions be studied.

(10) That the preparation and standardization of silver nitrate and thiocyanate solutions be studied.

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[1] For report of Subcommittee A and action by the Association, see *This Journal*, **22**, 49 (1939).

REPORT ON STANDARDIZATION OF ACIDIMETRIC SOLUTIONS

By R. L. VANDAVEER (U. S. Food and Drug Administration, New Orleans, La.), *Referee*

Of all the well-known procedures for standardizing acid solutions reported in the literature, the determination of the acid titer of a solution by means of borax ($Na_2B_4O_7.10H_2O$)[1] and of sodium carbonate (Na_2CO_3)[2] as primary standards appears to be foremost. These substances can be prepared pure, and they qualify in other respects as primary standard substances for acidimetry. If an accurate barometer is available, an accurate standard solution of hydrochloric acid may be prepared from the well-known constant boiling acid procedure. The determination of normality by precipitation and weighing as silver chloride also deserves consideration. Consequently, collaborative work (constant boiling HCl excepted), was limited to the above-mentioned methods.

To each collaborator was submitted a portion of a standard solution of hydrochloric acid prepared by the Referee from constant boiling HCl to be exactly 0.1 *N* at 20° C. The procedure recommended by Foulk and Hollingsworth[3] in obtaining constant boiling acid was followed. This 0.1 *N* factor on the collaborative solution was also checked through standard sodium hydroxide against acid potassium phthalate, *Methods of Analysis, A.O.A.C.*, 1935, 681, independently, by S. Alfend and the Referee.

In Table 1 are reported the collaborative efforts on this solution; each normality value was corrected to 20° C.

COMMENTS BY COLLABORATORS

G. M. Johnson.—There is considerable variation between the normality as obtained by precipitation as silver chloride and by direct titration against sodium carbonate or borax. This may be due to improper condition of precipitation and disregard for the solubility of the silver chloride. The standardization with borax was the most satisfactory. A large weight and a sharp end point combined to give good check determinations.

A. Alfend.—I checked a portion of the acid against Bureau of Standards acid potassium phthalate and against sulfuric acid made up determinately, through sodium hydroxide solution. The indicated strength was 0.1000 in each case. As for the silver chloride, I have no explanation to offer, nor do I have any confidence in the method.

J. P. Aumer.—No difficulty was experienced with the anhydrous sodium carbonate or the borax standardizations.

Jonas Carol.—No trouble was encountered in these standardizations. However, the borax method is apparently much easier to use, as the methyl red end point is much more definite than that obtained by using methyl orange.

E. H. Berry.— " . . . Of the two, I prefer borax, although equally accurate re-

[1] F. Hurley, *Ind. Eng. Chem. Anal. Ed.*, **8**, 220 (1936); **9**, 237 (1937).
[2] G. F. Smith and Croad, *Ibid.*, 141–42; Kolthoff and Furman, Volumetric Analysis, Vol. II, 87–93 (1929).
[3] *J. Am. Chem. Soc.*, **45**, 1223 (1923).

TABLE 1.—*Collaborative results on 0.1 N HCl solution. (All normalities are corrected to 20° C.)*

COLLABORATOR	PROCEDURE		
	SILVER CHLORIDE	SODIUM CARBONATE	BORAX
G. M. Johnson	0.09944	0.1000	0.1003
Minneapolis	0.09944	0.1001	0.1002
	0.09932	0.1002	0.1003
	0.09998	0.1001	0.1003
	0.09993		0.1003
			0.1003
Av.	0.09962	0.1001	0.1003
S. Alfend	0.0995	0.1003	0.1003
St. Louis	0.0995	0.1003	0.1002
	0.0996	0.1003	0.1002
Av.	0.0995	0.1003	0.1002
J. P. Aumer	0.1001	0.1002	0.1002
New Orleans	0.1001	0.1002	0.1002
		0.1002	0.1003
Av.	0.1001	0.1002	0.1002
J. T. Field[a]	0.0998	0.1000	0.1001
St. Louis	0.0997	0.1000	0.1002
Av.	0.09975	0.1000	0.10015
A. E. Plumb[a]	0.0996	0.1003	0.1002
St. Louis		0.1005	0.1004
Av.	0.0996	0.1004	0.1003
J. Carol	0.1000	0.1001	0.1000
Cincinnati	0.0999	0.1000	0.1001
	0.1000	0.0999	0.1001
Av.	0.1000	0.1000	0.1001
E. H. Berry	0.0999	0.1001	0.1001
Chicago	0.0999	0.1002	0.1001
Av.	0.0999	0.10015	0.1001
R. L. Vandaveer	0.0998	0.1001	0.1001
Chicago	0.0997	0.1000	0.1000
	0.0999	0.1000	0.1000
			0.1000
Av.	0.0998	0.1000	0.1000

[a] Used portions from same bottle.

sults were obtained with sodium carbonate. I believe that more accurate results can be obtained with either the borax or sodium carbonate than with the present silver chloride method, and would like to see standardization of acid solutions using these salts made official.

Table 2 summarizes the collaborative results based on each analyst's average normality value.

TABLE 2.—*Summary of Table 1 data based on each average result*

		METHOD		
		SILVER CHLORIDE	SODIUM CARBONATE	BORAX
Range of Collaborative Results	Max.	0.1001	0.1004	0.1003
	Min.	0.0995	0.1000	0.1000
Average (Mean)		0.09978	0.10014	0.10017
Mean Error of Mean—(Dm)—		0.00007	0.00006	0.00004
corresponding to an accuracy of (%)		0.07	0.06	0.04
Av. Deviation		0.00017	0.0001	0.00008

I. Sodium Tetraborate

The commercial preparations of this salt are readily obtained pure, and the product may also be easily prepared pure by recrystallization from water. The salt neither loses nor gains moisture during weighing. The large equivalent weight, which practically eliminates weighing error for 0.1 *N* solutions, and the absence of indicator error at the equivalence point when methyl red indicator is used[1] make this compound an extremely useful primary standard for acidimetry. According to the experimental evidence accumulated by F. Hurley,[2] borax may be stored for lengthy periods without change, if placed in a desiccator over a solution saturated with respect to sucrose and salt. The Referee has kept a sample of borax under these conditions for six months with no measurable change in neutralizing power by checking the sample against a standard hydrochloric acid solution. This method of keeping borax in an atmosphere of proper humidity overcomes its tendency to lose some of its molecular water, which may occur if the salt is allowed to remain in dry air for more than five days. The method presented for the standardization of acid solutions was published in *This Journal*, **22**, 102.

II. Anhydrous Sodium Carbonate

Here, as with borax, in order to obtain a salt of utmost purity, sodium bicarbonate (or sodium carbonate) is subjected to recrystallization, ignition at 290° C., and test for possible impurities before use. The sodium carbonate so prepared is a satisfactory primary standard for acid solu-

[1] Kolthoff and Furman, Volumetric Analysis, Vol. **II**, **95** (1929).
[2] *Ind. Eng. Chem. Anal. Ed.*, **9**, 237–38 (1937).

tions with methyl orange indicator. R. A. Osborn[1] has noted that this salt, if not heated above 290° C., is not hydroscopic during the normal period of weighing in open air.

The temperature at which the salt is completely decomposed into normal carbonate has a definite bearing upon its ultimate composition. G. F. Smith and Croad[2] have experimentally demonstrated that above 300° C. the rate of decomposition of sodium carbonate is proportional to the temperature. No decomposition of the salt was found at 300° C.

The only disadvantage of sodium carbonate as a primary standard under normal conditions is that titrations must be performed in the acid range with methyl orange or methyl yellow. The substance may be titrated to phenolphthalein if the carbon dioxide is removed by boiling. (NOTE: The working titer of the solution standardized is accurate only with the indicator with which the normality value was obtained. The correction to be applied may be determined. See *Methods of Analysis, A.O.A.C.*, 1935, 682.

The method presented for the standardization of acid solutions with sodium carbonate was published in *This Journal*, **22**, 103.

III. Precipitation as AgCl[3]

This well-known procedure measures the acid strength indirectly; the chloride content is calculated to its acid equivalent. However, it was believed desirable to submit the procedure to collaborative study.

DISCUSSION OF RESULTS

In any set of collaborative results, given a sufficient number, there is likely to be some deviation from the true value. It becomes then a matter of deciding whether the results obtained are sufficiently close to the absolute concentration (if such is known) to determine the accuracy of a method. Also to be taken into consideration is the standardization error that might affect the practical result of a determination in which the standard solution may be used.

The results obtained by both borax and sodium carbonate are in excellent agreement; those by silver chloride are materially lower. The greatest range of normalities determined by a single collaborator, in the case of borax and sodium carbonate, is two parts in a thousand; this is in contrast with the range of results of 0.6 per cent when the silver chloride method, as reported by one collaborator, was used.

If it is assumed that the solution, which was prepared from constant boiling HCl to be 0.1000 *N*, is such, then the collaborator whose determination with borax is farthest away from this value would be in error of no more than 3 parts in a thousand. With sodium carbonate the

[1] Private communication.
[2] *Ind. Eng. Chem. Anal. Ed.*, **9**, 141 (1937).
[3] *Methods of Analysis, A.O.A.C.*, 1935, 23.

greatest deviation is 4 parts in a thousand; while with silver chloride the maximum difference from 0.1 *N* is 0.5 per cent low.

CONCLUSIONS

On the basis of the collaborators' results and their comments, and because the silver chloride procedure has certain inherent errors due principally to solubility of silver chloride,[1] it is concluded that further study of silver chloride as a method for standardization is unwarranted.

With respect to standardization by means of borax and sodium carbonate, it is believed that these two well-recognized procedures will fill A.O.A.C. requirements for accurate and direct methods of assaying the strength of acid solutions.

It is recommended[2]—

(1) That the method for the standardization of acid solutions with borax be adopted as tentative.

(2) That the method for the standardization of acid solutions with sodium carbonate be adopted as tentative.

(3) That studies on the standardization of hydrochloric acid solutions by silver chloride be discontinued.

REPORT ON IODINE AND THIOSULFATE SOLUTIONS

By KENNETH L. MILSTEAD (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

The work of the Associate Referee this year on the preparation and standardization of iodine and thiosulfate was concerned with a preliminary study of existing and well-known methods. The work was confined to—

(1) Preparation of standard iodine solution by direct weighing of purified iodine.
(2) Standardization of iodine solution against Bureau of Standards arsenious oxide.
(3) Standardization of thiosulfate solution against standard iodine solution.
(4) Standardization of thiosulfate against potassium iodate.

Preparation of Iodine Solution by Direct Weighing

A good grade of resublimed iodine was resublimed once from a mixture of potassium iodide and ignited lime and three times from ignited lime alone. A standard solution of iodine was prepared from the purified crystals as follows:

A large weighing bottle containing 40 grams of potassium iodide and 10 cc. of water was accurately weighed after the contents had come to room temperature. To the weighing bottle was added approximately

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[1] Morey, *J. Am. Chem. Soc.*, 34, 1039 (1912).
[2] For report of Subcommittee A and action by the Association, see *This Journal*, 22, 49 (1939).

the amount of purified iodine required for 1000 cc. of a 0.1 *N* solution; the bottle was stoppered again and accurately weighed. The contents were transferred quantitatively to a 1000 cc. flask and made to the mark with water at 20° C. The actual weight of iodine added was 14.4130 grams. The calculated normality of this solution at 20° C. was 0.11356.

Standardization of Iodine Solution Against Arsenious Oxide

A standard arsenite solution was prepared with Bureau of Standards sample No. 83 and the method outlined in the Certificate of Analysis accompanying the standard sample; 4.5012 grams of arsenic trioxide was made to a volume of 1000 cc. at 20° C. The calculated normality of this solution was 0.90998 at 20° C.

Accurately measured portions of the standard arsenite solution were titrated with the iodine solution prepared by direct weighing following the Bureau of Standards directions. (The starch indicator used was prepared by triturating 2 grams of soluble starch and 10 mg. of mercuric iodide with a little water and adding the suspension to 1 liter of boiling water. The boiling was continued until the solution was clear; 5 cc. of this starch solution was used for each 100 cc. of solution to be titrated.) The results are given in Table 1.

TABLE 1.—*Standardization of iodine solution against arsenious oxide*

ARSENITE SOLN	TEMP.	ARSENITE SOLN 20° C.	IODINE SOLN	TEMP.	IODINE SOLN 20° C.	NORMALITY IODINE SOLN 20° C.	AV. NORMALITY BY ARSENITE SOLN	DEVIATION FROM MEAN	DIFF. FROM VALUE BY DIRECT WEIGHING
cc.	*° C.*	*cc.*	*cc.*	*° C.*	*cc.*	*cc.*			*per cent*
50	31	49.87	40.05	31	39.95	0.11358	0.11357	0.009	0.02
50	31	49.87	40.06	31	39.96	0.11355		0.02	0.01
50	31	49.87	40.03	31	39.93	0.11363		0.06	0.07
50	31	49.87	40.04	31	39.94	0.11361		0.04	0.05
50	31	49.87	40.08	31	39.98	0.11350		0.07	0.06
50	30	49.88	40.06	30	39.97	0.11354		0.03	0.02
50	30	49.88	40.04	30	39.95	0.11361		0.04	0.05
Av. Deviation								0.04	

The data indicate that the standardization of iodine solution by means of direct weighing and by the use of National Bureau of Standards standard sample of arsenious oxide No. 83 should agree within one part in 2000.

Standardization of Thiosulfate Against Standard Iodine Solution

The thiosulfate solution was prepared by dissolving approximately 25 grams of sodium thiosulfate in 1 liter of freshly boiled and cooled distilled water; 0.1 gram of sodium carbonate was added, and the solution was allowed to stand for 1 week before standardization.

Accurately measured portions of the iodine solution prepared by direct weighing and standardized against arsenious oxide were transferred to Erlenmeyer flasks, 5 cc. of 1 *N* sulfuric acid was added and the solution was titrated with thiosulfate solutions. Five cc. of starch indicator was added near the end point. The results are given in Table 2.

TABLE 2.—*Standardization of thiosulfate solution against standard iodine solution*

IODINE SOLN	TEMP.	IODINE SOLN 20° C.	THIOSULFATE SOLN	TEMP.	THIOSULFATE SOLN	NORMALITY THIOSULFATE 20° C.
cc.	° *C.*	*cc.*	*cc.*	° *C.*	*cc.*	
50.0	31	49.87	48.31	31	48.18	0.11756
50.0	31	49.87	48.33	31	48.20	0.11750
50.0	31	49.87	48.30	31	48.17	0.11757
50.0	31	49.87	48.30	31	48.17	0.11757
Av. normality thiosulfate against standard iodine						0.11755

Standardization of Thiosulfate Against Potassium Iodate

In this study attention was directed to the use of analytical grade potassium iodate without purification as a primary standard for thiosulfate because if offers many advantages over other substances that have been recommended for this purpose. Some attention was also given to the purification of potassium iodate by recrystallization from water, since it is reported in the literature that this procedure yields a pure product.

At the completion of the work on potassium iodate the thiosulfate solution being standardized was again titrated against the standard iodine. No change in the normality of the thiosulfate as determined against iodine solution occurred during the course of the work on potassium iodate.

TABLE 3.—*Standardization of thiosulfate against potassium iodate*

IODATE SOLN	TEMP.	IODATE SOLN 20° C.	THIOSULFATE SOLN	TEMP.	THIOSULFATE SOLN 20° C.	NORMALITY THIOSULFATE SOLN 20° C.	AV. NORMALITY BY IODATE	DIFF. FROM VALUE BY IODINE SOLN
cc.	° *C.*	*cc.*	*cc.*	° *C.*	*cc.*			*per cent*
50.0	31	49.87	42.90	31	42.77	0.11729	0.11728	0.2
50.0	31	49.87	42.90	31	42.77	0.11729		0.2
50.0	31	49.87	42.92	31	42.79	0.11723		0.3
50.0	31	49.87	42.90	31	42.77	0.11729		0.2
50.0	31	49.87	42.90	31	42.77	0.11729		0.2

Analytical grade of potassium iodate was dried at 180° C.; 3.5882 grams of the dried salt, weighed from a weighing bottle by difference, was transferred to a 1 liter volumetric flask and made to the mark with

water at 20° C. The calculated normality of this solution was 0.10059 at 20° C.

Accurately measured portions of this solution were transferred to Erlenmeyer flasks, 2 grams of potassium iodide (iodate-free) and 10 cc. of 1 *N* sulfuric acid were added, and the liberated iodine was titrated with thiosulfate. Five cc. of starch solution was added near the end point. The results are recorded in Table 3.

The results in Table 3 show that the normality of thiosulfate determined against unpurified analytical grade potassium iodate does not agree with the normality determined against iodine.

It would appear that the potassium iodate contains some impurity that liberates a larger amount of iodine than would be liberated by a corresponding amount of potassium iodate.

In an attempt to determine whether the discrepancy was due to an impurity in the iodate, a sample of the analytical grade was recrystallized from water twice and dried at 180° C.; 3.5025 grams of the purified and dried salt was dissolved in water and made to a liter at 20° C. The calculated normality of this solution was 0.09819 at 20° C. Accurately measured portions of this solution were titrated with the thiosulfate as previously described. The results are recorded in Table 4.

TABLE 4.—*Standardization of thiosulfate solution against purified potassium iodate*

IODATE SOLN	TEMP.	IODATE SOLN 20° C.	THIOSULFATE SOLN	TEMP.	THIOSULFATE 20° C.	NORMALITY THIOSULFATE 20° C.	DIFF. FROM VALUE BY IODINE
cc.	*° C.*	*cc.*	*cc.*	*° C.*	*cc.*		*per cent*
50.0	29.5	49.89	41.90	31	41.80	0.11720	0.3
50.0	29.5	49.89	41.90	31	41.80	0.11720	0.3
50.0	29.5	49.89	41.90	31	41.80	0.11720	0.3
Av. normality, by purified iodate						0.11720	

The results in Table 4 indicate that the impurity present in the potassium iodate is not eliminated by recrystallization from water, but that the purified salt contains a higher concentration of the impurity than the unpurified. This impurity is probably sodium iodate.

CONCLUSIONS

The normality of an iodine solution prepared by direct weighing of the purified iodine agrees closely with the normality determined by titration against Bureau of Standards arsenious oxide.

Analytical grade of potassium iodate is not a satisfactory primary standard for the standarization of thiosulfate, nor does recrystallization twice from water improve its quality for this purpose.

RECOMMENDATIONS[1]

It is recommended—

(1) That the method outlined in the certificate accompanying Bureau of Standards standard sample No. 83 of arsenic trioxide for the standardization of 0.1 *N* solution of iodine be made tentative with the view to official adoption.

(2) That the standardization of thiosulfate be studied further.

The method follows:

IODINE

REAGENTS

(a) *Arsenic trioxide.*—U. S. Bureau of Standards sample. Dry 1 hour at 105° C. immediately before using.

(b) *Starch indicator.*—Triturate 2 grams of soluble starch and 10 mg. of HgI_2 (preservative) with a little H_2O and add the suspension slowly to 1 liter of boiling H_2O. Continue boiling until solution is clear. Use 5 cc. of this starch solution for each 100 cc. of solution to be titrated.

(c) *Arsenite solution.*—Approximately 0.1 N. Preferably weigh the sample by difference in a small weighing bottle owing to the difficulty of completely brushing As_2O_3 from metallic or glass surfaces. Accurately weigh a stoppered weighing bottle containing approximately 4.95 grams of As_2O_3. Transfer without loss to a graduated liter flask and again weigh the bottle. Do not attempt to brush out the adhering oxide. Moisten the sample with H_2O, add 15 grams of pure NaOH and 100 cc. of distilled H_2O. Swirl the contents of the flask gently until the As_2O_3 is in solution. Dilute to 250 cc. with H_2O and saturate the solution with CO_2, thus converting all the NaOH to $NaHCO_3$. Dilute to the mark, mix thoroly, and stopper the flask. A solution thus prepared will preserve its titer almost indefinitely. If the solution is made up on a volume basis make corrections afterwards for temperature changes.

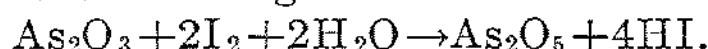
(d) *Iodine solution.*—Approximately 0.1*N*. Dissolve 12.7 grams of resublimed I and 20 grams of pure KI in 50 cc. of H_2O. When the I has dissolved transfer the solution to a glass-stoppered liter flask, dilute to mark with H_2O, mix thoroly, and stopper the flask.

STANDARDIZATION

Transfer an accurately measured portion (40–50 cc.) of the arsenite solution to a flask and titrate with the 0.1*N* iodine solution, using starch solution as indicator. To obtain accurate results it is absolutely necessary that the solution be saturated with CO_2 at the end of the titration. A current of CO_2 may be passed through the solution for a few minutes just before the end point is reached, or a few drops of HCl may be added to liberate sufficient CO_2 to saturate the solution. If the flask is stoppered immediately after the completion of the titration, the pink or rose-colored end point is stable for days.

From the quantities of I and arsenite solutions used calculate the titer of the I solution on the basis of the following relation:

$$As_2O_3 + 2I_2 + 2H_2O \rightarrow As_2O_5 + 4HI.$$



No report on insecticides, fungicides, and caustic poisons was given by the referee.

No report on fluorine compounds was given by the associate referee.

[1] For report of Subcommittee A and action by the Association, see *This Journal*, 22, 49 (1939).

REPORT ON PYRETHRUM PRODUCTS, DERRIS, AND CUBE

By J. J. T. GRAHAM (Food and Drug Administration, U. S. Department of Agriculture, Washington, D. C.), *Associate Referee*

During 1938 this Associate Referee gave his attention to methods for the analysis of pyrethrum products, derris, and cube powder.

DERRIS AND CUBE POWDER

The collaborators were asked to determine the rotenone and total ether extract. For the rotenone determination Method 1, the chloroform extraction or crystallization method adopted last year as tentative, *This Journal*, 21, 148, and Method 2, the Jones titration method,[1] were used.

METHOD 2, TITRATION METHOD

Extract the root sample and crystallize the rotenone from CCl_4 at 0° C. as described by Jones and Graham.[2] Filter and wash the precipitate as usual, and without further drying dissolve it in about 25 cc. of acetone in a 250 cc. flask. (This is readily accomplished by placing the crucible in a funnel and washing the contents through into the flask with small quantities of acetone.) Evaporate the solvent on the steam bath and treat the residue with 10 cc. of 80% (by volume) dichloroacetic acid, warming gently until the residue is just dissolved. Cool the solution for a few minutes in a bath of ice water. Add 10 cc. of cold water slowly while swirling the flask. Add a few seed crystals of rotenone-dichloroacetic acid solvate and again cool the flask in ice water 2 or 3 minutes. (Separation of a few small needle crystals will usually be noted at this point.) If no crystals separate, add water a drop or two at a time with intermittent cooling until a few crystals are seen, then add water 10–15 drops at a time with about 1 minute cooling periods between additions until 25 cc. has been added. Add 25 cc. additional water dropwise and again cool the solution. Finally add 50 cc. more water at a faster rate and again cool the solution. Filter the material through a Gooch crucible fitted with a disk of filter paper and wash with about 250 cc. of water in small portions. (It is well to remove the crucible from the holder after several washings and wash the outside of the crucible and the rubber holder and then replace the crucible and dissolve the contents in 25 cc. of $CHCl_3$.) If preferred, the crucible and contents may be placed in a beaker, the $CHCl_3$ added to dissolve the contents, and the crucible left in the beaker during the titration. Add 50 cc. of freshly boiled water and titrate the mixture with 0.1 N alkali, using phenolphthalein or bromothymol blue as indicator. Thoroughly agitate the mixture, particularly near the end point, to insure that all the acid is extracted from the $CHCl_3$ layer.

Each cc. of 0.1 N alkali is equivalent to 39.4 mg. of rotenone. Make the usual allowances, mentioned in the method cited, for added rotenone and for solubility of rotenone in CCl_4 and run a blank on the $CHCl_3$ used.

The results of the collaborators are given in Table 1.

DISCUSSION OF METHODS

Method 1, the tentative chloroform extraction method, gave good results on both the derris and cube samples.

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[1] *Ind. Eng. Chem. Anal. Ed.*, 10, 684 (1938).
[2] *Ibid.*, 19; *This Journal*, 21, 148 (1938).

TABLE 1.—*Collaborative results on derris and cube powder*

ANALYST	SAMPLE 1 ROTENONE METHOD 1	SAMPLE 1 ROTENONE METHOD 2	SAMPLE 1 ETHER EXTRACT	SAMPLE 2 ROTENONE METHOD 1	SAMPLE 2 ROTENONE METHOD 2	SAMPLE 2 ETHER EXTRACT
			per cent			
Analyst "A"	4.3		14.1	5.4		22.4
Sacramento	4.3			5.5		
Average	4.3			5.5		
Analyst "B"	4.3		14.0	5.4		22.0
Sacramento						
H. Bois		4.9	13.8		6.0	21.1
San Francisco		5.1	13.7		6.3	21.2
Average		5.0	13.8		6.2	21.2
C. G. Donovan	4.4			5.2		
Washington	4.4			5.2		
	4.4			5.4		
Average	4.4			5.3		
—. —. Feelemeyer	3.8		13.9	5.0		21.3
Baltimore	3.9		13.7	5.0		21.7
			14.0			21.8
			14.0			21.5
Average	3.9		13.9	5.0		21.6
J. J. T. Graham	4.6	4.0	14.5	5.7	5.3	23.3
	4.5	4.1		5.8	5.3	
	4.5	4.4		5.8	5.3	
		4.3		5.6	5.5	
		4.5				
Average	4.5	4.3		5.7	5.4	
D. G. Hoyer	4.4	4.7	13.8	5.5		21.4
New York	4.2	5.3	13.8	5.4		21.4
	4.5		13.8	5.7		21.4
Average	4.4	5.0	13.8	5.5		21.4
H. A. Jones	4.3			5.3		
Washington	4.6			5.2		
Average	4.5			5.3		
R. D. Stanley	4.1		14.1	5.1		22.2
Chicago	3.9		14.1	5.1		
Average	4.0		14.1	5.1		
A. Wolf	4.4			5.4		
Washington	4.7			5.4		
Average	4.6			5.4		

Method 2 is a new method developed by H. A. Jones. It is based on a sound principle and has given good results in the hands of Jones and his co-workers but several of the collaborating analysts had difficulty with it. One analyst commented that it appeared difficult, if not impossible, to wash out excess acid from such a sticky, gummy mass as that obtained in preparing the dichloracetic acid derivative. Another analyst reported that he obtained one or two results that checked with the crystallization method, but that subsequent assays gave results with no consistency in any direction. The rotenone acetic acid solvate forms small needle crystals that filter readily when properly precipitated. Apparently these analysts did not obtain the rotenone in this form. It is important that sufficient time be allowed between the additions of water during the crystallization to allow the crystals to form, otherwise the rotenone and impurities will be thrown down as an amorphous mass and incorrect results obtained. The method should receive further study.

PYRETHRUM PRODUCTS

Two samples were prepared for collaborative testing of the methods for the determination of pyrethrins. Sample 5 was a good grade of commercial pyrethrum powder. Sample 4 was a mineral oil extract prepared by dissolving the residue from a petroleum ether extract in a highly refined mineral oil base. To this was added, by volume oil of birch 1.5 per cent, Optone (a commercial preparation of rotenone in essential oils for incorporation in fly sprays) 1 per cent, and Lethane 384, 3 per cent. This sample had a theoretical pyrethrin content of 0.053 per cent calculated from the Associate Referee's analysis of the mineral oil-pyrethrum extract used in its preparation. These samples were sent to the collaborators with the following directions for analysis:

Pyrethrum Powder

PYRETHRIN I

Method 1.—(Seil, *Soap*, **10**, May 1934, pp. 89, 91, 111.)

Extract 12.5 grams of flowers ground to 30 mesh, or finer, in a Soxhlet extractor for 7 hours with petroleum ether (boiling range 30–60°). Evaporate the ether on a water bath, heating no longer than necessary to remove all the solvent. Add to the residue 20 cc. of 0.5 *N* alcoholic NaOH solution and boil gently under a reflux condenser 1–2 hours. Transfer the alkaline alcoholic solution to a 600 cc. beaker, washing the flask with water. Add sufficient water to bring the volume of liquid in the beaker to 200 cc. Add a few glass beads or introduce a boiling tube and boil to remove alcohol, using care to avoid boiling over due to saponification. When the volume has been reduced to 150 cc., cool and transfer to a 250 cc. volumetric flask to which has been added 1 gram of filter-cel. Add 10 cc. of 10% $BaCl_2$ solution, make to volume, and thoroughly mix the solution by shaking. Filter through a fluted paper, transfer 200 cc. of the filtrate to a 500 cc. flask, add 5 cc. of H_2SO_4 (1+4), and distil, using a distillation trap and an efficient condenser, until the volume has been reduced to about 20–30 cc. Put a low flame under the flask and pass steam through until 300–350 cc. of distillate has been collected. (The flask

should rest upon a piece of asbestos board in which a hole has been cut of sufficient size to allow the contents to be heated without super heating the sides of the flask above the liquid.) Receive the distillate in a 500 cc. separatory funnel. At this point the separatory funnel will contain the mono-carboxylic acid and the distillation flask the di-carboxylic acid.

To the separatory funnel add 50 cc. of petroleum ether and shake thoroughly. After the liquids have separated, draw off the aqueous layer into a second separatory funnel and again extract with 50 cc. of petroleum ether and discard the aqueous layer. Wash the extracts in the two funnels successively with 10 cc. of water and discard the water after it has passed through the second funnel. Wash again with another 10 cc. of water and discard as before. Combine the petroleum ether extracts. Neutralize 15 cc. of water containing 1 or 2 drops of phenolphthalein indicator solution with 0.02 N NaOH solution, add to it the combined petroleum ether solutions, and titrate with 0.02N NaOH solution, shaking after each addition, until the aqueous layer is just pink. Each cc. of 0.02 N NaOH solution consumed is equal to 0.0066 gram of Pyrethrin I.

Method 2.—Proceed as directed under the mercury reduction method, *This Journal*, **21**, 78 (1938).

PYRETHRIN II

Method 1

Allow the residue from the steam distillation to cool and filter through a Gooch crucible, washing the flask with a little water. Make the clear filtrate alkaline with $NaHCO_3$ transfer to a separatory funnel, and wash twice with $CHCl_3$. Wash the $CHCl_3$ extracts successively through one wash water of 10–15 cc. and combine the aqueous solutions. Acidify with about 10 cc. of HCl and saturate with salt, adding very cautiously at first to avoid excessive ebullition of CO_2. Extract with 50 cc. of ether, shaking for about 1 minute. Draw off the aqueous layer into a second funnel and again extract with 50 cc. of ether. Continue the extractions in a third and a fourth funnel, using in each case 35 cc. of ether. Each time that the aqueous solutions or washings are drawn off from the separatory funnels use care to see that the droplets adhering to the walls are removed as completely as possible. Wash the four ether extracts successively with two 10 cc. portions of water and then combine the ether solutions. Tap off any water that separates and filter the ether solution into a 500 cc. Erlenmeyer flask. Evaporate the ether on a water bath and dry the residue at 100° for 10 minutes. Add 2 cc. of neutral alcohol, warm gently, add 20 cc. of water, and heat to dissolve the acid. If a residue remains undissolved, cool and filter through a Gooch crucible. Add 1 or 2 drops of phenolphthalein indicator solution and titrate with 0.02 N NaOH solution. One cc. of 0.02 N NaOH solution is equivalent to 0.00374 gram of Pyrethrin II.

Method 2

Filter the aqueous residue from the petroleum ether extraction in the determination of Pyrethrin I by Method 2 (mercury reduction), through a Gooch crucible. Concentrate the filtrate to about 50 cc., transfer to a separatory funnel and proceed as directed in Method 1, beginning with "make alkaline with $NaHCO_3$."

PYRETHRUM EXTRACTS IN MINERAL OIL

PYRETHRIN I

Method 1. (Seil method modified.)

Transfer 100 cc. of the ordinary household extract, or less quantity of a concentrate, into a 500 cc. flask. If a volume less than 100 cc. is used, make up to that

volume with a highly refined mineral oil of the type used for the base of ordinary household extracts. Distil with steam until about 500 cc. has passed over, which should remove the perfume. Do not put a flame under the flask containing the sample during this distillation. Discard the distillate and transfer the residue in the flask to a 500 cc. separatory funnel, rinsing the flask with small portions of kerosene. Drain off the aqueous layer into another separatory funnel and wash with 25 cc. of a highly refined mineral oil. (It may be necessary to add a little saturated salt solution to get a clean separation. It may also be necessary to wash with several portions of the mineral oil to get complete separation of the oil-soluble portion from the aqueous solution.)

Transfer the oil containing the pyrethrins to an Erlenmeyer flask, add 20 cc. (or more if necessary) of 0.5 *N* alcoholic NaOH solution, and reflux 1–2 hours on a hot plate, using a vertical condenser.

Transfer the alkaline solution to a 600–800 cc. beaker. Wash the flask with water, adding the washings to the solution. Add sufficient water to bring the aqueous layer to 200 cc. or more if additional alcoholic NaOH has been used, and boil gently until the aqueous layer is reduced to 150 cc. to remove alcohol. Keep a boiling rod or glass beads in the beaker to prevent bumping. Cool, transfer to a separatory funnel and draw off the oil-free portion of the lower layer into a 250 cc. volumetric flask. Wash out the beaker with small additional quantities of water, adding the washings to the separatory funnel, mixing the contents, and drawing off the separated aqueous layers between washings. Finally, if some emulsion still persists, add a few drops of 10% $BaCl_2$ solution to break the remaining emulsion. Do not shake after adding the $BaCl_2$ to the separatory-funnel, because the reversed emulsion that may be formed cannot readily be broken. Discard the kerosene. Add 1 gram of filter-cel to the volumetric flask, then add 10 cc. of $BaCl_2$ solution, and shake thoroughly until the solution is clear, adding more $BaCl_2$ solution if necessary. When the solid material is coagulated, fill the flask to the mark, mix, and filter the solution through a fluted paper. Transfer 200 cc. of the filtrate to the flask of a steam distillation apparatus, add 5 cc. of H_2SO_4 (1+4), and proceed as directed under "Pyrethrum Powder, Method 1," beginning with the steam distillation following the addition of the dilute H_2SO_4.

PYRETHRIN II

Method 1.—Proceed as directed under Pyrethrum Powder, Method 1.

Method 2.—Proceed as directed in the mercury reduction method, *This Journal*, **21**, 78.

The results of the collaborators are given in Table 2.

DISCUSSION OF METHODS

On the pyrethrum powder, Sample 5, both methods gave results that agree fairly closely in most cases, although some variations are too great. On the mineral oil-pyrethrum extract, Method 2 (mercury reduction) gave results for Pyrethrin I that were more consistent than those by Method 1, the Seil method. The results for Pyrethrin II on the mineral oil-pyrethrum extract show a rather wide variation probably due to incomplete removal of the esters in the preliminary steam distillation. The collaborative work is insufficient to justify any recommendation other than that the work on pyrethrum products be continued.

Ripert[1] suggested that mineral oil-pyrethrum extracts should have free

[1] *Ann. fals.*, **27**, 577 (1934).

TABLE 2.—*Collaborative results on pyrethrum products*

ANALYST	SAMPLE 4 METHOD 1 PYRETHRIN I	SAMPLE 4 METHOD 1 PYRETHRIN II	SAMPLE 4 METHOD 2 PYRETHRIN I	SAMPLE 5 METHOD 1 PYRETHRIN I	SAMPLE 5 METHOD 1 PYRETHRIN II	SAMPLE 5 METHOD 2 PYRETHRIN I	SAMPLE 5 METHOD 2 PYRETHRIN II
			per cent				
J. A. Batscha	.073			.33	.34		
New York	.071			.32	.35		
	.072			.30	.41		
				.29	.38		
Average	.072			.31	.37		
J. C. Bubb	.066			.37	.47		
New York	.076	.25		.33	.45		
Average	.071			.35	.46		
C. G. Donovan			.049	.26	.41	.25	.41
J. J. T. Graham	.055	.094	.055	.28	.47	.27	.43
	.051	.094	.055	.28	.50	.27	.46
			.054			.27	
			.056			.27	
Average	.053	.094	.055	.28	.49	.27	.45
D. G. Hoyer	.069	.37		.26	.40	.21	.39
	.067	.18		.26	.40	.21	.42
	.062	.10					
Average	.066	.22		.26	.40	.21	.41
A. Wolf			.061				
			.063				
Average			.062				
H. Bois	.052	.069	.048	.26	.37	.21	.39
	.050	.076	.049	.26	.39	.22	.37
Average	.051	.073	.049	.26	.38	.22	.38

acids removed with aqueous normal alkali before the saponification with alcoholic alkali. Martin[1] applied this procedure to the petroleum ether solution in the analysis of pyrethrum flowers. Alvin J. Cox of the California Department of Agriculture has suggested the use of 50 per cent aqueous sodium hydroxide for extraction of acidic substances from commercial mineral oil-pyrethrum insecticides.

Wilcoxon[2] and also Pantsios[3] have shown that there is a heavy loss of

[1] *J. Agr. Sci.*, **28**, 456 (1938).
[2] *Contr. Boyce Thompson Inst.*, **8**, 175 (1936).
[3] *Ind. Eng. Chem. Anal. Ed.*, **10**, 386 (1935).

Pyrethrin I during the distillation of the acid solution in the Seil method. Martin investigated this loss and found that it depends largely upon the quantity of excess sulfuric acid present. He also found that by neutralizing the solution and then adding 1 cc. of normal acid in excess the results obtained check those obtained by the Wilcoxon mercury reduction method.

RECOMMENDATIONS[1]

It is recommended—

(1) That the study of methods for the analysis of derris and cube products be continued, and that special attention be given to the chloroform extraction and the Jones titration methods.

(2) That the study of methods of analysis of pyrethrum products be continued, with special attention given to the effect of a preliminary treatment for the removal of acidic substances, and also to a study of the effect of varying quantities of excess sulfuric acid on the decomposition of the pyrethrins during the distillation.

No report on naphthalene in poultry lice products was given by the associate referee.

No report on disinfectants was given by the referee.

REPORT ON SUGARS AND SUGAR PRODUCTS

By RICHARD F. JACKSON (National Bureau of Standards, Washington, D. C.), *Referee*

The Referee has studied in detail the methods of analysis of sugar products and is impressed by the care and assiduous attention to detail that have been exercised by previous referees and associate referees. The methods are well presented and in general reflect fairly present knowledge of the subject. Some comments on current problems suggest themselves.

For the analysis of reducing sugars it is probable that in spite of the great number of modifications that have been described most analyses are made by Munson and Walker's method. While many modifications are more rapid and convenient, there are but few that approach it in respect to precision. The comprehensiveness of the tables and the fact that the personal equation is not an important factor have added to its advantages. In view of its prevalence it has seemed important to verify the copper-sugar equivalents. Such an investigation has been completed by Dr. L. D. Hammond at the National Bureau of Standards and will be available for the next edition of *Methods of Analysis*. Dr. Hammond has determined reduced copper by electrolysis. A similar research has been

[1] For report of Subcommittee A and action by the Association, see *This Journal*, 22, 50 (1939).

partially completed by the Referee and Dr. Emma J. McDonald, who have determinationed copper by volumetric analysis.

An extension of Munson and Walker's table is highly to be recommended. There are two columns of copper-sugar equivalents adapted to sucrose-invert sugar mixtures in which 0.4 and 2.0 grams of total sugar, respectively, are taken for the analysis. These permit the determination of reducing sugars in mixtures in which a maximum of 60 per cent of the total sugar is reducing sugar. Above this maximum there are no tabulated data that are applicable, and the analyst is forced to the doubtful expedient of interpolating between pure invert sugar and the 0.4 gram total sugar column. Again at several intermediate ratios of invert sugar to total sugar, analysis is possible only by taking quantities of sample that reduce small weights of copper. Thus, of a mixture containing 13 per cent invert sugar and 87 per cent sucrose, a 0.4 gram sample containing 52 mg. of reducing sugar must be taken for analysis. This mixture can of course be analyzed, but with far less precision than a sample four times greater.

This situation will be partially rectified in Dr. Hammond's new table, but further extension of the principle is desirable. If a systematic series of experiments were made in which the sucrose were varied within very wide limits, it would be possible to construct tables or formulas for the evaluation of the effect of sucrose and thus increase greatly the flexibility of the method. Such a system would make it possible to replace the cumbersome Meissl and Hiller method by one based upon modern analyses. These investigations are in progress in the laboratories of the National Bureau of Standards.

The modern tendency has been to devise modifications of reducing sugar methods in which the entire analysis is completed in a single reaction vessel by volumetric procedure. Such a method is that of Lane and Eynon, in which the sugar solution is added to the point of complete reduction of the copper. In other modifications a portion of the copper is reduced under standard conditions and the reduced or unreduced copper determined by supplemental titration.

A method of this class was described by Shaffer and Hartmann, who suggested two modes of procedure, the one adapted to relatively large samples (200 mg. or less), the other to small samples (2 mg. or less). The latter has been extensively studied, particularly in its application to biological fluids. It has proved equally applicable to general chemical problems and the Referee believes it would serve a useful purpose in the analysis of materials with which this Association is concerned when it is necessary to analyze small samples. The macro method was not elaborated by Shaffer and Hartmann, but has been studied and modified by the Referee and Dr. McDonald. It is believed that both the Shaffer and Hartmann methods could advantageously be included.

The Clerget method, which in the present year has reached the ninetieth anniversary of its inception, is still beset with numerous problems, many of which are of a fundamental nature. In order that the method might rest upon a firm basis of accurately determined constants and correction coefficients, the Referee and Dr. McDonald have remeasured with care the basic values of the Clerget divisor under varied conditions of inversion, and the corrections to be applied for varied concentration and temperature. These experiments are described in a separate paper, which follows.

THE BASIC VALUES OF THE CLERGET DIVISOR AND THE CORRECTION COEFFICIENTS

By RICHARD F. JACKSON and EMMA J. McDONALD
(National Bureau of Standards, Washington, D. C.)

The present discussion is concerned with the basic values of the Clerget divisor that obtain under the conditions of Herzfeld's modification of the Clerget method. In devising the modification Herzfeld measured the direct polarization in the usual manner by taking the normal weight of pure sucrose in 100 cc. and observing its rotation in a 200 mm. column at 20° C. This rotation is by definition 100° S. For the invert polarization he took the half-normal weight (13 grams) in 75 cc. of solution, added 5 cc. of hydrochloric acid (38 per cent, or sp.gr. 1.188), immersed the flask in a water bath, and within 2 to 3 minutes warmed the contents to 67°–70° C. The solution having attained the temperature of the bath, he kept it as near 69° C. as possible for another 5 minutes, when it was quickly cooled, made to a volume of 100 cc. at 20° C., and polarized at the same temperature. Being that of a half-normal solution, the reading was multiplied by 2. Under these conditions the invert polarization was found by Dammüller[1] to be −32.66 at 20° C.

Later investigators have invariably obtained higher values; thus Tolman[2] found −32.88; Steuerwald[3] −33.05, Walker[4] −32.78, and Jackson and Gillis[5] a computed value of −33.08.

In 1920 Herzfeld published a posthumous work of Schrefeld, whose experiments were made in 1912. Schrefeld followed Herzfeld's procedure but defined the conditions of hydrolysis more precisely by specifying that a thermometer be inserted in the solution, which was agitated in a water bath at 70° C. until in $2\frac{1}{2}$–$2\frac{3}{4}$ minutes it had reached a temperature of 67°. From this moment he allowed it to remain in the bath for exactly 5 minutes, during which time the temperature gradually rose to 69.5°,

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[1] *Z. Ver. deut. Zucker-Ind.*, 38, 699, 746 (1888).
[2] *U. S. Dept. Agr. Bur. Chem. Bull.*, 73, 73 (1903).
[3] *Int. Sugar J.*, 16, 82 (1914).
[4] *Sugar*, 17, No. 2, 47 (1915).
[5] *Sci. Pap. BS*, 16, 153 (1920), S375.

whereupon it was rapidly cooled to 20°, diluted to 100 cc., and polarized after standing for at least ½ hour. As a mean of seven concordant measurements Schrefeld found the invert polarization to be −33.00. This value has been verified by Browne and Gamble.[1] Zerban and his co-workers[2] found −32.97, and Spengler, Zablinsky, and Wolf[3] −33.02. In a single measurement the present writers found the value −32.99. The mean polarization, −33.00, must be considered a well established value.

Jackson and Gillis showed that under the conditions of hydrolysis an appreciable destruction of invert sugar occurred. The writers have therefore sought to determine what the rotation would be if such destruction were avoided by shortening the final period of heating from 5 minutes by 1-minute intervals until a maximum negative rotation was reached. This maximum was attained by a 2-minute period of heating after the temperature of the solution reached 67° C., the reading being −33.08.

In 1920 Jackson and Gillis[4] published the results of a careful series of measurements made with solutions inverted in a bath at 60° C. instead of 70° C., the purpose being to avoid the destruction of invert sugar. Under these conditions they found the value −33.25. In order to eliminate instrument errors, however, they made their measurements on solutions more concentrated than 13 grams of sucrose in 100 cc. by comparing their rotations with those of such standard quartz plates as were available, and were thus obliged to correct their observations for the excess concentration, using the prevailing coefficient 0.0676 per gram of sugar in excess of 13 grams. Recent measurements discussed later show that this coefficient is too low and that a recalculation of their results is necessary. The recalculated value becomes −33.18.

The Arrhenius equation—

$$k_{T_1} = k_{T_2}\, e^{\frac{Q}{R}\left(\frac{T_2 - T_1}{T_2 T_1}\right)},$$

in which k is the velocity constant at the absolute temperature, T, and Q/R the "activation energy," permits the calculation of the velocity constant at any desired temperature if the velocity at any one temperature and the constant, Q/R, are known. Jackson and Gillis[5] determined that in the presence of 0.7925 N hydrochloric acid, k_{20} was 0.002161 (common logs and minutes) and Q/R 13087.6. With the aid of this equation the writers calculated the time required for 99.99 per cent hydrolysis at 49° and at 35° C., respectively, and found for both temperatures the value −33.25 for twice the rotation of the half-normal solution.

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[1] *J. Ind. Eng. Chem.*, 13, 793 (1921).
[2] Unpublished report to International Commission for Uniform Methods of Sugar Analysis.
[3] *Z. Wirtschaftsgruppe Zuckerind.*, 86, 670 (1936).
[4] *Sci. Pap. BS*, 16, 125 (1920), S375.
[5] *Ibid.*, 132.

Many analysts advocate inversion at room temperature as a safe means of avoiding the decomposition of invert sugar. The Arrhenius equation enables the analyst to calculate the velocities of inversion and times required for 99.99 per cent completion of the reaction at the temperatures that may be expected in uncontrolled laboratories. These periods of time vary considerably with small changes of temperature, as is shown in Table 1.

TABLE 1.—*Time of hydrolysis of cane sugar at room temperature*

TEMPERATURE	k (COMMON LOG, MINUTES)	TIME FOR 99.99 PER CENT INVERSION	TEMPERATURE	k (COMMON LOG, MINUTES)	TIME FOR 99.99 PER CENT INVERSION
°C.		*hours*	°C.		*hours*
18	0.001590	41.9	26	0.005290	12.6
20	0.002161	30.8	28	0.007073	9.4
22	0.002924	22.8	30	0.009421	7.08
24	0.003941	16.9	32	0.012502	5.33

While the velocities of many reactions double themselves with a rise of 10° in temperature, the velocity of inversion of cane sugar increases more than fourfold between 20° and 30°. Thus room temperature is safe only if such variations in temperature as inevitably occur are known to the analyst and are considered in calculating the time required for complete hydrolysis. It appears from Table 1 that 24 hours is insufficient for hydrolysis at 20° and that 16 or 17 hours for overnight inversion at 30° is excessive and serious decomposition of invert sugar can result. Evidently room temperature inversion must be carried out with considerable discretion.

For room temperature inversion the value of the negative constituent of the Clerget divisor adopted by this Association is −33.20. The writers measured this constant with care by inverting the half-normal solution for the calculated time in a thermostat that maintained a constant temperature within a few hundredths of a degree and observing its rotation at 20° C. The mean value found was −33.29 for solutions inverted at 25° C.

In recapitulation, Table 2 shows the values obtained under the conditions described in the previous paragraphs. These conditions, in all determinations except the first, were so adjusted that no decomposition of invert sugar occurred after the completion of the inversion. It is evident that the rotation is definitely a function of the temperature at which the sugar is inverted and that invert sugar is attacked by acid during the course of the inversion. This destructive reaction is apparently not the same in kind as that of acid upon ordinary invert sugar, for in additional experiments not tabulated the value remained unchanged when the time of heating at 60° C. was prolonged to 13 minutes. Conceivably furanoid

fructose, which has a transitory existence, is attacked by the acid the more strongly the higher the temperature.

TABLE 2.—*Variation of the negative constituent of the Clerget divisor with varied conditions of inversion*

TEMPERATURE OF INVERSION	TIME	ROTATION×2 AT 20° C.
°C.	*minutes*	°S.
67–69	5.0	−33.00
67–69	2.0	−33.08
60	9.5	−33.18
49	38.0	−33.25
35	205.0	−33.25
25	17.2 hrs.	−33.29

CONCENTRATION COEFFICIENT OF THE CLERGET DIVISOR

The specific rotation of both dextrose and levulose varies with the concentration of sugar, and that of invert sugar likewise varies with concentration, as is shown by Gubbe's[1] equation, $[\alpha]_D^{20} = -19.447 - 0.06068p + 0.000221\, p^2$, in which p is the per cent invert sugar. Thus the basic values discussed previously are valid only for a concentration of 13 grams of inverted sucrose in 100 cc.

Herzfeld applied to the basic value the correction $0.0676\ (m-13)$, in which m is the weight of inverted sucrose in 100 cc. of the solution taken for the invert polarization. This value of the coefficient has remained in general use to the present day, although Steuerwald found the slightly higher value 0.0717, Herles 0.067, and Sazavsky 0.0677. Zerban in his reports to the International Commission for Uniform Methods of Sugar Analysis has repeatedly recommended that both the concentration and temperature coefficients be reinvestigated.

The solutions for the determination of the concentration coefficient were prepared by dissolving 65 grams of pure sucrose in 310 cc. of water, or 130 grams in 269.2 cc., the total volume being in each case 350 cc. To each of these was added 50 cc. of hydrochloric acid (d 20/4 = 1.1029), and the sugar was inverted in a thermostat at 20°, or at 23.7° C. for the period of time calculated by means of the Arrhenius equation. The solutions were made to a volume of 500 cc. at 20° C., carefully weighed, and polarized at 20° C. Aliquot portions were then transferred to 100 cc. flasks, weighed, and made to volume after addition of such quantities of acid that each contained 10 cc. per 100 cc. All these solutions were polarized at 20°C. By this procedure assurance was had that all variables such as those arising from the inversion reaction itself were eliminated, the only

[1] *Ber.*, 18, 2207 (1885)

variable left being that caused by dilution. All readings were calculated to 26 grams of sucrose. The rotations were found by graphic plot and by mathematical analysis to diminish linearly with concentration for the entire range of concentrations between 26 and about 5 grams of sucrose. The values obtained in four series of measurements, adjusted by the method of least squares, yielded, respectively, the equations

$$\begin{aligned} P' &= -(32.244+0.0783\,s) \\ &= -(32.245+0.0803\,s) \\ &= -(32.265+0.0794\,s) \\ &= -(32.259+0.0794\,s) \\ \text{Mean } P' &= -(32.253+0.07935\,s) \qquad (1) \end{aligned}$$

in which s equals the grams of sucrose. The coefficient, 0.07935, is surprisingly higher than hitherto reported.

At the lower concentrations of sugar the small errors of observation are multiplied many fold in the calculation to 26 grams of sucrose. Hence a complete series of measurements was made by dilution of an acidified stock solution containing but 7 grams of sucrose in 100 cc. A least-square adjustment yielded the equation—

$$P' = -(32.243+0.0834\,s). \qquad (2)$$

The slightly higher coefficient indicates that a slightly increased slope occurs at very low concentrations, but the difference is within the experimental error of measurement. A solution of equations (1) and (2) yields identical values for 5 grams of sucrose and a difference for 2 grams corresponding to a difference in observation of 0.001° S. (200 mm.). For practical purposes Equation (1) can be extended to all concentrations of sugar below 26 grams.

The concentration coefficients just described were measured on solutions that contained 10 cc. of 6.34 N hydrochloric acid in accordance with the specifications of the "acid" Clerget method. It is now of interest to determine similar coefficients of invert sugar solutions in the absence of acid, since such is the condition in the enzyme Clerget method.

Invert sugar solutions were prepared by weighing out equal weights of very pure dextrose and levulose, the methods of purification of which are described elsewhere. A stock solution (500 cc.) was prepared containing 67.3702 grams each of pure dextrose and levulose, corresponding to 128.0032 grams of sucrose. The sugars were dissolved and made to volume after the completion of the mutarotation. As in the previous experiments, aliquot portions were taken, weighed, and made to volume. The solutions were allowed to stand overnight in a thermostat at 20° C. before polarization. All measurements were calculated to 26 grams of sucrose. A least-square adjustment yielded the equation—

$$P' = -(30.994+0.08241\,s) \qquad (3)$$

the relation being linear between 26 and 7 grams of sucrose.

Two typical series are given in Table 3. If the formula for the acidified solution is solved for 13 grams of sucrose, the divisor becomes −33.29, in agreement with the direct determination described previously. If that for the synthetic invert sugar is similarly solved, the divisor becomes −32.07, which is but slightly lower than Paine and Balch's value, −32.11, for invertase inversion. The difference between −33.29 and −32.07, or 1.22°, is due to the presence of 10 cc. of acid. Jackson and Gillis by a somewhat different method found 1.25.

TABLE 3.—*Typical measurements of the concentration coefficient of invert sugar*

10 cc. 6.34 *N* HCl $P' = -(32.253 + 0.07935\ s)$			SYNTHETIC INVERT SUGAR $P' = -(30.994 + 0.08241\ s)$		
WEIGHT OF SUCROSE	P' FOUND	P' CALCULATED	WEIGHT OF SUCROSE	P' FOUND	P' CALCULATED
grams			*grams*		
26.000	34.33	34.33	25.6001	33.11	33.10
23.6201	34.13	34.14	23.2542	32.91	32.91
20.7796	33.93	33.91	20.4571	32.66	32.68
18.1639	33.68	33.70	17.8814	32.47	32.47
15.5841	33.51	33.50	15.3376	32.25	32.26
12.9891	33.29	33.29	12.7940	32.08	32.05
10.6115	33.13	33.11	10.1919	31.85	32.84
7.7942	32.84	32.89	7.6688	31.61	31.63
5.1782	32.71	32.68	—	—	—

It is now of interest to calculate the value of the concentration coefficient that Gubbe's specific rotation equation would yield. Since in this formula concentrations are expressed as percentages, the calculation of concentration by volume requires a knowledge of the densities of the respective solutions. These densities are not well established, but if two solutions are selected differing by only 1 per cent concentration, say 13 and 14 per cent, the analyst is dealing merely with differences in concentration and any errors in absolute magnitudes are largely eliminated. It is then necessary to convert from circular degrees of sodium light to saccharimeter degrees with white light, and the conversion factor, 34.615, determined directly by Zerban,[1] is available for this purpose. The calculation carried out in this manner yielded the value 0.083 between 13 and 14 grams of sucrose, in good agreement with the directly determined coefficient of the writers, 0.0824. Between 26 and 27 grams of sucrose Gubbe's coefficient becomes 0.0687, which is in less satisfactory agreement.

[1] *J. Am. Chem. Soc.*, 47, 1110 (1925)

Zerban[1] has calculated from analyses previously published[2] the coefficient for the range of 6.5 to 13 grams of sucrose, finding 0.0831, and between 13 and 26 grams, 0.0781.

Thus the available data indicate that the higher value of the concentration coefficient is more nearly correct than the value, 0.0676, in present use.

A few experiments, preliminary in nature, were made to determine the temperature coefficient of the Clerget divisor. The coefficient that has been in use since Clerget devised the method is 0.5 t, although some doubt in respect to its validity has been expressed, particularly when it is applied to crude products.

The invert sugar solutions used by the present writers were prepared in much the same manner as those for the concentration coefficient. From 500 cc. of a weighed solution containing 143.279 grams of sucrose that had been inverted with 50 cc. of hydrochloric acid (d 20/4 1.1029) at 20° C., aliquot portions were transferred to 100 cc. flasks and weighed. These portions were so selected that the final solutions contained four concentrations of sugar, each in duplicate. Sufficient additional acid was added so that each solution contained 10 cc. One set of the four solutions was made to volume and polarized at 20° C., the other at 25° or 30° C., and polarized in a 400 mm. column in a thermostated room at the same temperature. The most reliable set of observations was obtained in a series polarized at 20° C. and at 30° C. One writer observed a mean coefficient of 0.4960, the other of 0.4963. For an interval of 10° this would make the corrected divisor 0.04° higher than would the coefficient 0.5, or about 0.03 per cent. Additional measurements in both acid and neutral solutions are in progress.

In summary, the present investigation has tended to show (1) that the negative constituent of the Clerget divisor in acid solution is a function of the temperature of inversion, (2) that the value of the divisor for room temperature inversion should be 133.29 instead of 133.20 at 20° C., (3) that the Jackson-Gillis value of the divisor for inversion at 60° C. should be 133.18 instead of 133.25, (4) that the concentration coefficient in acid solution should be 0.0794 instead of 0.0676, (5) that the concentration coefficient in neutral solution should be 0.0824, and (6) as a preliminary conclusion, that the temperature coefficient, 0.5 t, is very nearly correct, at least for pure invert sugar.

No report on honey was given by the associate referee.

No report on maple products was given by the associate referee.

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[1] Private communication.
[2] *This Journal*, 8, 384 (1925).

REPORT ON DRYING, DENSIMETRIC, AND REFRACTOMETRIC METHODS

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

It is recommended*—

(1) That the International Scale of Refractive Indices of Sucrose Solutions at 20° C., 1936, be adopted as official (final action).

(2) That the International Temperature Correction Table, 1936, be adopted as official (final action).

(3) That the vacuum drying method for the determination of moisture in cane and beet raw and refined sugars be adopted as official (final action), *This Journal* 21, 89 (1938).

(4) That the direct drying method, *Methods of Analysis, A.O.A.C.*, 1935, 462, **2**, now official, be made a tentative method.

No report on polariscopic methods (general) was given by the associate referee.

No report on chemical methods for reducing sugars was given by the associate referee.

REPORT ON ACETYLMETHYLCARBINOL AND DIACETYL IN FOOD PRODUCTS

By JOHN B. WILSON (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

Since last year's report was submitted, the attention of the Associate Referee has been directed to an article by Hooft and Leeuw,[1] who show that both acetylmethylcarbinol and diacetyl occur in commercial bread. The method used in the analytical work was the familiar precipitation as nickel dimethylglyoxime and a special type of apparatus was used to prevent loss of diacetyl.

The new colorimetric method of Pien, Baisse, and Martin[2] mentioned in last year's report, *This Journal*, **21**, 427, has now become available to the Associate Referee since after considerable difficulty a quantity of the reagent, diaminobenzidene, has been obtained.

Another colorimetric procedure based on the formation of a ferric iron-dimethylglyoxime complex is now recommended by Prill and Hammer.[3]

During the year some experimental work on determination of diacetyl

* For report of Subcommittee D and action by the Association, see *This Journal*, 22, 64 (1939).
[1] *Cereal Chem.*, 12, 213 (1935).
[2] *Lait*, 17, 673 (1937).
[3] *Iowa State Coll. J. Science*, 12, 385 (1938).

was carried out, diacetylmonoxine and diacetyldioxime (dimethylglyoxime) being used as the source of diacetyl, but no conclusion has as yet been reached as to the best procedure for quantities of diacetyl greater than 10 mg.

It is intended during the coming year to make a comparative study of the two outstanding colorimetric methods mentioned above, both of which are highly recommended by those who have investigated them.

It is recommended that investigations on methods for determination of acetylmethylcarbinol and diacetyl be continued.

No report on unfermentable sugars in molasses was given by the associate referee.

No report on refractive indices of sugar solutions was given by the associate referee.

REPORT ON VINEGARS

By A. M. HENRY (U. S. Food and Drug Administration, Atlanta, Ga.), *Referee*

It is recommended*—

(1) That the method proposed by the Associate Referee for the determination of ash in vinegar, *This Journal*, **21**, 89, and adopted as official (first action) last year, be adopted as official (final action).

(2) That methods for the determination of total phosphoric acid be studied.

(3) That the official method for the determination of solids in vinegar be studied, especially with reference to its applicability to vinegars high in solids, such as malt vinegar.

(4) That methods for the detection of caramel in vinegar be continued.

(5) That methods for the determination of dilute acetic acid in vinegar be studied.

PRELIMINARY INVESTIGATION OF THE LICHTHARDT TEST FOR THE DETECTION OF CARAMEL IN CIDER AND DISTILLED VINEGARS

By FRANK E. COOK and HARVEY MILLER (Eighth Corps Area General Depot, Inspection Laboratory, Fort Sam Houston, Texas)

The Lichthardt test[1] was applied to various pure cider and distilled vinegars, to which had been added solutions of caramel from various

* For report of Subcommittee C and action by the Association, see *This Journal*, 22, 63 (1939).
[1] *J. Ind. Eng. Chem.*, 2, 389 (1910).

sources. The writers desire to thank Mr. Etter of the San Antonio Brewing Co., and Mr. Fetzer of the Union Starch and Refining Co., for the samples of caramel coloring which they so kindly furnished.

The Lichthardt test follows:

Dissolve 1 gram of tannic acid in 30 cc. of water. Add 0.75 gram of H_2SO_4 (sp.g. 1.84). (A precipitate formed on the first addition of H_2SO_4 will dissolve.) Make up the volume to 50 cc. with water, let stand 24 hours, and filter. The reagent keeps well. To 5 cc. of vinegar add 5 cc. of reagent. Heat the mixture gently until the precipitate at first formed is nearly dissolved. Set aside 12 hours (overnight). A brown precipitate adhering closely to the bottom of the tube indicates caramel, and a flocculent, non-adhering precipitate is to be ignored.

An initial examination of the Lichthardt tannic acid reagent showed that the volume produced by the formula would be insufficient for any series of tests, and also that it is difficult to weigh concentrated sulfuric acid but easy to measure it.

The following formula, which it is believed keeps the original characteristics of the reagent, was used:

Add 4 cc. of H_2SO_4 (sp.g. 1.84) to 9.8 grams of tannic acid dissolved in 294 cc. of water. Make to 490 cc. with water. (The H_2SO_4 was added from a 4 cc. pipet, and the pipet was washed to remove the remaining acid.)

The first tests showed that often no precipitate was formed on the addition of the reagent to the vinegar, whether it contained caramel or not. This raised the question of how much heat, if any, should be applied. Various degrees of heating were tried, as shown in Table 2. Except for the above noted changes, the method used was as originally outlined.

TABLE 1.—*Analyses of vinegars used*

TESTS	I	II	III	IV	V	VI	VII	VIII
Acidity (g./100 cc. as acetic)	5.16	6.18	4.74	4.00	5.07	4.05	4.40	4.05
Total Solids (g./100 cc.)	1.45	2.47	1.74		1.47	1.48	1.75	2.10
Ash (g./100 cc.)	0.28	0.32	0.28		0.27	0.47	0.31	0.29
Alkalinity of water-soluble ash $\frac{\text{cc. 0.1 N Acid}}{\text{100 cc.}}$	28.0	30.0	26.0		24.0	48.0	27.0	27.0
Qualitative Tests								
Sulfates	trace	trace	trace		trace	trace	trace	trace
Calcium	trace	trace	trace		trace	trace	trace	trace
Chlorides	none	none	none		none	present	none	none
Ppt. w/neutral Pb(AC)	O.K.	O.K.	O.K.		O.K.	slight	O.K.	O.K.

TABLE 2.—*Effect of varying conditions on caramels and vinegars*

KIND OF VINEGAR	CONDITION	SOURCE OF CARAMEL							
		0	1	2	3	4	5	6	7
I	a	neg. 3*	pos.	pos.	pos.	pos. 2			
		sl. ppt. 1							
	b	neg. 1	pos. 2		pos. 2	pos. 2			
	c		pos.		pos.	pos.			
	d	neg.	pos.		pos.	pos.			
		sl. ppt. 1							
	e-40	neg. 1	pos.		pos.	pos. 2	pos.	pos.	
		sl. ppt. 1							
	e-44	neg. 1	pos.		pos.	pos. 2	pos.	pos.	
		sl. ppt. 1							
	e-48	neg. 1	pos.		pos.	pos. 2	pos.	pos.	
						poor 2			
II	a	neg. 2	pos.	pos.	pos.	settling			
III	a	neg.	pos.	pos.	pos.	pos.			
IV	a	pos. 3	(Colored distilled vinegar)						
		sl. ppt. 3				poor 2			
V	a					settling			
		sl. ppt. 2							
	b		pos. 2		pos. 2	pos. 2			
	e-40	sl. ppt. 2			pos.	pos.	pos.	pos.	
	e-44	sl. ppt. 2			pos.	pos.	pos.	pos.	
	e-48	sl. ppt. 2			pos.	pos.	pos.	pos.	
VI	a	neg. 2				cloudy 2			
	b	neg. 3	pos. 3		pos. 3	pos. 3			
	c		pos.		pos.	cloudy			
	d		pos.		cloudy	cloudy			
	e-40	neg. 2	pos.		pos.	pos. 2	pos.	pos.	
		sl. ppt. 1							
	e-44	neg. 2	pos.		pos.	pos. 2	pos.	pos.	pos. 2
	e-48	neg. 2	pos.		pos.	pos. 2	pos.	pos.	
VII	a	neg. 2				cloudy			
	b	neg. 3	pos. 3		pos. 3	pos. 3			
	c		pos.		pos.	cloudy			
	d	neg.	pos.		pos.	cloudy			
	e-40	neg. 2	pos.		pos.	pos. 2	pos.	pos.	
	e-44	neg. 3	pos.		pos.	pos. 2	pos.	pos.	pos. 2
	e-48	neg. 2	pos.		pos.	pos. 2	pos.	pos.	
VIII	a	neg. 2				cloudy			
	b	neg. 3	pos. 3		pos. 3	pos. 3			
	c		pos.		pos.	cloudy			
	d		pos.		pos.	cloudy			
	e-40	neg. 2	pos.		pos.	pos. 2	pos.	pos.	
	e-44	neg. 3	pos.		pos.	pos. 2	pos.	pos.	pos. 2
	e-48	neg. 2	pos.		pos.	pos. 2	pos.	pos.	
Water	a	neg. 2			pos. 4	pos. 4			
& H(AC)	e-44	neg.							pos. 2

The A.O.A.C. modification of Amthor's test for caramel was made on vinegar No. V, as this vinegar gave a slight precipitate under all conditions when the Lichthardt test was applied. The results were unsatisfactory and inconclusive.

RESULTS

(1) Some caramels gave no immediate precipitate when the tannic acid reagent was added.

(2) Uncontrolled heating of the vinegars and caramel with the reagent gave unsatisfactory results.

(3) Temperatures of 50° C. and above interfere with the formation and settling of the precipitate.

(4) Temperatures up to 48° C. held for not over 4 minutes do not interfere with the formation and settling of the precipitate.

(5) Vinegars Nos. I and VI, after standing in the laboratory for 3 to 4 months, and being opened repeatedly, commenced to show slight precipitates with the tannic acid reagent, where previously they had shown none.

(6) Vinegars Nos. I and VI also showed a marked progressive darkening in color on standing through the 3–4 month period.

(7) Vinegar No. 5 showed a slight precipitate with the tannic acid reagent under all conditions of test.

(8) The precipitate termed "positive," when allowed to form unmolested while settling, would spread itself over the side walls and bottom of the test tube, but a slight twist of the tube would cause it to settle to the bottom.

(9) No precipitate was noted actually to adhere to the side walls or bottom of the tube.

(10) Very little difference was noted in the nature of the precipitate obtained from vinegars containing added caramel and those which had no caramel added.

DISCUSSION

The vinegars were the unsolicited samples that were "on hand" at

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* Source of caramel and designation:
0.—No caramel, 1.—Imitation maple flavoring, 2.—Fictitious vanilla extract, 3.—Imitation vanilla extract, 4.—Caramel malt coloring (from San Antonio Brewing Co.), 5.—Caramel Coloring (Drug Store), 6.—Caramel—Acid Proof—"Pennant" (Union Starch & Refining Co.), 7.—Laboratory-prepared caramel.
The caramels from the sources designated as 1, 2, and 3 were added to the vinegars in the proportion of 1 cc. of solution to 4 cc. of vinegar. The caramel concentrates designated as 4, 5, 6, and 7 were first diluted to approximately 1:200 with water and then added to the vinegar in the proportion to 1 cc. of diluted caramel to 4 cc. of vinegar.
Subscripts indicate number of tests run.
Negative (neg.) indicates no precipitate formed.
Positive (pos.) indicates more than slight precipitate.
Sl. ppt. indicates slight precipitate.
Conditions under which tests were run:
(a) Heated—no specific control (as no precipitate formed)
(b) Not heated—room temperature (30°–40° C.).
(c) Heated to 50° C., held for 5 minutes, removed, cooled in air.
(d) Heated to boiling, removed, cooled in air.
(e) Placed in water held at 40, 44, and 48° C., respectively, for 4 minutes, removed, cooled in air.

the time of this investigation. They were all, with the exception of No. IV (colored distilled), considered to be pure cider.

Vinegars I, II, III, and IV showed expected reactions under varying conditions, and were considered normal.

Vinegars V, VI, VII, and VIII failed to give precipitates under all conditions, and were, therefore, selected for the remaining tests because of their erratic behavior.

Vinegar I was considered representative of the first group and was also used in the remaining tests, however it also turned out to be erratic in that it finally gave precipitates when no caramel was present.

Vinegars I and VI were very light in color when received. On standing in the laboratory in the presence of (among other things) sunlight and air, a very marked progressive darkening occurred. As received, these vinegars gave no precipitate with Lichthardt's tannic acid reagent, but after standing 3–4 months both gave slight precipitates.

The original instructions directed that gentle heat be applied until the precipitate that forms is nearly dissolved. Caramel coloring No. 5 was the only caramel, or vinegar, that gave an immediate noticeable precipitate and to which the original instructions were applicable. An attempt was made to obtain selective precipitation through control of the heating. This was only partially successful as some vinegars gave a slight precipitate when no caramel was present.

Temperatures of 40–48° C. for 4 minutes did not inhibit precipitation, as the higher temperatures did. Although these temperatures were used for the majority of the tests, they showed no specific advantage over room temperature (30–40° C.)

The precipitate produced when caramel had been added to the vinegars was slightly less flocculent and less easily disturbed from the sides and bottom of the tube than the precipitate produced when there was no caramel present. There was, of course, less precipitate when no caramel was present.

CONCLUSIONS

1. The difficulty of this method lies in the differentiation between the types of precipitates obtained from pure vinegars and those from vinegars containing added caramel.

2. Some vinegars will develop bodies on standing (perhaps caramel-like), which will give a precipitate with Lichthardt's tannic acid reagent.

3. It may be the import of the original method to apply no heat to the vinegar and tannic acid reagent unless a precipitate is immediately formed.

4. The method shows sufficient promise to warrant further investigation into the conditions under which the precipitates formed can be readily differentiated.

No report on ash in vinegar was given by the associate referee.

REPORT ON FLAVORS AND NON-ALCOHOLIC BEVERAGES

By John B. Wilson (U. S. Food and Drug Administration, Washington, D. C.), *General Referee*

No collaborative work was done on this subject during the past year. However, at this meeting the Referee is presenting papers on a quick method for determination of coumarin in imitation vanilla, and a method for determination of beta-ionone, which are worthy of collaborative study. See *This Journal*, **22**, 378–96.

In reviewing the methods contained in the chapter on flavoring extracts in *Methods of Analysis, A.O.A.C.*, 1935, the thought occurred to the Referee that several of the methods in current use might be subjected to modern improvements to bring them up to date in regard to the type of apparatus used. For example, an automatic extractor might be applied to the gravimetric determination of vanillin and coumarin, and the spectrophotometer might be applied in the methods for the colorimetric determination of vanillin, aldehydes, citral, etc.

This year the associate referee appointed on the general subject of organic solvents has successfully investigated a chemical method for the determination of isopropyl alcohol. The Referee concurs in the associate referee's recommendation that the proposed method be further studied collaboratively.

RECOMMENDATIONS

It is recommended[1]—

(1) That the proposed chemical method for determination of isopropyl alcohol be studied further collaboratively.

(2) That the proposed chemical method for determination of isopropyl alcohol be applied to mixtures containing essential oils.

(3) That the photometric method for coumarin in imitation vanilla be subjected to collaborative study.

(4) That the proposed method for quantitative determination of beta-ionone be studied collaboratively.

(5) That the Referee study the advantages of the automatic extraction of vanillin and coumarin.

(6) That the Referee study the application of the photometer to the present colorimetric methods in the chapters on Flavors and Non-alcoholic Beverages.

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[1] For report of Subcommittee D and action by the Association, see *This Journal*, **22**, 67 (1939).

REPORT ON ORGANIC SOLVENTS IN FLAVORS

By R. D. STANLEY (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

A search was made of manufacturers' catalogs and booklets to determine what solvents are available and the uses for which they are recommended. Basing his conclusions partly on this search and eliminating those unlikely to appear in foods or drugs and those low-boiling products used principally for extraction with later elimination of the solvent, the Associate Referee decided that the most important solvents likely to be found in food or drug products are water soluble and include ethyl and isopropyl alcohol, acetone, glycerol, the glycols, and glycol-ethers. The water-soluble solvents are considered of first importance since the most general use of an organic solvent in food and drug products, and particularly in flavors, is to hold in solution with water those materials otherwise insoluble. Some also are of importance because of their poisonous nature, for example diethylene glycol, which was found in elixir sulfanilamide and which has also been used in some flavors.

Isopropyl alcohol has been found in flavors, in drugs, and in insecticides. There is no method for its estimation among the methods of the A.O.A.C., in the United States Pharmacopoeia or in the National Formulary. Therefore it was decided that methods for the determination of isopropyl alcohol should be studied.

Adams and Nicholls[1] determined isopropyl alcohol colorimetrically after oxidation to acetone; and Cassar[2] determined it in the presence of acetone by oxidation with dichromate and titration of the excess dichromate with sodium thiosulfate. Estimation by density and refractive index measurements has been recommended and the literature contains the tables of Lebo,[3] of Bennett and Garret,[4] and those recently compiled by Batsche and Reznek of the U. S. Food and Drug Administration, *This Journal*, **20**, 107.

An ideal method would provide for the estimation of isopropyl alcohol in the presence of any or all of those compounds that might be present in the distillate of the usual alcohol determination, and could include volatile water-soluble alcohols, aldehydes, and ketones. Density and refractometric procedures would not be applicable in such a mixture. Oxidation, wherein the isopropyl alcohol is oxidized to acetone, and aldehydes and primary alcohols to the corresponding acids, and determination of the acetone formed seems to be the method of choice. Such a method was devised by Stanley and Vandaveer[5] of the U. S. Food and Drug Ad-

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[1] *Analyst*, 54, 2 (1929).
[2] *Ind. Eng. Chem.*, 19, 1060 (1927).
[3] *J. Am. Chem. Soc.*, 43, 1006 (1921).
[4] *Perfumery and Essential Oil Record*, 16, 18 (1925
[5] Unpublished.

ministration in 1935, and was used by them in official work, but this method is not applicable if both acetone and isopropyl alcohol are present.

Experimental work this year was limited to isopropyl-alcohol, ethyl-alcohol, acetone, and water mixtures. The results are encouraging, and it is believed that the method proposed can be extended to commercial samples containing isopropyl alcohol if appropriate extraction and distillation procedures are supplied. Further work along this line is planned.

The method involves oxidation with dichromate, distillation, and determination of the acetone formed by the procedure given in U.S.P.XI for acetone. A qualitative test is made for acetone in the original sample, and if present it is removed with paraformaldehyde.[1] The methods used follow:

QUALITATIVE TEST FOR ACETONE

Distil a portion of the sample and collect the first 2 cc. Add 5 cc. of an alcoholic solution of o-nitrobenzaldehyde (5%), and 1 cc. of NaOH solution (10%). Mix, then shake with a small quantity of $CHCl_3$. A blue color in the $CHCl_3$ shows the presence of acetone.

ISOPROPYL ALCOHOL IN WATER MIXTURES

(Use foil-wrapped stoppers)

I. Acetone Present.—Place 1.5 grams of paraformaldehyde in a 200 cc. volumetric flask and add about 20 cc. of normal NaOH solution. Pipet an aliquot containing not over 0.8 gram of alcohol into the flask. (An approximation to the alcoholic content may be had by a specific gravity determination and reference to the ethyl alcohol tables.) Dilute the mixture with water to about 100 cc. Connect the flask to a reflux condenser and heat slowly on a hot plate just to boiling. Remove the hot plate, wash down the condenser, and when cool make to the mark and mix. Filter through a dry filter, if necessary pouring the solution back through the filter until a clear filtrate is obtained. Pipet 100 cc. into a 500 cc. Erlenmeyer flask, add 5 grams of $K_2Cr_2O_7$, and when most of the salt has dissolved, add 100 cc. of H_2SO_4 (1+3). Stopper the flask, swirl, and let stand 30 minutes. Add 100 cc. of $FeSO_4$ solution (25%). Connect the flask to a vertical condenser through a foam trap. Slowly distil about 100 cc. into a 500 cc. volumetric flask containing 200–300 cc. of cold water. Dilute to the mark, mix and pipet 25 cc. into a glass-stoppered flask containing 25 cc. of normal NaOH; add 50 cc. of standard 0.1*N* I_2 solution while swirling the flask. Allow to stand 15 minutes. Add 26 cc. of normal HCl and at once titrate the residual I_2 with standard 0.1*N* $Na_2S_2O_3$ solution, adding starch solution when the I_2 color is nearly discharged. Each cc. of 0.1*N* I_2 consumed in the reaction corresponds to 0.001001 gram of isopropyl alcohol ($CH_3CHOHCH_3$).

II. Acetone Absent.—Into a 500 cc. Erlenmeyer flask containing 50 cc. of approximately 2*N* $K_2Cr_2O_7$, pipet an aliquot containing not over 0.8 gram of alcohol. Dilute to about 100 cc. with H_2O and continue as directed previously, beginning with "Add 100 cc. of H_2SO_4 (1+3). . . ."

EXPERIMENTAL WORK

Anhydrous isopropyl alcohol was prepared by refluxing 500 cc. of reagent-grade isopropyl alcohol labeled 98% with 200 grams of lime for 1.5 hours. The alcohol was then distilled. Constants found were: Density

[1] *Analyst*, 58, 749 (1933).

(20/4) 0.7846, boiling point 82.2° C., refractive index (20° C.) 1.3774. The acetone was dried over calcium chloride and distilled. Reagent "absolute" ethyl was used.

The samples were prepared by weighing the alcohols and acetone in small glass-stoppered flasks and transferring with water to 100 cc. or 200 cc. volumetric flasks. Suitable aliquots were measured out for analysis, and the following results were obtained:

WEIGHED			FOUND ISOPROPYL ALCOHOL	RECOVERY
ISOPROPYL ALCOHOL	ETHYL ALCOHOL	ACETONE		
grams	*grams*	*grams*	*grams*	*per cent*
3.7474			3.7413	99.8
3.7474			3.7163	99.2
3.0877			3.1070	100.6
3.1533	3.1710		3.1740	100.6
3.1533	3.1710	0.8 cc.*	3.1460	99.8
4.5612	4.0953	4.4666	4.5680	100.1

* 0.8 cc. acetone added to aliquot taken for analysis.

It is recommended[1] that the method submitted be studied further and extended to include isopropyl alcohol in mixtures containing essential oils, and that the methods be submitted to collaborative study.

REPORT ON MEAT AND MEAT PRODUCTS

By R. H. Kerr (U. S. Bureau of Animal Industry, Washington, D. C.), *Referee*

A collaborative study was made of the method for nitrates in meat and meat food products, which was previously studied in 1937. Consideration was also given to the methods for coagulable nitrogen in meat and for copper in gelatin. The method for coagulable nitrogen has been criticized, in that directions for the use of the indicator are such as are likely to lead to interference when the filtrate is subsequently used for the determination of creatin. A clarifying change is recommended. The methods for copper and zinc in gelatin are not satisfactory for the quantities of those metals ordinarily occurring in commercial gelatin of good quality. Substitution of the methods used in the Bureau of Animal Industry laboratories for many years is recommended.

For the study of the nitrate method a mixture containing a known quantity of sodium nitrate was prepared and distributed to the collaborators, with instructions to mix thoroughly one gram of the mixture with 200 grams each of finely comminuted, uncooked, fresh meat, of

[1] For report of Subcommittee D and action by the Association, see *This Journal*, 22, 67 (1939).

smoked and cooked sausage, such as bologna or frankfurter style, and of canned corned beef, and to determine nitrates in each mixture in accordance with the method studied.

Reports were received from six collaborators, and the results are shown in the table.

Collaborative results

ANALYST BUREAU OF ANIMAL INDUSTRY	W. C. MCVEY, WASHINGTON	I. M. MYERS, OMAHA	W. B. FROMER, OMAHA	F. B. HILTY, CHICAGO	W. F. SCHROEDER CHICAGO	A. E. GRAHAM, SAN FRANCISCO
I. Test sample mixed with fresh meat						
$NaNO_3$, p.p.m.—						
Found in mix-	565	525	550	506	536	600
ture	580	525	550			530
Found in meat	30	28	26	trace	trace	53
	30	19	23			
Amount recov-	535	497	524	506	536	547
ered	530	506	527			477
II. Test sample mixed with bologna style sausage						
$NaNO_3$, p.p.m.—						
Found in mix-	890	743	796	532	552	795
ture	860	743	796			719
Found in sau-	360	240	240	30	30	267
sage	345	240	240			
Amount recov-	530	503	556	502	522	528
ered	515	503	556			452
III. Test sample mixed with canned corned beef						
$NaNO_3$, p.p.m.—						
Found in mix-	730	721	721	598	604	719
ture	720	728	721			682
Found in meat	180	228	228	75	80	227
	165	228	228			189
Amount recov-	550	493	493	523	524	492
ered	555	493	493			493

An analysis of the original mixture showed it to contain 10.2 per cent sodium nitrate. One gram added to 200 grams of meat was, therefore, equivalent to 510 p.p.m. in the mixture. The maximum quantity found by any analyst was 556 p.p.m., or 109 per cent of the amount added. The minimum quantity found was 452 p.p.m., corresponding to 90 per cent of the quantity added. Average of all determinations reported was 515 p.p.m., or 100.9 per cent of the quantity added. The variation in results includes all discrepancies due to possible lack of uniformity in the nitrate mixture distributed as well as imperfect mixing of the sample

with the meat, in addition to analytical discrepancies. The results appear to warrant adoption of the method as a tentative method in place of the phenolsulfonic acid method.

RECOMMENDATIONS[1]

It is recommended—

(1) That the method for nitrates studied be adopted as a tentative method. This method was published in *This Journal*, **22**, 82.

(2) That the phenoldisulfonic method, Chapter *XXVIII*, **14** and **15**, be dropped.

(3) That the words, "using the indicator outside the solution to avoid subsequent interference with the determination of creatin," under **31**, be added immediately following the words "neutralizing to phenolphthalein," in **28**, XXVIII.

(4) That paragraphs **64** and **65**, XXVIII, be dropped and the methods submitted by the Referee be substituted. (These methods were published in *This Journal*, **22**, 84.)

(5) That the words, "neutralize with 10 per cent NaOH solution," occurring in the last sentence of paragraph **18** be changed to read, "neutralize with NaOH solution (1+1)."

REPORT ON SPICES

ASSAY OF SAGE

By J. F. CLEVENGER (U. S. Food and Drug Administration, New York, N. Y.), *Referee*

Work was continued in accordance with the recommendations approved last year. W. H. Headley of the H. J. Heinz Company, Pittsburgh, Pa., Bernard J. Thiegs of the North Dakota Regulatory Department, Bismarck, N. D., and John Molitor of this Station collaborated.

Samples of coarsely ground Dalmatian sage stored in air-tight glass containers were given to the collaborators, who were directed to assay the sample by the method as outlined in *Method of Analysis, A.O.A.C.*, 1935, pp. 447–49.

Results of assay

	Clevenger	*Headley*	*Theigs*	*Molitor*
Volatile oil (cc. per 100 grams)	1.6	1.45	1.45	1.55
Specific gravity (25°/25°)	0.923	0.922	0.922	0.9225
Optical rotation*	+12.11	+11.7	+12.1	+13.6
Refractive index (20° C.)	1.464	1.464	1.461	1.462
Acid number	1.22	1.25	1.3	1.3
Ester number	16.24	16.45	16.8	24.3

* Angular degrees 25° C., 100 mm. tube, white light.

The results reported are considered satisfactory. Variations are prob-

[1] For report of Subcommittee C and action by the Association, see *This Journal*, 22, 62 (1939).

ably accounted for, in part, by the different periods of time elapsing between grinding and assaying the sage.

It is recommended that this method,[1] which is now tentative, be made official, first action.

REPORT ON BAKING POWDERS

CREAM OF TARTAR AND TARTARIC ACID IN TARTATE BAKING POWDERS

By B. G. HARTMANN (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

The methods for the determination of cream of tartar and tartaric acid in tartrate baking powders, described in *This Journal*, **13**, 386, were subjected to collaborative study.

Four members of Food Division, U. S. Food and Drug Administration, Washington, D. C., participated in the work: R. A. Osborn, V. E. Munsey, W. O. Winkler, and B. G. Hartmann. With the exception of the Referee, none of these analysts had had previous experience with the method.

The two samples submitted were prepared in the laboratory and had the following compositions: Sample No. 1 contained 44.9 per cent cream of tartar, and 5.5 per cent free tartaric acid; Sample No. 2 contained 59.86 per cent cream of tartar, and no free tartaric acid. The samples were carefully checked for tartaric acid, and the cream of tartar and tartaric acid used were purified by recrystallization.

METHODS

The texts of the methods submitted to the collaborators were changed from those given in the original paper. The revised methods were published in *This Journal*, **22**, 74.

The results follow:

Collaborative results

COLLABORATOR	SAMPLE 1			SAMPLE 2			SAMPLE 1	SAMPLE 2
	INDIRECT METHOD			INDIRECT METHOD			DIRECT METHOD	
	TOTAL TARTARIC (41.4)	CREAM OF TARTAR (44.9)	FREE TARTARIC (5.5)	TOTAL TARTARIC (47.9)	CREAM OF TARTAR (59.9)	FREE TARTARIC (0)	FREE TARTARIC (5.5)	FREE TARTARIC (0)
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	41.5	46.0	5.1 4.5	46.8	58.1	0.5	5.3	0.1
2	41.5	44.6	5.9	47.1	57.9	0.9	5.4	0.2
3	42.1	45.9	5.5	48.3	58.4	1.6	5.3	0.0
4	41.4	43.9	6.4	47.6	58.1	1.2	5.4	0.3

[1] For report of Subcommittee C and action by the Association, see *This Journal*, 22, 63 (1939).

DISCUSSION OF RESULTS

The method for the determination of cream of tartar and total tartaric acid is satisfactory. The indirect method for free tartaric acid is not reliable, particularly when applied to powders containing no free tartaric acid, and therefore its use for determining this constituent cannot be recommended. The direct method for free tartaric acid is accurate. An error of −3.5 per cent on a mixture containing approximately 50 per cent of cream of tartar cannot be considered serious.

No adverse criticisms were received from any one of the collaborators; the general opinion was that the methods are simple and rapid.

It is recommended[1] that the methods here discussed be adopted as official, first action.

No report on fish and other marine products was given by the referee.

The informal discussion by Fred Hillig on volatile acids as an approach to the evaluation of spoilage in canned fish will not be published.

REPORT ON CACAO PRODUCTS

By W. O. Winkler (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

Study of the pectic acid method for shell was continued, and some work was done on the determination of milk protein.

PECTIC ACID METHOD FOR SHELL

It was indicated last year that the method for separating the gums from the pectin was not entirely satisfactory. A simple way of more effectively accomplishing this separation was found this year. The new procedure, which will take the place of the partial precipitation of the gums, proteins, etc. (which were extracted with the ammonium oxalate solution) with tannic acid is based upon the observation that these materials did not readily dissolve after dehydration on the steam bath. The new procedure following the precipitation of the pectins, etc., with 80 per cent acidified alcohol is as follows:

Dissolve, and with hot water from a wash bottle transfer the precipitate back to 800 cc. beaker from the centrifuge bottle. Avoid an excess of water. Add a few drops of phenolphthalein and then concentrated NH_4OH dropwise with stirring until the solution has a basic reaction. Stir if any undissolved gelatinous material remains. Add dropwise with stirring until the pink color disappears a 10% solution of citric acid and then add a few drops in excess. Evaporate the sample to about 20 cc. on a hot plate (to speed up evaporation) and then to dryness on a steam bath. Allow the beaker to remain on the bath at least 15 minutes after the sample appears

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[1] For report of Subcommittee C and action by the Association, see *This Journal*, 22, 74 (1939).

completely dry; remove from the steam bath and add 70 cc. of cold distilled water. Stir with a policeman to remove the material from the bottom of the beaker and let stand 15 minutes with frequent stirring.

Filter off the insoluble matter on a 7 cm. Büchner funnel in a Büchner flask (500 cc. or smaller), using a filter paper overlain with paper pulp about $\frac{1}{16}$–$\frac{1}{8}$ inch thick. Wash the beaker and filter with two or three 15 cc. portions of water, washing out any residue. Transfer the filtrate to a 800 cc. beaker and dilute to 190–200 cc.

Cool the solution below 25° C. and proceed with the saponification and the precipitation of the calcium pectate, etc.

Experiments with the procedure showed that commercial fruit pectins previously dissolved and precipitated redissolved completely after the dehydration treatment. The shell extract also dissolved almost completely, and the portions that did not dissolve were in all probability not pectin. On the other hand, a large portion of the material extracted from nibs and precipitated by alcohol did not redissolve after the treatment. Moreover, separations of the calcium precipitate and "pectic acid" precipitate, which follow, were clean and sharp, and filtered without clogging or gumming.

PECTIC ACID BY CALCIUM PECTATE AND ACIDIFICATION VS. A.O.A.C. PECTIC ACID

The fact that the material resulting from this method is the same as that from the A.O.A.C. procedure for pectic acid in fruits is shown by the analysis of two commercial citrus pectins. The results are given in Table 1.

TABLE 1.—*Pectic acid in commercial citrus pectins*

PECTIN NO.	SAMPLE	BY SHELL METHOD		BY A.O.A.C. METHOD	
	mg.	*mg.*	*per cent*	*mg.*	*per cent*
1	200	83.2	41.6	82.6	41.3
2	200	62.0	31.0	61.1	30.5

The results (Table 1) indicate that the methods determine the same thing, namely, di-galacturonic acid.

INDICATIONS OF SHELL DURING DETERMINATION

There is often an indication of the presence of shell in a chocolate sample at the beginning of the determination. Following the centrifuging in the extractions with ether and acid alcohol, there is usually a considerable amount of darker and coarser material at the bottom of the bottle. The higher layers of the cacao mass are lighter in color and finer in division. The darker and coarser material in samples containing appreciable quantities of shell is more pronounced and can usually be easily recognized by comparison with samples that are practically shell-free. This

fact is generally observed in authentic samples of liquor of known shell content and in commercial samples giving a high pectic acid figure.

This observation suggested the possibility of further increasing the sensitivity of the method, or any method, by separating the top layers of the sample and using the bottom ones for the determination. In the case of a sample containing only a small amount of shell in which the figure obtained was due primarily to the liquor itself, the figure obtained on the lower layers would not be materially increased. However, on a sample containing appreciable amounts of shell, the majority of this would be found in the lower layers and the figure for pectic acid on this portion would be correspondingly increased. A fairly good separation of the lower layers might possibly be effected by the use of the proper shaped centrifuge bottle or tube.

PECTIC ACID IN AUTHENTIC CACAO SAMPLES

The results of pectic acid determinations on a number of authentic chocolate liquors (commercially prepared) are given in Table 2. The shell content of the samples was ascertained by hand picking the nibs used in their manufacture and by the addition, to such liquors, of known quantities of shell.

TABLE 2.—*Pectic acid in chocolate liquors*

SAMPLE NO.	SHELL, HAND PICKED (fat-free basis)	SHELL ADDED (fat-free basis)	PECTIC ACID (fat-free dry basis)
	per cent	*per cent*	*per cent*
1	0.49	0	0.11
2	0.91	0	0.14
3	0.45		0.10
4	0.95	0	0.16
5	0.95	4.0	0.32
6	0.95	9.85	0.66
7	0.95	14.2	0.87
8		100	5.18, 4.92

The results in Table 2 show that the method will readily distinguish the samples containing varying amounts of shell. The same thing is indicated in the analysis of a number of commercial cocoas. The samples represent the varieties of cocoas on the local market. The results are given in Table 3, and the various kinds of cocoa are distinguished by number. The fat content was determined for basis of calculation and is also listed.

The results (Table 3) show plainly that one sample, No. 3, contained an amount of shell in excess of that consistent with good commercial practice. The figure obtained on this sample is almost three times as high as the other samples and evidently contains nearly 8.5 per cent of shell on the fat-free basis. Sample 8 was prepared by adding 0.5 gram of shell

to 6.0 grams of Sample 4. The figure of pectic acid on this sample is practically equal to that of No. 3. Moreover, the presence of a considerable amount of the darker and coarser material previously referred to was evident in this sample during centrifuging. The Referee is convinced of

TABLE 3.—*Fat and pectic acid in commercial cocoas*

NUMBER OR DESCRIPTION	FAT	PECTIC ACID (dry fat-free basis)
	per cent	*per cent*
1	21.93	0.15
2	15.49	0.18
3	15.21	0.54
4	11.99	0.18
5	12.36	0.17
6*	11.56	0.26
7	14.20	0.21
8—6 grams of No. 4 with 0.5 gram added shell	11.1	0.57
9—100% shell		4.50

* Probably contained more shell than is permitted in good factory practice.

its high shell content. The shell added to Sample 8 was that determined in No. 9. The fairly exact nature of the determination is shown by the fact that the calculated pectic acid in Sample 8 based on its ingredients is almost exactly 0.57, the figure found. The Referee believes that the method will give a close estimation of the shell content. From the results so far obtained, any sample that yields a greater figure than 0.25 per cent pectic acid is questionable. The Referee has considered making the method more sensitive by oxidizing the product obtained with chromic acid.

MILK PROTEIN IN MILK CHOCOLATE

Several analysts have complained of the length of the A.O.A.C. method for milk protein in milk chocolate and also in regard to the difficulty of the digestion, which is slow because of incessant foaming in the beginning. The Referee found that the milk protein was readily and easily dissolved by shaking the fat-free sample first with water, followed by 1 per cent sodium oxalate. The water dispersed or emulsified the milk protein, which immediately dissolved when the sodium oxalate was added. The following method appears to be both rapid and accurate:

Place 10 grams of milk chocolate in a centrifuge bottle (250 cc. or larger), and extract twice with about 100 cc. of ether by shaking until uniform, centrifuging, and decanting the supernatant ether layer each time. Place in the bottle a perforated stopper carrying a bent glass tube, and a straight glass tube that extends about one-third the way down into the bottle. Expel the ether by attaching the bent tube to the vacuum and drawing a moderate current of air through the bottle

while the latter is placed in a moderately warm (not hot) place. When the ether is expelled, measure 100 cc. of distilled water into the bottle with a bulb pipet. Stopper the bottle and shake vigorously for 3 minutes. Measure in with a pipet 100 cc. of 1% $Na_2C_2O_4$ solution. Stopper, and shake vigorously 2 minutes. Allow the bottle to stand about 10 minutes and again shake 1–2 minutes. Place the sample in the centrifuge and whirl for about 15 minutes at high speed (1800 r.p.m. if No. 1 type Sb is used).

Remove the bottle from the centrifuge and decant the supernatant liquid into a beaker. Pipet 100 cc. into a dry 250 cc. beaker and add 1 cc. of glacial acetic acid while stirring gently. Let the sample stand for a few minutes for the precipitate to partly separate and add 4 cc. of 10% tannic acid solution with stirring (solution should not be more than a week old). Allow the precipitate to separate and settle a few minutes, then filter on a Büchner funnel (7 cm. size), using moderate suction. Use as a filter a No. 589 white ribbon paper overlain with a medium layer of paper pulp prepared by shaking No. 1 Whatman filter paper with water. Transfer all the precipitate to the funnel with the aid of a policeman and a wash solution composed of 1% $Na_2C_2O_4$ to which 1 cc. of glacial acetic acid and 2 cc. of 10% tannic acid per 100 cc. have been added. Wash on the filter 1 or 2 times. Loosen the filter around the edge with a spatula. Carefully roll up and remove the filter and precipitate to a Kjeldahl flask. Transfer to the flask any particles of precipitate clinging to the funnel or spatula with small pieces of damp filter paper, add 20 cc. of H_2SO_4, 15 grams of Na_2SO_4 and 1 gram of catalyst (1 gram of Se to 5 grams of HgO). Digest after the solution clears, about $\frac{1}{2}$ to $\frac{3}{4}$ as long as the time required for the solution to become clear. (Digestion should then be complete.) Distil off the nitrogen by the usual A.O.A.C. method, except to use 50 cc. of a solution containing 300 grams of NaOH and 10 grams of $Na_2S_2O_3 \cdot 5H_2O$ in 500 cc. volume instead of the solution specified in this method. Multiply the nitrogen found by 6.38 to obtain the casein and albumin and this product by the factor 1.07 to obtain the total protein.

The factor 1.07 was arrived at after the analysis of several samples of skimmed milk powder for protein both by direct nitrogen determination and by the method given above.

When the method was applied to an authentic sample of milk chocolate of which the protein content was known, almost perfect recovery resulted. The calculated protein present was 5.60 per cent and that found by the above procedure was 5.62 per cent.

Analyses of a number of skimmed milk powders are given in Table 4.

TABLE 4.—*Protein in skimmed milk powder*

SAMPLE NO.	(1) BY DIRECT *N* DETERMINATION	(2) BY METHOD PRESENTED	FACTOR (COL. 1 ÷ COL. 2)
	per cent	*per cent*	
1	32.74	30.69	1.065
2	32.74	30.39	1.07
3	32.85	30.41	1.08
4	35.62	33.13	1.07
5	35.62	32.95	1.08

The results in Table 4 indicate that a rather constant ratio exists between the nitrogen determined on the sample directly and that deter-

mined by the method. The Referee believes that the factor is more constant than the one now used, namely, 1.25×casein, which is obtained by precipitation with acetic acid alone.

From experiments conducted the Referee is convinced that all protein in solution is precipitated by this method. Although nitrogen is still present in the solution after the precipitation, this nitrogen appears to be due to substances other than protein, such as inorganic salts, amino acids, creatin, creatinin, uric acid, and organic bases. No protein was precipitated from this solution by boiling or by phosphotungstic acid, saturated ammonium sulfate, magnesium sulfate, or other protein precipitants. It is therefore believed that the nitrogen present is non-protein nitrogen.

LECITHIN

No work was done on lecithin because of insufficient time and the fact that no associate referee was appointed. It is hoped that work can be done on this subject next year.

RECOMMENDATIONS[1]

It is recommended—

(1) That further collaborative work be done on the pectic acid method for shell.

(2) That collaborative work be done on the method for milk protein given in this report.

(3) That work be done on the determination of lecithin in cacao products.

REPORT ON GUMS IN FOOD PRODUCTS

MAYONNAISE AND FRENCH DRESSING

By F. Leslie Hart (U. S. Food and Drug Administration, Los Angeles, Calif.), *Referee*

Edible gums are being increasingly used in the manufacture of prepared foods. While in some cases they have a legitimate use, in many cases they serve to mask adulteration. Gums have been advertised as "permitting the addition of much more water to your food product than would otherwise be possible."

The field now includes not only the true gums, such as locust bean and tragacanth, but also gel-forming substances obtained from marine algae, such as agar-agar, Irish moss, and sodium alginate. These substances are all classified chemically as polysaccharides. For convenience they will all be designated "gums," and will be included in this study. Certain other

[1] For report of Subcommittee D and action by the Association, see *This Journal*, 22, 67 (1939).

TABLE 1.—*Collaborative results on salad dressings*

COLLABORATOR	PRODUCT	KIND AND AMOUNT OF GUM ADDED		CHARACTER OF ALCOHOL PRECIPITATE	RESULTS OF QUALITATIVE SUGAR TESTS			
					BENEDICT	MOLISCH	SELIWANOFF	TOLLEN
			per cent					
Doris H. Tilden U. S. Food & Drug Adm., San Francisco	Mayonnaise	Locust bean	0.2	white, opaque, partly stringy, partly granular	positive	positive	pink color	—
	French dressing	Karaya	0.2	finely flocculent, translucent	positive	positive	negative	amber
	Mayonnaise	Ghatti	0.2	flocculent, white translucent	positive sl. prec.	positive	pale pink	negative
	French dressing	Tragacanth	0.2	colorless, translucent gelatinous	positive	positive	negative	—
J. A. Kime U. S. Food & Drug Adm., Washington	Mayonnaise	Locust bean	0.15	heavy granular	positive	positive	negative	positive
	Mayonnaise	Karaya	0.15	immediate flocculent precipitate	—	positive	negative	negative
T. E. Strange U. S. Food & Drug Adm., Seattle	Mayonnaise	Ghatti	0.2	light, coherent translucent, colorless	positive	positive	pale pink	negative
	Mayonnaise	Mixture: locust bean and agar agar	0.15	white, granular, voluminous	positive	positive	positive	positive
F. Leslie Hart	Mayonnaise	Karaya	0.1	flocculent	—	—	sl. pos.	negative
	Mayonnaise	Karaya	0.2	flocculent	—	—	positive	negative
	Mayonnaise	Karaya, from diff. source	0.2	faint cloud, insufficient for further tests	negative	negative	negative	negative
	Mayonnaise	Agar-agar	0.2	blue-white jell	positive	positive	positive	positive
	Mayonnaise	Agar-agar	0.1	colorless jell	positive	positive	positive	doubtful
	French dressing	Locust bean	0.1	stringy, white	—	positive	positive	negative
	Mayonnaise	Ghatti	0.2	white, translucent	positive	positive	doubtful	positive
L. W. Ferris U. S. Food & Drug Adm., Buffalo	Mayonnaise[1]	Dextrin	0.2	granular precipitate	positive	—	negative	negative
	Mayonnaise	Locust bean	0.2	No comments	positive	positive	positive	—
	Mayonnaise	Karaya	0.15	No comments	sl. prec.	negative	negative	—

[1] Reddish-blue color with iodine.

substances, such as pectin, dextrin, and gelatin, used occasionally by food manufactuers as "thickeners," will not be considered at this time.

Methods for the detection of these gums are of interest to food chemists, particularly those engaged in regulatory activities. Due to the complexity of many prepared foods, and the varying nature of the gums themselves, it is impossible to devise one method for all products and conditions. Therefore, as methods are devised for the detection of gums in certain foods they will be reported to the Association.

Gums may be encountered in a variety of prepared foods. Among these are mayonnaise and French dressing, fruit spreads and jellies, sirups, cold-packed berries, bottlers' fruit concentrates, chocolate concentrates (powder or liquid) for dairy drinks, soft cheeses and cheese spreads, ice cream, whipping cream, catsup, prepared mustard, flavor emulsions, pudding powders, ice cream powders, and confectionery.

The work during the past year was confined to the detection of gums in mayonnaise and French dressing. A method published previously by the Referee in *This Journal*, **20**, 527, with certain modifications, was used for the work reported here. In the earlier work it was found that trichloracetic acid is the best reagent to use as a protein precipitant in separating egg or other proteins from gums. The method given herein specifies this reagent. It is the hope of the Referee that methods for the detection of gums in other food products, based on this separation, will be presented to the Association.

Relatively little work has been done on the detection of gums in food products. A bibliography on this subject, to 1937, is given in the contributed paper referred to above. Since then, a method for the detection of gums in dairy products, also specifying trichloracetic acid, was published by Racicot and Ferguson in *This Journal*, **21**, 110.

METHOD

Transfer 100 grams into a 250 cc. beaker, add 35–40 cc. of hot water, and mix thoroly. Heat to 65°–70° C. in a water bath, add 10 cc. of 50% trichloracetic acid solution in water, and maintain at 65°–70° C. until the emulsion shows signs of breaking (in no case over 10 minutes). Transfer the mixture to an 8 oz. nursing bottle, insert a pipet guard* and centrifuge 15–20 minutes at about 1200 r.p.m. (This should separate the mixture into a lower aqueous layer and an upper oily layer, with a layer of curd between. If separation does not occur, add 30–40 cc. of toluene, mix, and repeat the centrifuging.) By means of a pipet inserted through the pipet guard, remove as much of the aqueous layer as possible and filter it into a 600 cc. beaker. Add 5 volumes of alcohol and allow the mixture to stand overnight to precipitate the gums.

Decant or pipet off sufficient alcohol to leave not over 225 cc., transfer the con-

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* The device described by the writer in *This Journal*, **20**, 529, 2nd par., may be used as a pipet guard. In lieu of this a piece of glass tubing, 8 mm. inside diameter and about 7½" long, may be used. The tube should be flared at the upper end, and a small cork stopper fitted onto the lower end. This stopper should be of sufficient size so that it does not rise up into the tube when the nursing bottles are centrifuged. The tube is supported in the neck of the bottle by a slotted rubber stopper. After the material in the bottle is centrifuged the cork stopper is pushed through by means of a solid glass rod and the lower layer is removed by means of a pipet inserted through the pipet guard.

tents of the beaker to an 8 oz. nursing bottle, centrifuge until the gum settles to the bottom, and decant the supernatant alcohol as completely as possible. Dissolve the residue in not over 1.5 oz. of hot water, add 1 or 2 cc. of acetic acid, and reprecipitate by adding alcohol to the 8 oz. mark on the nursing bottle. Let stand overnight, or until the precipitate becomes flocculent, centrifuge at 1200 r.p.m., and decant the alcohol. A precipitate at this point indicates gums. This may be confirmed by the following procedure.

Add 35 cc. of hot water to the precipitate in the nursing bottle, transfer to a small beaker, add 5 cc. of concentrated HCl, and boil gently for 2 minutes to hydrolyze the gums to sugars. This solution may now be used for various qualitative tests for monosaccharide sugars, as follows:

1. Transfer 1 cc. of the hydrolyzed gum solution to a test tube, neutralize with approximately $2N$ NaOH solution, using litmus paper as a reagent, remove the litmus paper, add 5 cc. of Benedict's qualitative sugar solution,† and boil vigorously 1–2 minutes. Allow to cool spontaneously. A voluminous precipitate, which may be green, yellow, or red, indicates reducing sugars.

2. *Molisch test for carbohydrates.*—Transfer 5 cc. of the hydrolyzed gum solution to a test tube, and add 2 drops of a 15% solution of alpha naphthol in alcohol. Incline the tube and slowly pour down the inner side 3–5 cc. of concentrated H_2SO_4 so that the two layers will not mix. A reddish-violet zone at the point of contact indicates carbohydrates. (A 5% solution of thymol in alcohol may be substituted for alpha naphthol.)

If sufficient solution remains, divide into two equal parts and apply the following tests:

3. *Seliwanoff test.*—Heat the hydrolyzed gum solution to boiling, and add a few milligrams of resorcinol. A red color indicates hexoses.

4. *Tollens test.*—Heat the hydrolyzed gum solution to boiling, and drop in a few crystals of phloroglucinol. A red or deep amber color indicates pentoses. Certain other sugars (as galactose) also give a positive reaction.

Samples of mayonnaise made from salad oil, egg, and vinegar, and from salad oil, egg, and lemon juice, each containing small amounts of gum, were sent to collaborators. Table 1 lists the kind and amounts of gum present in these samples, and the results submitted by the collaborators.

DISCUSSION

With the exception of Karaya all the gums used gave positive tests, in amounts as low as 0.10 per cent or 0.15 per cent. Karaya, for some reason at present unknown, gave variable results. Certain specimens of karaya gum invariably gave positive results, others either negative or doubtful. The gums used in the preparation of these mayonnaise and French dressing samples were bought from reliable importers. U.S.P. gums, acacia, agar-agar, and tragacanth complied with the criteria for these products laid down by the United States Pharmacopoeia. There is no reason to doubt the authenticity of any of the gums used.

All gums tested by the writer responded to the Benedict and Molisch

† Dissolve 17.3 grams of Na citrate and 10 grams of anhydrous Na_2CO_3 in about 80 cc. of hot water; dissolve 1.73 grams of crystalline Cu sulfate in 10 cc. of water. Filter the alkaline citrate solution, add the Cu sulfate solution slowly, with constant stirring, and make up to 100 cc.

tests after hydrolysis. The Seliwanoff test reacted positively to karaya, agar-agar, Irish moss, and locust bean (slowly), and negatively or doubtfully to acacia, tragacanth, dextrin, sodium alginate, ghatti, and sometimes locust bean. The Tollens test gave positive reactions for karaya, tragacanth (slowly), agar-agar, locust bean, and gum ghatti. Sodium alginate turned yellow, then purple. The only gum tested showing negative or doubtful reactions to this reagent was acacia. It is noted that collaborators' reports at times show variance with these results. It is believed that this may be due to incomplete hydrolysis of the gum precipitate, or to the use of insufficient solution to respond positively.

The previous work cited showed that sugar up to 6 per cent in mayonnaise did not interfere. At that time the method was found to react positively to 0.2 per cent agar. The present investigation shows that amounts as low as 0.1 per cent gums may be detected. This is below the amount expected in commercial practice.

In the absence of starch, dextrin, or appreciable amounts of pectin, the presence of gums is indicated by an alcohol precipitate soluble in water and reprecipitated with alcohol, responding to tests for monosaccharides after hydrolysis.

The Referee has tested this method on four samples of gum-free mayonnaises, containing 8–12 per cent egg yolk, with negative results. However, time did not permit collaborative studies of gum-free mayonnaises. Samples known to be free from gums will be submitted for collaborative work next year to establish the negative application of this method.

It is recommended[1] that work on the detection of gums in mayonnaise be continued and that methods of detection of gums in other food products be studied.

REPORT ON OILS, FATS, AND WAXES

By G. S. Jamieson (Bureau of Chemistry and Soils, Washington, D. C.), *Referee*

During the past year a collaborative study was undertaken with reference to the effect upon the results obtained for the Polenske number determination of the size of the pumice which is used to aid in the distillation of volatile acids in fats and oils. The results of this study, which has been under the direct supervision of Associate Referee R. S. McKinney, will be the subject of his report. Associate Referee Lawrence Zeleny will present a report which he and M. H. Neustadt have prepared covering the work accomplished on the refractometric determination of oil in oil seeds.

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[1]For report of Subcommittee C and action by the Association, see *This Journal*, 22, 62 (1939).

It is recommended[1]—

(1) That changes be made in the Association's specifications for the titer thermometer, *Methods of Analysis, A.O.A.C.*, 1935, 408, so as to conform to the latest specification of the National Bureau of Standards (final action).

(2) That the Kaufmann thiocyanogen method, *This Journal*, **21**, 87, be made official (final action).

(3) That the methods of the National Cottonseed Products Association for the determination of free fatty acids in crude and refined oils, *This Journal*, **21**, 88, be made official (final action).

(4) That further work be done on the refractometric method for the determination of oil in oil seeds.

(5) That further study be made of the Polenske method and the use of powdered pumice, with a view to finding a procedure for eliminating the troublesome bumping during the distillation.

(6) That the refractive index method for the determination of oil in flaxseed, *This Journal*, **22**, 74, be made official (final action).

REPORT ON REFRACTOMETRIC DETERMINATION OF OIL IN SEEDS (SOYBEANS)

By LAWRENCE ZELENY (*Associate Referee*) and M. H. NEUSTADT
(Agricultural Marketing Service, Washington, D. C.)

In a previous report by the Associate Referee, *This Journal*, **20**, 74, 421, a refractometric method was applied to the determination of the oil content of flaxseed. Collaborative study indicates that the method is fully as accurate and reliable as the conventional petroleum-ether extraction method, and it has the advantage of being much more rapid than any previously proposed acceptable method.

The method was adopted by the Association as an official method (final action) for the determination of oil in flaxseed, and it has subsequently been used successfully as a routine procedure in a number of commercial laboratories.

The method is based upon the principle that the refractive index of the filtrate from an intimate mixture of a fat solvent and the ground flaxseed is a function of the oil content of the seed and its refractive index. Thus, if the refractive indices of both the solvent and the oil are known, the refractive index of such an extract is a measure of the oil content of the seed.

ADAPTATION OF THE REFRACTOMETRIC METHOD TO SOYBEANS

Using a mixture of halowax (α-chloronaphthalene) and α-bromonaphthalene adjusted to a refractive index of 1.63940 at 25° C. as a standard

[1] For report of Subcommittee C and action by the Association, see *This Journal*, **22**, 63 (1939).

solvent, the writers determined the refractive indices of mixtures of this solvent in various proportions with a composite sample of freshly prepared soybean oil having a refractive index, $n_D^{25}=1.47302$ (Table 1).

TABLE 1.—*Refractive indices at 25° C. of known mixtures of the halowax-α-bromonaphthalene solvent with a composite sample of soybean oil*

OIL IN MIXTURE	n_D^{25}
per cent	
0.000	1.63940
3.961	1.63031
4.898	1.62817
5.705	1.62646
6.569	1.62458
7.266	1.62297
9.154	1.61891
12.991	1.61078
16.647	1.60311
20.186	1.59612
88.879	1.48675
100.000	1.47302

The percentage of oil in the mixture obtained in the actual analysis of soybeans may be calculated from the formula:

$$\frac{100\ Wx}{W'+Wx}$$

where W = weight of ground soybeans in grams,
W′ = weight of solvent in grams, and
x = weight of oil in grams in 1 gram of the ground soybeans.

It may be shown with this formula that when 2 grams of ground soybeans are mixed with 5 cc. of the standard solvent, a range in soybean oil content of 10–26 per cent will correspond approximately to a range in oil content of the solvent-oil mixture of 3–7 per cent. Thus, by plotting the values shown in Table 1, the refractive index of the solvent extract corresponding to any value for oil content of soybeans may be determined. In this manner a conversion table was prepared for converting refractive index readings into soybean oil content percentages (Table 2).

Obviously this table will be strictly valid only for the analysis of soybeans whose oil has the refractive index, $n_D^{25}=1.47302$, since an oil having that value was used in the preparation of the table. A correction table (Table 3) was therefore prepared to indicate the values to be added or subtracted from the values obtained by the conversion table for soybeans whose oils have refractive indices other than the index used as a standard. In actual practice, however, soybeans that have oils with refractive indices differing sufficiently from this standard to cause a significant error

TABLE 2.—*Conversion table for determining the percentage of oil in soybeans from the refractive index of the halowax-α-bromonaphthalene extract*

(1.2900 grams (5 cc.) of solvent to extract 2.00 grams of meal)

OIL	n_D^{25}	OIL	n_D^{25}	OIL	n_D^{25}	OIL	n_D^{25}	OIL	n_D^{25}	OIL	n_D^{25}
per cent		*per cent*		*per cent*		*per cent*		*per cent*		*per cent*	
10.0	1.63250	12.7	1.63069	15.4	1.62895	18.1	1.62727	20.8	1.62563	.5	1.62402
.1	1.63243	.8	1.63062	.5	1.62889	.2	1.62721	.9	1.62557	.6	1.62396
.2	1.63236	.9	1.63056	.6	1.62882	.3	1.62715	21.0	1.62551	.7	1.62390
.3	1.63230	13.0	1.63049	.7	1.62876	.4	1.62709	.1	1.62545	.8	1.62384
.4	1.63223	.1	1.63043	.8	1.62870	.5	1.62703	.2	1.62539	.9	1.62378
.5	1.63216	.2	1.63036	.9	1.62863	.6	1.62696	.3	1.62533	24.0	1.62372
.6	1.63209	.3	1.63030	16.0	1.62857	.7	1.62690	.4	1.62527	.1	1.62366
.7	1.63202	.4	1.63023	.1	1.62851	.8	1.62684	.5	1.62521	.2	1.62360
.8	1.63196	.5	1.63017	.2	1.62845	.9	1.62678	.6	1.62515	.3	1.62354
.9	1.63189	.6	1.63010	.3	1.62838	19.0	1.62672	.7	1.62509	.4	1.62348
11.0	1.63182	.7	1.63004	.4	1.62832	.1	1.62666	.8	1.62503	.5	1.62343
.1	1.63175	.8	1.62997	.5	1.62826	.2	1.62660	.9	1.62497	.6	1.62337
.2	1.63169	.9	1.62991	.6	1.62820	.3	1.62654	22.0	1.62491	.7	1.62331
.3	1.63162	14.0	1.62984	.7	1.62814	.4	1.62648	.1	1.62485	.8	1.62325
.4	1.63155	.1	1.62978	.8	1.62807	.5	1.62642	.2	1.62479	.9	1.62319
.5	1.63149	.2	1.62971	.9	1.62801	.6	1.62635	.3	1.62473	25.0	1.62313
.6	1.63142	.3	1.62965	17.0	1.62795	.7	1.62629	.4	1.62467	.1	1.62307
.7	1.63135	.4	1.62958	.1	1.62789	.8	1.62623	.5	1.62462	.2	1.62301
.8	1.63128	.5	1.62952	.2	1.62783	.9	1.62617	.6	1.62456	.3	1.62296
.9	1.63122	.6	1.62946	.3	1.62776	20.0	1.62611	.7	1.62450	.4	1.62290
12.0	1.63115	.7	1.62939	.4	1.62770	.1	1.62605	.8	1.62444	.5	1.62284
.1	1.63108	.8	1.62933	.5	1.62764	.2	1.62599	.9	1.62438	.6	1.62278
.2	1.63102	.9	1.62926	.6	1.62758	.3	1.62593	23.0	1.62432	.7	1.62272
.3	1.63095	15.0	1.62920	.7	1.62752	.4	1.62587	.1	1.62426	.8	1.62267
.4	1.63089	.1	1.62914	.8	1.62745	.5	1.62581	.2	1.62420	.9	1.62261
.5	1.63082	.2	1.62907	.9	1.62739	.6	1.62575	.3	1.62414	26.0	1.62255
.6	1.63075	.3	1.62901	18.0	1.62733	.7	1.62569	.4	1.62408		

TABLE 3.—*Correction table to be applied to results from conversion table (Table 2).* Standard* $n_D^{25} = 1.4730$.
(For higher values of n_D^{25}, add correction; for lower values, subtract correction)

$n_D^{25}-1.4730$	OIL *per cent* 10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
0.0001	.00	.01	.01	.01	.01	.01	.01	.01	.01	.01	.01	.01	.01	.01	.01	.01	.01
2	.01	.01	.01	.01	.01	.01	.02	.02	.02	.02	.02	.02	.02	.02	.02	.02	.03
3	.01	.02	.02	.02	.02	.02	.02	.02	.03	.03	.03	.03	.03	.03	.04	.04	.04
4	.02	.02	.02	.02	.03	.03	.03	.03	.03	.04	.04	.04	.04	.05	.05	.05	.05
5	.02	.03	.03	.03	.03	.04	.04	.04	.04	.05	.05	.05	.05	.06	.06	.06	.06
6	.03	.03	.03	.04	.04	.04	.05	.05	.05	.06	.06	.06	.06	.07	.07	.07	.08
7	.03	.04	.04	.04	.05	.05	.05	.06	.06	.06	.07	.07	.08	.08	.08	.09	.09
8	.04	.04	.05	.05	.05	.06	.06	.07	.07	.07	.08	.08	.09	.09	.09	.10	.10
9	.04	.05	.05	.06	.06	.06	.07	.07	.08	.08	.09	.09	.10	.10	.11	.11	.12
10	.05	.05	.06	.06	.07	.07	.08	.08	.09	.09	.10	.10	.11	.11	.12	.12	.13
11	.05	.06	.06	.07	.07	.08	.08	.09	.10	.10	.11	.11	.12	.12	.13	.14	.14
12	.06	.06	.07	.07	.08	.09	.09	.10	.10	.11	.12	.12	.13	.14	.14	.15	.15
13	.06	.07	.07	.08	.09	.09	.10	.11	.11	.12	.13	.13	.14	.15	.15	.16	.17
14	.07	.07	.08	.09	.09	.10	.11	.12	.12	.13	.14	.14	.15	.16	.16	.17	.18
15	.07	.08	.09	.09	.10	.11	.12	.12	.13	.14	.15	.15	.16	.17	.18	.18	.19
16	.07	.08	.09	.10	.11	.12	.12	.13	.14	.15	.16	.16	.17	.18	.19	.20	.20
17	.08	.09	.10	.11	.11	.12	.13	.14	.15	.16	.17	.17	.18	.19	.20	.21	.22
18	.08	.09	.10	.11	.12	.13	.14	.15	.16	.17	.18	.18	.19	.20	.21	.22	.23
19	.09	.10	.11	.12	.13	.14	.15	.16	.17	.18	.19	.19	.20	.21	.22	.23	.24
20	.09	.10	.11	.12	.13	.14	.15	.17	.18	.19	.20	.21	.22	.23	.24	.25	.26
21	.10	.11	.12	.13	.14	.15	.16	.17	.18	.19	.20	.22	.23	.24	.25	.26	.27
22	.10	.11	.12	.14	.15	.16	.17	.18	.19	.20	.21	.23	.24	.25	.26	.27	.28
23	.11	.12	.13	.14	.15	.17	.18	.19	.20	.21	.22	.24	.25	.26	.27	.28	.29
24	.11	.12	.14	.15	.16	.17	.19	.20	.21	.22	.23	.25	.26	.27	.28	.29	.31
25	.12	.13	.14	.15	.17	.18	.19	.21	.22	.23	.24	.26	.27	.28	.29	.31	.32

* Per cent oil as determined from Table 2.

in the uncorrected oil content value taken from the conversion table, are seldom, if ever found.

The procedure follows:

ANALYTICAL PROCEDURE

(1) Obtain a representative sample of about 50 grams of the soybeans either by hand quartering or by use of a mechanical sampling device.

(2) Grind the beans as fine as practicable with the equipment available. The experimental roller flour mill described later is recommended for this purpose. (It is usually best to crack the beans in a coarse mill before grinding.)

(3) Weight out accurately 2 grams of the well-mixed meal and transfer the weighed sample to a 3-inch porcelain mortar that has been previously warmed to about 55° C.

(4) Add about 1.5 grams of reagent-quality sea sand and exactly 5 cc. of a halowax-α-bromonaphthalene mixture having a refractive index of $n_D^{25}=1.63940$ ± 2. Use the utmost care in the measurement of this solution. (It is best accomplished by using an accurately calibrated pipet having a delivery time of not less than 15 seconds.)

(5) Grind the mixture in the mortar vigorously for 3 minutes, constantly scraping into the bottom the particles of meal that are thrown against the sides of the mortar.

(6) Filter the mixture into a test tube through a good quality fat-free folded filter paper of sufficiently fine porosity to yield a clear filtrate.

(7) Determine the refractive index of the filtrate at 25.0° C. to an accuracy of ± 0.00002. If the reading is made at any temperature other than 25.0° C., add 0.00043 for each degree above 25.0° C. (It is important that all temperature readings be made to the nearest 0.1°.)

(8) Using Table 2, note the percentage of oil corresponding to the refractive index reading obtained in (7). This is the uncorrected value for oil content.

(9)* In a flask shake for a minute or two about 5 grams of the meal with about 25 cc. of a good grade of low-boiling petroleum ether and filter into a small shallow evaporating dish. Carefully evaporate off the solvent on a steam bath or hot plate at low heat, and place the dish in an oven at 105° C. for 20 minutes. Cool the oil thus prepared to room temperature and determine its refractive index at 25.0° C. (The refractive index correction for temperature for soybean oil is 0.000364 per 1° C., to be added if the temperature at which the reading is taken is above 25.0°, and subtracted if below that temperature. If preferred, the sample of oil may be prepared by pressing a small sample of the ground seed in a laboratory hydraulic press and filtering the oil so obtained if it is not entirely clear.)

(10)* From the refractive index of the oil as determined in (9), subtract the value 1.4730 (refractive index at 25.0° C. of the composite sample of soybean oil used in obtaining data for the conversion table). Using this difference, determine from Table 3 the correction to be applied to the uncorrected value for oil content as determined in (8). If the difference is positive, add the correction; if negative, subtract it.

EXPERIMENTAL

As a check with which to compare results obtained by the refractometric method, the finely ground soybeans were extracted for approximately 17 hours with a petroleum ether conforming to the official spec-

* For practical purposes Steps 9 and 10 may usually be omitted, as they add very little to the accuracy of the determination.

TABLE 4.—*Comparison of the oil content of 61 samples of soybeans as determined by the petroleum ether extraction method and by the proposed refractometric method*

SAMPLE NUMBER	DESCRIPTION OF SAMPLE	A PETROLEUM ETHER EXTRACTION METHOD (DRY-MATTER BASIS)	B REFRACTOMETRIC METHOD (DRY-MATTER BASIS)	DIFFERENCE B−A
		per cent	*per cent*	
A-5	Laredo	15.39	15.23	−0.16
B-19	Laredo, 1937; Monetta, S.C.	15.47	15.17	−0.30
B-14	Wilson—Five, 1935; Holgate, Ohio	15.99	15.99	0.00
B- 4	Haberlandt	16.20	16.20	0.00
B-21	Monetta, 1937; Monetta, S.C.	16.38	16.36	−0.02
V-49	Laredo	16.60	16.18	−0.42
V-46	Medium green	16.68	16.64	−0.04
B-34	Peking, 1936; Ohio	16.69	17.07	+0.38
A- 4	Monetta	16.76	16.39	−0.37
B-42	Avoyelles, 1937; Monetta, S.C.	17.33	17.09	−0.24
A- 6	Mammoth yellow	17.39	17.18	−0.21
C-44	Commercial	17.48	17.60	+0.12
B-41	Biloxi, 1937; Monetta, S.C.	17.64	17.47	−0.17
V-50	Otootan	17.65	17.57	−0.08
V-47	Biloxi	17.73	17.46	−0.27
B-5	Mammoth yellow	17.73	17.58	−0.15
B- 1	Virginia	17.94	18.13	+0.19
B-36	Otootan, 1937; Monetta, S.C.	18.01	18.03	+0.02
B-37	Georgian, 1937; Monetta, S.C.	18.05	18.04	−0.01
B- 3	Easycook	18.15	18.00	−0.15
B-38	Creole, 1937; Monetta, S.C.	18.19	18.19	0.00
B-20	Cayuga, 1937; Ithaca, N.Y.	18.42	18.09	−0.33
B-10	Tarheel, 1937; N.Car.	18.51	18.44	−0.07
B- 6	Tokyo	18.57	18.61	+0.04
B-11	Mandarin, 1937; Ames, Iowa	18.79	18.81	+0.02
B-29	Palmetto, 1936; Monetta, S.C.	18.80	18.38	−0.42
C-47	Commercial	18.82	18.82	0.00
B-35	Mandarin, 1936; Ohio	18.94	19.17	+0.23
B-27	White Biloxi, 1936; Monetta, S.C.	18.97	18.97	0.00
B- 8	Spooner Mandarin, 1937; Spooner, Wis.	19.03	19.27	+0.24
B-40	Charlee, 1937; Monetta, S.C.	19.06	19.12	+0.06
B- 2	Hollybrook	19.17	19.23	+0.06
V-45	Tarheel	19.17	19.39	+0.22
V-48	Virginia	19.41	19.21	−0.20
B-26	Mamloxi, 1937; Stoneville, Miss.	19.50	19.38	−0.12
B-16	Mukden, 1936; Iowa	19.70	20.00	+0.30
B-15	Mandell, 1936; Illinois	19.86	20.10	+0.24
B-28	Mamredo, 1937; Stoneville, Miss.	19.94	19.64	−0.30
A- 2	Commercial	20.12	19.95	−0.18
B-30	Hayseed, 1937; Monetta, S.C.	20.13	19.95	−0.17
C-43	Commercial	20.17	20.41	+0.24
B-25	Delsta, 1937; Stoneville, Miss.	20.21	19.90	−0.31

TABLE 4.—(*Continued*)

SAMPLE NUMBER	DESCRIPTION OF SAMPLE	A PETROLEUM ETHER EXTRACTION METHOD (DRY-MATTER BASIS)	B REFRACTOMETRIC METHOD (DRY-MATTER BASIS)	DIFFERENCE B−A
		per cent	*per cent*	
C-48	Commercial	20.22	20.41	+0.19
B-18	Missoy, 1937; West Point, Miss.	20.24	20.47	+0.23
B- 9	Harbinsay, 1937; Urbana, Ill.	20.26	20.32	+0.06
B-13	Habaro, 1937; N.D.	20.29	20.47	+0.18
B-24	Wisconsin Early Black, 1937; Ames, Ia.	20.50	20.51	+0.01
B-23	Manchuria (13177), 1935; Holgate, Ohio	20.57	20.54	−0.03
B-17	Macoupin, 1935; Holgate, Ohio	20.64	20.94	+0.30
B-22	Scioto, 1936; Scioto, Ohio	20.79	20.99	+0.20
B- 7	Minsoy, 1937; Ames, Iowa	21.24	21.07	−0.17
B-31	Illini, 1936; Ohio	21.38	21.08	−0.30
B-39	A.K., 1936; Arlington, Va.	21.43	21.26	−0.17
A- 1	Commercial	21.51	21.22	−0.29
A- 3	Commercial	21.74	21.50	−0.24
B-32	Dunfield, 1936; Ohio	21.77	22.02	+0.25
D-53	Commercial	21.80	21.78	−0.02
E-58	Commercial	21.83	21.45	−0.38
B-12	Hudson Manchu, 1937; Vt.	21.89	21.86	−0.03
C-49	Commercial	22.07	22.08	+0.01
B-33	Manchu, 1936; Ohio	23.91	23.75	−0.16
	Average	19.16	19.12	

ifications for petroleum ether for cottonseed extraction.[1] The beans were coarsely ground and then passed three times through a roller-type experimental flour mill having two 6×6 inch rolls (40 corrugations to the inch), a speed differential between rolls of 9:7, and a speed of approximately 900 r.p.m. for the faster roll. The extraction of oil from soybeans ground in this manner is practically complete in 17 hours without the customary regrinding of the sample in a mortar. Tests were made on the extracted meal by regrinding it in a mortar with sand and extracting the reground meal for an additional 17 hours. In most cases less than 0.1 per cent of additional extract was obtained, and it appeared to consist largely of some substance other than a true oil.

Sixty-one samples of domestic soybeans were analyzed for oil content by both the petroleum ether extraction method and the refractometric method. The results of these analyses are listed in Table 4 in the order of increasing oil content. Between the two methods the average oil content for the entire series differed by only 0.04 per cent. The maximum discrepancy between the two methods was 0.42 per cent oil, and the average

[1] U. S. Dept. Agr. Service and Regulatory Announcement No. 133 (1932).

of individual discrepancies was 0.17 per cent oil. In the case of the 42 samples of this series on which duplicate determinations were made by both methods, the average difference between duplicates was 0.15 per cent oil for the extraction method, and 0.07 per cent oil for the refractometric method.

COLLABORATIVE STUDY OF THE REFRACTOMETRIC METHOD

Six samples of soybeans were analyzed by both the petroleum ether extraction method and the refractometric method by the following five collaborators.

Mayne R. Coe, Bureau of Chemistry and Soils, Washington, D. C.
R. T. Milner, U. S. Regional Soybean Industrial Products Laboratory, Urbana, Ill.
M. H. Neustadt, Agricultural Marketing Service, Washington, D. C.
W. F. Geddes, Dominion Grain Research Laboratory, Winnipeg, Canada.
The Associate Referee.
These names are not listed in the order used in Table 5.

The results of this collaborative study are shown in Table 5.

TABLE 5.—*Oil content of 6 samples of soybeans as determined by 5 collaborators using the refractometric method and the petroleum ether extraction method*
(All data are averages of duplicate determinations, and are expressed as per cent oil on a dry-matter basis.)

SAMPLE NUMBER	COLLABORATOR				
	A	B	C*	D	E
Refractometric Method					
1	20.92	21.22	19.95	21.13	19.39
2	19.79	19.95	17.57	18.82	18.72
3	21.86	21.50	20.20	20.19	21.44
4	16.62	16.39	14.72	19.92	13.50
5	15.58	15.23	—	13.49	12.44
6	17.27	17.18	—	16.22	17.03
Extraction Method					
1	21.17	21.51	21.51	—	20.86
2	20.31	20.12	19.83	—	20.38
3	21.63	21.74	21.56	—	22.42
4	16.75	16.76	15.89	—	16.26
5	15.90	15.39	14.09	—	15.04
6	17.19	17.39	16.59	—	17.72

* Refractometric determinations were made with an instrument not covering the required range in refractive index at ordinary temperatures. Readings were taken at 50.75° C. and converted to 25° C., and thus the chance for error was increased appreciably.

RECOMMENDATIONS[1]

It is recommended that further work on the refractometric method be directed toward the development of a general method for determining the oil content of oil-bearing seeds and possibly other agricultural products.

Such a method conceivably could be as broad in its scope as the present extraction procedure for determining crude fat, and would be distinctly advantageous for routine work in which prompt results are required. A general method, to be of practical value, would involve the preparation of a master conversion table based upon a careful study of the relationship between the composition of solvent-oil mixtures and their refractive indices for fats and oils of different refractive index throughout the entire range encountered in nature.

No report on thiocyanogen number was given by the associate referee.

REPORT ON THE POLENSKE VALUE OF FATS AND OILS

By R. S. McKinney (Bureau of Chemistry and Soils, Washington, D. C.), *Associate Referee*

In accordance with the recommendations adopted by the Association, a collaborative study was made of two methods for determining the Polenske value of fats and oils. In this study Samples A and B were the fats from blue cheese, Samples D and E were the fats from Roquefort cheese, and Sample C was 50 per cent by weight blue cheese fat and 50 per cent coconut oil. The two methods used are similar to the one described in *Methods of Analysis, A.O.A.C.*, 1935, p. 414, except that a Florence flask was specified, wash water at a temperature of 20° was used, and in Method II, 0.1 gram of powdered pumice stone was used instead of the broken pieces.

The samples were submitted to the following collaborators:

(1) I. D. Garard, New Jersey College for Women, New Brunswick, N. J.

(2) R. S. McKinney.

(3) K. R. Majors, U. S. Regional Soybean Industrial Products Laboratory, Urbana, Ill.

(4) F. H. Lehberg, Grain Research Laboratory Board of Grain Commissioners, Winnipeg, Canada.

(5) Burton Jordan, State Chemical Laboratory, Vermillion, S. Dak.

(6) M. L. Offutt, U. S. Food and Drug Administration, New York, N. Y.

(7) J. L. Perlman, State Food Laboratory, Albany, N. Y.

COMMENTS AND CONCLUSIONS OF THE ASSOCIATE REFEREE

Although this study was primarily on the Polenske value determination, it was thought worth while to include also the Reichert-Meissl value

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[1] For report of Subcommittee C and action by the Association, see *This Journal*, **22**, 63 (1939).

TABLE 1.—*Reichert-Meissl values*

COLLABORATOR	METHOD I (PIECES OF PUMICE)		METHOD II (FINE PUMICE)	
		Sample A		
		av.		*av.*
1	26.15; 25.02	25.6	25.52; 24.68	25.1
2	25.3 ; 25.1	25.2	25.6 ; 25.7 ; 25.8	25.7
3	27.1 ; 27.0 ; 27.1	27.1	26.6 ; 26.3	26.5
4		26.2		26.31
5	25.6 ; 25.7 ; 25.9	25.7	25.7 ; 26.1 ; 26.2	25.6
6	25.9	25.9		25.6
7	25.9 ; 26.5	26.2	26.7 ; 26.2	26.5
	Av.	26.3		25.9
	Av. variation	0.5		0.4
		Sample B		
		av.		*av.*
1	23.12; 22.73	23.0	22.97; 23.2	23.1
2	22.7 ; 22.5	22.6	22.0 ; 22.3 ; 22.3 ; 22.9	22.4
3	22.6 ; 22.8	22.7	23.2 ; 23.4	23.3
4		23.77		23.4
5	22.83; 23.10; 23.21; 23.07; 23.43; 22.88	23.1	23.02; 22.91; 23.05	23.0
6		22.6		23.2
7	21.9 ; 22.5	22.2	22.9 ; 22.1	22.5
	Av.	22.9		23.0
	Av. Variation	0.4		0.3
		Sample C		
		av.		*av.*
1	10.50; 18.83	10.7	10.65; 10.58	10.62
2	10.5 ; 10.6 ; 10.7	10.6	10.6 ; 10.8	10.7
3	10.8 ; 10.9	10.85	11.0 ; 11.0 ; 11.2	11.1
4		10.83		11.09
5	11.63; 11.30; 11.63; 11.52; 11.77	11.56	11.58; 11.52; 11.52; 11.58	11.55
6		11.1		11.2
7	10.6 ; 10.7	10.7	10.7 ; 11.1	10.9
	Av.	11.0		11.0
	Av. Variation	0.4		0.3
		Sample D		
		av.		*av.*
1	27.22; 26.65	26.9	27.46; 27.52	27.5
2	28.1 ; 28.3 ; 28.5	28.3	27.4 ; 27.4	27.4
3	29.0 ; 29.0	29.0	28.8 ; 29.3 ; 29.4	29.2
4		30.14		30.21
5	29.14; 29.21; 29.45	29.3	29.38; 29.28; 29.13; 29.61	30.4

TABLE 1.—*Reichert-Meissl values*—(*Continued*)

COLLABORATOR	METHOD I (PIECES OF PUMICE)		METHOD II (FINE PUMICE)	
		av.		*av.*
6		28.2		28.4
7	29.3 ; 29.6	29.5	28.9 ; 29.3	29.1
	Av.	28.8		29.9
	Av. Variation	0.8		0.9
		Sample E		
		av.		*av.*
1	29.82; 29.65	29.7	29.61; 30.44	30.0
2	29.1 ; 29.3 ; 29.8	29.4	28.9 ; 29.1	29.0
3	30.3 ; 29.3 ; 29.8 ; 29.6	29.8	29.6 ; 30.2	29.9
4		30.51		30.18
5	30.82; 30.82; 30.76	30.8	30.26; 30.71; 30.71; 30.54	30.7
6		29.9		30.0
7	30.1 ; 30.2	30.2	29.8 ; 30.0	29.9
	Av.	30.0		30.0
	Av. Variation	0.4		0.3

TABLE 2.—*Polenske values*

COLLABORATOR	METHOD I (PIECES OF PUMICE)		METHOD II (FINE PUMICE)	
		Sample A		
		av.		*av.*
1	1.88; 1.75	1.82	2.42; 2.47	2.45
2	1.37; 1.39	1.38	1.65; 1.78; 1.92	1.79
3	2.1 ; 2.0 ; 1.9	2.0	2.1 ; 2.4	2.25
4		2.45		2.56
5	1.58; 1.48; 1.75	1.60	1.92; 1.94; 1.96	1.94
6		1.63		1.80
7	2.1 ; 2.1	2.1	2.2 ; 2.4	2.3
	Av.	1.85		2.16
	Av. Variation	0.28		0.25
		Sample B		
		av.		*av.*
1	2.12; 1.93	2.03	2.73; 2.84	2.79
2	1.89; 1.91	1.90	1.80; 1.92; 2.22	1.98
3	1.90; 2.0 ; 2.1	2.0	1.8 ; 1.8	1.80
4		2.45		2.42
5	1.82; 1.95; 1.85; 1.80	1.86	2.12; 2.82	2.47
6		2.03		2.03
7	2.4 ; 2.2	2.3	2.8 ; 2.5	2.65
	Av.	2.05		2.30
	Av. Variation	0.16		0.31

TABLE 2.—*Polenske values*—(*Continued*)

COLLABORATOR	METHOD I (PIECES OF PUMICE)	av.	METHOD II (FINE PUMICE)	av.
		Sample C		
		av.		*av.*
1	7.13; 7.21	7.17	8.26; 8.76	8.51
2	6.22; 6.24	6.23	7.21; 7.44	7.33
3	5.1 ; 5.6	5.35	7.1 ; 7.5 ; 7.4	7.3
4		7.7		7.7
5	7.50; 5.66; 5.85; 6.42; 5.83; 6.80	6.34	6.95; 7.30; 7.75; 7.10	7.23
6		5.76		6.12
7	7.2 ; 8.4	7.8	7.1 ; 8.2	7.65
	Av.	6.62		7.41
	Av. Variation	0.80		0.47
		Sample D		
		av.		*av.*
1	3.03; 3.36	3.20	4.42; 4.51	4.47
2	2.45; 3.12; 3.75	3.12	3.70; 3.71	3.71
3	3.7 ; 3.2	3.45	3.6 ; 3.9 ; 4.6	4.40
4		5.08		5.13
5	3.40; 3.45; 3.25	3.67	4.10; 4.55; 2.65; 4.20	3.88
6		4.04		4.75
7	3.6 ; 3.4	3.5	4.6 ; 4.8	4.7
	Av.	3.72		4.43
	Av. Variation	0.48		0.39
		Sample E		
		av.		*av.*
1	4.32; 6.39	5.36	5.15; 4.49	4.82
2	3.64; 3.64; 3.72	3.67	4.52; 4.54	4.53
3	5.4 ; 4.7 ; 4.0 ; 4.6	4.7	5.0 ; 5.0	5.0
4		4.23		5.10
5	4.55; 4.35; 4.55	4.48	3.2 ; 5.1	4.15
6		4.10		4.67
7	4.0 ; 4.1	4.05	5.2 ; 5.3	5.25
	Av.	4.37		4.79
	Av. Variation	0.41		0.29

results. These same results are being used by I. D. Garard, Associate Referee on Cheese and also on the Difference between Dairy Products from Cow's Milk and those from Milk of other Animals. Garard prepared the samples and sent them to the collaborators for study.

In regard to the Reichert-Meissl value determination, it was found that the same results were obtained with both methods, although slightly better agreement was obtained when the fine pumice was used.

In regard to the Polenske value determination, it was found that

Method II, with the fine pumice, gave results about 0.50 per cent higher than did Method I, with the pieces of pumice. The former method gave results showing a slightly better agreement between individual analysts, 0.34 per cent instead of 0.43 per cent average variation. However, any better agreement was overshadowed by the fact that more than half of the collaborators had considerable trouble with bumping.

It is therefore recommended[1] that further study be made of the Polenske method, with powdered pumice, with a view to finding a procedure for eliminating the troublesome bumping during the distillation.

REPORT ON MICROCHEMICAL METHODS

ALKOXYL DETERMINATION

By E. P. CLARK (Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Washington, D. C.), *Referee*

The past year has been the first period since the appointment of the Referee on Microchemical Methods that interest in or cooperation on the subject has been secured. Advantage of this fact was immediately taken, and as a start the methoxyl and ethoxyl method previously reported by the Referee, *This Journal*, **15**, 136, was submitted to collaborative study. There were several reasons for this: First, the method has been recommended for adoption; second, it has been used by Milstead, *Ibid.*, **21**, 543, for estimation of the purity of guaiacol and its derivatives; and third, the method is precise and has been largely adopted in research and industrial laboratories.

Samples of two preparations, lignin and rotenone, and directions for conducting the determination were submitted to a number of analysts, nine of whom reported. Their findings are given in Table 1.

From these results the following conclusions are drawn:

The variations in the checks on lignin can be accounted for, to some extent at least, by the fact that its moisture content varies with the relative humidity, and consequently the different times and places at which the samples are weighed influence the values obtained unless special precautions are taken. In the case of the samples submitted no special instructions of this type were given. With rotenone the moisture factor did not enter, and the values obtained are consequently more consistent.

The outstanding result of the study, however, is that most collaborators obtained close agreements in their check analyses and the average results of all analyses are very near the true values of the compounds submitted.

Aside from this study, the Referee's experience in analyzing over 500 pure compounds, together with private reports from other laboratories in which at least several thousand determinations have been made, indicates that the variations in relative results are not due inherently to the

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[1] For report of Subcommittee C and action by the Association, see *This Journal*, **22**, 63 (1939).

method, but rather to the balances, methods of weighing, and the standardization of the volumetric solutions employed. It is therefore suggested that the method be adopted as official.

The procedure recommended is, with the exception of a few minor changes, the same as previously published. A change in the apparatus is

TABLE 1.—*Collaborative results on determination of methoxyl in lignin and rotenone*

ANALYST	LIGNIN		ROTENONE	
	OCH_3 FOUND	DIFFERENCE	OCH_3 FOUND	DIFFERENCE
	per cent	*per cent*	*per cent*	*per cent*
1	12.81 12.78	0.03	15.71 15.67	0.04
2	12.90 12.72	0.18	15.90 15.93	0.03
3	12.50 12.50	0	15.6 15.6	0
4	12.91 12.75	0.16	15.78 15.74	0.04
5	15.61 12.65	0.04	15.73 15.70	0.03
6	12.83 12.93	0.10	15.83 15.97	0.14
7	12.73 12.73	0	15.71 15.67	0.04
8	12.62 12.42	0.20	15.37 15.41	0.04
9	12.34 12.38	0.04	15.37 15.45	0.09
Average	12.67	0.08	15.68	0.05
Calculated	12.79		15.7	

made with the hope that apparatus manufacturers will follow the specifications of the more simply fabricated design more closely than they did with the previous apparatus. Furthermore, the new design can readily be adapted to the determination of high alkoxyls by slipping a water jacket over the air condenser and scrubber and heating the entire apparatus to the necessary temperature. The use of less potassium acetate-

acetic acid solution than formerly used is recommended, as it insures a clearer and sharper end point.

This method was published in *This Journal*, 22, 100.

REPORT ON MICROBIOLOGICAL METHODS

By ALBERT C. HUNTER (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

The program planned for the development of microbiological methods is progressing, even though slowly. Those who have followed the program in this field will recall that at the beginning some time was devoted to a study of fundamental procedures and matters of technic involved in the bacteriological examination of canned foods, following which attention was turned toward the development of proposed methods for the microbiological examination of specific classes of products. Methods have now been set up for the bacteriological examination of canned meats, canned fish, canned vegetables, canned tomato products, and sugar. These methods are now being subjected to collaborative study. Inasmuch as these studies have not yet been carried far enough to provide bases for reports, there are no statements to be made this year by the Associate Referees in charge of those methods which have been presented heretofore, and have been published.

Last year in expansion of the program, the appointment of an associate referee on microbiological methods for the examination of eggs and egg products was recommended, and as a result, at this meeting a proposed method for the bacteriological examination of frozen eggs is being presented.

While it is believed that the methods for canned meats and for sugar have probably been studied sufficiently to warrant a recommendation for their adoption as tentative methods, it appears to be the better policy to withhold such recommendation until further collaborative work on the closely related methods for canned foods other than meats can be completed. The program then for the ensuing year calls for extensive collaborative work on the methods now set up without expanding the field further at this time.

It is recommended that the present Associate Referees on Canned Vegetables, Canned Tomato Products, Canned Fish Products, Canned Meats, Sugar, and Eggs and Egg Products be reappointed.

No reports were given on the following subjects relating to canned products: Fish, meats, vegetables, tomato products, and sugar.

REPORT ON MICROBIOLOGICAL METHODS FOR EXAMINATION OF FROZEN EGG PRODUCTS

By Roy Schneiter (Bacteriological Section, U. S. Food and Drug Administration, Washington, D. C.),
Associate Referee

Egg products may be classified as follows:

1. Shell eggs
2. Broken out eggs (liquid):
   - (a) Whole eggs
   - (b) Egg whites
   - (c) Egg yolks
3. Dried eggs:
   - (a) Albumin or whites
   - (b) Yolk
   - (c) Whole or mixed eggs
4. Frozen eggs:
   - (a) Albumin or whites
   - (b) Plain yolk
   - (c) Sugar yolk
   - (d) Salt yolk
   - (e) Glycerine yolk
   - (f) Whole or mixed eggs.

The bacteriological examination of egg products involves different factors for each different class, but only methods for the microbiological examination of frozen egg products are to be considered at this time.

The major portion of the output of the frozen egg industry is utilized in the preparation of food products that receive little or no heat processing during their manufacture. It is, therefore, essential that the frozen egg products used be free from large numbers and types of viable microorganisms, which may induce rapid spoilage or be of potential danger to health when incorporated in foods.

Microbiological methods for the examination of frozen egg products should include procedures for the determination of (1) the total numbers of microorganisms and (2) the incidence of types of microorganisms that may be dangerous to health or conducive to food spoilage.

While the presence of large numbers of viable microorganisms in frozen egg products is indicative of the use of poor quality shelf stock, unsatisfactory manufacturing procedures, or insanitary plant conditions, a high incidence of coliform organisms and hemolytic types of bacteria may be, in addition, indicative of potential danger to health.

The following microbiological methods are proposed:

I. SAMPLING

EQUIPMENT

(1) Electric drill with auger (12″×1″), (2) alcohol burner, (3) alcohol (95% C_2H_5OH), (4) absorbent cotton, (5) two tablespoons, (6) sample containers (sterile

1 qt. or 1 pt. Mason jars), (7) hammer and steel strip (12″×2″×0.25″) or other tool for opening cans, (8) water pail, (9) towels, and (10) record book, pencils, etc.

PROCEDURE

Select a representative number of cans from lot (square root of total). Note and record all marks of identification, for example: firm name and location, brand, type product, code or lot numbers, etc. Sterilize auger and tablespoons by sponging off with alcohol-soaked cotton and heating in flame of alcohol burner. Wash drill and spoons in pail of water and re-sterilize after each container sampled. Open the containers aseptically. Drill three cores equidistant between side and center of can and one-third of periphery apart. Transfer drillings from can to sample container with sterile tablespoon. Examine product organoleptically by smelling at opening of drill-hole after sample is removed. (Heat produced by electric drill intensifies odor of egg material, thus facilitating the organoleptic examination.) Record odors as normal, putrid, sour, or musty.

Refrigerate samples with dry ice if analysis is to be delayed or sampling point is at some distance from laboratory. Carry out sampling procedure under as nearly aseptic conditions as possible.

II. ANALYTICAL PROCEDURE

PREPARATION OF SAMPLE

Thaw frozen egg material as rapidly as possible in order to prevent an increase in the numbers of microorganisms present and at temperatures sufficiently low to prevent destruction of microorganisms (20°–30° C.). (Frequent shaking aids in thawing the frozen material. Thawing temperatures may be maintained by the use of a water bath.)

Thoroughly mix each thawed sample with spoon or electric stirrer before analysis. Prepare a 1–10 dilution by aseptically weighing 5.0 grams of egg material into a wide-mouthed glass-stoppered bottle containing 45 grams of sterile physiological salt solution (0.85% NaCl per 1,000 cc. distilled water) and 1 tablespoonful of glass shot. Agitate the 1–10 dilution thoroughly to insure complete solution or distribution of the egg material in the diluent. Prepare serial dilutions from 1–100 to 1–100,000,000 for inoculation into various culture media. Inoculate all media within 15 minutes after the sample is prepared.

PLATE COUNTS

Inoculate duplicate plates with 1 cc. portions of all dilutions from 1–1,000 to 1–1,000,000. (Nutrient agar or dextrose agar may be employed as plating media.) Incubate one set of plates at 20°–30° C. for 3 days and the second set of plates at 37° C. for 3 days. Express final results as numbers of viable microorganisms per gram of egg material. (Uniformly higher counts are always obtained on plates incubated at 20°–30° C.)

INCIDENCE OF COLIFORM GROUP

Inoculate 1.0 cc. portions from suitable dilutions (1–10 to 1–100,000,000) of egg material into fermentation tubes of lactose broth. Incubate at 37° C. for 24–48 hours.

Streak Levine's eosin methylene blue agar plates from all lactose broth cultures showing gas production. Incubate plates at 37° C. for 24–48 hours. Examine E. M. B. agar plates for colonies of microorganisms of the coliform group.

Inoculate from colonies of the coliform types of bacteria appearing on E. M. B. agar plates to nutrient agar slants. Incubate at 37° C. for 24 hours. Purify cultures for further study.

Obtain biochemical reactions of purified cultures by Kovac's test, indol production; methyl red (M. R.) and Voges Proskauer (V. P.) tests; and Koser's sodium citrate test, utilization of sodium citrate as the sole source of carbon.

NOTE: Follow procedure recommended in *Standard Methods of Water Analysis*, 8th Ed., 1936, of The American Public Health Association for Biochemical Reactions.

HEMOLYTIC TYPES OF MICROORGANISMS

Inoculate petri plates with 1 cc. portions of all dilutions from 1–100 to 1–1,000,000. Pour plates with veal-infusion agar containing 6% of defibrinated horse, sheep, or rabbit blood (0.6 cc. of blood per 10 cc. of media). Cool agar to 40° C. and add blood just prior to pouring plates. Incubate plates for 24 hours at 37° C. Express final results as numbers per gram.

ANAEROBIC TYPES OF MICROORGANISMS

Inoculate tubes containing chopped meat media with 1 cc. portions of all dilutions from 1–10 to 1–100,000. Incubate for 3 days at 37° C.

DIRECT MICROSCOPIC COUNTS

Place 0.01 cc. of the 1–10 or 1–100 dilutions of egg material on a clean microsopic slide and spread over an area of 1.0 sq. cm. Permit the smear preparation to dry on a level surface at 30°–40° C. Proceed as directed in *Standard Methods of Milk Analysis* of the American Public Health Association (latest edition). Multiply total count by 10 or 100, since the original smear preparation was made from a 1–10 or 1–100 dilution in order to obtain the numbers of bacteria per gram of egg material.

III. CULTURE MEDIA

STANDARD METHODS MEDIA

Prepare as recommended in *Standard Methods of Water Analysis*, 8th Ed., 1936, of The American Public Health Association.

Nutrient agar, lactose broth, Levine's eosin methylene blue agar, tryptophane broth, methyl red-Voges Proeskauer peptone medium, and Koser's sodium citrate medium.

Other media include (a) *Dextrose agar.*—Standard nutrient agar plus 1.0% dextrose. (b) *Veal infusion agar.*—Ground lean veal, 500.0 grams, and distilled water, 1000.0 cc. Infuse overnight in refrigerator and strain through cheesecloth without pressure. Make up to original volume and skim off any fat. Steam in Arnold 30 minutes and filter through paper. Add:

	per cent		*grams*
Peptone (Difco)	1.0	or	10.0
NaCl	0.5	or	5.0
Agar	1.5	or	15.0

Steam in Arnold to dissolve. Adjust reaction to *p*H 7.6 and steam in Arnold 15 minutes. Filter through Büchner funnel with paper pulp mat, by the aid of suction. Use egg albumin for clarification when necessary. Distribute 10 cc. quantities into test tubes or 80 cc. quantities into bottles. Sterilize at 15 lbs. pressure for 20 minutes. Final *p*H 7.4. (For hemolytic tests agar should be cooled to 40° C. and 6% of defibrinated horse, sheep, or rabbit blood added prior to pouring plates (0.6 cc. of blood per 10 cc. of media.)

(c) *Holman's cooked meat medium (alkaline).*—Distilled water, 1000 cc., ground fresh lean beef, 500 grams, bacto-peptone, 5 grams, and C. P. NaCl, 5 grams. Infuse the beef-water mixture overnight in refrigerator. Strain through several layers of cheesecloth and press out broth, retaining the meat press cake. Add distilled

water to the infusion to make 1 liter. Add the peptone and heat in the Arnold or boil 10 minutes. Filter, and add salt. Add normal NaOH until alkaline to phenolphthalein. Heat in Arnold 15 minutes to clear and filter. Distribute the pressed-out beef remaining from the infusion into medium sized tubes (150×20 mm.), approximately 2 grams into each tube, and add 10 cc. of the cleared alkaline broth. Sterilize in the autoclave at 15 lbs. pressure for 15 minutes. Final reaction should be *p*H 7.2–7.4. Store in refrigerator. Prior to using, boil the tubed medium for at least 10 minutes to expel adsorbed oxygen and cool promptly in a water bath.

The Manual Methods for Pure Culture Study of Bacteria of the Society of American Bacteriologists should be used as a guide for the further study of microorganisms obtained in the cultural procedures described.

No report on feeding stuffs was given by the associate referee.

REPORT ON SAMPLING FEEDING STUFFS

By L. M. JEFFERS (Bureau of Field Crops, California Department of Agriculture, Sacramento, Calif.), *Associate Referee*

In connection with regular feeding work, the Associate Referee carried on limited tests for the purpose of determining the efficiency of different types of samplers and different methods of sampling. In these tests the inspector was instructed to attempt to get a representative sample under each method. The analytical results obtained in these comparative methods of sampling are surprisingly close, but data obtained are not sufficiently extensive to warrant introduction at this time.

There may be certain limits in the setting up of definite sampling instructions, and the attempt of the inspector to secure truly representative samples is most important.

No information has been secured from other agencies relative to sampling methods and suggestions for definite instructions. The gathering of such information is desirable.

It is recommended,[1] therefore, that the study be continued with the hope that there may be developed standard methods of sampling feeding stuffs.

REPORT ON ASH IN FEEDING STUFFS

By J. L. ST. JOHN (Division of Chemistry, Agricultural Experiment Station, Pullman, Wash.), *Associate Referee*

It has been observed that difficulty is experienced in determining the ash of mixed feeds that contain added calcium carbonate in the form of oyster shell or limestone. Results obtained by different laboratories have

[1] For report of Subcommittee A and action by the Association, see *This Journal*, 22, 50 (1939).

been found to be irregular. The Association therefore appointed a referee to study methods of determining ash in feeds. The work this year was confined to feeds to which calcium carbonate had been added.

The major part of the work done on the determination of ash during recent years appears to relate to methods useful for the determination of ash in flour, and most of the papers were published in *Cereal Chemistry.* L. H. Bailey (1937) summarized the work on methods of ashing cereal products, much of which has been confined to a study of flour. He made a further study of the application of certain of these methods to the determination of flour ash and also to a limited extent the ashing of bran and low-grade flour. He concluded that the most satisfactory method was a modification of the magnesium-acetate method where, with bran, a two gram sample was used, three times the amount of magnesium-acetate solution was added, and ashing was continued for 1.5 hours. He states that the results duplicated those obtained by the official A.O.A.C. method. Six grams of anhydrous magnesium-acetate per liter of alcohol was used; 9 cc. of this solution was used with bran. The temperature of the muffle furnace was 700° C.

The magnesium-acetate method and other modified methods of ashing are described in *Cereal Laboratory Methods,* and in *This Journal,* **20**, 69. It was recommended by this Association that this method be adopted as official (first action), *Ibid.,* **21**, 389, for the determination of ash in flour, macaroni products, and baked products.

Blasdale, in his text on quantitative analysis, gives the following table for the dissociation pressure of calcium carbonate:

°C.	*mm.*
20—	2.2×10^{20}
200—	7.8×10^{9}
400—	0.3×10^{3}
500—	0.15
600—	2.98
700—	31.2
800—	208
882—	760
900—	984

He also states that "although precipitated calcium carbonate rapidly attains true equilibrium when heated, this is not true of naturally occurring samples. The latter can be heated to temperatures in excess of that named for several hours before appreciable decomposition occurs."

Willard and Furman, in their text on quantitative analysis, make the following statements in discussing the determination of calcium:

$$CaCO_3 \text{ (heated to 850° C.)} = CaO + CO_2.$$

This reaction is rapid at 850°, but begins at about 600°. At 550° the carbonate loses some carbon dioxide. The dissociation of the carbonate is a true equilibrium reaction. At 500° the dissociation pressure of calcium carbonate is 0.11 mm., a

value less than the partial pressure of carbon dioxide in ordinary air. Consequently, no dissociation of the carbonate will occur at this temperature. At 600° the dissociation pressure of calcium carbonate is 2.35 mm., and therefore carbon dioxide will slowly be lost. At 890° the dissociation pressure is 690 mm. A method of determining calcium as calcium carbonate by heating the oxalate to about 700° C. in an atmosphere of carbon dioxide has been described. Since calcium oxide is not reduced by carbon, no special care is necessary in burning off the filter. Care must be taken, however, to maintain a very high temperature during ignition and to heat the material sufficiently long to decompose all calcium carbonate. The dissociation is assisted by the removal of the carbon dioxide evolved, and it can be completed below 800° C. if the calcium carbonate is heated in a current of air free from carbon dioxide.

The A.O.A.C. method for feeds specifies that the material shall be burned at a low red heat not exceeding dull redness until free from carbon, but does not define these statements. The A.A.C.C. method specifies ignition at approximately 550° C. (dull red) until a light gray ash results, or until no further loss in weight occurs. The term "dull red heat" is indefinite. The ignition of a sample until it ceases to lose weight is impractical for many laboratories. Also, there is a question of the meaning of "free from carbon" when calcium carbonate is included in the sample. It is believed that the need for standardization of the ash method is evident, particularly in view of the results presented in this paper.

To collect further information regarding the results obtained by using different conditions in ashing the same material, the Associate Referee sent three samples to forty collaborators throughout the country, and after correspondence with them regarding the method to be used in the study this year, furnished the following method for this survey:

Weigh 2 gram samples of feed in porcelain crucibles, place in a cold muffle furnace, bring rapidly to the desired temperature, and continue at that temperature for the specified time.

The collaborators were requested to make duplicate determinations and report to the second decimal place. Each of the three samples was to be ashed at 550, 600, 650, and 700° C. for 2, 5, and 16 hours after the furnace reached temperature. It was specified that the temperature should be accurately controlled and measured with a pyrometer. Somewhat detailed information was requested regarding the kind of muffle used, the type of crucible, the accuracy with which it was possible to control the temperature, and other observations.

The three samples of feed submitted to the collaborators were based on a poultry and a dairy mixed ration recommended by the departments of the State College of Washington that work along these lines. The poultry ration was a chick starting mash with the following ingredients:

per cent
40 Ground yellow corn
10 Ground wheat

12 Finely ground heavy oats
15 Wheat bran
5 Dehydrated alfalfa
5 Meat scrap
5 Fish meal
5 Skim milk powder
2 Ground oyster shell or limestone
1 Salt
1 Biologically tested cod-liver oil or fish oil or its equivalent in concentrated Vitamin D

This ration, mixed by the poultry department, constituted Sample 1. It will be noted that it contains 2 per cent of calcium carbonate as ground oyster shell. Sample 2 was composed of an additional portion of this same ration to which a further 4 per cent of precipitated calcium carbonate had been added. Sample 3 was the dairy ration, composed of one-third peas, one-third barley, one-third oats, plus 1 per cent bone meal and 1 per cent sodium chloride. To this basic ration was added 10 per cent of precipitated calcium carbonate. The ration represented by Sample 1 ordinarily runs 6.5 per cent of ash, and that represented by Sample 3, without the calcium carbonate, ordinarily runs around 5.5 per cent of ash. If completely decomposed to calcium oxide during ashing the calcium carbonate incorporated in Sample 2 would add 2.24 per cent ash to the sample, while in Sample 3, 5.6 per cent ash would be added.

TABLE 1.—*Results on screened samples*

SAMPLE NO.	1	2	3
	per cent	*per cent*	*per cent*
20 mesh	2.7	4.7	7.1
40 mesh	30.0	36.7	49.3
60 mesh	20.9	17.0	16.2
80 mesh	26.3	17.8	12.5
100 mesh	8.7	5.0	3.1
Less than 100 mesh	10.1	17.3	9.8
	98.7	98.5	98.0

The poultry ration used in Samples 1 and 2 was very thoroughly ground through a Wiley mill with the coarse screen. It was then thoroughly mixed on a cloth and divided into two portions; the 4 per cent calcium carbonate was added to one portion, which was again very thoroughly mixed on a mixing cloth. Particular attention was given to the mixing of all samples. The dairy ration was ground through the Wiley mill, mixed, and then mixed with the calcium carbonate. Samples were placed in 4 ounce bottles with screw tops and shipped to the collaborators.

Recently weighed portions of all three samples were put through a screen. The results are given in Table 1.

TABLE 2.—*Collaborative results*

	Collaborator No.	550—2 hrs.			550—5 hrs.			550—16 hrs.			600—2 hrs.		
1. Adams	22	8.34	11.15	9.94	8.38	10.16	8.95				7.91	10.02	7.96
2. Becknell	30				9.28	12.21	11.11	9.21	11.86	9.63	9.00	11.83	10.50
3. Brooke	2	9.58	12.30	11.41	8.72	11.54	10.55	8.20	10.00	8.94	8.90	11.84	10.60
4. Clulow	33	9.61	12.34	11.50	8.35	11.93	8.31	9.11	11.22	8.06	8.46	10.00	7.82
5. Geagley	9	9.24	11.86	11.34	8.77	11.55	10.53	8.26	9.76	7.95	8.39	10.84	9.54
6. Grattan	10	9.92	12.57	11.82	9.88	12.53	11.72	9.88	12.51	11.71	9.71	12.50	11.45
7. Halvorsen	12							9.23	12.51	11.41	9.32	12.30	11.22
8. Hand	13	9.61	12.86	11.04	9.30	12.05	10.85	9.23	12.08	10.30	8.50	11.58	9.49
9. Haskins	15	9.60	11.98	11.17	8.43	10.12	8.67	8.48	10.13	7.98	8.19	10.08	7.92
10. King	17	8.75	11.85	10.15	8.52	11.07	9.43	7.82	10.07	7.77	7.73	11.24	
13. Montzheimer	34	8.27	10.84	9.56	8.30	10.11	8.06	8.33	10.16	8.16	8.21	9.81	8.18
14. Nixon	6	9.28	12.27	10.05	9.21	12.04	11.11	8.74	11.20	9.30	9.25	12.10	11.02
15. Randall	29	9.40	12.44	10.76	9.40	12.34	10.63	9.29	12.16	10.65	8.94	11.95	10.88
16. Struve	23	9.60	12.64	11.26	9.52	12.50	10.44	8.78	11.43	9.31	9.57	12.45	11.17
17. Tobey	24	9.84	12.36	11.22	8.40	10.78	9.67	8.33	10.19	8.01	8.76	11.78	9.78
18. Walker	25	9.54	12.93	11.35	9.48	12.44	11.18						
19. Ziegler	27	9.21	12.14	10.96	8.90	11.54	10.01	8.15	10.21	9.33	8.34	9.94	8.26
20. Walls											8.10	12.35	10.80
Averages		9.34	12.21	10.93	8.96	11.63	10.16	8.78	11.14	9.38	8.66	11.33	9.79

TABLE 2.—*Collaborative results*—(*Continued*)

	600—5 HRS.			600—16 HRS.			650—2 HRS.			650—5 HRS.			650—16 HRS.		
1.	8.16	10.04	8.04				8.21	9.76	7.85	7.85	9.85	7.62			
2.	8.27	11.41	8.19	8.22	9.87	7.88	8.26	10.01	8.16	8.12	9.99	8.01	7.93	9.73	7.47
3.	8.45	9.96	7.90	7.54	9.81	8.30	8.95	11.23	9.40	8.26	9.69	7.62	7.55	9.33	7.65
4.	8.20	10.03	7.83	7.75	9.40	7.32	7.81	10.04	7.82	7.30	9.43	7.62	7.17	8.65	7.43
5.	8.33	9.91	7.89	8.05	9.62	7.60	8.35	9.81	7.72	8.08	9.78	7.61	7.73	9.25	7.51
6.	9.26	12.13	10.93	8.50	11.11	9.69	8.67	11.60	10.53	8.41	10.45	8.58	8.43	10.27	8.48
7.	8.34	9.67	7.51	8.72	11.16	9.99	8.70	11.56	10.36	7.82	9.80	7.47	8.23	10.12	7.78
8.	8.29	10.02	7.91	7.88	9.94	7.71	8.51	10.37	7.79	8.14	10.04	7.77	7.99	9.70	7.60
9.	8.04	9.93	7.77	8.27	10.03	7.93	8.43	10.18	8.19	7.98	9.59	7.87	7.44	9.69	7.38
10.	7.87	10.43		7.99	10.84		7.93	10.27	7.17	7.82	10.22	8.08	7.54	10.03	7.91
13.	8.15	9.80	8.04	7.85	9.49	7.78	7.86	9.78	7.73	7.60	9.51	7.69	7.33	8.88	7.60
14.	8.66	12.02	11.03	8.01	9.91	7.93	8.97	11.79	10.83	8.08	10.06	7.75	7.39	9.43	7.63
15.	8.87	11.88	10.45	8.60	11.18	10.10	8.57	10.70	9.51	8.16	10.06	8.06	8.01	9.93	7.91
16.	9.23	11.71	10.47	9.27	11.40	8.38	9.40	12.22	10.92	8.35	10.56	8.34	8.45	10.25	8.16
17.	8.05	9.89	7.81	8.08	10.04	7.99	8.57	10.02	8.01	7.83	9.60	7.61	6.65	8.36	7.17
18.							8.50	10.16	9.25	8.29	10.09	8.59	7.75	9.77	7.84
19.	8.36	9.83	7.93	8.08	9.88	7.85	8.08	9.75	8.04	7.72	9.71	7.53	7.59	9.50	7.77
20.	8.30	11.15	9.38				8.01	10.07	8.02	8.00	10.37	8.00			
Av.	8.45	10.67	8.84	8.23	10.34	8.50	8.43	10.52	8.74	7.99	9.93	7.88	7.72	9.58	7.73

TABLE 2.—*Collaborative results*—(*Continued*)

	700—2 HRS.			700—5 HRS.			700—16 HRS.			750—2 HRS.			750—5 HRS.		
1.	8.19	9.69	7.88	7.75	9.62	7.41									
2.	8.18	9.85	7.74	8.04	9.88	7.74	7.31	9.22	7.49						
3.	8.58	10.12	7.53	8.24	9.59	7.32									
4.	7.16	8.86	7.78	7.24	8.73	7.48	6.95	8.64	7.11						
5.	7.90	9.69	7.71	7.62	9.45	7.60	7.28	8.85	7.50	7.63	8.94	7.32			
6.	8.67	10.00	8.04	8.45	10.00	8.00	8.24	9.80	7.85	8.52	9.92	7.98	8.19	9.86	7.92
7.	8.18	9.54	7.76	7.15	9.32	7.34	7.35	9.34	7.64						
8.	8.45	10.40	7.73	8.06	10.07	7.60									
9.	8.21	10.03	7.80	7.51	9.17	7.61	7.01	8.93	7.61						
10.	7.72	10.20	8.36	7.38	9.88	8.18	6.68	8.96	8.40						
13.	7.88	9.47	7.63	7.48	9.12	7.44	7.08	8.81	7.36						
14.	8.18	9.75	7.64	7.54	9.50	7.50	7.07	8.65	7.43						
15.	8.03	9.93	7.58	7.87	9.90	7.37	7.58	9.60	7.20	7.89	9.50	7.27	7.52	9.23	7.21
16.	8.68	11.10	9.43	8.02	9.80	7.36	8.01	9.80	7.35						
17.	7.10	9.00	7.44	6.78	8.53	7.37				7.16	9.03	7.38	6.85	8.43	7.27
18.															
19.	8.88	9.65	7.56	7.12	9.11	7.65									
20.	8.48	9.84	7.83	8.22	9.42	7.53									
Av.	8.15	9.83	7.84	7.73	9.53	7.66	7.37	9.20	7.55	7.80	9.35	7.50	7.52	9.17	7.47

Table 2.—*Collaborative results*—(*Continued*)

	750—16 hrs			800—2 hrs			800—5 hrs			800—16 hrs			850—2 hrs*		
1.															
2.															
3.															
4.															
5.										7.14	8.97	7.68			
6.	7.75	9.48	7.77	7.91	9.75	7.90	7.65	9.49	7.82	7.50	9.28	7.77	7.48	9.45	7.96
7.													7.16	8.90	7.43
8.															
9.															
10.															
13.													7.10	8.81	7.11
14.															
15.															
16.															
17.				6.82	8.56	7.32	6.78	8.57	7.44				6.78	8.43	7.23
18.															
19.															
20.															
Av.													7.13	8.90	7.43

* At 850—5 hrs., Collaborator 6 obtained 7.39, 9.28, and 7.95, and Collaborator 17 obtained 6.90, 8.58, and 7.37.
At 500—2 hrs., Collaborator 5 obtained 9.85, 11.80, and 10.20.

The results submitted by the 18 collaborators who reported are presented in Table 2. Each result is the average of the duplicate determinations submitted by a collaborator. The data in each vertical column, that is the average results of all collaborators on each sample for each condition under which ash was determined, are averaged, and these averages are collected in Table 3 for comparison. A major portion of the data covers temperatures from 550° through 700° for 2, 5, and 16 hours. Each average under these conditions represents the results obtained by fifteen to eighteen different collaborators, the number being sufficiently large so that it probably has some statistical significance. Since some data

TABLE 3.—*Average of collaborative results on ash*

TEMP.	TIME	1	2	3
°C.	*hours*	*per cent*	*per cent*	*per cent*
550	2	9.34	12.21	10.93
	5	8.96	11.63	10.16
	16	8.78	11.14	9.38
600	2	8.66	11.33	9.79
	5	8.45	10.67	8.84
	16	8.23	10.34	8.50
650	2	8.43	10.52	8.74
	5	7.99	9.93	7.88
	16	7.72	9.58	7.73
700	2	8.15	9.83	7.84
	5	7.73	9.53	7.66
	16	7.37	9.20	7.55
750	2	7.80	9.35	7.50
	5	7.52	9.17	7.47
850	2	7.13	8.90	7.43

were obtained from collaborators who also ashed at 750° and 850°, the averages are also presented in Table 3, the results being the average of the work of three or four collaborators in each case.

In Table 3 certain rather definite trends are evident. In practically all cases for each length of time and for all samples, the percentage of ash decreases as the temperature increases. In addition to this, at each temperature an increase in the length of time of ashing produces a lower percentage of ash. The percentage of ash in Sample 2 is consistently higher than it is in Sample 1, as would be expected. Sample 3 was intermediate in ash level between Samples 1 and 2, although for conditions beyond 600° at 5 hours the differences between Samples 1 and 3 are not large. Increasing the temperature to 750° and 850° further reduces the ash percentage.

In general, the results presented in Table 3 might be anticipated. However, they do not indicate the set of conditions most suitable and most practical for ash determination. They raise the question, "What is the percentage of ash in these three samples?" In an endeavor to approach an answer to this problem, certain comparatively simple statistical studies were made, including the probable error of the mean (PEm) of these averages, which is presented in Table 4. The probable errors indicate that it would not be difficult to duplicate the averages in Table 3 within 0.1 per cent if this study were repeated in the case of Sample 1, and with Samples 2 and 3 for conditions beyond 650° at 5 hours. For Samples 2 and 3, from 550° at 2 hours through 650° at 2 hours, the consistency in the results obtained by different investigators is not so desirable as indicated by the probable error of the mean, which varies from well over 0.1 per cent of about 0.25 per cent.

TABLE 4.—*Probable error of the mean of ash determination*

TEMP.	TIME	1	2	3
°C.	*hours*	*per cent*	*per cent*	*per cent*
550	2	.087	.102	.118
	5	.088	.153	.194
	16	.096	.172	.230
600	2	.095	.163	.238
	5	.070	.153	.226
	16	.077	.135	.197
650	2	.067	.123	.195
	5	.048	.055	.059
	16	.076	.088	.053
700	2	.081	.087	.075
	5	.081	.090	.079
	16	.091	.091	.074

The range in the results obtained by the different collaborators is also indicated in Table 5, where the difference between the maximum and minimum result obtained under each condition of ashing is tabulated. These differences between maximum and minimum results seem comparatively large, varying from slightly over 1 per cent to about 4 per cent. Here again it will be noted that the maximum ranges between collaborators are found in the case of Samples 2 and 3 under conditions ranging from 550° at 2 hours through 650° at 2 hours.

The Associate Referee also studied two or three methods of measuring the accuracy obtained by each collaborator. Each man was asked to make each determination in duplicate and to carry his results to the

second decimal place. The differences between duplicates for each condition under which ashing was done was determined for each one of the collaborators and is shown in Table 6. How well each collaborator was able to check his own results throughout this series of determinations by averages of the difference between duplicates for each condition of ashing is shown in Table 7. These averages vary from .04 to .64, with a fairly even distribution between these two values, as shown by the following series: .04, .07, .10, .13, .15, .15, .15, .16, .19, .20, .21, .24, .27, .29, .31, .44, .64.

In view of the range of results shown in Tables 3, 4, and 5, a second average of the differences between duplicates for all of the collaborators

TABLE 5.—*Maximum range between results obtained by collaborators*

TEMP.	TIME	1	2	3
°C.	*hours*	*per cent*	*per cent*	*per cent*
550	2	1.65	2.02	2.26
	5	1.58	2.55	3.66
	16	2.06	2.96	4.06
600	2	1.84	2.69	3.53
	5	1.48	2.55	3.71
	16	1.73	2.72	3.75
650	2	1.59	2.47	3.75
	5	1.11	1.13	1.12
	16	1.28	1.91	1.31
700	2	1.78	2.24	1.99
	5	1.93	1.96	1.93
	16	1.56	1.24	1.29

was made; it included mainly the results from 650° at 16 hours through 700° at 16 hours, together with those at 650° at 2 and 5 hours and 600° at 5 and 16 hours for Sample 1. These averages are presented in the second column of Table 7. The range of differences between duplicates was from .04 to .34. Those who obtained the greatest differences for the group as a whole showed a materially smaller result when the latter average is considered. A number of the remaining collaborators secured essentially as great an accuracy in the results at lower temperatures, so far as this is shown by the difference between the duplicate determinations, although in several cases improvement was shown.

From Table 6 averages were also made of the difference between duplicates for each condition of ashing. These were combined in Table 8 to indicate the facility with which the collaborators, as a group, may check their own results when determining ash under each of the specified conditions. While in general the variations between duplicates for Samples 2

Table 6.—*Differences in duplicate results of collaborators*

	COLLABORATOR NO.	550—2 HRS			550—5 HRS			550—16 HRS			600—2 HRS		
1. Adams	22	.02	.07	.01	.09	.05	.01				.03	.05	.04
2. Becknell	30				.03	.15	.24	.29	.84	.51	.36	.69	.59
3. Brooke	2	.01	.33	.14	.08	.34	.20	.20	.15	.23	.14	.06	.23
4. Clulow	33	.35	.21	.18	.03	.05	.31	.08	.33	.66	.16	.05	.00
5. Geagley	9							.22	.16	.12	.08		
6. Grattan	10	.43	.06	.47	.42	.06	.48	.43	.06	.47	.18	.22	.08
7. Halvorsen	12				.34	.20	.02		.08	.48	.04	.06	.42
8. Hand	13	.17	.24	.36	.33	.20	.02	.65	.19	.23	.13	.17	.33
9. Haskins	15	.26	.19	.26	.34	.33	.42	.32	.04	.02	.33	.34	.04
10. King	17	.51	.10	1.15	.19	.65	1.11	.34	.13	.87	.31	1.12	3.82
13. Montzheimer	34	.05	.52	.39	.10	.12	.20	.07	.12	.08	.03	.01	.05
14. Nixon	6	.21	.31	.16	.05	.40	.17	.33	.23	.00	.18	.42	.11
15. Randall	29	.05	.01	.05	.07	.02	.07	.04	.12	.16	.00	.02	.08
16. Struve	23	.34	.40	.06	.13	.14	.02	.23	.80	.96	.04	.28	.06
17. Tobey	24	.03	.32	.00	.10	.09	.43	.07	.26	.21	.19	.49	.09
18. Walker	25												
19. Ziegler	27							.00	.02	.55	.33	.15	.12
Averages		.22	.23	.25	.16	.19	.27	.24	.23	.37	.21	.34	.42

TABLE 6.—*Differences in duplicate results of collaborators*—(*Continued*)

	600—5 HRS.			600—16 HRS.			650—2 HRS.			650—5 HRS.			650—16 HRS.		
1.	.04	.04	.01				.02	.04	.01	.05	.06	.04			
2.	.04	.35	.24	.14	.05	.19	.22	.13	.48	.08	.08	.25	.21	.10	.21
3.	.11	.19	.01	.25	.15	.10	.29	.03	.08	.21	.19	.10	.20	.17	.03
4.	.01	.22	.12	.13	.24	.12	.14	.31	.07	.22	.13	.18	.11	.11	.20
5.	.16	.30	.12	.54	.02	.14	.28	.15	.11	.17	.17	.04	.19	.12	.03
6.	.07	.22	.34	.12	.65	1.22	.04	.54	.58	.02	.46	.75	.02	.15	.69
7.	.16	.01	.64	.18	1.28	1.45	.78	1.35	1.23	1.41	.04	.33	.12	.65	.15
8.	.13	.08	.19	.04	.02	.16	.23	.45	.13	.16	.17	.02	.13	.19	.04
9.	.09	.08	.09	.31	.17	.13	.28	.10	.44	.12	.31	.28	.25	.12	.01
10.	.24	.40	3.25	.02	.87	1.58	.10	.35	.96	.07	.37	.95	.18	.45	.89
13.	.09	.00	.07	.03	.02	.04	.01	.04	.04	.01	.02	.02	.05	.03	.01
14.	.35	.37	.62	.06	.17	.00	.21	.00	.01	.13	.38	.17	.26	.17	.06
15.	.00	.05	.16	.24	.35	.63	.02	.30	.08	.03	.10	.07	.04	.05	.08
16.	.11	.08	.58	.13	.22	.12	.32	.09	.03	.19	.72	1.04	.43	.00	.03
17.	.11	.33	.11	.18	.32	.08	.12	.16	.02	.19	.15	.09	.00	.12	.10
18.							.18	.34	.26	.17	.32	.32	.08	.19	.02
19.	.28	.05	.05	.15	.03	.10	.07	.05	.07	.20	.28	.05	.15	.20	.20
Av.	.13	.18	.40	.16	.30	.39	.20	.30	.27	.19	.24	.27	.15	.18	.17

TABLE 6.—*Differences in duplicate results of collaborators*—(*Continued*)

	700—2 HRS.			700—5 HRS.			700—16 HRS.			750—2 HRS.			750—5 HRS.			750—16 HRS.		
1.	.08	.07	.03	.01	.01	.07												
2.	.14	.33	.14	.12	.23	.10	.14	.03	.09									
3.	.10	.22	.09	.14	.08	.17												
4.	.16	.06	.03	.03	.00	.20	.02	.16	.05									
5.	.31	.10	.03	.33	.17	.00	.01	.08	.00	.01								
6.	.33	.19	.17	.35	.18	.15	.34	.15	.15	.54	.03	.10	.46	.03	.08	.43	.04	.06
7.	.01	.54	.01	.26	.37	.51	.05	.24	.02									
8.	.29	.34	.31	.26	.22	.09												
9.	.02	.08	.08	.12	.06	.17	.04	.39	.26									
10.	.01	.10	.62	.11	.25	.70	.03	.03	.12									
13.	.04	.05	.06	.03	.05	.08	.03	.03	.03									
14.	.31	.20	.04	.00	.09	.16	.08	.18	.08									
15.	.00	.05	.13	.01	.01	.16	.06	.01	.13	.06	.01	.01	.03	.07	.03			
16.	.09	.19	1.43	.05	.15	.05	.10	.02	.25									
17.	.11	.21	.13	.09	.11	.13				.11	.03	.19	.00	.24	.18			
18.																		
19.	.23	.00	.02	.00	.08	.10												
Av.	.14	.17	.22	.13	.16	.17	.08	.13	.11									

TABLE 7.—*Average difference between duplicates for each collaborator*

COLLABORATOR	TOTAL	LIMITED
22	.04	.04
30	.24	.15
2	.15	.16
33	.15	.10
9	.15	.16
10	.31	.19
12	.44	.34
13	.20	.19
15	.19	.14
17	.64	.25
32	.29	.19
34	.07	.04
6	.19	.15
29	.10	.06
23	.27	.14
24	.16	.12
27	.13	.13

and 3 under conditions ranging from 550° at 2 hours to 650° at 5 hours may be somewhat greater than in the remainder of the cases, one might conclude from inspection of this table, in comparison with Tables 3, 4, and 5, that the individual collaborators checked their own results for the different conditions more satisfactorily or with a greater consistency than is the case when the results of the different collaborators are compared. However, in the opinion of the Associate Referee the average difference between duplicates, as indicated by Column 2 in Table 7 and also by Table 8, is materially larger than is desirable.

TABLE 8.—*Average difference between duplicates for each set of conditions under which ashing is done*

TEMP.	TIME	1	2	3
°C.	*hours*	*per cent*	*per cent*	*per cent*
550	2	.22	.23	.25
	5	.16	.19	.27
	16	.24	.23	.37
600	2	.21	.34	.42
	5	.13	.18	.40
	16	.16	.30	.39
650	2	.20	.30	.27
	5	.19	.24	.27
	16	.15	.18	.17
700	2	.14	.17	.22
	5	.13	.16	.17
	16	.08	.13	.11

It is anticipated that with the same method the experience of various laboratories would be the same as that in this laboratory, where an average of the differences between duplicates in ashing 70 samples selected at random from a regular run of control samples showed an average variation between duplicates of .060, very few results running above .10. Over a third of this group of samples of concentrated commercial feeding stuffs contained shell flour or calcium carbonate in some form or other.

The second method of determining the facility or accuracy with which the different collaborators obtained ash results under each of the specified conditions may be measured by a determination of the probable error of the average results obtained by each collaborator for each of the

TABLE 9.—*Probable error of the average of ash collaborators' results for each ashing condition*

TEMP.	TIME	1	2	3
°C.	*hours*	*per cent*	*per cent*	*per cent*
550	2	.337	.394	.455
	5	.354	.612	.778
	16	.406	.731	.975
600	2	.391	.672	.951
	5	.298	.648	.932
	16	.307	.541	.763
650	2	.285	.524	.826
	5	.196	.228	.241
	16	.314	.364	.217
700	2	.335	.358	.310
	5	.344	.330	.309
	16	.317	.314	.226

specified conditions. The probable error of a single determination (PEs), that is the probable error of each man's average, is presented in Table 9. The probable error of a single determination in this instance indicates that the average obtained by any one collaborator has a fifty-fifty chance of coming within the plus or minus range of the quantity in Table 9 of the average. Again, in the opinion of the Associate Referee, these results are greater than the allowable variation.

Considering Table 9, one might conclude from the results for Sample 1 that the accuracy of any one man's result for the different conditions of ashing was essentially the same, and also that these accuracies were essentially the same as those for Samples 2 and 3 for the conditions between 650° at 5 hours and 700° at 16 hours. However, the facility with which the different collaborators may determine the ash in Samples 2 and 3, containing comparatively large quantities of calcium carbonate, under conditions varying from 550° for 2 hours through 650° for 2 hours,

is decidedly less. The probable error of a single determination in this latter group varies from about .40 to nearly 1.0, indicating rather definitely that with the large amounts of calcium carbonate in the samples different collaborators may not be expected to obtain satisfactory ash results. Other forms of calcium carbonate may show less variation.

A rather simple comparison is made in Table 10, which presents the difference between the percentage of ash for these samples as shown in Table 3. In Table 10 the difference in the percentage of ash between Samples 1 and 2, 2 and 3, and 1 and 3 is shown. It will be noted here that the percentage of ash in Samples 1 and 3 is very nearly the same when comparison is made of the results obtained by ashing under conditions varying from 600° at 5 hours to 700° at 16 hours. It is believed that this

TABLE 10.—*Difference in amount of ash between three samples for each condition*

TEMP.	TIME	2–1	2–3	3–1
°C.	*hours*	*per cent*	*per cent*	*per cent*
550	2	2.87	1.28	1.59
	5	2.67	1.47	1.20
	16	2.36	1.76	.60
600	2	2.67	1.54	1.13
	5	2.22	1.83	.39
	16	2.11	1.84	.27
650	2	2.09	1.78	.31
	5	1.94	2.05	−.11
	16	1.86	1.85	.01
700	2	1.68	1.99	−.31
	5	1.80	1.87	.07
	16	1.83	1.65	.18

tends to confirm the observation that ashing conditions of 600° at 5 hours and beyond are more satisfactory for determinations on samples containing calcium carbonate.

It is also interesting to compare the results obtained in this laboratory on these samples when determinations were made by the method in use. Two gram samples are placed in a cold muffle at night, and the rheostat is set so that the furnace reaches a temperature of 650° and maintains this temperature throughout the night. These determinations were made in the regular course of control work and placed in the muffle with control samples. This was the first and only set of determinations made under these conditions. The results are as follows:

	1	2	3
	8.29	10.21	8.13
	8.44	10.26	8.14
	8.37	10.24	8.14
Av. difference	.15	.25	.01

In addition to the work suggested two collaborators made ash determinations at the different temperatures, placing the samples in a hot muffle and ashing for 2 hours. These results are presented in Table 11. In general it appears from these results that this method gives a percentage of ash lower than is obtained under the same condition when the sample is put in a cold muffle, and results are more nearly equal to the results obtained when ashing is continued for a much longer period of time if the sample is put into a cold muffle.

TABLE 11.—*Results obtained by two collaborators placing sample in hot oven*

TEMP.	TIME	1	2	3	$CaCO_3$
°C.	*hours*	*per cent*	*per cent*	*per cent*	*per cent*
550	2	9.69	12.47	10.25	
600	2	8.33	9.83	7.92	97.07
		8.92	11.44	10.23	97.32
650	2	8.18	9.60	7.56	92.40
		8.58	10.02	8.27	93.24
700	2	7.14	8.93	7.63	58.32
		8.27	10.06	8.31	62.64

TABLE 12.—*Percentage of moisture reported by collaborators*

COLLABORATOR	1	2	3
22	8.78	4.05	8.43
2	7.95	7.64	8.42
33	9.0	8.65	9.12
9	8.46	8.45	8.92
10	9.53	8.45	9.50
17	9.27	11.18	8.19
32	—	—	—
34	7.8	7.6	8.6
6	9.78	9.26	9.21
24	7.86	7.30	8.34
25	8.96	8.49	8.93
27	8.65	8.25	9.00

The collaborators were asked to determine the percentage of moisture in the samples at the time they were weighed out for the ash determination. The results are reported in Table 12. In view of the rather wide variations in the moisture determinations themselves no attempt was made to convert the ash results to the same moisture basis in all cases. It is possible that further work needs to be done on the moisture determination.

In view of the results presented in this paper a number of points seem evident to the Associate Referee.

(1) There is a decided need for a standardization of conditions under which ash determinations are made.

(2) The conditions to which the method should be standardized are not sufficiently evident.

(3) Further work may be needed on the standardization of the method for ash determination on samples that do not contain calcium carbonate as well as those that do.

(4) Especially where larger quantities of calcium carbonate are present the results suggest that the ashing should be done at a temperature of 650° or above for at least 5 hours.

(5) Because of the variation in results obtained by different collaborators, it is probable that a much closer adherence to the specified conditions is essential.

(6) The placing of the samples in a hot muffle and the use of methods such as the magnesium acetate and other modified methods should be investigated.

(7) It is recommended[1] that the collaborative work on ash determination in feeds both with and without calcium carbonate be continued.

TABLE 13.—*Coefficient of variations*

TEMP.	TIME	1	2	3
°C.	*hours*	*per cent*	*per cent*	*per cent*
550	2	5.35	4.79	6.17
	5	5.84	7.80	11.3
	16	6.85	9.73	15.4
600	2	6.69	8.79	14.4
	5	5.21	9.00	15.6
	16	5.54	7.75	13.3
650	2	5.00	7.38	14.0
	5	3.64	3.40	4.54
	16	6.02	5.64	4.15
700	2	6.09	5.40	5.85
	5	6.59	5.15	5.99
	16	6.36	5.05	4.42

TABLE 14.—*T test*

TEMP.	TIME	1	2	3
°C.	*hours*	*per cent*	*per cent*	*per cent*
550	2	.467	.546	.630
	5	.492	.851	1.080
	16	.569	1.024	1.365
600	2	.546	.938	1.321
	5	.417	.907	1.300
	16	.427	.752	1.056
650	2	.398	.733	1.156
	5	.274	.317	.336
	16	.438	.509	.302
700	2	.4679	.500	.433
	5	.482	.462	.433
	16	.428	.4271	.310

[1] For report of Subcommittee A and action by the Association, see *This Journal*, **22**, 50 (1939).

TABLE 15.—*Sigma*

TEMP.	TIME	1	2	3
°C.	hours	per cent	per cent	per cent
550	2	.500	.585	.675
	5	.524	.908	1.153
	16	.602	1.084	1.446
600	2	.580	.997	1.410
	5	.441	.961	1.382
	16	.456	.802	1.132
650	2	.422	.777	1.224
	5	.291	.338	.358
	16	.465	.540	.321
700	2	.497	.531	.459
	5	.510	.490	.459
	16	.469	.465	.334

REPORT ON MINERAL MIXED FEEDS*

By ALFRED T. PERKINS, *Associate Referee,* and B. W. BEADLE
(Kansas Agricultural Experiment Station, Manhattan, Kan.)

The major part of the Associate Referee's work was devoted to the tentative method for calcium oxide in mineral feeds. Some progress was also made on an iodine method, but at present the data do not warrant a report.

The calcium oxide method, *Methods of Analysis, A.O.A.C.*, 1935, 347, 44, corrected in line 8 by changing "(pH 3.0−4.4)" to "(pH 2.5−3.0)" and in line 9 by changing "20–30 cc." to "10 cc.," was tested by the collaboration of over 20 chemists.

The analyses were made on three samples of high-lime feeds to which impurities had been added in order to give the method a severe test. Sample A consisted of pieces of limestone gathered at random from the surrounding terrain. The rocks were crushed and then ground in a Braun mill, equipped with steel disks, to pass an 80-mesh sieve. After sieving, the sample was thoroughly mixed, reground, and remixed, and after a third grinding and mixing was bottled in 4 oz. sample bottles. Sample B consisted of 56 per cent of Sample A and 44 per cent of impurities as $CuSO_4$, $Fe_2(SO_4)_3$, $KMnO_4$, Derby Soil, KI, $Al_2(SO_4)_3 \cdot (NH_4)_2SO_4 \cdot 24H_2O$, and Filtercel. The non-limestone portion was handled as a unit, ground to pass an 80-mesh sieve, thoroughly mixed, and then added to the limestone.

* Contribution No. 240 from the Department of Chemistry.

After the two portions had been mixed, the sample was reground and remixed three times and then bottled in 4 oz. sample bottles. Sample C consisted of 32 per cent of Sample A and 68 per cent of impurities such as $MgSO_4 \cdot 7H_2O$, Na_2HPO_4, $Fe_2(SO_4)_3$, bone charcoal, boiler scale, cottonseed meal, and wheat mill tailings. The same mechanical methods that were used in preparing Sample B were used for Sample C. Considerable supervised N.Y.A. labor was used in preparing the samples.

TABLE 1.—*Collaborators**

1. B. W. Beadle, 2. A. T. Perkins, 3. L. I. Miller, Kansas State College, Manhattan, Kan.
4. Oscar I. Struve, Eastern States Cooperative Milling Corp. Buffalo, N. Y.
5. H. R. Kraybill and P. B. Curtis, Purdue University, Lafayette, Ind.
6. Geo. E. Grattan and C. V. Marshall, Department of Agriculture, Ottawa, Canada.
7. E. R. Tobey and C. Harry White, Maine Agricultural Experiment Station, Orono, Me.
8. Richard O. Brooke, Wirthmore Research Laboratory, Malden, Mass.
9. Geo. H. Marsh and W. J. Marsh, Alabama Department of Agriculture and Industries, Montgomery, Ala.
10. Arthur L. Haskins, Pennsylvania State College, State College, Penn.
11. W. F. Hand, Mississippi State College, State College, Miss.
12. J. Frampton King, Department of Agriculture, Atlanta, Ga.
13. G. S. Fraps and J. F. Fudge, Agricultural and Mechanical College of Texas, College Station, Tex.
14. C. C. Zeigler, Swift and Company, Union Stock Yards, Chicago, Ill.
15. H. H. Hanson, State Board of Agriculture, Dover, Del.
16. W. B. Griem and Wenzel S. Thompson, Wisconsin Department of Agriculture and Markets, Madison, Wis.
17. Hugo W. Nilson and Arthur L. Fowler, Bureau of Fisheries, College Park, Md.
18. F. E. Randall, Cooperative G. L. F. Mills, Inc., Buffalo, N. Y.
19. T. H. Burton, Alabama Department of Agriculture and Industries, Auburn, Ala.
20. W. C. Geagley and M. M. Nasif, Department of Agriculture, Lansing, Mich.
21. L. S. Walker and E. F. Boyce, Vermont Agricultural Experiment Station, Burlington, Vt.
22. L. M. Nixon and H. D. Matheson, North Carolina Department of Agriculture, Raleigh, N. C.

* Collaborators are listed in the order in which the reports were received.

The three samples were sent out to 27 chemists who had signified willingness to collaborate. Twenty-one had reported at the time of writing this report, and these results have been considered in the discussion. Those reporting are listed in Table 1 with an identifying number.

DISCUSSION

The results submitted by the 21 collaborators reporting by the time the paper was prepared have been considered in the calculations and discussion.

The three sets of results obtained in the Kansas State College Laboratory were obtained entirely independently, except that the same apparatus and stock chemicals were used. Different sources of sodium oxalate were used as a standard for the potassium permanganate, but they had been compared with a sample of Bureau of Standards sodium oxalate. One of the three analysts was an undergraduate chemist.

TABLE 2.—*Collaborative results on calcium oxide and deviation from average**

COLLABORATION NO.	CaO (PER CENT)			DEVIATION FROM AVERAGE (PER CENT)			AV.
	SAMPLE			SAMPLE			
	A	B	C	A	B	C	
8	49.62	28.40	20.25	−0.56	−0.46	−0.33	−0.45
5	49.83	28.13	20.33	−0.35	−0.73	−0.25	−0.44
18	49.85	28.41	20.14	−0.33	−0.45	−0.44	−0.41
16	50.07	28.36	20.24	−0.11	−0.50	−0.34	−0.32
2	49.81	28.49	20.40	−0.37	−0.37	−0.18	−0.31
3	49.87	28.48	20.44	−0.31	−0.38	−0.14	−0.28
20	50.10	28.60	20.24	−0.08	−0.26	−0.34	−0.23
6	49.89	28.64	20.45	−0.29	−0.22	−0.13	−0.21
10	49.91	28.57	20.51	−0.27	−0.29	−0.07	−0.21
1	49.85	28.59	20.57	−0.33	−0.27	−0.01	−0.20
21	50.27	28.66	20.40	+0.09	−0.20	−0.18	±0.16
19	50.12	28.98	20.45	+0.06	+0.12	−0.13	±0.10
9	50.22	28.88	20.56	+0.04	+0.02	−0.02	±0.03
11	50.30	28.93	20.55	+0.12	+0.07	−0.03	±0.07
14	50.20	29.28	20.92	+0.02	+0.42	+0.34	+0.26
15	50.40	29.30	20.76	+0.22	+0.44	+0.18	+0.28
4	50.73	29.25	20.76	+0.55	+0.39	+0.18	+0.37
12	50.57	29.24	21.19	+0.39	+0.38	+0.61	+0.46
17	50.43	29.98	20.76	+0.25	+1.12	+0.18	+0.52
7	50.68	29.31	21.21	+0.50	+0.45	+0.63	+0.53
13	51.10	29.60	20.96	+0.92	+0.74	+0.38	+0.68
Av.	50.18	28.86	20.58	±0.29	±0.39	±0.24	±0.31
22	50.13	28.78	20.42	−0.05	−0.08	−0.12	−0.08

* Listed in order of average plus and minus deviations from average.

The average of all 21 analyses reported for each sample was made. No satisfactory method was apparent to eliminate certain figures that were rather far from the average.

Table 2 shows that, of the 63 individual calcium oxide reports received, seven varied from the average more than 0.5 per cent and 34 varied more than 0.25 per cent. All collaborators showed remarkable consistency in reporting high or low results. Of the 21 analysts reporting, 10 reported all

their results below the average, seven reported all their results above the average, and only four reported results above and below the average. These four had the lowest average deviations; *i.e.*, 0.03 per cent, 0.07 per cent, 0.10 per cent, and 0.16 per cent, and thus reported most nearly average results. The average deviation of all reports was 0.31 per cent, the maximum average deviation for one chemist was 0.68 per cent, and the maximum single deviation was 1.12 per cent. These figures show that a laboratory was apt to report high, low, or average results on all three samples. This seems to indicate that where laboratories do not check, there is a constant difference in the laboratories rather than a fault in the method.

It is believed that the main inaccuracies of the method are those that are inherent in any method and that the greatest improvement in the method can be made by the use of a uniform standard.

The results reported by the collaborators with the deviations from the average are given in Table 2.

COMMENTS OF COLLABORATORS

No. 13.—Suggests use of 100 cc. volumetric and 10 cc. aliquot and 0.07143 *N* $KMnO_4$ instead of 0.1000 *N*. Also suggests combustion temperature of 600°.

No. 15.—Checked Samples A by a CO_2 method and reported 50.41 per cent compared to 50.40 per cent.

No. 16.—Mentions method of adjusting *p*H caused difficulty as Samples A and B became alkaline on standing. Also reported somewhat higher results by method of Clifcorn, *This Journal*, **16**, 240.

No. 17.—Filtered samples before taking aliquots. Reported use of $600° \pm 10°$ for ignition.

RECOMMENDATIONS[1]

It is recommended—

(1) That preliminary work be continued on method for small amounts of iodine in mineral feeds.

(2) That study of the method for the determination of calcium in mineral feeds be continued. Additional collaborative work should be done and the potassium permanganate used should be standardized by the usual method in each laboratory and against a sample of sodium oxalate submitted with the samples. Results to be on moisture basis as received and on dry basis.

REPORT ON LACTOSE IN MIXED FEEDS

By D. A. MAGRAW (American Dry Milk Institute, Inc., Chicago, Ill.), *Associate Referee*

The collaborative work done during 1937 indicated that it was necessary to formulate a more satisfactory correction blank, determine the maxi-

[1] For report of Subcommittee A and action by the Association, see *This Journal*, **22**, 50 (1939).

mum and minimum fermentation temperatures, and ascertain whether alcohol can be used to eliminate the interference of peanut meal and low-grade tankage.

To study these phases of the method five samples of feed were hand mixed. They contained the more common feed ingredients used in commercial feed mixes. The dry skim milk used contained 51.4 per cent lactose as determined by the Munson and Walker method.

The five samples were then sent to the collaborators with the following instructions:

Directions for Collaborative Study on Determination of Lactose in Mixed Feed (1938)

The study involves the use of five samples, which will be investigated as follows:

I. *Maximum and minimum fermentation temperature.*
   a. Samples 1 and 2, follow Method I, which is attached, using a fermentation temperature falling in the range of 75°–80° F.
   b. Samples 1 and 2, follow Method I, using a fermentation temperature falling in the range of 80°–85° F.
   c. Samples 1 and 2, follow Method I, using a fermentation temperature falling in the range of 85–90° F.

II. *Alcohol as precipitating agent for possible elimination of interference by peanut meal and low-grade tankage.*
   a. All five samples, follow Method I, using a fermentation temperature of 80°–85° F.
   b. All five samples, follow Method II, using a fermentation temperature of 80°–85° F.

III. *Reporting of data*

Report all determination, giving (1) mg. of CuO, (2) percentage of lactose as found by formula, (3) temperature of fermentation used on each determination, (4) any abnormalities, and (5) comments.

TABLE 1.—*Feed formulas*

SAMPLE NO.	1	2	3	4	5
Ground corn	27½	27½	22½	22½	22½
Fine ground oats	10	10	10	10	10
Wheat bran	10	10	10	10	10
Ordinary flour middlings	20	20	20	20	20
Alfalfa leaf meal	5	5	5	5	5
Fish meal	2½	2½	2½	2½	2½
Soybean oil meal	5	5	5	5	5
Limestone	2	2	2	2	2
Steamed bone meal	2	2	2	2	2
Salt	1	1	1	1	1
Meat scraps	5	5	—	—	—
Cottonseed meal	5	—	5	4½	—
Gluten feed	5	—	5	4½	—
Peanut oil meal	—	—	5	5	5
Low grade tankage	—	—	5	5	5
Dry skim milk	—	10	—	1	10

Method 1 was published in *This Journal*, **19**, 605. Method 2 is changed as follows: An equal amount of 95 per cent ethyl alcohol is added to the filtrate after the water extraction, the precipitate formed is removed by centrifugalization, and the alcohol is boiled off and the solution washed into a 200 cc. volumetric flask. The appropriate changes are made in the final calculations to allow for changes in volume, and no blank is taken off.

TABLE 2.—*Various fermentation temperatures*

METHOD		NO. 1 NO ALCOHOL 6 MG. LACTOSE BLANK			NO. 2 WITH ALCOHOL NO BLANK SUBTRACTED		
SAMPLE NO.	TEMP. OF FERMENTATION / COLLABORATOR NO.	75°–80° F.	80°–85° F.	85°–90° F.	75°–80° F.	80°–85° F.	85°–90° F.
		LACTOSE (PER CENT)					
1	1	.27	.19	0		.13	
Blank						.00	
	2					.00	
	3	.75	.456	.42		.88	
	4		.0	.0			
			.0	.0			
	5		.72				
			.61				
			.15				
	6		.85	.26		.14	
			.0	.22		.14	
			.83	.27			
	7			.0		.0	
				.0			
2	1	5.18	5.53	4.84		3.21	
5.14%	2					4.96	
						4.96	
	3	6.16	4.94	5.23		5.99	
		6.25	4.86	4.67		5.47	
	4		4.12	3.91			
			4.30	4.05			
	5		5.21				
			5.36				
			4.38				
	6		5.28	4.74		4.42	
			5.15	4.63		4.17	
				4.57		4.54	
	7		5.44			5.37	
			5.57				

RESULTS

The results of the analyses made by the seven collaborators are presented in Tables 2 and 3. The following are the collaborators:

1. A. M. Besemer, San Francisco, Calif.
2. A. H. Johnson, Baltimore, Md.
3. O. I. Struve, Buffalo, N. Y.
4. S. E. Danielson, Akron, Ohio.
5. P. B. Curtis, Lafayette, Ind.
6. W. S. Thompson, Madison, Wis.
7. L. L. Lachat, Minneapolis, Minn.

The results obtained by the collaborators are not sufficiently complete to warrant definite conclusions as to the effect on the recovery of the lactose of different temperatures of fermentation. This was undoubtedly due to work required. At the time the instruction sheets were sent out it was not realized that the amount of work involved was so great.

TABLE 3.—*Methods 1 and 2 at 80°–85° F. fermentation temperature on Samples 3, 4, and 5*

COLLABORATOR	SAMPLE 3 BLANK		SAMPLE 4 .51% LACTOSE		SAMPLE 5 5.14% LACTOSE	
	METHOD 1	METHOD 2	METHOD 1	METHOD 2	METHOD 1	METHOD 2
1	.65	0	1.09	.17	5.23	3.32
2		0		.34		4.68
		0		.30		4.71
3	.45	.52	.95	.98	5.68	5.85
	.32	.39	.82	.96	5.59	5.46
4	.02	.04			3.37	4.68
5	.07		.20		3.81	
	.29		.36		3.79	
			.07		3.89	
6	.98	0	1.26	.23	4.99	4.84
	.53	0	1.40	.41	5.14	4.76
	.95	0	1.52	.17		
7	0	.07	.63	.57	5.59	5.74
	0		.65		5.24	

The collaborative results are erratic and in many cases are too low, and they may be accounted for by the following comments made by W. S. Thompson:

No difficulty was encountered in clarification of samples. Variations in results might be explained by the poor quality of some of the yeast used. As large cakes of yeast are not necessary and are uneconomical to buy, and because it is not known under what conditions the yeast has been kept at the bakers (who usually sell the scraps of the cake left from baking), it appears that the method should be

worked out for the small cakes. These are always covered and kept cold and are more convenient to obtain than the other type of yeast. It seems that the yeast used may be the source of greatest error.****

Owing to this comment a letter was sent to the collaborators asking for information on the types of yeast used, and it was found that in many cases old baker's yeast or Fleischmann's small cake yeast (which

TABLE 4.—*A.D.M.I. results by use of Fleischmann's small cake yeast*

METHOD	NO. 1, NO ALCOHOL 6 MG. LACTOSE BLANK SUBTRACTED			NO. 2, WITH ALCOHOL NO BLANK SUBTRACTED		
TEMP. OF FERMENTATION	75°–80° F.	80°–85° F.	85°–90° F.	75°–80° F.	80°–85° F.	85°–90° F.
SAMPLE	LACTOSE (PER CENT)					
1			0		.12	0
Blank			0		.16	0
					.10	
					.12	
2			4.93		4.75	4.68
5.14%			5.06		4.79	4.46
					4.87	
					4.73	
3			0		.11	.05
Blank			0		.09	.05
4			.41–.65		.58	.53
.51%			.42–.55		.51	
5		4.72	4.77		4.59	4.56
5.14%		5.10	4.67		4.84	4.69
					4.77	
					5.07	

can be bought in drug and grocery stores) was used. The effect on the recovery of lactose of the use of the small cakes of yeast was also verified in this laboratory quite by accident since the small yeast cakes were used during a period when the refrigerator was being repaired and it was not possible to keep the large one-pound cakes of Fleischmann's baker's yeast. These results are given in Table 4.

Table 5 gives the results obtained in the American Dry Milk Institute laboratories when only Fleischmann's fresh baker's yeast was used.

CONCLUSIONS

The following conclusions seem to be consistent with the results obtained this year:

(1) The fermentation temperature of 75°–80° F. with Method I is too low and gives higher results.

(2) 80°–85° F. is satisfactory with Method I except in the presence of peanut meal.

(3) 85°–90° F. with Method I appears to be too high in some cases, and therefore unsatisfactory.

(4) The fermentation temperature of 75°–80° F. with Method II is fairly satisfactory.

(5) 80°–85° F. with Method II is satisfactory.

(6) 85°–90° F. with Method II is fairly satisfactory.

(7) Peanut meal appears to present an interference with Method I, which is eliminated in Method II.

(8) The small Fleischmann yeast cakes usually give low results with the present correction factors.

(9) Old yeast, either baker's yeast or the small cakes, is unsatisfactory.

TABLE 5.—*A.D.M.I. results with Fleischmann's baker's yeast*

METHOD	NO. 1, NO ALCOHOL 6 MG. OF LACTOSE BLANK SUBTRACTED			NO. 2, WITH ALCOHOL NO BLANK SUBTRACTED		
TEMP. FERMENTATION	75°–80° F.	80°–85° F.	85°–90° F	75°–80° F.	80°–85° F.	85°–90° F.
SAMPLE	LACTOSE (PER CENT)					
1	.67	.26	.007	.34	.05	.15
Blank	.73	.26	.10	.00	.10	.0
	.74	.34		.38	.08	
	.47	.17			.11	
2	5.95	5.28	4.87	5.28	5.01	5.17
5.14%	5.48	5.15	5.16	5.20	5.05	5.13
	4.94	4.97		5.10		
	5.35			5.14		
3	1.01	.53	0	.09	.08	.20
Blank	.62	.52	0	.00	.10	.00
	.29	.25		.26	.11	.11
	.37	.25		.26		
4	1.01	1.11	.33	.78	.67	.55
.51%	1.21	1.11	.13	.78	.86	.53
	.78	1.19		.58		.75
		.58				.76
5	5.13	5.05	5.12	5.14	5.08	4.70
5.14%	5.37	5.12	5.17	5.03	4.63	4.75
	5.01			4.94	4.93	

It is recommended that the study be continued and that consideration be given to working out a correction factor whereby the Fleischmann's

[1] For report of Subcommittee A and action by the Association, see *This Journal*, 22, 51 (1939).

small yeast cakes or some other small yeast cakes may be used and to the use of alcohol for elimination of interference from peanut meal and low-grade tankage.

No report on moisture in feeding stuffs was given by the associate referee.

REPORT ON BIOLOGICAL METHODS FOR ASSAY OF VITAMIN D CARRIERS

By W. B. Griem (Wisconsin Department of Agriculture and Markets, Madison, Wis.), *Associate Referee*

Last year collaborative work was conducted for the first time on the revised tentative A.O.A.C. method as adopted in 1936, *This Journal*, 20, 72. Thirteen collaborators assayed a sample of cod liver oil submitted to them. They were asked to interpret their own results. There was evidence of far better agreement in interpretation of results than agreement in actual ash values. In other words, the responses to vitamin D intake by the birds varied greatly among laboratories.

It was fortunate that a large number of the collaborators and others interested in this work were present at last year's meeting. Many hours were spent in informal discussion of the collaborative results and of the need for future work. It was quite generally agreed that the variations in the vitamin D reserves in the chicks were responsible for much of this variation in response. It was suggested that if all chicks were obtained from a few hatcheries maintaining controlled flocks, a far better agreement would be expected between laboratories.

In order to obtain information on several of the important details of the tentative method, a collaborative study was designed which was intended to clarify some of these factors. Because of several physical aspects of the work, it was deemed necessary to limit the activity to a relatively small group of collaborators. The collaborators are as follows:

(1) E. M. Bailey and R. B. Hubbell, Conn. Agr. Exp. Station, New Haven, Conn.
(2) L. E. Bopst, Maryland Regulatory Service, College Park, Md.
(3) F. D. Baird, National Oil Products Co., Harrison, N. J.
(4) R. F. Mann, White Laboratory, Newark, N. J.
(5) W. B. Griem.

In brief, the work consisted of a feeding trial conducted simultaneously in five laboratories. A basal rachitogenic ration and U.S.P. Reference Cod Liver Oil dilutions prepared by the Associate Referee were fed along with the collaborator's own basal and oil dilutions.

The chicks used in the trial were obtained from a hatchery making a

specialty of supplying experimental birds. The parent flock was confined indoors without benefit of sunlight. The flock received a pelleted mash as the only source of feed. The feed intake would be relatively equal between individuals. Such a flock would be closely culled for egg production. It was assumed that the chicks would be relatively equal, as nearly as practical, in vitamin D reserve. Each collaborator was supplied with birds impartially selected from the hatch.

Sufficient basal ration to furnish each laboratory with a supply for five groups was prepared by the Associate Referee in one batch. The calcium content was .88 of 1 per cent and the phosphorus content was .66 of 1 per cent. The calcium-phosphorus ratio was 1.33. This feed was carefully divided into five equal portions, and extreme precautions were taken to avoid any appreciable difference in the lots. Corn oil dilutions of the reference oil were prepared and subdivided. They supplied 5, 10, 15, and 20 U.S.P. or A.O.A.C. chick units per gram or supplied additions of this amount when 1 gram was added to 100 grams of basal ration. Instructions were submitted for the incorporation of these dilutions with the basal ration so that all laboratories would use an identical method.

At the four other laboratories there was prepared the basal ration from the sources of ingredients customarily used at the laboratories. These collaborators prepared their own comparable oil dilutions from the reference oil.

All oil incorporations for the various groups were made on the same day. Groups of sixteen birds were started instead of the minimum of ten. Collaborators were asked to darken the pens at night for the sake of uniformity.

Relative to the preparation of the bones for analysis, collaborators were asked to remove both tibiae, one to be used for individual ashing and the other for composite group ashing. A somewhat detailed method of preparing the bones was outlined to reduce irregularities to a minimum. A change in the present tentative procedure was incorporated in the instructions. This called for the extracting of the uncrushed bones rather than the crushed bones. It has become common practice in most laboratories to eliminate the crushing of the bones. They are far easier to handle uncrushed and they can be just as thoroughly extracted. The collaborators were asked to extract them for 30 hours with each solvent instead of the 20 hours specified. The Associate Referee is of the opinion that in most details a quite general agreement in procedure was obtained.

COLLABORATIVE EXPERIMENTAL RESULTS

The tabulation accompanying this report includes all the information requested by the Associate Referee with the exception of the individual tibia ash percentages. Instead of listing all of the individuals there is shown only the maximum and the minimum of each group.

Since the experiment did not involve the actual assay of a sample of oil, comparisons can only be made with ash values. With one exception of the possible 36, there was a distinct increase in tibia ash percentage with each increase in vitamin D. Collaborator No. 5, at the 20 unit level, obtained a value which was no higher than that obtained at the 15 unit level. The oil dilution used at this level was a replacement of the original dilution so that there is the possibility of an error in its preparation.

The regular increments in ash values is the encouraging part of the study. It was, however, anticipated that far better agreement between laboratories would have been obtained on that part of the experiment in which the subdivided basal and oil dilutions were used. The maximum differences in individual ash averages for each feeding level of the subdivided rations were 3.81, 8.16, 4.77, 4.89, and 8.90. The averages of Collaborator No. 4 were highest in the five instances, those of Collaborator No. 1 lowest in one instance, and those of Collaborator No. 2 and No. 5 each lowest in two instances.

In the second part of the experiment relating to the individually prepared rations the maximum ash differences were 5.27, 6.77, 8.89, 4.23, and 3.00. It is interesting to note that in this part of the experiment Collaborator No. 1 obtained a considerably lower response in calcification at the low vitamin D levels than was obtained on the subdivided rations, and Collaborator No. 2 obtained a considerably higher response. This indicates ration differences which both collaborators had previously observed* and which could not be explained by variations in total calcium and phosphorus content of the feed. It is suggested that the differences are due to variations in available phosphorus.

The variations in the individual ash percentages of all groups were large. In 24 of the 45 groups reported there were maximum differences of more than 10 per cent in the individual ash percentages. In 17 groups such differences exceeded 12 per cent, and in 4 groups such individual differences exceeded 15 per cent.

It could be expected that under controlled flock conditions as described previously, variations should be small. Narrowing the maximum spread with the groups tends to increase accuracy when relatively small numbers of birds are used. In the laboratory of the Associate Referee, greater differences between individuals have never been observed even when chicks from uncontrolled flocks have been used. Chicks such as were employed in this experiment may tend to give responses of a more uniform nature between different series of tests but they will not improve the accuracy of the method.

There is fair agreement in average chick weights for most vitamin D feeding levels with the exception of Collaborator No. 5 and a few other isolated instances. The chicks were all from the same source so that total

* Private communication.

feed consumption and insufficient vitamin D should in a large measure be responsible for the variations obtained. Total feed intake varies with laboratory conditions. It may be necessary eventually to specify such conditions as type of cage, temperature control, and period and intensity of lighting, if it can be shown that average weights seriously affect the accuracy of the test.

The collaborative work of last year, *This Journal*, **21**, 607, indicated that composite ashing of the tibiae of the groups gives good agreement with the values obtained by averaging the individual tibia ash percentages. These present results again demonstrate the feasibility of such composite ashing. Only two of the forty-five groups indicate differences exceeding 1 per cent. In 27 instances the differences did not exceed .50 per cent. It should be remembered that individual variations were very large in this experiment. These differences are smaller than the differences that can usually be expected when duplicate groups are fed.

OBSERVATIONS

The basic soundness of the method is again demonstrated by the fact that there are quite definite increases in calcification with increased vitamin D intake.

As the individual variations in all groups were large, the Associate Referee is of the opinion that the most important problem to be studied for improving the accuracy of the method is that of reducing these variations. Until these individual variations within each group can be minimized it seems desirable to increase the size of the groups.

Comments from those using the method and others qualified in this field suggest revision of the basal ration so as to increase the manganese content and the ribo-flavin (vitamin G) content. There is a possibility of obtaining more uniform results between laboratories if the inorganic phosphorus content would be increased.

Composite ashing should be permitted and provision should be made to permit the extraction of uncrushed bones.

It seems desirable to rewrite the method at this time and to retain the fundamental features as now tentatively established.

RECOMMENDATIONS[1]

It is recommended—

(1) That investigational and collaborative work be continued.

(2) That the text of the tentative method be revised. The revision was published in *This Journal*, **22**, 81.

No report on hydrocyanic acid in glucoside-bearing materials was given by the associate referee.

[1] For report of Subcommittee A and action by the Association, see *This Journal*, **22**, 55 (1939).

RATION	FEED AND OIL DILUTIONS PREPARED BY ASSOCIATE REFEREE					BY COLLABORATORS			
	COLLABORATOR					COLLABORATOR			
	1	2	3	4	5	1	2	3	4
Basal Negative Control									
No. of birds	14	16	14	15	18	15	16	14	16
Av. weight (g.)	112	120	128	125	105	116	114	128	126
Min. indiv. ash (%)	26.68	28.30	29.00	29.19	27.80	27.52	29.40	28.70	30.04
Max. indiv. ash (%)	32.46	36.58	36.60	42.72	37.80	33.32	37.89	36.80	40.67
Av. of indiv. ash (%)	30.60	31.25	32.30	34.41	32.67	30.07	32.63	33.20	35.34
Composite ash (%)	30.53	30.39	32.20	33.53	32.59	29.70	32.82	33.00	34.77
Diff. indiv. − comp.	+.07	+.86	+.10	+.88	+.08	+.37	−.19	+.20	+.57
Basal+5 A.O.A.C./100 g.									
No. of birds	15	15	15	16	16	15	16	15	15
Av. weight (g.)	116	142	136	144	122	110	128	133	135
Min. indiv. ash (%)	30.39	28.06	32.00	34.35	32.00	28.23	32.63	31.60	31.81
Max. indiv. ash (%)	40.23	37.56	40.00	47.15	40.40	36.50	45.75	49.40	44.26
Av. of indiv. ash (%)	34.61	32.73	36.10	40.89	35.34	31.59	38.36	36.60	38.29
Composite ash (%)	34.10	32.54	35.70	40.48	35.18	31.98	37.60	35.20	38.42
Diff. indiv. − comp.	+.51	+.19	+.40	+.41	+.16	−.39	+.76	+1.40	−.13
Basal+10 A.O.A.C./100 g.									
No. of birds	15	16	15	16	16	15	16	15	17
Av. weight (g.)	143	141	146	145	127	145	141	142	151
Min. indiv. ash (%)	31.94	32.17	30.50	34.17	33.30	27.20	36.91	32.60	37.94
Max. indiv. ash (%)	47.23	46.26	46.50	48.17	43.10	40.62	45.38	47.50	48.77
Av. of indiv. ash (%)	40.94	37.36	38.30	42.13	37.87	34.54	41.61	38.50	43.43
Composite ash (%)	40.92	36.48	37.50	41.60	37.79	35.51	42.77	38.60	43.17
Diff. indiv. − comp.	+.02	+.88	+.80	+.53	+.10	−.97	+1.16	−.10	+.26
Basal+15 A.O.A.C./100 g.									
No. of birds	16	15	15	16	16	15	16	15	16
Av. weight (g.)	151	148	147	148	121	142	151	149	162
Min. indiv. ash (%)	35.66	37.72	36.60	34.91	35.60	33.01	38.06	34.10	34.00
Max. indiv. ash (%)	47.10	44.85	48.90	49.55	46.60	47.88	48.31	47.70	49.74
Av. of indiv. ash (%)	42.75	41.92	42.00	44.77	39.88	40.58	42.02	42.00	44.81
Composite ash (%)	43.29	42.42	41.20	44.71	39.05	40.38	42.16	42.20	44.52
Diff. indiv. − comp.	−.54	−.50	+.80	+.06	+.83	+.20	−.14	−.20	+.29
Basal+20 A.O.A.C./100 g.									
No. of birds	16	15	12	15	15	15	15	15	17
Av. weight (g.)	151	183	156	155	129	173	148	161	147
Min. indiv. ash (%)	42.86	38.79	38.60	45.98	34.20	33.98	42.75	37.70	38.05
Max. indiv. ash (%)	50.12	46.26	49.70	51.76	45.60	47.92	47.91	47.30	49.34
Av. of indiv. ash (%)	46.52	43.44	43.30	48.20	39.30	43.05	45.47	44.60	46.05
Composite ash (%)	47.06	43.80	43.50	47.47	38.55	42.87	45.06	44.60	46.67

REPORT ON FAT IN FISH MEAL

By R. W. HARRISON (U. S. Bureau of Fisheries, Seattle, Wash.), *Associate Referee*

At the last meeting, *This Journal*, **21**, 618, data were given showing the rate of extraction and percentage of extract obtained with 12 different fat solvents when used on several samples of fish meal. The solvents tested were petroleum ether, hexane (pract.), heptane (pract.), ethyl ether, carbon bisulfide, cyclohexane, benzene, methylene chloride, trichloroethylene, chloroform, acetone, and 1.4 dioxane. With the exception of acetone, there was practically no increase in extract value after 4 hours' extraction. The gross extraction values for the various solvents increased in the above order, although solvents of similar general chemical structure gave quite similar results.

TABLE 1.—*Decrease in extraction values of various solvents due to oxidation of fish meal*

SOLVENT	B.P.	SOLVENT EXTRACT VALUE				DECREASE IN EXTRACT VALUE DUE TO OXIDATION	
		MEAL NO. 3 SPECIAL SALMON MEAL		MEAL NO. 4 COMMERCIAL SARDINE MEAL			
		AS RECEIVED	OXIDIZED	AS RECEIVED	OXIDIZED	MEAL NO. 3	MEAL NO. 4
	°C.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Petroleum ether	35–60	7.8	5.9	8.8	3.1	24.4	64.8
Hexane (pract.)	62–67	7.5	6.6	9.8	3.4	12.0	65.3
Heptane (pract.)	91–96	7.7	6.8	10.1	3.7	11.7	63.4
Ethyl ether	34.5	7.8	7.3	10.1	4.4	2.7	56.5
Isopropyl ether	63–69		7.1		4.1		
Carbon bisulfide	46.3	8.1	6.8	10.5	3.5	16.1	66.7
Cyclohexane	78–81	8.1	6.9	10.5	3.6	14.8	65.7
Benzene	79.6	8.4	7.5	11.0	6.1	10.7	44.6
Toluene	110–111				8.5		
Methylene chloride	40–41	8.8	7.7	11.4	5.3	12.5	53.5
Trichloroethylene	83–87	9.2	7.6	11.6	6.7	17.4	42.2
Chloroform	61.2	9.1	8.2	11.7	7.6	9.9	35.1
Carbon tetrachloride	76.8				4.2		
Acetone	56.1	9.0	7.9	11.9	6.7	12.3	43.7
Methyl isobutyl ketone	111–117		9.7		11.3		
1-4 Dioxane	91–101	11.4	9.9	16.2	12.9	13.2	20.4

Because of the limitations in laboratory equipment, it was impossible to continue an active study of the problem during the past year. However, two samples of meal, which had been placed in storage under conditions designed to accelerate oxidation, were analyzed to determine the effect of oxidation on the extract value given by the various solvents. These

values, together with the percentage decrease caused by oxidation, are given in Table 1. Generally speaking, all solvents tested were found to give definitely lower extract values after the meal had been oxidized. The unusually efficient recovery obtained with ethyl ether in the case of Meal No. 3 must be considered as particular to the sample of meal in question because in numerous other instances not reported here, ethyl ether extract has been found to decrease markedly as a result of oxidation. In fact, the present study has resulted from this limitation of ether extract as a measure of the true fat content of fish meal. If time permits, the work will be continued during the coming year.

REPORT ON BIOLOGICAL METHODS FOR COMPONENTS OF THE VITAMIN B COMPLEX

By O. L. KLINE (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

In the Associate Referee's report of last year, written by C. A. Elvehjem, *This Journal*, **21**, 622, further work with the chick method of assay for vitamin B_1 was described. The chick method is a prophylactic type of assay in which the incidence of polyneuritis in groups of chicks fed varying levels of the assay material is determined. The chicks are maintained on a vitamin-B_1-low diet consisting of a mixture of corn, wheat-middlings, and casein that has been autoclaved to destroy the B_1, with additions of salts, dried liver, and cod-liver oil. This method is specific for vitamin B_1 and is of particular advantage in the assay of low potency materials. However, in view of the limited experience with the chick method, further study was recommended by the Referee at that time.

During the past year this method has been used with good results in this laboratory. Characteristic polyneuritis was observed, and there was no difficulty in interpreting the results obtained with different levels of the material being assayed. No further collaborative work on this method has been carried out.

At last year's meeting this Associate Referee's work on a rat-curative procedure for vitamin B_1 assay was presented, *This Journal*, **21**, 305. This method is essentially a modified Smith curative technic.[1] Changes in the diet allow for inclusion of the necessary components of the vitamin B complex, except B_1. This is accomplished by furnishing autoclaved peanuts and purified liver extract in addition to the usual supplements. By modifying the method of interpreting results a comparison of standard and unknown is made in the same animal by determining the length of curative response to each. In this procedure animals have consistently

[1] U. S. Public Health Rpt., **45**, 116 (1930).

developed uncomplicated polyneuritis in a 25–50 day depletion period and have been used for as many as 10–20 curvative responses when crystalline vitamin B_1 was administered.

During the past year this method was subjected to critical collaborative study in a number of laboratories by members of the U.S.P. Vitamin Committee, under direction of the Pharmacopoeia Revision Committee, and is now under consideration for inclusion in the Pharmacopoeia. In view of this consideration and since there is no advantage to be gained in duplication, no recommendation with respect to the rat-curative method will be made at this time.

A note to be published in the forthcoming issue of *Science*[1] describes briefly studies on the destruction of vitamin B_1 by the use of sulfite. This is an application of the original observation of Williams and associates,[2] who found that the vitamin is destroyed by cleavage of the molecule in the presence of sulfite. A vitamin-B_1-free basal diet of sucrose, purified casein, salts, fat, and cod-liver oil was used in these studies. Fifty grams of dried yeast was treated with 400 cc. of a 0.1 per cent solution of sodium sulfite, and sulfur dioxide was introduced until a pH 4.0 was reached. The material was allowed to stand in a tightly stoppered bottle for 5 days at room temperature, then dried at 60°C. Animals fed the basal diet supplemented with 15 per cent sulfite-treated yeast developed characteristic polyneuritis in 30–35 days. Animals that received the basal diet containing sulfite-treated yeast plus crystalline vitamin B_1 grew as rapidly as animals fed the basal diet supplemented with untreated yeast, showing average daily weight gains of 3.6 and 3.8 grams. Normal reproduction occurred in females in the group receiving sulfite-treated yeast plus vitamin B_1. These and other studies indicate that other components of the vitamin B complex are apparently unaffected by sulfur dioxide.

Sulfite treatment offers a practical and simple means of preparation of a diet satisfactory for the determination of vitamin B_1, and appears to have definite advantage over any procedure of purification previously used.

Modifications involving use of sulfite destruction may make for improvement in the chick method and may also give a more accurate rat growth procedure, either of which would be useful in the assay of low potency materials. This will require further study.

Methods for other components of the vitamin B complex have received considerable attention. Riboflavin has been measured by means of the chick test, the Sherman-Borquin rat-growth method, and by chemical means. Nicotinic acid determinations have been carried out with dogs. Rapid advances in this field have made these two substances, as well as vitamin B_6, available in crystalline form. With these in hand further

[1] *Science*, 88, 508 (1939).
[2] *J. Am. Chem. Soc.*, 57, 536 (1935).

improvements in assay methods may be expected. Since the attention of the Associate Referee has been focused on the vitamin B_1 determination during the past year, no recommendations with respect to methods for other B complex factors are offered at this time.

It is recommended[1] that further study of vitamin B methods be carried out, and that the recent developments in this field be utilized, with particular emphasis given to their application to low-potency materials.

No report on technic and details of biological methods, vitamin D carriers was given by the associate referee.

REPORT ON CAROTENE

By V. E. MUNSEY (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

The study on carotene during the past three years has been on modifications of the Guilbert method. The essential difference in the modified methods is the use of petroleum ether for extraction of carotene in one case and ethyl ether in the other. The principle of the subsequent separation of the carotene and xanthophyll requires the removal of ethyl ether, another step that involves time and difficulty. The majority of the collaborators the past two years favored the petroleum ether modification, but since some preferred the ethyl ether procedure a modification in the method for removal of the ethyl ether was made, and this method was again included in the collaborative study this year. In this modification the ethyl ether is removed by addition of methyl alcohol, followed by evaporation of the mixture to a small volume, which results in removal of the ether and leaves the pigment in methyl alcohol rather than a nearly solid mass dissolved with difficulty, as in the procedure previously studied.

The collaborators this year were asked to analyze two samples of feed, No. 1, alfalfa meal, and No. 2, a mixed feed, by the petroleum ether procedure of Peterson and Hughes and the ethyl ether Fraps procedure with the slight modification just referred to. The choice of three procedures was submitted for measurement of the carotene concentration in solution, namely, the Peterson and Hughes spectrophotometric procedure, the Fraps 0.1 per cent potassium dichromate, and Russell's 0.036 per cent potassium dichromate. Some of the collaborators were also asked to analyze the samples by the Peterson and Hughes procedure, with and

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[1] For report of Subcommittee A and action by the Association, see *This Journal*, 22, 55 (1939).

without washing the petroleum ether free of alkali. Their results and the results of the Associate Referee show that leaving out the water washing has no effect, since the alkali is removed by the subsequent methyl alcohol extractions. The results obtained are included in Tables 1 and 2. The essential difference is that the results are lower by the modified Fraps procedure. In addition to the results requested, some of the collaborators reported results by methods used in their laboratories and others made their measurements by photoelectric colorimeters.

The following methods were studied:

Peterson-Hughes modification of Guilbert method.—This method was adopted as tentative and published in *This Journal*, **22**, 79.

MEASUREMENT OF CAROTENE IN SOLUTION

No. 1. Peterson and Hughes Procedure.—After making the carotene solution up to volume, determine the concentration by the spectrophotometric method. For each determination make optical density measurements at wave lengths of 4500, 4700, and 4800 Å.U. Using the absorption coefficients calculated for beta carotene at these wave lengths, determine the carotene concentration for each wave length and take the average.

Extinction Coefficients

Wave Length Å	*Skellysolve* *b. 60–70°*	*Petroleum Ether* *b. 40–60°*
4500	238	243
4550		231
4700	200	207
4800	212	212

No. 2. Fraps Procedure.—Estimate the amount of carotene in the sample by comparing it colorimetrically against 0.1% $K_2Cr_2O_7$. Put the solution of the sample in the left-hand cup of the colorimeter and set the scale at 0.5 cm., 1 cm., 2 cm., 3 cm., or 4 cm., according to the amount of color present. Vary the depth of the dichromate solution in the right-hand cup until the density of color in both cups is equal, and make eight independent readings, putting them down in millimeters. Average the readings. Make the dichromate readings between 4 mm. and 12 mm. on the colorimeter. If a reading below 4 mm. can not be avoided, make it, but repeat the analysis with a larger sample.

By use of the table transform the millimeter depth of 0.1% dichromate in p.p.m. of carotene. Then calculate the p.p.m. of carotene actually in the sample by use of the formula. The formula and table were published in *This Journal*, **22**, 79.

No. 3. Russell Procedure.—Estimate the amount of carotene in the sample by comparing it colorimetrically against 0.036% $K_2Cr_2O_7$. (The concentration of the solution before being compared colorimetrically should be about one-half as much as when compared against the 0.1% $K_2Cr_2O_7$.) Set the standard 0.036% $K_2Cr_2O_7$ at 10 mm. and match against the carotene solution by taking eight independent readings. The carotene solution readings should be between 7 and 14 mm. The 0.036% $K_2Cr_2O_7$ = 2.06 mg. per liter of carotene.

TABLE 1.—*Results on carotene on Sample 1 by the two methods referred to in this report (p.p.m.)*

COLLABORATOR	PHOTOELECTRIC COLORIMETER	SPECTROPHOTOMETER[1]	0.1% $K_2Cr_2O_7$[1]	0.036% $K_2Cr_2O_7$[2]
		Procedure No. 1		
1		239	247	215
		228	241	215
		253	235	214
		257	230	212
		257	255	206
		266	267	214
		Av. 250	Av. 246	Av. 213
2			221	
			249	
			Av. 235	
3		248		252
		257		259
		254		256
		Av. 253		Av. 256
4		255	264	255
5	275			
6	246			240
	246			239
	Av. 246			Av. 240
7		266	224	222
		252	240	215
		Av. 259	Av. 232	Av. 219
8		262		258
		254		
		Av. 258		
9				215
10	227	217	207	189
11			225	202
12			235	224
13			250	
			248	
			Av. 249	

[1] *This Journal*, **20**, 459 (1937).
[2] *Plant Physiology*, **10**, 325 (1935).

TABLE 1.—(*Continued*)

COLLABORATOR	PHOTOELECTRIC COLORIMETER	SPECTROPHOTOMETER	0.1% $K_2Cr_2O_7$	0.036% $K_2Cr_2O_7$
		Procedure No. 1—(*Continued*)		
14			245	261
			248	280
			Av. 247	Av. 272
16[3]			245	242
			243	246
			Av. 244	Av. 244
17[3]			221	
			221	
			Av. 221	
Av.	249	247	238	231
Max.	275	259	264	272
Min.	227	217	207	189
Range	48	42	57	83
		Procedure No. 2		
1				
2			203	
			201	
			Av. 202	
3		228		229
		227		232
		233		232
		Av. 229		Av. 231
4		244	256	243
5	290			
6	237			231
	235			232
	Av. 236			Av. 232
7		229	219	204
		239	214	198
		Av. 234	Av. 217	Av. 201
8		247		
		238		
		Av. 243		

[3] Received too late to include in average.

TABLE 1.—(*Continued*)

COLLABORATOR	PHOTOELECTRIC COLORIMETER	SPECTROPHOTOMETER	0.1% $K_2Cr_2O_7$	0.036% $K_2Cr_2O_7$
		Procedure No. 2—(*Continued*)		
9				219
10	220	219	220	208
11			225	191
12			235	226
13			225	
			227	
			Av. 226	
14				
16[3]				
17[3]			167.5	
			159.2	
			169.5	
			Av. 165.4	
Av.	248	234	226	219
Max.	290	244	256	243
Min.	220	219	217	191
Range	70	25	39	52

TABLE 2.—*Results on carotene on Sample 2 by the same methods used in Table 1 (p.p.m.)*

COLLABORATOR	PHOTOELECTRIC COLORIMETER	SPECTROPHOTOMETER[1]	0.1% $K_2Cr_2O^1$	0.036% $K_2Cr_2O_7$[2]
		Procedure No. 1		
1		85.2	90.0	79.0
		86.4	90.0	79.0
		82.5	84.3	73.8
		82.4	85.7	69.0
		86.7	89.6	71.8
		86.7	86.6	71.9
		Av. 85.0	Av. 87.7	Av. 74.1
2			106	
			83	
			66	
			Av. 85	
3		83.5		82.2
		83.6		83.3
		83.4		84.3
		Av. 83.5		Av. 83.3

[1] *This Journal*, 20, 459 (1937).
[2] *Plant Physiology*, 10, 325 (1935).

TABLE 2.—(*Continued*)

COLLABORATOR	PHOTOELECTRIC COLORIMETER	SPECTROPHOTOMETER	0.1% $K_2Cr_2O_7$	0.036% $K_2Cr_2O_7$
		Procedure No. 1—(*Continued*)		
4		86	81	83
5	92			
6	85.5			82.6
	85.8			82.5
	Av. 85.7			Av. 82.6
7		88.3	76.0	75.5
		85.1	82.2	71.5
		Av. 86.7	Av. 79.1	Av. 73.5
8		91		84
		95		
		Av. 93		
9				72.5
10	81.3	78.1	71.5	69.3
11			76.2	67.8
12			78.0	75.0
13			72.2	
			71.1	
			Av. 71.7	
14		87.5	91.5	
		85.0	91.5	
		Av. 86.3	Av. 91.5	
16[3]			97.5	99.3
			98.3	101.6
			Av. 97.9	Av. 100.5
17[3]			61.0	
			60.6	
			Av. 60.8	
Av.	86.3	85.4	79.6	77.8
Max.	92.0	93.0	87.7	91.5
Min.	81.3	78.1	71.5	67.8
Range	10.7	14.9	16.2	23.7
		Procedure No. 2		
1				
2			76	
			66	
			Av. 71	

[3] Received too late to include in average.

TABLE 2.—(*Continued*)

COLLABORATOR	PHOTOELECTRIC COLORIMETER	SPECTROPHOTOMETER	0.1% $K_2Cr_2O_7$	0.036% $K_2Cr_2O_7$
		Procedure No. 2—(*Continued*)		
3		78.3		77.4
		80.6		77.7
		77.6		77.3
		Av. 78.8		Av. 77.5
4		85	79	81
5	100			
6	82.0			79.5
	82.0			79.3
	Av. 82.0			79.4
7		82.1	76.7	68.7
		84.7	81.0	70.8
		Av. 83.4	Av. 78.9	Av. 69.8
8		88		
		73		
		Av. 81		
9				75
10	81.7	79.8	81.7	70.7
11			75.8	63.5
12			78.0	75.0
13			70.0	
			67.5	
			Av. 68.8	
14			77.5	87.3
15[4]	47.5			
	49.5			
	48.2			
	Av. 48.4			
16				
17[4]			57.9	
			58.6	
			Av. 58.3	
Av.	87.9	81.6	76.3	75.4
Max.	100.0	85.0	81.7	87.3
Min.	81.7	78.8	68.8	63.5
Range	18.3	6.2	12.9	23.8

[4] Not included in average.

DISCUSSION OF RESULTS

Excluding those obtained by the photoelectric colorimeter, the results on both Samples 1 and 2 by both procedures are highest by the spectrophotometer and also show best agreement among collaborators, as was the case the past two years. The results by 0.1 per cent potassium dichromate are somewhat lower and show less agreement, but they are in better agreement, on the whole, than those by the 0.036 per cent potassium dichromate. The results with the 0.036 per cent potassium dichromate are lowest and they show a greater spread. The results by the modified Fraps procedure are the lowest. The results by the photoelectric colorimeter this year, as in previous years, indicate satisfactory agreement with the results by the spectrophotometer.

Under directions for use of 0.036 per cent potassium dichromate it is necessary to work within a very limited range of concentration, since the carotene reading should be between 7 and 14 mm., with the potassium dichromate reading set at 10 mm. On the basis of this limitation and the results obtained in these studies it seems preferable to choose the 0.1 per cent potassium dichromate as reference standard, but for greater reliance it should be standardized by several chemists by comparison against pure beta carotene and the average value taken, since the values obtained are too low, according to several of the chemists, which fact is confirmed by collaborative results.

No collaborative work was done with the neutral wedge photometer since none was available to the collaborators. However, the Associate Referee obtained precision comparable with the spectrophotometer, and the results agree well with those obtained by other means of measurement.

On the basis of these collaborative studies the Peterson and Hughes extraction procedure and measurement of carotene in solution by the spectrophotometer and the photoelectric colorimeter seem satisfactory for carotene determination. In the absence of these means of measurement, the 0.1 per cent potassium dichromate may be used.

The carotene reported by the present extraction procedures may be considerably in error, due to varying amounts of impurities measured as carotene. The Bureau of Dairy Industry has done considerable investigating on the amounts of these impurities, which have been determined by an adsorption procedure, with magnesium oxide used as an adsorbing agent. The amount of these impurities may be around 30 per cent, depending on the type of material, being much greater on low-grade hays than in the case of fresh-cut grasses, which may be very low. In view of the existence of this varying amount of impurities measured as carotene by the methods studied the past few years it would seem logical to give consideration to the application of the adsorption principle. Theoretically, such a plan seemed desirable, but as a practical routine operation for this year's study it did not seem advisable at this stage of development. The

application of the chromatrographic principle requires special equipment and experience. Its application is difficult enough from a qualitative standpoint and even more so from the quantitative aspect. The adsorption agent must be activated by specially controlled conditions and be packed in the adsorption tower in such a way that filtration will not be too slow and at the same time not too loose to cause channeling. After the solution is poured on the adsorbing agent the chromatogram must be developed, followed by removal of the different zones and elution of the adsorbed pigment. The reference, "Die Chromatographische Adsorptionsmethode," Wien, 1937, by L. Zechmeister and L. V. Cholnoky, gives valuable information on this subject. Also in a recent reference Hoppe-Seyler,[1] using aluminum oxide as adsorbing agent for chromatographic adsorption analysis of small amounts of carotenoid, gives further evidence to indicate the possibility of the application of this principle. It would seem possible to make a direct adsorption of the solution containing the total carotenoid pigments without first removing the xanthopyll by use of the aqueous methyl alcohol. Further work on this subject of carotene determination should be devoted to the development of a method based on the adsorption principle.

Appreciation of the generous cooperation of the following collaborators is herewith expressed:

G. C. Crooks, Burlington, Vt.
D. S. Binnington, Board of Grain Commissioners, Winnipeg, Man.
J. M. Kniseley, Seattle, Wash.
Virgil Wodicka, Ralston Purina Co., St. Louis, Mo.
W. J. Peterson, Manhattan, Kan.
H. Boeddeker, Rossford, Ohio.
R. O. Brooke, Malden, Mass.
S. M. Greenberg, College Station, Tex.
C. H. Haurand, Bayonne, N. J.
W. Zimmerman, Geneva, N. Y.
F. Kopko, Geneva, N. Y.
Lyle Swift, Lafayette, Ind.
F. E. Randall, Buffalo, N. Y.
O. I. Struve, Buffalo, N. Y.
R. W. Caldwell, Davis, Calif.
A. J. Soderberg, Davis, Calif.
H. R. Bicknell, Sacramento, Calif.
A. L. Haskins, State College, Pa.

RECOMMENDATIONS[2]

It is recommended—

(1) That no more collaborative work be done on the Peterson-Hughes method for the present.

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[1] *Z. physiol. chem.*, 253, 40 (1938).
[2] For report of Subcommittee A and action by the Association, see *This Journal*, 22, 50 (1939).

(2) That the Peterson-Hughes method be adopted as a tentative method for the determination of carotene, and that the spectrophotometer be used or the 0.1 per cent potassium dichromate reference standard, preferably the former.

(3) That the potassium dichromate standard be rechecked by several chemists against pure beta carotene and the best conditions for accurate application be established before further collaborative work is done involving the use of this reference standard.

(4) That study of the application of the neutral wedge photometer and the photoelectric colorimeter be continued.

(5) That study be made on the application of a quantitative adsorption procedure for the determination of carotene.

REPORT ON MANGANESE IN STOCK FEEDS*

By John B. Smith, *Associate Referee*, and E. J. Deszyck
(Agricultural Experiment Station, Kingston, R. I.)

Manganese deficiency apparently is an important cause of perosis, or slipped tendon, in chicks, and the use of various inorganic manganese compounds as mineral supplements is becoming a common practice. Several compounds have been found effective, notably the sulfate, the carbonate, oxides, certain manganese ores, etc. Rhodonite, a silicate ore, and rhodochrosite, a carbonate ore, proved less satisfactory. The topic has been summarized, very recently, by Schaible, Bandemer and Davidson.[1]

In attempting to write an official method for manganese in feeds the writers recognized that knowledge of the subject is increasing rapidly, but that the background is far from complete. Doubtless further work will show that some supplements are more effective than others. At the moment, however, the demand seems to be for a simple, rapid, comprehensive method for manganese, rather than for differentiation among compounds. The method proposed includes all acid-soluble forms of manganese in the ash of feeds and it seems unlikely that compounds excluded by this treatment are of nutritional value. It is more probable that some of the less valuable forms may be included. The method does not differentiate between manganese added as a supplement and that in other ingredients.

Correspondence with the collaborators who have had experience with this work, led to the selection of the colorimetric potassium periodate method for measuring the small quantities of manganese involved. This

* Contribution No. 556 of this Station.
[1] Mich. Expt. Sta., Tech. Bull. 159 (1937).

well-known procedure was published originally by Willard and Greathouse,[2] and has been reviewed comprehensively by Smith.[3] The details were selected from procedures that were in use by the collaborators. Perhaps it is well to note that the method is intended for measuring small quantities of manganese, and may not apply to the analysis of manganese salts and other carriers.

The procedure was published in *This Journal,* **22**, 78.

Six samples, based on two formulas popular in this region, were sent to collaborators. Manganese sulfate and manganese carbonate were added in the customary proportions and the sulfate in a considerably larger proportion. The common practice is to use about four ounces of these compounds in a ton of feed. It would seem almost impossible to mix such a small quantity commercially with sufficient accuracy to permit satisfactory sampling. In making up these samples, the manganese carrier was mixed thoroughly with another ingredient, and then combined with the remainder of the mixture by prolonged mixing. The samples were then ground to pass a 1 mm. sieve and again mixed very thoroughly. Despite storage of the ingredients during a very wet period before mixing, the ingredients appeared normal and mixed readily. However, moisture must have increased from exposure during the long mixing at a period of high humidity, for the samples showed abnormally high moisture and a little mold upon standing tightly sealed. For this reason, all results were calculated on the oven-dry basis in accordance with the moisture results reported by each collaborator.

Results for manganese are expressed in this report as parts per million. In work that is entirely scientific, the designation of units is not so important as are determinations intended for the layman. Guarantees and determinations as percentages would appear as small decimals, difficult to comprehend. Whole numbers are easier to compare. Milligrams per cent and milligrams per kilogram mean little to the feeder and require too much printing on tags. Ounces per ton is understandable, but would be a figure of low magnitude and require too much printing. The writers prefer parts per million abbreviated to p.p.m. It is the same type of unit as percentage, is easily translated into percentage units, and results are whole numbers within the limits of accuracy of the method. In accord with the trend in similar work, results are expressed as Mn rather than as manganese oxides.

The periodate colorimetric method for manganese appears twice among the methods of this organization, *Methods of Analysis, A.O.A.C.,* 1935, XII, **13**; XXVI, **17**, but in the judgment of the writers these procedures are so different from the procedure presented here that a separate section in the publication is justified. The Associate Referee is also responsible

[2] *J. Am. Chem. Soc.,* 39, 2366–2377 (1917).
[3] G. Frederick Smith Chemical Co. Publications, Vol. 1, 2nd ed., Aug. 1933.

for a method for manganese in fertilizers, *This Journal*, **22**, 270. The procedure to be remembered eventually is similar to that for feeds and will be written to conserve space in the chapter on fertilizers.

TABLE 1.—*Description of samples and collaborative results, calculated as p.p.m. Mn on the oven-dry basis*

COLLABORATOR	SAMPLES*					
	A	A-1	A-2	B	B-1	B-2
H. Boeddeker, Larrowe Milling Co. Photoelectric colorimeter	45	92	151	46	88	106
Richard O. Brooke, Wirthmore Research Laboratory Photoelectric colorimeter	47	93	142	48	94	102
E. J. Deszyck Duboscq type colorimeter	50	104	153	49	91	112
Arthur L. Haskins, Penn State Coll. Duboscq type colorimeter	53	100	159	56	100	112
Oscar I. Struve, Eastern States Coop. Milling Corp. Duboscq type colorimeter	52	102	149	51	102	111
F. E. Randall, Coop. G. L. F. Mills, Inc. Duboscq type colorimeter	55	101	151	62	104	115
Average	50	99	151	52	97	110
Calculated	—	102	148	—	101	108

* *Sample A.*—Yellow corn meal, flour middlings, ground oats, dried skimmilk, alfalfa leaf meal, meat scraps, fish meal, wheat bran, limestone, salt, dicalcium phosphate, cod-liver oil.
Sample A1.—Sample A plus 52 p.p.m. Mn in $MnSO_4$.
Sample A2.—Sample A plus 98 p.p.m. Mn in $MnSO_4$.
Sample B.—Ingredients as in Sample A, but different proportions, without wheat bran and $CaHPO_4$.
Sample B1.—Sample B plus 49 p.p.m. Mn in $MnSO_4$.
Sample B2.—Sample B plus 56 p.p.m. Mn in $MnCO_3$.

Results submitted by collaborators appear in Table 1. The analysts had had previous experience with similar procedures, and the results are remarkably consistant. This is especially true, in view of the minute quantities of manganese involved, possible variation in sample uniformity, and unavoidable errors for colorimetric methods. Reports in the literature and experience with the method show that the ratio between acidity and manganese concentration is important, but the method as written appears to control this factor within the necessary limits.

In the judgment of the Associate Referee, the results submitted justify recommendation of the procedure as a tentative method. Minor changes

to avoid the time lost in evaporation after filtration of the insoluble residue, and the use of potassium nitrate with the acid mixture to aid in the destruction of the last traces of organic matter are possible, and have worked well in this laboratory. These should be tried collaboratively next year, also different feed formulas, and other carriers such as pyrolusite.

The writers express their appreciation of the advice received from several investigators prominent in the field of poultry nutrition, as well as that of the collaborators listed in this report.

It is recommended[1] that the method presented by the associate referee for the determination of manganese in grain and stock feeds be adopted as tentative, and that the study be continued.

REPORT ON ADULTERATION OF CONDENSED MILK PRODUCTS AND COD LIVER OIL

By P. B. Curtis (Department of Agricultural Chemistry, Purdue University, Lafayette, Ind.), *Associate Referee*

At the 1937 meeting of this Association the Associate Referee on Stock Feed Adulteration presented a method for the detection of starch or starchy materials in condensed milk products. Due to the simplicity and short time required to make the test it was deemed advisable to conduct a collaborative study of the method. This was done the past year with six laboratories participating in the work.

On June 23 four samples of condensed skimmed milk, three of which contained known amounts of cornstarch, were sent to the collaborators with the request that the samples be tested for starch according to the method outlined last year, and published in *This Journal*, **21**, 596. A request was also made that the samples be listed in order of their predominance in starch content as revealed by the test.

A summary of the results is given in the following table.

Detection of starch in condensed milk products

COLLABORATOR	SAMPLES			
	1	2	3	4
1	++	−	+	trace
2	++	−	+	−
3	++++	−	+	trace
4	++	−	+	−
5	+++	−	++	+
6	+++++	−	+	−
Starch content (as prepared)	5%	none	1%	0.1%

[1] For report of Subcommittee A and action by the Association, see *This Journal*, **22**, 50 (1939).

The results (Table 1) show that the samples as prepared had the following composition: Sample 1, condensed skimmed milk containing 5 per cent starch; Sample 2, condensed skimmed milk with no starch added; Sample 3, condensed skimmed milk with 1 per cent starch; and Sample 4, condensed skimmed milk with 0.1 per cent starch. While all the collaborators agreed on the relative quantities of starch present some of them reported no starch in Sample 4, which was prepared to contain 0.1 per cent starch. This discrepancy may be due to the difficulty involved in obtaining a uniform mixture of the condensed skimmed milk with the small amount of starch added.

When the samples were sent out each collaborator was asked to submit any comments or criticisms of the method. Some of the comments received follow:

Analyst 3.—The method can be worked just as satisfactorily on the dried milk product as on the condensed milk product. I would recommend that the method be brought forward with a view towards making it official.

Analyst 4.—Suggests the following procedure in order to eliminate the greenish effect sometimes produced in the spot plate test: Dip a starch-free smooth texture filter paper into the I-KI test solution, or drop some of the test solution on the paper. Allow this paper to dry to apparent dryness in the air or in an air oven (at first while moist and heavy with iodine the paper is dark brown, but on drying the color becomes yellowish brown). Allow a drop or two of the suspected solution, prepared as indicated in the method, to fall upon the test paper. In the presence of much starch a clear blue spot will appear on the paper, free from excess iodine or other colored solution.

Analyst 5.—Likewise found that the spot plate test on Sample 4 gave a greenish brown color. He suggests that the iodine solution be added to the milk solution in a test tube rather than on the spot plate.

Analyst 6.—Believes that the method which has been in use for a number of years in his laboratory is much better adapted to the detection of starch than the one proposed. A brief outline of this method is as follows: Place a small amount of the sample on a slide, add one drop of the I-KI solution, mix well, and cover with a cover-glass. Examine under a microscope. In semi-solid or condensed milk products the individual starch grains are easily noted while in the dried materials the blue or purplish color is apparent.

In view of these various comments and criticisms the Associate Referee recommends[1]—

(1) That further study be made on the detection of starch or starchy materials as an adulterant of condensed milk products.

(2) That a study be made of methods for the detection of adulteration of cod liver oil.

[1] For report of Subcommittee A and action by the Association, see *This Journal*, 22, 50 (1939).

CONTRIBUTED PAPERS

DETERMINATION OF AMINOPYRINE IN THE PRESENCE OF ANTIPYRINE AND CAFFEINE[1]

By F. C. Sinton, New York, N. Y., and F. A. Rotondaro, Philadelphia, Pa. (U. S. Food and Drug Administration)

Methods for the determination of aminopyrine in mixture with antipyrine are given in the literature, but they depend essentially on titration or colorimetric procedures. During the course of regulatory work the writers were given a mixture of aminopyrine, antipyrine, and caffeine, and as a result of experimental work devised a method for the separation of the aminopyrine from the antipyrine and caffeine by means of extraction from a solution of controlled acid strength. It was found that from a 3.5–5 per cent solution of sulfuric acid, by weight, antipyrine plus caffeine could be quantitatively extracted with chloroform. On being made ammoniacal subsequently, the aminopyrine was quantitatively removed. It was also found that on saturating with anhydrous sodium sulfate the limits of acid strength necessary for quantitative separation were raised and broadened.

Determinations were made on a solution containing in 100 cc. 3 grams of aminopyrine, 2 grams of antipyrine, and 0.5 gram of caffeine. The following procedure was used:

Transfer to a separator a 10 cc. sample, add 5 cc. of the acid, and extract with 25 cc. portions of $CHCl_3$. Wash the extracts in a second separator with 5 cc. of 3.5% H_2SO_4. After making five extractions, test the next for complete extraction. Evaporate the $CHCl_3$, dry the residue at 80°–100° C. for 10 minutes, and weigh. Transfer the wash water to the original separator, make ammoniacal, and extract the aminopyrine with $CHCl_3$. Evaporate the combined $CHCl_3$ extracts, dry at 80°–100° C. for 10 minutes, and weigh.

The procedure involving the use of anhydrous Na_2SO_4 is the same except that preliminary to the $CHCl_3$ extraction the solution is saturated with the Na_2SO_4.

It is to be noted that the strength only of the 5 cc. of acid added has been determined. Since this is diluted with 10 cc. of water, the resulting solution is approximately one-third the strength, at least where the acid is low in concentration.

The results obtained show that antipyrine and caffeine are quantitatively removed from a sulfuric acid solution of 3.5–5 per cent. If the concentration is much less, aminopyrine is partly removed; if the concentration is increased, the antipyrine tends to be held in the acid. The same separation can be made on adding sodium sulfate to saturation, but the concentration of the acid appears to be less critical. The 5 cc. of acid added to 10 cc. of water must, however, be 30 per cent or more, since in

[1] Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., November 14, 15, 16, 1938.

weaker strengths the aminopyrine tends to be thrown out of the acid. The sodium sulfate appears to depress the ionization of the acid and a stronger acid strength must be used than in the case of the simple acid.

RESULTS

10 cc. solution, no sodium sulfate

NUMBER	STRENGTH OF THE 5 CC. OF ACID BY WT.	ANTIPYRINE AND CAFFEINE (5 EXTRACTIONS)	RECOVERY	AMINOPYRINE	RECOVERY
	per cent		*per cent*		*per cent*
1	1.7	0.2696	107.8		
2	5	0.2595	103.8		
3	10	0.2501	100.0		
4	10	0.2487	99.5		
5	10	0.2514	100.7	0.2994	99.8
6	10	0.2496	99.8	0.3001	100.0
7	10	0.2503	100.1	0.2990	99.7
8	10	0.2504	100.2	0.2998	99.9
9	10	0.2496	99.8	0.3005	100.2
10	15	0.2486	99.4	0.2969	99.0
11	21.4	0.2396	95.8		
12	30	0.2211	88.4		
13	60.8	0.0725	30.1		

10 cc. solution, anhydrous sodium sulfate to saturation

NUMBER	STRENGTH OF THE 5 CC. OF ACID BY WT.	ANTIPYRINE AND CAFFEINE RECOVERY	AMINOPYRINE RECOVERY
	per cent	*per cent*	*per cent*
1	10	111.5	
2	15	114.2	
3	22.5	103.5	96.3
4	22.5	104.5	95.8
5	30	101.9	98.6
6	30	100.7	96.7
7	30	101.4	97.0
8	45	100.9	98.4
9	60	100.7	99.2
10	60	99.0	98.6
11	60	99.2	98.5

The aminopyrine residues obtained in both procedures had melting points generally within the U.S.P. range of 107°–109° C., showing a complete separation. In a few cases when the simple acid procedure was used, the melting point was a degree or two low, but still showing the product practically pure.

CONCLUSION

The method presented effectively separates aminopyrine from antipyrine and caffeine. The same method of procedure might presumably be applied to other combinations containing aminopyrine.

DETERMINATION OF ARSENIC IN SOIL TREATED WITH ACID LEAD ARSENATE

By LOUIS KOBLITSKY (Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Washington, D. C.)

For several years acid lead arsenate has been used at the rate of 1,500 pounds per acre to free the soil of the immature larvae of the Japanese beetle, *Popillia japonica* (Newm.).[1] Because it is necessary to maintain this dosage to assure their elimination and permit certification of plants for shipment outside the area infested with the Japanese beetle, a method of analyzing soil to determine the arsenical concentration had to be developed.

It was believed that the method should involve removal of the arsenic from the soil, and furthermore, that it should detect from a trace up to 0.06 per cent of arsenic in the soil and lend itself to speedy manipulation, because of the large number of samples to be analyzed in a short time.

EXPERIMENTAL

To avoid the interference of some of the constituents of the soil, both a partial digestion with nitric acid[2] and a fusion with sodium carbonate and potassium nitrate[2] were investigated. The soil was filtered or the melt dissolved to bring the arsenic into a solution.

Gravimetric methods for evaluating the arsenic were not investigated, owing to the general slowness of such procedures. The volumetric procedures investigated, namely, those involving titration with potassium iodate or iodine,[3] require the arsenic to be in the reduced state. After its removal from the soil the arsenic can be reduced by distillation from a hydrochloric acid solution and the use of hydrazine sulfate and sodium bromide as reducing agents,[4] or by heating with potassium iodide in an acid solution.[2]

Combinations of the methods mentioned above were tried, but none was completely satisfactory. Because a large sample is necessary to obtain a measurable quantity of arsenic, the fusion procedure required too much time and attention to obtain a satisfactory melt. The partial digestion method was simpler and faster, but an equilibrium was reached wherein

[1] U. S. Dept. Agr., Plant Quarantine and Control Admin. Service and Regulatory Announcement No. 100, 1929, pp. 133–134.
[2] Scott, W. W. *Standard Methods of Chemical Analysis*, 3rd ed., 1922, Vol. 1, pp. 34–55.
[3] Jamieson, G. S. *Ind. Eng. Chem.*, **10**, 290–292 (1918).
[4] Graham and Smith, *Ind. Eng. Chem.*, **14**, 207–209 (1922).

not more than 90 per cent of the arsenic could be recovered. It was thought that if the digestion were carried to completion and the organic matter in the soil destroyed complete recovery would be possible. Attempts were made to rid the soil of organic matter by using (a) nascent chlorine liberated from potassium chlorate,[2] (b) liquid bromine,[2] and (c) carbon tetrachloride and potassium bromide,[2] and also by taking the soil to dryness three times with nitric acid.[5] Although the results obtained by these methods were satisfactory, the procedures were too complicated and time-consuming.

With the idea of reducing and removing the arsenic as soon as it goes into solution, direct distillation of the soil from a hydrochloric acid solution with hydrazine sulfate-sodium bromide solution as the reducing agent was tried. In following this procedure there was a tendency for the contents of the distilling flask to boil over at the outset of the distillation, but this difficulty was finally overcome by careful regulation of the temperature. Moreover, instead of a sharp blue color, various shades of purple were obtained in titrating some samples with iodine. Use of the sodium bromate titration for arsenic[6] gave a satisfactory end point, but too much time was required to heat the sample prior to titration, and the bromine fumes liberated were very annoying to the analyst when a large number of samples were analyzed daily.

In experiments conducted in 1937 it was found that no discoloration of the distillate and interference at the end point occurred if 10 ml. of 30 per cent hydrogen peroxide and sufficient water to moisten were added to 50 grams of soil in the distillation flask, the oxidation reaction was allowed to go to completion, and distillation was carried out in the usual

TABLE 1.—*Recovery obtained on laboratory-prepared samples of soil containing acid lead arsenate*

ACID LEAD ARSENATE ADDED TO SOIL—		SAMPLES ANALYZED	MEAN RECOVERY	STANDARD DEVIATION	RANGE OF RECOVERIES	
PER 3 KG. OF MIXTURE	PER 50 GRAM SAMPLE					
grams	*grams*		*gram*	*gram*	*gram*	*per cent*
9	0.15	45	0.1498	0.0008	0.1482–0.1519	98.8–100.3
12	0.20	50	0.1999	0.0009	0.1978–0.2015	98.8–100.7
15	0.25	25	0.2487	0.0015	0.2456–0.2530	98.1–101.2

manner. However, this peroxide treatment increases the time of a determination so much that it is impracticable when a large number of samples are being analyzed. Also, by conducting the distillation so that not more than 150 ml. was distilled in 2 hours, and making additions of

[5] Greaves, J. E., *J. Am. Chem. Soc.*, 35, 150–156 (1913).
[6] *Methods of Analysis, A.O.A.C.*, 1935, 41–43.

hydrochloric acid before the volume in the distillation flask was less than 75 ml., the discoloration of the distillate was reduced sufficiently to prevent obscuring of the end point.

In testing the method, three samples were prepared in the laboratory by thoroughly mixing 9, 12, and 15 grams of acid lead arsenate with enough air-dry soil to give 3 kg. of mixture; 50 gram samples were analyzed, and suitable aliquots were titrated with iodine of a known titer. The results were calculated as grams of acid lead arsenate per 50 grams of mixture. The results are shown in Table 1.

Another series of 20 samples was prepared by weighing 0.2000–0.2036 gram of acid lead arsenate directly into the distilling flask and adding 50 grams of air-dry soil. The recoveries ranged from 97.7 to 100.8 per cent of the added acid lead arsenate.

In the first year (1930) the distillation procedure was used, 746 samples of soil from nursery sections treated with acid lead arsenate were analyzed for arsenic. It was necessary to reanalyze about 3 per cent because duplicates did not check within 50 pounds of acid lead arsenate per 3-inch acre. After the technic had been mastered, practically no analyses had to be repeated.

METHOD OF ANALYSIS

Directions for sampling.—To obtain a representative sample in areas of 20,000 square feet or less, make, preferably, a composite of 50 borings.

Preparation of samples.—Take samples directly from the composite, air-dry, and store until analyzed.

Reagents.—Use the reagents described in *Methods of Analysis, A.O.A.C.*, 1935, 41, 43, **3**, **6**.

Apparatus.—Use the apparatus shown in **4** of the same chapter of the A.O.A.C. methods cited above, with the following exceptions: (a) Use a spiral instead of a straight condenser, and (b) bend the delivery tube of the distillation flask so that when it is in position and connected to the condenser the condenser remains vertical. (This is done primarily to save space.)

Determination.—Place in the distilling flask 50 grams of air-dry soil containing arsenic. Add through the separatory funnel 50 ml. of the $N_2H_4 \cdot H_2SO_4 - NaBr$ solution and 50 ml. of HCl, close the flask, and heat very gently to boiling. Take considerable care at the beginning of the distillation to prevent the contents of the flask from boiling over. After the distillate has begun to pass over, increase the heat and conduct the distillation so that approximately 150 ml. passes over in 2 hours. When the volume in the flask is reduced to about 75 ml., add through the funnel 50 ml. more HCl and continue the distillation, repeating the addition of portions of HCl whenever the volume in the distilling flask is reduced to 75 ml. until 150–200 ml. of distillate has passed over. Wash down the condenser and all connecting tubes. Carefully transfer the distillate and washings to a liter volumetric flask, dilute to the mark, and mix thoroughly. Titrate the distillate with iodine as directed in **5**(a), p. 42, of the A.O.A.C. methods. Calculate the results as pounds of acid Pb arsenate per 3-inch acre and round to the nearest 50 pounds.

SUMMARY

The distillation procedure specifying hydrazine sulfate-sodium bromide solution as the reducing agent, followed by an iodine titration, is a satis-

factory method for determining arsenic in soils treated with acid lead arsenate. The distillation is conducted so that approximately 150 ml. is distilled in 2 hours, and additions of hydrochloric acid are made before the volume is reduced to 75 ml. This reduces the color of the distillate so that the end point is not obscured. The appearance of color can be prevented entirely by oxidizing the organic matter in the distillation flask with 30 per cent hydrogen peroxide prior to distillation.

VOLATILE OIL IN SAGE

By J. F. Clevenger (U. S. Food and Drug Administration, New York, N. Y.)

Most of the sage leaves used are grown in Jugoslavia and imported from Trieste. These are usually referred to as "Dalmatian" sage (*Salvia officinalis*). Limited amounts of sage herb are grown in Greece and imported from Athens. This is another species of sage, and it is usually referred to as Greek sage (*Salvia triloba*).

Dalmatian Sage

YIELD v/w*	SP. GR. 25°/25° C.	OP. ROT. 25° C.	REF. IND. 20° C.	AC. NO.	EST. NO.
1.9	0.925	+ 5.0	1.467	1.57	28.74
1.6	0.921	+ 5.2	1.465	2.23	20.71
1.6	0.923	+12.1	1.466	1.50	16.60
1.6	0.923	+13.5	1.463	1.50	19.12
1.8	0.923	+12.8	1.466	2.58	14.24
1.44	0.922	+ 4.5	1.462	2.2	21.30
2.3	0.917	+ 3.8	1.461	1.1	13.2
2.1	0.928	+15.2	1.464	3.5	21.5
1.7	0.937	+ 8.6	1.469	2.1	14.0
1.5	0.931	+ 7.9	1.464	0.9	10.8
1.2	0.935	+11.4	1.466	1.8	16.0
		Greek Sage			
2.4	0.917	−21.0	1.469	1.2	
2.6	0.913	− 4.1	1.464	1.0	20.0
2.5	0.917	− 6.2	1.473	1.5	19.7
2.5	0.915	−17.3	1.468	0.57	15.8
2.45	0.915	−16.3	1.469	0.5	14.6
2.3	0.918	−13.1	1.466	0.9	13.0
2.2	0.917	−11.4	1.470	1.4	13.0
2.4	0.909	−12.4	1.469	1.35	21.4
2.5	0.911	−12.4	1.469	0.98	14.7
2.4	0.907	−13.8	1.470	0.87	25.2
2.1	0.916	−16.6	1.471	1.75	24.1

* cc. per 100 grams of sage.

During the past seven years many of the importations of sage leaves in New York have been analyzed for the yield of volatile oil. Determinations have also been made of some of the physical and chemical characteristics of these oils.

The results reported here were obtained by the method outlined in *Methods of Analysis, A.O.A.C.*, 1935, 447–449.

To determine the extent of loss of volatile oil in sage leaves upon exposure in the laboratory, a portion of some coarsely ground uniformly mixed sage leaves was analyzed. The remaining portion of the sample was stored in the laboratory in an open shallow pan. Seven months later the remaining portion was analyzed. The results follow:

DATE	YIELD v./w.	SP. GR. 25°/25°C.	OP. ROT. 25°C.	REF. IND. 20°C.	AC. NO.	EST. NO.
1/25/38	1.6	0.923	+12.11	1.464	1.22	16.24
8/15/38	1.2	0.929	+13.15	1.461	1.23	14.6

CONCLUSIONS

(1) This investigation was limited to a study of the volatile oil in Dalmatian and Greek sage. The volatile oil from Dalmatian sage always gave a positive rotation and that from Greek sage always gave a negative rotation. This characteristic would be of value in distinguishing between the two varieties studied.

(2) The yield of volatile oil is generally greater for the Greek sage than for the Dalmatian sage.

(3) The variations in the acid and ester numbers are not considered significant.

(4) After coarsely ground sage leaves had stood seven months in the laboratory in an open pan, it was found that a significant loss in volatile oil resulted. This determination indicates that on long exposure a material proportion of the volatile oil may be lost.

OBSERVATIONS ON THE COLORIMETRIC METHOD FOR VANILLIN*

By A. L. Curl and E. K. Nelson (Food Research Division, Bureau of Agricultural Chemistry and Engineering, Washington, D. C.)

In collaboration with the Federal Agricultural Experiment Station at Mayaguez, Puerto Rico, the junior author attempted to use the colorimetric method described in *Methods of Analysis, A.O.A.C.*, 1935, 307, in determining the vanillin content of Puerto Rico vanilla.

The results obtained by the colorimetric method were found to be much

* Food Research Division Contribution No. 437.

higher than those by the gravimetric method, and a colorimetric determination made on the residue from the extraction of vanillin in the gravimetric method showed that a substance not vanillin remained and reacted with the reagent.

Accordingly, a study of the method was undertaken to find the cause of the discrepancy. The following table shows the results obtained when the lead reagent used to precipitate resins and tannins was a solution of 5 per cent neutral lead acetate and 5 per cent basic lead acetate for sugar analysis by the Horne method (later found to assay 68 per cent total lead and 16.8 per cent basic lead,[1] giving a ratio of neutral to basic lead in the final reagent of 6.3 to 1). Unless given otherwise, the vanillin results presented in this paper are expressed as grams per 100 cc.

	I GRAVIMETRIC	II COLORIMETRIC	III COLORIMETRIC AFTER EXTRACTION OF VANILLIN	IV SUM OF I AND III
10% Extract P.R. I	0.346	0.476	0.124	0.470
10% Extract P.R. II	0.206	0.336	0.134	0.340

In order to find whether this discrepancy in the results was due solely to the characteristics of Puerto Rico vanilla, 10 per cent extracts made from Bourbon, Java, Mexican, South American, and Tahiti vanilla beans* were examined. The following results were obtained:

	GRAVIMETRIC	COLORIMETRIC	DEVIATION
Bourbon	0.260	0.360	+0.100
Java	0.292	0.412	+0.120
Mexican	0.160	0.240	+0.080
South American	0.224	0.367	+0.143
Tahiti	0.168	0.218	+0.050

As it was then suspected that the basic lead acetate used was not sufficiently basic to precipitate all the interfering substances, a lead reagent was prepared with 10 per cent of the basic lead acetate containing 16.8 per cent basic lead (ratio of neutral to basic 3:1), No neutral lead acetate was added. The results are as follows:

	COLORIMETRIC	GRAVIMETRIC	DEVIATION
P.R.I.	0.356	0.346	+0.010
Bourbon	0.249	0.260	−0.011
Java	0.278	0.292	−0.014
Mexican	0.178	0.160	+0.018
South American	0.235	0.224	+0.011
Tahiti	0.159	0.168	−0.009

[1] Rosin, Reagent Chemicals and Standards, p. 224. D. Van Nostrand & Co. (1937).
* Specially made by David Michael & Co., Front & Master Sts., Philadelphia.

Then there was obtained a sample of basic lead acetate that assayed 73.4 per cent total lead and 33.5 per cent basic lead, a ratio of neutral to basic of 1.2 to 1. Using this sample the senior author made a lead reagent with 5 per cent each of neutral and basic, giving a ratio of neutral to basic lead of 2.8 to 1. This was used in the colorimetric determination on two extracts. The results follow:

	COLORIMETRIC	GRAVIMETRIC	DEVIATION
Mexican	0.171	0.160	+0.011
Tahiti	0.160	0.168	−0.008

To determine the effect of using a higher concentration of basic lead acetate, a 10 per cent solution with a ratio of neutral to basic lead of 1.2 to 1 was substituted for the 5 per cent neutral and 5 per cent basic. The results follow:

	COLORIMETRIC	GRAVIMETRIC	DEVIATION
Mexican	0.119	0.160	−0.041
Tahiti	0.104	0.168	−0.064

As described in *Methods of Analysis, A.O.A.C.*, 1935, and by Folin and Denis,[1] Leach,[2] or Snell,[3] the method gives no specifications for the basic lead acetate to be used, but these experiments show that the ratio of neutral to basic lead acetate should not be much greater or less than 3 to 1. A ratio of 6.3 to 1 was unsatisfactory, especially in vanillas of higher resin content, as it failed to precipitate interfering substances and caused high results. On the other hand, a reagent with a ratio of neutral to basic lead of 1.2 to 1 (10 per cent solution) carried down vanillin and led to low results.

The basic lead acetate used in making up the reagent for the colorimetric method for vanillin should therefore be assayed, and if the ratio of neutral to basic lead is much greater than 1 to 1 a new supply should be obtained.

The preservation of the standard vanillin solution used in the colorimetric method also requires attention. It was found that a standard vanillin solution increases in apparent strength when allowed to stand in a partially filled bottle. This is probably due to oxidation, as a full bottle, kept in the refrigerator, checked with a freshly made solution. A partially filled bottle kept several months at room temperature gave a color increase of 19 per cent over a freshly made standard.

[1] *J. Ind. Eng. Chem.*, 4, 671 (1912).
[2] Food Inspection and Analysis, 4th Ed., 1920, p. 922.
[3] Colorimetric Methods of Analysis, 2, 86–7 (1937).

In a colorimetric test on a solution of 0.1 gram of vanillic acid to the liter, 13 mm. depth matched a 0.1 gram/liter vanillin of 20 mm. depth, the vanillic acid giving about 50 per cent more color than the vanillin.

Folin and Denis[1] state that with the colorimetric method, with the standard set at 20 mm., no readings should be accepted as final if they fall much outside the limits of 15–30 mm.

In colorimetric determinations it is sometimes necessary to make a 1:2 dilution to bring the readings within the 15–30 mm. limit as shown in the following results:

	1ST READING	VANILLIN	2ND READING	VANILLIN	DEVIATION OF 1ST
					per cent
P.R. I	9.2	0.435	16.8	0.476	8.6
P.R. II	12.1	0.331	23.8	0.336	1.5

Snell[4] specifies a 20 per cent solution of sodium carbonate in place of the saturated solution used in the colorimetric method. This was found by the writers to be more satisfactory, as it causes less trouble with precipitation and is more convenient to prepare.

The solutions should be allowed to stand long enough to effect precipitation before being filtered, as a turbidity in the colorimeter will completely vitiate the results. Fifteen or twenty minutes is usually sufficient.

In carrying out the gravimetric vanillin method on extracts of pure vanilla beans, the ammonia extraction (designed to separate vanillin from coumarin) was dispensed with. Petroleum ether extractions of the vanillin residues were made, and the insoluble impurities, amounting to 3 to 14 mg., deducted from the weight of the crude vanillin.

The following table shows the effect of omitting the ammonia extraction in the analysis of authentic extracts of vanilla beans:

EXTRACT	NH_4OH EXTRACTION OMITTED	NH_4OH EXTRACTION PERFORMED	DEVIATION
Java	0.284	0.272	−0.012
South American	0.228	0.224	−0.004
Puerto Rico	0.332	0.340	+0.008
Bourbon	0.252	0.244	−0.008
Tahiti	0.152	0.147	−0.005
Mexican	0.156	0.147	−0.009
Average	0.234	0.229	−0.005

SUMMARY

(1) The basicity of the lead reagent should be high enough to equal a ratio of neutral to basic lead of approximately 3 to 1.

(2) The vanillin standard should be freshly prepared or kept in full bottles in a refrigerator. Old standards that have stood around in partly filled bottles should be discarded.

(3) To develop the color in the colorimetric method a 20 per cent solution of sodium carbonate is preferable to a saturated solution.

IDENTIFICATION OF FLAVORING CONSTITUENTS OF COMMERCIAL FLAVORS

VIII. SEMI-MICRO DETERMINATION OF THE AMIDO NITROGEN ATOM IN SEMICARBAZONES

By John B. Wilson*

While engaged in the work of identifying the volatile flavoring ingredients of a commercial flavor the writer encountered an aldehyde of unknown composition. A semicarbazone was formed, but the properties did not coincide with those of any known substance, and the quantity of material available was insufficient for the determination of nitrogen by Veibel's method,[1] which was used in Part I of this series[2] to establish the composition of the semicarbazones of several aldehydes and ketones. It seemed advisable to adapt the procedure to semi-micro proportions to permit such determinations to be made when 0.1–0.2 gram of sample is not available for the purpose, as required in the original procedure.

After some experimentation the procedure given below was worked out and found applicable to 10–25 mg. of sample, depending upon the content of nitrogen.

SEMI-MICRO DETERMINATION OF THE AMIDO NITROGEN ATOM IN SEMICARBAZONES

APPARATUS

Micro-Kjeldahl digestor.—Designed by E. P. Clark.[3]

Micro-Kjeldahl distilling apparatus.—Designed by E. P. Clark.[3]

REAGENTS

(a) *Potassium iodate solution.*—Dissolve 5 grams of KIO_3 in water and dilute to 100 cc.

(b) *Standard nitrogen solution.*—Place a quantity of NH_3 equivalent to about 40 mg. of N in a 100 cc. volumetric flask, add 25 cc. of water and a few drops of methyl red indicator, titrate with 0.1 *N* acid, and dilute to the mark with water (1 cc. of $N/10$ acid = 1.4 mg. of nitrogen in the standard solution.)

(c) *Sodium hydroxide solution.*—Dissolve 400 grams of NaOH in water and dilute to 1 liter.

(d) *Standard alkali solution.*—Use $N/70$ solution of NaOH, KOH, or $Ba(OH)_2$ (1 cc. of $N/70$ solution = 0.2 mg. of nitrogen).

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* Contribution from the Beverage Section of the Food Division, U. S. Food and Drug Administration, Washington, D. C.

[1] *Bull. soc. chim.*, 4th ser., **41**, 1410 (1937).

[2] Wilson and Keenan, *This Journal*, **13**, 389 (1933).

[3] *This Journal*, **16**, 255 (1933).

DETERMINATION

On a tared piece of cigarette paper about 35×40 mm., weigh 10–25 mg. of sample expected to contain 1–2 mg. of nitrogen. Place paper and sample in a micro-Kjeldahl flask, add 5 cc. of H_2SO_4 (1+4), and heat just to boiling on the digestor for 15–25 minutes, or until the sample is completely hydrolyzed. During hydrolysis, note the odor of the aldehyde or ketone as a key to its identity. If the mixture begins to darken (due to charring) dilute with 1–2 cc. of water and continue the hydrolysis.

When the hydrolysis is complete, wash down the neck of the flask with 1–2 cc. of water, using a dropper; add 1 cc. of the KIO_3 solution, mix, return to the digestor, and boil until the iodine vapor is completely removed (about 10 minutes). Wash down the neck of the flask as before and cool.

Set the resistance connected to the steam generator of the micro-Kjeldahl distilling apparatus so that 10 cc. will distil in 6–7 minutes. Transfer the solution from the digestion flask to the distilling apparatus, washing the flask 4–5 times with small quantities of water (about 10 cc. in all). Seal the outlet of the condenser with a 125 cc. Erlenmeyer flask containing 10 cc. of ±0.02 *N* acid and 0.1 cc. of methyl red indicator. Add 5 cc. of the NaOH solution to the distilling flask and steam distil for 4–5 minutes. Lower the receiving flask so that the outlet of the condenser no longer dips below the surface of the acid solution and continue the distillation for 1.5–2 minutes to wash out the condenser tube.

When the distillation is complete, heat the receiver on a hot plate just to boiling for 1 minute. Titrate the excess acid while hot with standard alkali solution. Just before the end point is reached, boil again for 1 minute and finish the titration with 1 or 2 drops of alkali. If more alkali is needed, return to the hot plate for further heating until not more than 2 drops are required to make the color change. Find the titer of 10 cc. of acid solution in the same way as for the distillate from the sample and subtract the latter to obtain the acid equivalent to the nitrogen present.

To standardize the alkali solution, pipet 5 cc. of the standard nitrogen solution into the distilling flask, add 10 cc. of water and 5 cc. of the NaOH solution, and distil as with the sample into 10 cc. of the same ±0.02 *N* acid and 0.1 cc. methyl red indicator.

The procedure was applied to several semicarbazones with the results given in Table 1.

The data show about the same degree of accuracy for the semi-micro method as for the macro method, but the tendency is toward high results rather than toward low results as in the case of the work reported in Part I of this series. Results obtained by the method may be used in the calculation of the molecular weight semicarbazones by the formula given in that paper as follows:

$$\text{Mol. wt.} = \frac{1400.8}{\text{percentage of nitrogen found}} - 57.05.$$

SUMMARY

Veibel's method for determining one atom of nitrogen in semicarbazones has been adapted to a semi-micro scale.

Results obtained on several semicarbazones are of the same order as those obtained by the macro method.

TABLE 1.—*Nitrogen in semicarbazones by the semi-micro method*

SEMICARBAZONE	SAMPLE	NITROGEN FOUND		THEORY
	mg.	*mg.*	*per cent*	*per cent*
Acetone	11.2	1.37	12.23	12.17
	16.0	1.96	12.25	
Benzaldehyde	10.2	0.88	8.62	8.59
	15.7	1.38	8.79	
	19.2	1.68	8.75	
	20.0	1.74	8.70	
Heliotropin	16.5	1.11	6.72	6.76
	25.4	1.70	6.69	
	21.5	1.47	6.84	
p-Methyl acetophenone	13.8	1.01	7.32	7.32
	20.6	1.52	7.38	
Vanillin	21.6	1.45	6.71	6.70
	24.8	1.65	6.65	

IDENTIFICATION OF FLAVORING CONSTITUENTS OF COMMERCIAL FLAVORS

IX. DETERMINATION OF β-IONONE, WHEN 1–10 MG. IS PRESENT

By JOHN B. WILSON*

In Part V[1] of this series the writer presented a method for the quantitative determination of β-ionone and the results of testing it out upon quantities as low as 10 mg. The procedure has now been successfully applied to commercial flavors containing quantities of β-ionone ranging from 50 to 200 mg. per liter of sample.

With products containing less than 10 mg. of β-ionone it was found that slight variations in the procedure simplified the technic without affecting the accuracy of the method. As precipitation of β-ionone-*m*-nitrobenzhydrazide does not occur readily in 10 cc. of alcohol (1+1) unless 20 mg. or more of the ionone is present, it is unnecessary to await the beginning of crystal formation before diluting the solution to an alcohol content of 30 per cent by volume. When 10 mg. or less is present, the hot solution of the ionone and the *m*-nitrobenzhydrazide reagent in 5 cc. of alcohol may be diluted at once with 10 cc. of hot water, acidified, and set aside for the beginning of crystallization. When this procedure was followed, quanti-

* Contribution from the Beverage Section of the Food Division, U. S. Food and Drug Administration, Washington, D. C.

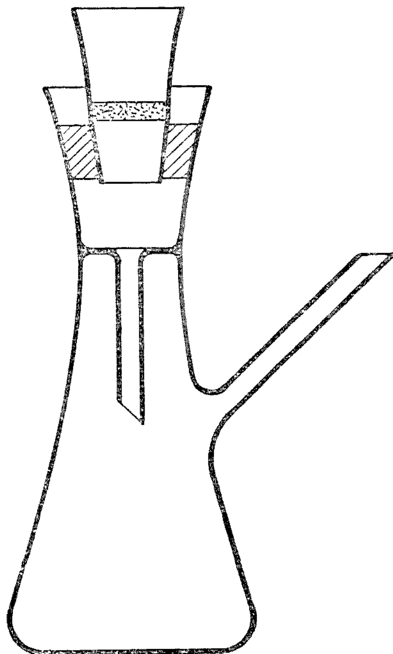
[1] *This Journal*, 22, 378 (1939).

ties of β-ionone as low as 2 mg. showed crystal formation within 30 minutes after being removed from the heat.

As a further aid in maintaining the accuracy of the method when such small quantities are to be precipitated, a number of ½ inch sintered glass disks were made from Pyrex glass according to the directions given by Kirk et al.[2] The disks were sealed into Pyrex tubing, which was then cut and fire polished so that crucibles 3 cm. long were formed with the disk about 1.5 cm. from the top. The crucibles weigh from 5 to 7 grams each and have a capacity of about 3 cc. It was found that 1 mg. of material could easily be removed from the disk for microscopic identification.

To accomplish filtration by suction a small flask was made from a heavy walled 50 cc. Erlenmeyer flask by adding a side tube of 5 mm. o. d. tubing about 50 mm. in length, at a point about 50 mm. above and ending about 80 mm. above the bottom of the flask. The neck of the flask was sealed off and built up for about 35 mm. above the seal. A drainage tube 5 mm. o. d. was then sealed in and the built-up portion flared to fit the crucible. A small piece of rubber tubing 1–1.5 cm. long was used to adapt the crucible to the flask (see figure).

Several solutions of known β-ionone content were prepared from a stock



SUCTION FLASK USED WITH SINTERED GLASS CRUCIBLES

[2] *Ind. Eng. Chem. Anal. Ed.*, **6**, 154 (1934).

solution, and the ionone was precipitated as β-ionone-*m*-nitrobenzhydrazide according to the following directions.

QUANTITATIVE PRECIPITATION OF β-IONONE WHEN 1–10 MG. IS PRESENT

Place 5 cc. of alcohol containing 1–10 mg. of β-ionone in a 50 cc. conical flask, add 90–95 mg. of solid *m*-nitrobenzhydrazide, and dissolve by warming the solution on the steam bath, taking precautions to prevent loss of alcohol through evaporation. Add 10 cc. of warm water, and if the solution becomes cloudy warm until clear. Remove the solution from the steam bath, add 0.2 cc. of glacial acetic acid, stopper the flask lightly, and place upon a wooden surface to prevent too rapid cooling. If 2 mg. or more of β-ionone is present, crystals begin to form withint 30 minutes after the contents of the flask have reached room temperature. Let stand in the room for at least 2 hours (overnight does no harm). Place in the refrigerator and leave overnight or up to 48 hours. Filter through one of the small sintered glass crucibles; wash with 15 cc. of cold 30% alcohol, using a wet policeman to remove precipitate adhering to the flask; and dry in a vacuum oven at 70° C. Weight of precipitate $\times$0.541 = corresponding weight of β-ionone.

TABLE 1.—*β-ionone in varying dilutions of stock solution*

SOLUTION	PRESENT	FOUND BY DETERMINATION				RECOVERY
		1	2	3	AV.	
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*per cent*
A	7.7	7.4	7.2	7.0	7.2	92
B	5.8	5.4	5.1	5.1	5.2	90
C	3.8	3.4	3.4	3.5	3.4	89
D	1.8	1.8	1.9	1.5	1.7	90
E	0.96	0.8	lost	0.8	0.8	83

To establish the effect of the volumes of sample and distillate upon the recovery of β-ionone, a number of steam distillations of known quantities of β-ionone were made. Both the liquid in the distillation flask and the distillate collected varied in volume. These data are given in Table 2.

The distillations were carried out as described in Part V of this series except for the volume of distillate collected. The distillates were extracted with 60, 30, and 30 cc. portions of ether. Before the distillate was extracted the first portion of ether was poured through the condenser to obviate mechanical loss of β-ionone. The *m*-nitrobenzhydrazide (90–95 mg.) was placed in a 50 cc. conical flask, and the three ether extracts and 0.2 cc. of acetic acid were added and were evaporated in rotation. The residue was taken up in 5 cc. of alcohol, and the precipitation carried out as described previously.

The data in Table 2 show that while in some cases most of the β-ionone in a 250 cc. sample is contained in the first 100 cc. of distillate, 150 cc.

TABLE 2.—*Recovery of β-ionone from varying volumes of solution and distillate*

EXPERIMENT NUMBER	VOLUME OF—		β-IONONE	
	SOLUTION	DISTILLATE	PRESENT	FOUND
	cc.	*cc.*	*mg.*	*mg.*
1	250	150	8	8.2
2	250	100	8	7.4
3	250	50	8	6.8
4	250	100	8	7.0
5	250	100	8	6.7
6	250	100	8	6.4
7	250	100	10	8.7
8	250	100	10	8.4
9	250	100	10	9.8
10	250	150	8.7	8.5
11	250	150	8.7	8.4
12	500	150	8.7	8.2
13	750	150	8.7	8.0

should be collected in order to be sure of complete recovery. When the volume of sample is increased there is a gradual falling off in the recovery (11–12–13), again indicating the necessity of collecting larger quantities of distillate in the case of larger volumes of sample.

To test the applicability of the procedure to flavored food products, two 100 gram portions of a commercial strawberry flavored gelatin dessert were placed in liter flasks; 5 cc. of a 40 per cent alcohol solution containing 8.7 mg. of β-ionone was added to one and 5 cc. alcohol solutions containing 1.9 mg. of β-ionone was added to the other. Each sample was then dissolved in 250 cc. of water, and the determination of β-ionone was made as directed previously. The results are given in Table 3.

TABLE 3.—*Recovery of β-ionone from gelatin dessert solutions*

EXPERIMENT NO.	GELATIN DESSERT	WATER ADDED	DISTILLATE	PRECIPITATE	β-IONONE—	
					FOUND	PRESENT
	grams	*cc.*	*cc.*	*mg.*	*mg.*	*mg.*
1	100	250	150	16.1	8.7	8.7
2	100	250	150	3.2	1.7	1.9

The results reported in Table 3 show that the procedure recommended can be depended upon to give good recoveries of β-ionone when 1–10 mg. is present.

SUMMARY

The method for the quantitative determination of β-ionone given in Part V of this series was modified to permit its use for quantities of less than 10 mg. of β-ionone. The procedure gave good results when applied to solutions of known quantities of β-ionone even when interfering agents were present.

BOOK REVIEWS

The Chemical Formulary. A Collection of Valuable, Timely, Practical Commercial Formulae and Recipes for Making Thousands of Products in Many Fields of Industry. Volume IV. Editor-in-Chief, H. Bennett. Chemical Publishing Company of New York, Inc., New York, N. Y. 1939. 638 pp. Price $6.00.

Undoubtedly the majority of chemists are acquainted with the preceding volumes of this work. The current volume comprises 572 pages of new and additional formulae in the same fields included in the earlier volumes. It also contains a new introductory chapter for beginners in the art of compounding chemicals. The omission from this volume of the useful table of common names of chemical products is perhaps more to be regretted than the further omission of the alcohol, temperature, and weights and measures tables.

To those who are not acquainted with this work, it may be stated that the sub-title of the book describes it very well. It is in fact more than the title indicates for in addition to formulae it has throughout its text concise but very informative explanations of the principles and practice of numerous useful arts. Its comprehensive character is indicated by the following titles picked at random from the index. "Artists' Crayons," "Retarding Staling of Bread," "Dog Bath Powder," "Vacuum Tube 'Getter'," "Destroying Yellow Jackets," "Worcestershire Sauce," "Stopcock Lubricants." The book is indeed an encyclopedia of chemical compounding.

The sections entitled "Beverages, Liquors and Flavors," "Cosmetics and Drugs," and "Food Products" should be of particular interest to members of the A.O.A.C. engaged in regulatory work. They contain clues to substances that are likely to be found in new products which present to the chemist analytical problems in the enforcement of laws relating to these products. It is regrettable that there is no warning in this volume to prospective manufacturers in regard to the applicability of the Federal Food, Drug, and Cosmetic Act and similar laws to many of the products described.

Though its primary appeal is to those engaged in the chemical arts, The Chemical Formulary contains abundant material of interest and practical usefulness to the worker in any field of chemistry.—EDWARD O. HAENNI.